THE REGULATION OF GENE EXPRESSION DURING MEMORY CONSOLIDATION IN THE HIPPOCAMPUS

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ABSTRACT

THE REGULATION OF GENE EXPRESSION DURING MEMORY CONSOLIDATION IN THE HIPPOCAMPUS

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Memory consolidation is the process through which short-term memories are stabilized for long-term retention. New gene expression is required for this process to occur successfully. Although gene expression is a necessary component for memory consolidation, the targets and regulation of this gene expression are not well understood. The advent of next-generation sequencing technologies has provided a tremendous resource to probe important questions genome-wide in ways that were previously impossible. In this dissertation, I use next-generation sequencing to investigate the transcriptional targets of learning in the hippocampus. **Chapter 1** reviews the previous research on the regulation of gene expression during memory consolidation. Previous work has implicated histone acetylation as an epigenomic modification that regulates long-term memory. In **Chapter 2**, I use RNA-seq to investigate the gene expression changes that occur 30 minutes after contextual fear conditioning. I use recently developed analysis techniques to improve our ability to detect changes and study alternative splicing genome-wide for the first time after learning. **Chapter 3** investigates whether these gene expression changes are specific to contextual fear conditioning or shared with other hippocampus-dependent learning tasks such as object-location memory. I find that the transcriptional targets are similar between training paradigms, but their temporal activation differs. In **Chapter 4**, we use ChIP-seq, Sono-seq and MNase-

seq to determine changes in histone acetylation, chromatin accessibility and nucleosome positioning that occur in response to learning. I find only small changes in H3K9/14ac, but large changes in chromatin accessibility. This may suggest that a multitude of histone modifications act in concert to regulate chromatin accessibility during memory consolidation.

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CHAPTER 1: Epigenomic Regulation Of Gene Expression In Long-term Memory

Abstract

Long-term contextual memory is formed in the hippocampus through a transcription-dependent process known as memory consolidation. This transcription is regulated by histone acetylation, an activating epigenetic mark. Determining the specific lysine residues and genetic targets of histone acetylation during learning is crucial to understanding the transcriptional regulation that occurs in the hippocampus. Chromatin accessibility, the result of histone acetylation changes, is also poorly studied during memory consolidation. Next-generation sequencing technology has fostered unprecedented discoveries in the field of gene expression and epigenetic regulation, but has yet to uncover the genetic regulation that occurs in response to learning. In this work, I use high-throughput techniques to investigate the gene expression and epigenetic changes that occur in the hippocampus during memory consolidation.

1.1 The hippocampus and contextual memory

A role for the hippocampus in forming episodic memories in humans was first described by Scoville and Milner over 50 years ago [1]. Our understanding of the role of the hippocampus in long-term memory has since been greatly supported by rodent studies. Long-term contextual memories are formed in the hippocampus of the rodent through a process involving distinct stages of acquisition, consolidation, maintenance and retrieval [2]. Contextual fear conditioning is a behavior that allows for dissection of particular phases of memory in the hippocampus due to the temporal resolution of using only one brief training session [3, 4]. Contextual fear conditioning consists of placing a mouse in a novel context that is paired with an aversive foot shock. The memory is then tested by measuring freezing, a species-specific fear response, at 1 hour after training for short-term memory and 24 hours after training for long-term memory (**Figure 1.1**). Contextual fear memory formation depends on at least two brain systems: the hippocampus and the amygdala [4]. Hippocampal lesions impair acquisition [5] and produce temporally graded retrograde amnesia [6, 7] for contextual fear memory. Thus, components of contextual fear memory traces appear to be formed and stored in the hippocampus for weeks prior to systems-level consolidation into the cortex [8].

1.2 Long-term contextual memory and CREB-dependent transcription

Memory consolidation, the process by which memories become stored [9, 10], requires transcription to convert labile short-term memories into stable long-term memories. The need for transcription in this process was first demonstrated over 40 years ago through the ability of actinomycin D, a transcription inhibitor, to block long-term memory in goldfish [11]. This finding was extended by studies demonstrating that two necessary

waves of gene transcription and protein translation occur in the hippocampus after memory formation and correlate with previously determined waves of PKA activity [12, 13]. Although transcription has been known to be involved in memory formation for many years, the processes regulating this transcription have only recently begun to be unraveled [14].

In the hippocampus, there are two time windows after learning that exhibit increased phosphorylation of the transcription factor cAMP-response element-binding protein (CREB), which activates this protein and is thought to mediate memory-promoting transcription [15]. These two windows of CREB phosphorylation, 0-30 min and 3-6 hr after training, coincide strikingly well with the two time windows during which inhibition of transcription or translation impairs memory storage [13, 16]. These gene expression changes after learning occur in between 18-40% of the neurons in the pyramidal cell layers of the hippocampus, depending on the specific subregion observed [17, 18]. Importantly, long-term memory is affected in both flies [19] and mice [20, 21] bearing mutations in CREB, highlighting the importance of this transcription factor. Although CREB is a necessary transcription factor regulating the transcription required for long-term memory formation, studies have shown that CREB phosphorylation alone is not sufficient to drive expression of target genes [22], indicating that additional coactivators of CREB are required for transcription of target genes. One of these coactivators, CREB-binding protein (CBP), has been shown by our lab and others to be crucial for long-term memory [23-28]. CBP is a histone acetyltransferase (HAT), an enzyme that adds acetyl groups to the N-terminal tails of histones to activate transcription.

1.3 Epigenetic regulation of transcription

Substantial advances in our understanding of the regulation of transcription have been made in the field of epigenetics, which is the study of stable alterations of gene expression that do not involve changes in the underlying DNA sequence. The fundamental unit of chromatin is the repeated nucleosome structure, which consists of the four core histones, each in duplicate, tightly encircled by genomic DNA. The amino-terminal tails of histone proteins protrude out of the nucleosome and are sites for post-translational modifications that regulate the ability of the transcriptional machinery to bind to and transcribe the underlying genes. Specific amino acid residues on histone tails are targets for acetylation, methylation, phosphorylation, sumoylation, and/or ubiquitination among other modifications [29]. These modifications are postulated to form a combinatorial code that defines the transcription status of a given loci. This is known as the “histone code” hypothesis [30]. The epigenetic landscape at promoters can regulate accessibility of chromatin for current or future transcriptional activation [31, 32].

In general, histone acetylation and phosphorylation are activating marks while histone methylation can be activating or repressing depending on the lysine residue targeted [29]. Each modification is expected to have a “writer” that deposits the mark, a “reader” that binds to the mark for a given function, and an “eraser” that removes the mark [31, 33]. In the case of histone acetylation, histone acetyltransferases (HATs) are the “writers” that add acetyl marks, histone deacetylases (HDACs) are the “erasers” that remove acetyl marks, and bromodomain-containing proteins can target and bind histone acetylation as “readers” [33]. Histone modifications also often associate with another epigenomic mark, DNA methylation [34], to facilitate or repress transcription. The epigenetic code at a given locus is thought to regulate the accessibility of chromatin in that region. The accessibility of chromatin regulates whether transcriptional machinery

will bind [35, 36]. Given this complexity, it is important to determine which chromatin modifying enzymes are required for a process such as memory before studying the epigenetic code resulting from learning.

1.4 The role of histone acetyltransferases in long-term contextual memory

The concept of DNA-histone complexes regulating memory was first speculated as early as 1975 [37]. Several proteins that interact with phosphorylated CREB are histone acetyltransferases (HATs), including CBP, p300 and p300/CBP associated factor (PCAF). Histone acetylation appears to play a critical role in a number of psychiatric and neurological disorders including depression, schizophrenia and intellectual disability [38, 39]. Therefore, these histone acetyltransferases may be crucial regulators of the transcription necessary for long-term memory. Studies have shown that each of these HATs serves a role in distinct types of memory formation (**Table 1.1**). Mice lacking *Pcaf* display short-term memory deficits that gradually worsen with age [40]. Although this finding may appear to link PCAF histone acetyltransferase function with memory, short-term memory is transcription-independent and therefore this deficit is likely due to developmental defects in the knockout animals. Morphological analysis of the mutant mice showed loss of cells in the CA1 and CA3 subfields of the hippocampus, further supporting the notion of developmental defects causing memory impairments in these mice [40]. Mice overexpressing a truncated form of *p300* [41] and mice with conditional *p300* deletion [42] demonstrate selective long-term memory deficits in both contextual fear conditioning, which is hippocampus- and amygdala-dependent and object recognition memory, which is hippocampus-independent [43]. Hippocampus-dependent spatial memory is not affected in either of these mice, which indicates p300 is not

required for this form of memory and may suggest that the memory deficits in the p300 mutant mice are due to transcriptional effects in brain regions outside the hippocampus.

The most thoroughly studied histone acetyltransferase in memory is CREB binding protein (CBP). CBP is a transcriptional coactivator that is recruited to phosphorylated CREB [44], as well as other transcription factors [45]. Mutations of *Cbp* or *p300* in the human population cause a form of intellectual disability termed Rubinstein-Taybi syndrome [46]. Recent studies by our lab and others have demonstrated that the histone acetyltransferase CBP is required for efficient long-term memory consolidation [23-27]. Six independently generated *Cbp* alleles disrupt long-term memory without impairing short-term memory [23-27, 47]. We have produced mice in which CBP activity in neurons is reduced by the transgenic expression of an inhibitory form of CBP lacking the HAT domain [26]. These mice exhibit selective deficits in long-term contextual memory, underscoring the importance of the histone acetyltransferase activity of CBP for memory consolidation. As a coactivator with intrinsic histone acetyltransferase activity, CBP interacts with numerous transcription factors and contains multiple functional domains. Importantly, mice homozygous for a mutation in the kinase-inducible interaction (KIX) domain of CBP, in which CBP is unable to interact specifically with the CREB/ATF transcription factor family, are impaired in long-term memory formation [27, 48]. It was reported that mice with forebrain-specific full *Cbp* deletion displayed both short- and long-term memory deficits [49], a result that has not been observed with any previous C*bp* mutant mice. However, deletion of CBP in adult mice using viral Cre expression causes deficits in synaptic plasticity and a long-term form of hippocampus-dependent memory selectively [28]. This finding would indicate that the deficits in short-term memory observed in mice with the CaMKIIα-driven

forebrain deletion were probably due to developmental defects, rather than due to a role of CBP in short-term memory. Taken together, the studies of the role of the histone acetyltransferase CBP in long-term memory consolidation suggest that histone acetylation may be a crucial regulator of transcription during memory consolidation.

1.5 Acetylation of specific lysine residues on histone tails may be important during memory consolidation

Because individual HATs are known to acetylate specific lysine residues on histone tails, the possibility exists that acetylation of particular lysine residues controls the transcription necessary for long-term memory (**Table 1.2**). Although the first evidence for histone acetylation occurring during memory consolidation came by studying the incorporation of radioactive acetyl-CoA into histones in 1979 [50], the first study to demonstrate changes in particular histone acetylation marks was made in 2004 with the finding that acetylation of lysine 14 on histone H3 increases in bulk histone extracts one hour after contextual learning [51]. This mark returns to baseline levels by 2 hours after fear conditioning [52]. A study by Peleg et al. [53] has found multiple acetylation marks upregulated one hour after contextual fear conditioning including lysine 9 of histone H3 and lysines 5, 8 and 12 of histone H4. These changes were studied in both young mice, which are able to properly form long-term memories, and aged (16 month old) mice, which have long-term memory deficits. Lysine 12 of histone H4 was the only mark specifically increased in young mice but not in aged mice, indicating that this acetylation mark may be a key regulator of the transcription necessary for long-term memory formation.

The most direct method to determine the histone acetylation marks pertinent to memory formation would be to study those affected by the histone acetyltransferases known to be required during memory consolidation, such as CBP. *In vitro*, CBP and p300 have multiple overlapping lysine targets on histone tails. However, recent *in vivo* work has suggested a more circumscribed role for individual HATs in regulating acetylation of specific lysine residues. Work by Jin et al. [54] has found that knockout of PCAF in mouse embryonic fibroblast cells only decreases acetylation at lysine 9 of histone H3, whereas loss of CBP/p300 showed remarkable specificity for decreasing acetylation of lysines 18 and 27 of histone H3. It is important to note that changes in acetylation of lysines 18 and 27 of histone H3 were not examined in previous studies of bulk histone acetylation changes after learning. The observed specificity of CBP/p300 histone acetylation in fibroblasts could be due to compensation by other histone acetyltransferases at a subset of CBP/p300 target lysines or the guiding of HAT activity by accessory components that provide target specificity. In either scenario, the targets of specific HATs may be different in the hippocampus, the site where memory is consolidated. To bypass these problems, Barrett et al. have used a focal viral deletion of CBP to study the histone marks regulated by CBP in the adult mouse hippocampus [28]. Viral deletion has the benefit of temporal control, which limits compensation by other histone acetyltransferases, as well as stereotaxic control to only affect the brain region of interest. Using this technique, acetylation differences were observed on lysine 14 of histone H3, lysine 12 of histone H2B, and lysine 8 of histone H4, but not the mark decreased in aged mice, lysine 12 of histone H4 [28]. Lysines 18 and 27 of histone H3 were not studied in this work. Determining the genes regulated by these acetylation marks during memory consolidation promises to uncover interesting targets that are important for long-term memory, and future methods targeting these specific lysine

residues could hold potential for novel therapeutics that would improve memory formation while limiting side effects.

1.6 Histone deacetylase inhibitors increase long-term memory

The increases in histone acetylation during memory consolidation suggest that artificially increasing histone acetylation could enhance long-term memory. Histone acetylation at a promoter is controlled through a delicate balance of HATs, such as CBP, that add acetyl groups to specific lysine residues on histone tails, and histone deacetylases (HDACs), which remove acetyl groups from these lysines (**Figure 1.2**). Increasing histone acetylation could be achieved either through enhancing HAT activity or by reducing memory-suppressing HDAC activity. HDAC inhibitors, which increase histone acetylation, given during memory consolidation enhance long-term memory [55, 56]. Our work has shown that the memory enhancement by the HDAC inhibitor trichostatin A (TSA) requires the CREB-CBP interaction, indicating that CREB target genes are those required for the memory enhancement. Surprisingly, TSA increased expression of only two out of fourteen CREB target genes examined, the orphan nuclear receptors *Nr4a1* and *Nr4a2* [55]. It was previously shown that knock-down of *Nr4a2* expression impairs long-term memory formation in a spatial discrimination task [57, 58], and global constitutive, heterozygous *Nr4a2* knockout mice are impaired in long-term memory formation in the hippocampus-dependent passive avoidance task [59] indicating that this gene expression change may be important for the memory enhancing effects of HDAC inhibitors [60]. A study by our lab found that mice expressing a dominant negative form of NR4A to block all family members display long-term memory deficits [61]. Excitingly,

these mice also do not respond to TSA, indicating this gene family is a crucial regulator of memory enhancement by TSA.

Determining the molecular targets and pathways affected by HDAC inhibitor treatment promises to uncover new genes necessary for contextual memory as well as providing novel avenues for therapeutic intervention for diseases in which memory is affected. Studying the role of particular HDACs in memory formation is another strategy that could lead to development of selective pharmacological agents that cause memory enhancement. HDACs are classified into four families (I-IV) based on sequence homology and structure [62]. Current studies indicate a role for class I HDACs in long-term memory formation (**Table 1.3**). Class I HDACs act in the nucleus and include HDACs 1-3 and 8. Genetic evidence has demonstrated that both HDAC2 and HDAC3, but not HDAC1, are required for long-term memory consolidation [58, 63]. Targeting only those specific HDAC proteins that dampen memory formation could lead to the application of selective therapeutics with greatly reduced side effect profiles compared to broad-spectrum HDAC inhibitors. This is especially important considering that chronic treatment of broad-spectrum HDAC inhibitors, such as TSA, causes synaptic dysfunction [64].

1.7 Hippocampal gene regulation after contextual fear conditioning

The genes regulated by contextual fear conditioning have received tremendous attention as potential targets to modulate memory. Early investigations discovered a set of inducible transcription factors, known as immediate early genes (IEGs), regulated by learning [65]. These include the genes *c-fos* and *Egr1* (*zif268*) that are induced by neuronal activity. Also, brain-derived neurotrophic factor (*Bdnf*) expression levels

increase in CA1 after contextual fear conditioning [66]. Mutant mice with any of these genes knocked out demonstrate they participate in long-term memory [67-69]. Microarray analyses after contextual fear conditioning discovered additional phosphatases (*Dusp1*) and nuclear receptors (*Nr4a1*) among other genes, but the genes regulated by learning did not show much overlap between labs [70, 71]. Our lab has focused on CREB-dependent genes [72], including *Nr4a1* and *Nr4a2*, which both increase after contextual fear conditioning and are required for long-term memory [61]. We have also found that CREB-dependent *Gadd45b* and *Gadd45g* increase after contextual learning and that *Gadd45b* knockout mice show selective long-term memory deficits [73].

Despite this progress, discovery of the genes regulated by contextual learning remains challenging. This is because of the heterogeneity of the hippocampus, which dilutes real signal from activated excitatory neurons with signal from unactivated excitatory neurons, glia and inhibitory neurons. Thus, the gene expression changes observed are often small. Statistical methods for removing variance have begun to correct this problem, but have yet to be applied genome-wide to data after learning.

1.8 Hippocampal gene regulation after spatial memory tasks

Although the targets and temporal dynamics of gene expression after contextual fear conditioning have been well studied, transcription after other spatial memory tasks is less understood. Object-location memory is a hippocampus-dependent spatial memory task in which a mouse learns the spatial orientation of objects within a novel chamber (**Figure 1.3**). Unlike contextual fear conditioning, object-location memory does not rely on a noxious footshock to induce a learning event. This is important because there is a

concern that some gene expression changes in the hippocampus could be caused by fear instead of learning [74]. Because of the propensity for mice to explore novelty, when an object is moved to a new location in the box 24 hours later, the mouse will preferentially explore that object over the other non-displaced objects [43, 56, 75]. Spatial memory tasks such as object-location memory induce gene expression of many of the same genes observed to change after fear conditioning [76], but there have been few large-scale studies of the targets and temporal dynamics of gene expression after these tasks.

Histone acetylation has also been implicated in long-term spatial memory. Viral deletion of the histone acetyltransferase CBP in the adult hippocampus blocks long-term (but not short-term) object-location memory [28]. Acetylation of lysine 12 of histone H2B and lysine 12 of histone H4 are both increased in bulk histone extracts during spatial memory consolidation [77]. Our lab has found that class I HDAC inhibition using MS275 enhances long-term object-location memory [56]. MS275 has highest affinity for HDACs 1 and 2, moderate affinity for HDAC3, and low affinity for HDAC 8. Another HDAC inhibitor that preferentially binds to HDAC3, RGFP136, has also been shown to enhance long-term memory using this task [58]. It is currently unclear whether HDAC2 and HDAC3 have overlapping genetic targets important for memory formation, or whether each regulates expression of distinct genes.

1.9 Next-generation sequencing technology and gene regulation in the hippocampus

Research into gene expression and chromatin regulation has been revolutionized by the advent of next-generation sequencing technology. RNA-seq takes advantage of high-

throughput sequencing to quantify gene expression levels of the entire transcriptome. RNA-seq provides increased sensitivity over microarrays, allows for discovery of novel gene isoforms, and can be used to study alternative splicing [78]. ChIP-seq couples chromatin immunoprecipitation (ChIP) with next generation high throughput sequencing to determine the location of chromatin bound proteins genome wide. ChIP-seq can be used to study histone modifications or transcription factor binding. DNase-seq [79], FAIRE [80], ATAC-seq [81], and Sono-seq [82] are all methods to isolate "open" regions of chromatin. Chromatin being described as "open" or "loosened" means that these regions are thought to be accessible for binding of transcription or epigenetic factors. It is expected that a change in epigenetic modifications would result in alterations in this accessibility, with activating marks such as histone acetylation causing more opening. These powerful sequencing techniques are only recently being applied to studies of long-term memory [53, 76, 83], but often with too few biological replicates to be statistically meaningful in the brain. Bioinformatic analysis of high-throughput sequencing data is a rapidly evolving field and results obtained are highly dependent on analysis method, so implementation of the proper algorithms is necessary to gain an understanding of biological phenomena.

In this work, I use next-generation sequencing technology to understand gene regulation in the hippocampus after learning and stress. **Chapter 2** utilizes RNA-seq to determine the gene and exon-level gene expression changes that occur 30 minutes after contextual fear conditioning. **Chapter 3** uses a microfluidic high-throughput qPCR system to study gene expression after object-location memory, a spatial memory task that does not rely on a noxious footshock to study memory. **Chapter 4** utilizes ChIP-seq

and Sono-seq to study the chromatin regulation that occurs 30 minutes after fear conditioning to regulate gene expression.

Figure Legends

Figure 1.1. Contextual Fear Conditioning Paradigm. In contextual fear conditioning, a mouse is placed into a novel context, allowed to explore, and delivered a mild footshock. The mouse is then returned to the homecage. Upon being reintroduced to the training context, the mouse will exhibit the species-specific fear response of freezing. The time spent freezing can be measured as a correlate for how well the mouse associates the context with the footshock.

Figure 1.2. Regulation of histone acetylation at promoters of genes necessary for long-term memory. After learning, CREB is activated by phosphorylation, binds to CREB response elements (CREs) in the genome, and recruits the coactivator CBP to the region. Acetyl groups are added to lysine residues on histone tails by the histone acetyltransferase function of CBP. Acetylation is removed by class I histone deacetylase (HDAC) proteins.

Figure 1.3. Object-location memory paradigm. In object-location memory, mice are trained to learn the spatial configuration of 3 objects relative to a cue on one wall. After 3 training sessions, mice are removed to their homecage. 24 hours later, mice are placed back in the context with one object displaced. Because mice naturally prefer novelty, they will explore the displaced object more than non-displaced objects.

Table 1.1. Summary of hippocampus-dependent memory in HAT mutant mice. PCAF mutant mice had impaired shock sensitivity, so fear conditioning was not measured. Impaired short- and long-term spatial memory in these mice suggests a

transcription-independent mechanism. Both p300 mutant mice show impaired long-term contextual fear conditioning, but normal spatial memory suggesting a non-hippocampal effect. CBP mutant mice generally show selective hippocampal long-term memory deficits.

Table 1.2. Summary of HAT homology and lysine modifications. Each HAT in the mouse genome is shown within a homology family with known targeted lysines. If a predominant mark is known, it is shown in bold. Clock has homology to both the SRC and MYST family, but is shown in the SRC family in this table.

Table 1.3. Summary of HDAC classes and relation to long-term memory. Class I HDACs have been highly implicated in long-term memory. HDACs 4, 5, and 6 also have evidence linking them to memory but this may or may not be through regulating histones. HDAC3 was shown to regulate memory in a CBP-dependent manner.

Contributions

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Figures

Figure 1.1

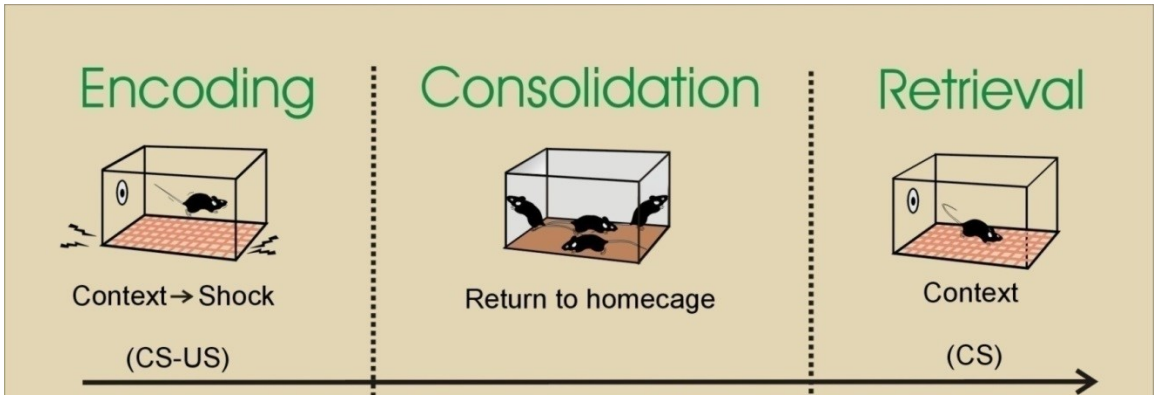
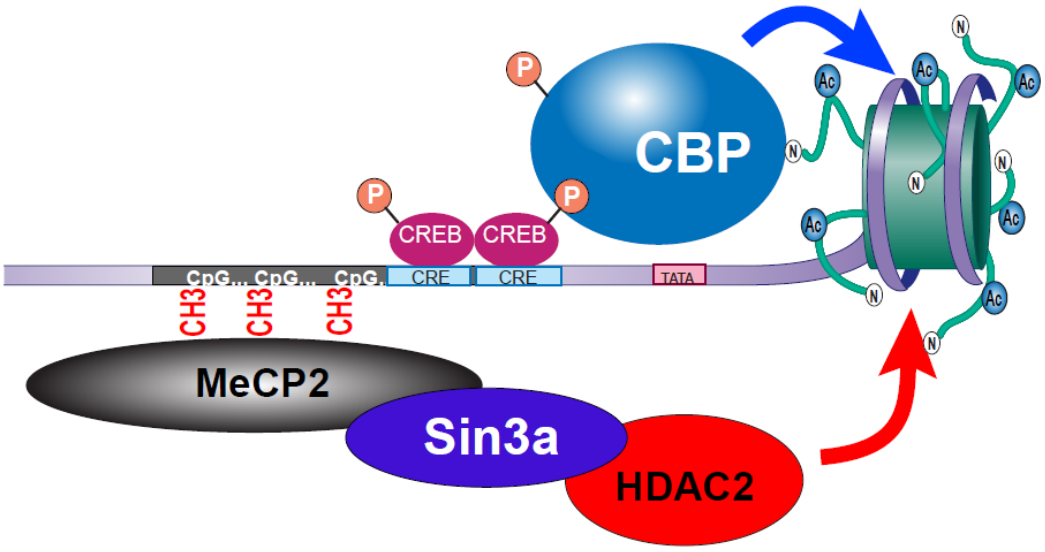


Figure 1.2


CBP
P
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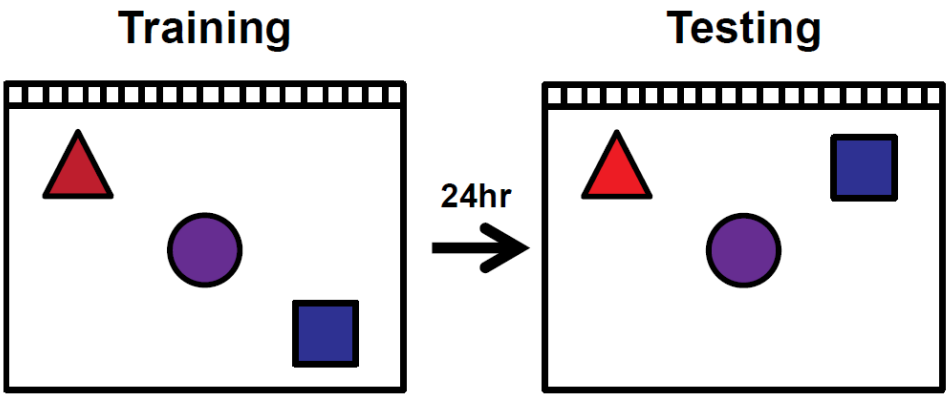
CREB
CREB
CpG... CpG... CpG...
CRE
CRE
TATA
CH3
CH3
CH3
MeCP2
Sin3a
HDAC2
Ac
N

Figure 1.3



Tables

Table 1.1

Mouse model	Short-term Contextual FC	Long-term Contextual FC	Spatial Memory
PCAF KO	?	?	Impaired (short and long-term, progresses with age)
Truncated p300	Normal	Impaired	Normal
Conditional p300 KO	Normal	Impaired	Normal
Truncated CBP (CBP+/-)	Normal	Impaired	?
CBP-kix mutant	Normal	Impaired	Impaired (water maze)
Truncated CBP (CBPΔ1)	Normal	Impaired	?
CBP+/-	Normal	Impaired	Normal (water maze)
CBP HAT-	Normal	Normal	Impaired (water maze)
Floxed CBP (CaMKIIα-cre)	Normal [48] Impaired [50]	Normal [48] Impaired [50]	Normal (water maze) [48] Impaired [50]
Hippocampal CBP knockout (Cre virus)	Normal	Impaired	Impaired (OLM)

Table 1.2

GCN-related Family		
Name	***Other Names***	***Lysine Modified***
Gcn5	Kat2a	**H3K14,** H3K18 [84]
Pcaf	Kat2b	**H3K9** [54]
Hat1	Kat1	**H4K5, H4K12**, H2AK5 [85]
Elp3	Kat9	H3K14, H4K8 [86]
Atf2		H4, H2B [87]
MYST Family		
Name	***Other Names***	***Lysine Modified***
Myst1	MOF, Kat8	**H4K16** [88]
Myst2	Hbo1, Kat7	**H3K14** [89]
Myst3	MOZ, Kat6a	**H3K9** [90]
Myst4	Morf, Kat6b	H3/H4 [91]
TIP60	Kat5	H2AK5, H3K14, H4K5, H4K8, H4K12, H4K16 [92]
CBP/p300 family		
Name	***Other Names***	***Lysine Modified***
Crebbp	CBP, Kat3a	H3K14, **H3K18**, **H3K27**, H4K5, H4K8, H4K12, H4K16 [54, 93]
EP300	p300, Kat3b	H3K14, H3K18, H4K5, H4K8, H4K12, **H4K16** [54, 93]
SRC (Nuclear Receptor Coactivators)		
Name	***Other Names***	***Lysine Modified***
Ncoa1	Src1, Kat13a	H3K9, H3K14, H4 [94]
Ncoa2	Src2, Tif2, Kat13c	Unknown

Ncoa3	Src3, Actr	Unkown
Clock	Kat13d	H3/H4 [95]
Other		
Name	***Other Names***	***Lysine Modified***
TAF1	TAFII250, Kat4	H3K14, H4 [96]
Gtf3c1	TFIIIC90, Kat12	H3K14 [97]
Cdyl		H4, H2A [98]
Mgea5	NCOAT	H3K14, H4K8 [99]
Naa50		Unknown
Naa60	Nat15, Hat4	**H4K20, H4K79, H4K91** [100]

Table 1.3

Class I			
Name	***Typical Location***	***Histone Target***	***Relation to Long-term Memory***
HDAC1	Nucleus	Yes	May regulate fear extinction [101]
HDAC2	Nucleus	Yes	Negative regulator of fear memory [63] and extinction [102]
HDAC3	Nucleus/cytoplasm	Yes	Negative regulator of spatial memory [58]
HDAC8	Nucleus	Yes	Unknown
Class IIA			
Name	***Typical Location***	***Histone Target***	***Relation to Long-term Memory***
HDAC4	Nucleus/cytoplasm	Yes	Positive regulator of memory in Drosophilia [103]
HDAC5	Nucleus/cytoplasm	Yes	HDAC5 -/- mice have impaired spatial memory [104]
HDAC7	Nucleus/cytoplasm	Yes	Unknown
HDAC9	Nucleus/cytoplasm	Yes	Unknown
Class IIB			
Name	***Typical Location***	***Histone Target***	***Relation to Long-term Memory***
HDAC6	Cytoplasm	No	HDAC6 reduction improves memory in Alzheimer's model [105, 106]
HDAC10	Nucleus/cytoplasm	Yes	Unknown
Class III			

Name	***Typical Location***	***Histone Target***	***Relation to Long-term Memory***
SIRT 1-7	Nucleus/cytoplasm	Yes	SIRT1 loss impairs memory by increasing miR-134 [107]
Class IV			
Name	***Typical Location***	***Histone Target***	***Relation to Long-term Memory***
HDAC11	Nucleus/cytoplasm	Yes	Unknown

CHAPTER 2: Determining The Transcriptional Targets Of Contextual Fear Conditioning

Abstract

Memory consolidation requires transcription to form long-term memories. Significant effort has been dedicated to characterizing the genes that change expression in the hippocampus in response to contextual fear conditioning. However, the genome-wide targets of this transcription have not been thoroughly studied. Previous work studying gene expression on a genome-wide scale after contextual fear conditioning has identified only a small number of genes and has not revealed consistent results between labs. In this work, we use RNA-seq in combination with a novel normalization method that allows reliable and consistent expression differences to be discovered. Using this method, we identified 823 genes regulated after contextual fear memory. These genes cluster with transcriptional regulation, MAPK signaling, calcium signaling and synaptic transmission – functional classes known to be involved in learning and memory. In addition to gene expression differences, RNA-seq also allows exon-level transcription analysis. Studying alternative splicing by differential exon usage during memory consolidation has not been previously attempted genome-wide. Here, we use RNA-seq to determine exon-level changes in expression after contextual fear conditioning. We discovered that *Ania-3*, a short variant of *Homer1* which has not been studied after learning, is regulated by contextual fear conditioning. The ribosome biogenesis regulator *Las1l* and the RNA-binding protein *Rbm3* also change specific exon usage after fear

conditioning. This analysis discovered novel gene and exon regulation after learning that is unlikely to be detected by other methods.

Introduction

Contextual fear conditioning requires two waves of transcription and protein synthesis to form long-term memory in the hippocampus [16, 108]. Our lab and others have focused on discovering the genetic targets of these transcriptional waves using both candidate gene and genome-wide approaches. Our research has indicated that the first wave of transcription induces the largest change in gene expression at 30 minutes after contextual learning [61, 109]. However, previous genome-wide efforts to study the gene expression during memory consolidation have led to largely inconsistent findings across labs [70, 71, 109-113]. This is due both to study design and restraints of microarray technology, which can introduce variability in hybridization between runs [78]. To resolve the genetic targets of the transcription that occur immediately after contextual fear conditioning, we used RNA-seq. RNA-seq is a recent technology that allows all polyadenylated mRNA within a sample to be sequenced, mapped to the transcriptome, and quantified. RNA-seq has a better dynamic range than microarrays and consistent technical reproducibility [78], so this technology provides the best opportunity to discover reliable transcriptional targets. In addition, we designed our study in such a way that mice from each group were dissected at the same time over multiple days, limiting the effects of circadian rhymicity and differences such as odors or loud noises that could occur on one particular day.

Contextual learning occurs throughout the lifetime of an animal, so isolating the effects of a specific contextual memory can be difficult. Standard RNA-seq normalization is unable to resolve small differences within a complex signal [114]. This is because the signal contains both variation caused by the condition being tested as well as variation caused by unwanted effects. Ideally, RNA-seq analysis would account for this unwanted variation to reveal the true signature of the condition being tested. To this end, we

applied a recently designed normalization method, termed remove unwanted variation (RUV) that accounts for unwanted variation within an RNA-seq data set [114]. Unwanted variation would be any variation in gene expression not caused by the treatment being tested. In the case of our experiment, this could include previous contextual information, individual effects of stress on mice, or even variation caused by RNA extraction and library preparation. This method is much better at resolving complex signals, such as those from the brain, than standard upper quantile normalization, which primarily normalizes for read count differences between samples.

Gene expression is a complex process with multiple layers of regulation. Expression levels of particular isoforms of transcripts can be regulated by alternative start sites, differential splicing including exon skipping and intron retention, and alternative poly(A) site selection [115]. Alternative splicing can lead to distinct protein function and interactions [116] or regulate mRNA localization [117-119], and thus is expected to be particularly important in neurons with long processes. Previous research studying genome-wide gene expression in the hippocampus after contextual learning has relied on microarray technology [70, 71, 109-113]. Although microarrays are good at identifying gene expression differences, they are often unable to distinguish exon-level effects that are indicative of alternative splicing. RNA-seq provides numerous advantages over microarrays, including the ability to study exon-level changes in gene expression. Isoform-specific gene expression changes are known to occur after contextual fear conditioning, including specific upregulation of *Bdnf IV*, but not other *Bdnf* isoforms [120], and *Homer1a*, but not *Homer1c* [121]. These examples indicate that transcriptional changes after learning may be more complex than gene-level differences and can be highly selective for particular exons of a gene.

In this study, we used RNA-seq to study gene expression 30 minutes after contextual fear conditioning in the hippocampus. Applying RUV, a recently designed normalization algorithm, to our data, we find that the number of differentially regulated novel and positive control genes dramatically increase. This results in interesting functional classes of genes being identified. We also tested differential alternative splicing 30 minutes after contextual fear conditioning. We discovered 118 exons across 97 genes that showed differential expression of an exon independent of gene-level expression differences. These differences confirmed changes in *Snord14e*, a small nucleolar RNA that our lab has previously shown to be regulated at this time point [109]. Sno-RNAs regulate RNA processing and have been implicated in memory consolidation [122]. *Ania-3*, an alternative short form of Homer1 that has not previously been linked to learning, was also shown to be regulated by fear conditioning. These findings demonstrate for the first time on a genome-wide scale that alternative splicing is regulated by contextual learning and also provide novel transcriptional targets that are regulated during memory consolidation.

Materials and Methods

Subjects

C57BL/6J mice were maintained under standard conditions with food and water available *ad libitum*. Adult male mice 2 months of age were kept on a 12-hr light/12-hr dark cycle with lights on at 7AM. All behavioral and biochemical experiments were performed during the light cycle with training starting at 10AM (ZT3).

Behavior

Fear conditioning was performed as previously described [55, 61] with handling for 3 days prior to conditioning. Briefly, the conditioning protocol entailed a single 2-sec, 1.5 mA footshock terminating at 2.5 minutes after placement of the mouse in the novel chamber. Mice were left in the chamber for an additional 30 seconds and then returned to their homecage. One mouse per behavioral group (homecage and fear conditioned) was trained per day over 5 days. This allowed dissections to occur at the same time and reduce circadian effects.

RNA isolation

Hippocampi were dissected from homecage mice or fear conditioned mice 30 minutes after the training into RNAlater (Qiagen, Valencia, CA) and frozen on dry ice. Tissue was homogenized using a TissueLyser system and RNA was extracted using the RNAeasy Microarray Tissue kit (Qiagen) according to the manufacturer's instructions. Samples were DNase treated using the RNase-Free DNase kit (Qiagen) off-column by incubating 5 µl DNase and 35 µl Buffer RDD for 25 min at RT with each sample. Samples were then ethanol precipitated and resuspended in water.

RNA-seq Library Preparation and Sequencing

2 µg of RNA from n=5 homecage and fear conditioned mice was used in the TruSeq RNA Sample Prep Kit (Illumina, San Diego, CA) according to the manufacturer's instructions with polyA selection. Completed libraries were size-selected on an agarose gel to remove any high basepair fragments, quantified by qPCR (KAPA Biosystems, Boston, MA), and submitted to the PGFI sequencing core at UPENN. An Illumina HiSeq 2000 sequenced the libraries in paired-end 100bp reads. 3 libraries were sequenced per lane on an Illumina HiSeq 2000, resulting in an average of 67,011,105 reads per sample

in the homecage mice and 62,115,805 reads per sample after fear conditioning. Reads had good unique concordance (86.9% in homecage, 85.5% after fear conditioning) and mapping (90.7% of unique concordant reads in homecage and 93.1% after fear conditioning).

Data Analysis

Sequencing reads were aligned to the mouse mm9 genome using GSNAP [123] (http://share.gene.com/gmap). A gene count table was produced using HTSeq [124] (http://www-huber.embl.de/users/anders/HTSeq/doc/overview.html). Gene counts were normalized either using standard EdgeR normalization [125] or RUVneg, which corrects for unwanted variation using negative controls [114]. We used 625 genes identified as unchanged from a previous microarray experiment as negative controls for RUV [109]. We discovered that five principal components (k=5) need to be normalized to resolve the differences caused by contextual fear. Significance was calculated using EdgeR [125]. Functional annotation was performed through DAVID [126, 127] (http://david.abcc.ncifcrf.gov/). The annotation was limited to the following sources: GO Biological process, GO Molecular Function, KEGG pathways, and SwissProt and Protein Information Resource keywords and an EASE score restriction of 0.1.

Exons were separated into unique, non-overlapping “bins” using Ensembl gene models and DEXseq [128]. A “bin” can either be part of an exon or an entire exon depending on the uniqueness of the region. A bin read count table was produced using HTSeq. Samples were normalized using RUV. We operated under the assumption that exons from housekeeper genes selected from a previous microarray study did not change. Exon-level analysis was performed using the Bioconductor package voom and diffSplice.

qPCR analysis

RNA was extracted from a separate cohort of fear conditioned mice as described above. RNA was converted to cDNA using the RETROscript kit (Ambion) according to the manufacturer's instructions. cDNA reactions were diluted to 200 µl and 2.25 µl was combined with 0.25 µl 5µM primer mix and 2.5 µl SYBR Select Master Mix (Life Technologies) and run on a Viia7 Real Time PCR system. The ΔΔCt method was used for analysis as previously described [55]. The primers used for exon-level analysis were: Ania-3 F: 5'- AGTGGCTGGTTTTCTTGGACT-3', Ania-3 R: 5'-GGGAGGTGGATTGGTGACAA-3', Homer1 Bin21 F: 5'-CTGGAGTCCACTGCCAATGT-3', Homer1 Bin21 R: 5'- CTCTGCTTCCTCCTGGTACG-3', Las1l Bin15 F: 5'- TCAAAGTCAGAGGGGTCGGA-3', La1l Bin15 R: 5'-AGACTTCGCTCTTGCTGCTT-3', Las1l Bin17 F: 5'- TGCTGGAGAAACACAGGCAT-3', Las1l Bin17 R: 5'- ACATTGTACACGTGGGGAAAGA-3', Rbm3 Bin2 F: 5'-ACCTGAGTTTTGGAGGCTGG-3', Rbm3 Bin2 R: 5'- ACAACAGCGGACACCATAGG-3', Rbm3 Bin7 F: 5'-GGTGGCTATGACCGCTACTC-3', Rbm3 Bin7 R: 5'-TTTTGTGTGCATGCCCCATC-3', Rbm3 Bin22 F: 5'-TGCCCCTGGCAGACATAGAG-3', Rbm3 Bin22 R: 5'-GTCTGCCACTTTCTTCGTTCTTT-3'.

Results

To study gene expression after learning using RNA-seq, we applied a recently created algorithm, termed remove unwanted variance (RUV) [114], that allows a user to correct for unwanted variance. RUV uses negative control genes (or samples) to test for sources of variation that do not correlate with the factor of interest and correct for that

variation. We used 625 negative control genes from a previous microarray study [109] as controls for this normalization and therefore will refer to the normalized data as RUVneg. There are a number of ways to test whether data normalization is performing its function, but two major quality control tests for RNA-seq data analysis are relative log expression (RLE) plots and principal component analysis. RLE plots measure the count data for a gene in a sample relative to the median of all samples and plot this over all genes. We would expect the mean to be close to 0 and variability between all samples to be minimal on this plot. RUVneg restores this uniformity, while upper quantile normalization does not (**Figure 2.1A**). Principal component analysis determines which components of the data are the major drivers of variability in a data set. Ideally, the difference being tested (in our case learning) would be the major driver of variability. However, upper quantile normalization shows no clustering of samples in the first two principal components (**Figure 2.1B**), indicating that learning is not the major driver of differences between groups. RUVneg with normalization of five principal components introduces proper clustering (**Figure 2.1B**), indicating that any differences observed in this data will primarily be the result of differences induced by contextual learning.

We then applied upper quantile and RUVneg normalization to our data to identify differentially regulated genes. We would expect that the p-value distribution of the results would have a uniform value, with peaks at either end for genes that are obviously changed or unchanged [129]. In other words, there is no logical reason that more genes would have a p-value of 0.8 than a p-value of 0.2. However, we see that upper quantile normalization does not have a uniform p-value distribution, while RUVneg does (**Figure 2.2A**). RUVneg also introduces a large peak at the lowest p-value bin, indicating a greater power to detect changes. A major test of this normalization is that it is not introducing artifacts into our data. To test this, we produced a volcano plot showing p-

value vs. fold change ($-\log_{10}$p-value vs. $\log_2$FC/CC) (**Figure 2.2B**). While RUVneg produces many more significant genes than upper quantile normalization (blue), it also greatly improves the number of 18 positive control genes selected from the literature (red) that are detected as significant. This gives us confidence that the genes detected by RUVneg are indeed genes regulated by contextual fear conditioning.

Upper quantile normalization detected 56 genes as differentially regulated (**Table 2.1**), while RUVneg identified 823 genes (**Table 2.2**). The 823 genes identified by RUVneg include all 56 genes identified by upper quantile normalization (**Figure 2.3**). Functional annotation of these gene lists by DAVID [126, 127] also demonstrates the improved ability of RUVneg to identify changes important for learning. Upper quantile normalization produced 7 significant clusters mapping to transcriptional regulation, embryo/blood vessel development and vision. RUVneg produced 20 clusters mapping to transcriptional regulation, synaptic transmission, MAPK activity and calcium homeostasis among other functions (**Figure 2.3**). Because of the known importance of synaptic changes, MAPK signaling, and calcium signaling to memory [130, 131], this further demonstrates the power of RUVneg. RUVneg, therefore, is a useful tool that identified numerous genes and processes that are regulated during memory consolidation.

RNA-seq also has the advantage of distinguishing exon-level reads that are difficult to detect by any other method, and therefore is an ideal technique to study alternative splicing. We used GSNAP [123] to align reads to the mm9 mouse genome and HTSeq [124] to count reads by DEXSeq bins [128] using Ensembl gene models. Bins are separated based on overlap of Ensembl gene models, with exons that have variable sizes between different transcripts split into multiple bins. Therefore, a bin can represent either a whole exon or part of an exon. RUVneg normalization was performed as described [114], using removal of 5 principle components of variance, which we

discovered is optimal for this dataset. Bioconductor packages voom and diffSplice were then used to determine differential exon usage independent of gene-level changes. We identified 118 bins across 97 genes that displayed differential usage after contextual fear conditioning (**Table 2.3**). 87 of these exons were upregulated and 31 were downregulated, consistent with the general increase in gene expression after fear conditioning (**Figure 2.2**). We performed functional classification of genes showing at least 1 exon-specific change after fear conditioning. The SwissProt and Protein Information Resource keyword "alternative splicing" was enriched in our data set, indicating that our exon-level analysis discovers alternative splicing as expected. However, no functional clusters were enriched in either the upregulated or downregulated exon lists.

Upregulated exons included *Snord14e*, which reside in the introns of the *Hspa8* gene. We have recently validated *Snord14e* upregulation after detecting differences by microarray [109]. We also discovered that a poorly studied short isoform of *Homer1* known as *Ania-3* is upregulated after contextual fear conditioning. *Homer1a* has previously been shown to be upregulated by fear conditioning, but *Ania-3* has not been studied [121]. To validate our results, we performed qPCR in a separate cohort of mice, comparing the bins observed to change in these genes to an exon of the same gene that was unchanged. *Ania-3* was found to be upregulated independently of *Homer1* (**Figure 2.4**). Ribosome biogenesis protein *Las1l* exhibits bin-specific downregulation in response to contextual fear conditioning (**Figure 2.5**). RNA-binding protein *Rbm3*, which our lab has shown to change in the hippocampus after sleep deprivation [132], displays complex regulation with both upregulated and downregulated bins after learning (**Figure 2.6**). In all cases, the exon predicted to change was significantly regulated while a

control exon in the same gene was unchanged. This indicates that alternative splicing does occur on a genome-wide scale in response to contextual fear conditioning.

Discussion

Although gene expression has been recognized to be crucial for long-term memory for many years [11, 108], identifying the genome-wide targets of this transcription has proven difficult. This is due both to the limitations of technology used in previous experiments and the inability to distinguish signal from noise. The brain is a heterogenous tissue with only a fraction of excitatory neurons responding transcriptionally to a learning event [17]. This heterogeneity dilutes changes and makes it difficult to identify changes caused by contextual learning. In this study, we used RNA-seq to study gene expression in the hippocampus 30 minutes after contextual fear conditioning, a time point our lab has previously determined to show the most expression changes after fear conditioning [61, 109]. We used a recently developed normalization algorithm, RUV, in combination with negative controls identified from a previous microarray experiment to reduce the effects of unwanted variation. We found that RUVneg normalization greatly improves our ability to detect both novel and expected changes with RNA-seq. We also provide the first evidence of genome-wide regulation of alternative splicing after learning in the hippocampus.

RUVneg normalization was able to normalize a number of factors that standard upper quantile normalization does not. This is an important because a major problem with studying gene expression after learning is the heterogeneity of the brain. The brain contains excitatory neurons, inhibitory neurons, glia, and epithelial cells. Only a fraction (18-35% [17]) of excitatory neurons respond transcriptionally to any memory trace.

Therefore, the signal caused by contextual fear conditioning is highly diluted by surrounding cells, making it small and hard to observe. After RUVneg, the RLE plot was much less variable between samples and principal component analysis clustered samples according to treatment. These normalization measures are essential for producing a reliable list of genes that are caused by the learning event in question. The gene list produced by RUVneg showed a uniform p-value distribution, which would be expected, and identified more novel and positive control genes than standard upper quantile normalization. The larger gene list allowed for identification of more functional classes of genes being regulated that could be followed up for future study. Immediate shock and context only controls will be needed to test whether these gene expression changes are caused by the association between context and shock or caused by either stimulus alone. Importantly, because the goal of RUV is to normalize for sources of unwanted variation, this normalization should be broadly applicable to future studies of this type and greatly enhance consistency and reliability between experiments. We believe that the gene list produced from our data will be highly reproducible by other labs studying contextual fear conditioning.

Because RNA-seq provides the additional advantage of studying alternative splicing, we also used RUV to look at gene counts broken down by bins corresponding to whole or parts of exons. We were able to detect gene expression changes at particular bins occurring in response to contextual fear conditioning at 97 genes. Although individual examples of alternative splicing have been observed during memory consolidation [120, 121, 133], nothing has ever been discovered at this genome-wide level. We confirmed [109] that *Snord14e*, which exists within an intron of *Hspa8*, is regulated by fear conditioning. We also implicate the selective expression of particular bins of *Homer1* isoform *Ania-3,* RNA-binding protein *Rbm3* and ribosome biogenesis

regulator *Las1l* in learning for the first time. This discovery would not have been possible looking at gene-level expression. The mechanism that drives this alternative splicing is unclear, although transcription of certain splicing proteins is known to change after fear conditioning [134]. Our data also indicates regulation of a specific isoform of splicing factor *Sfpq* (data not shown). However, it is unclear whether these transcriptional changes would have time to translate into protein and effect splicing by 30 minutes. It is possible that changes in epigenetic modifications are regulating this selective exon usage [135], including H3K36me3 and H4K20me1 [136, 137]. Future studies can determine whether the differential bins discovered in this study show differential histone modifications as well. Although the exact function of the exons discovered to be differentially regulated by fear conditioning in this study remains unclear, we hope that these findings drive further study into the mechanisms of isoform specific transcriptional effects during memory consolidation.

Figure Legends

Figure 2.1. RUV normalization allows proper grouping of replicates. A) Relative log expression (RLE) plot of all samples following either traditional upper-quantile normalization or normalization with RUV using negative controls (RUVneg). This plot compares the ratio between the gene counts for each sample and the median gene counts for all samples and plots the data over all genes. We expect RLE distributions to be centered around zero and as similar as possible to each other. Red samples are controls matched for time of day (CC), blue samples were obtained 30 minutes after memory acquisition (FC), and green samples were obtained 30 minutes after memory retrieval (RT). The RLE boxplots clearly show the need for additional normalization following upper quantile normalization. B) Scatterplot of first two principal components (PC1 and PC2) log-scaled and centered following traditional upper-quantile normalization (left) or normalization with RUV using negative controls (right). Samples do not cluster according to treatment following UQ normalization but do after applying RUV normalization.

Figure 2.2. Normalization impacts differential expression after contextual fear conditioning. A) Distribution of edgeR uncorrected p-values for tests of differential expression between control (CC) and fear conditioned (FC) samples for upper quantile normalized counts and RUVneg normalized counts. The y-axis is frequency of each p-value bin occurring and x-axis is increasing p-values. The distribution of upper quantile normalized counts is far from the expected uniform distribution. RUVneg returns uniformity to the p-value distribution and increases discovery of differentially expressed

genes (genes that have a low p-value). B) Volcano plot of differential expression ($-\log_{10}$ p-value vs $\log_2$ fold change) of upper quantile normalized samples and RUVneg normalized samples. Genes with and FDR <0.1 are highlighted in blue. Genes known to be regulated by FC (positive controls) are outlined in red. RUV increases the significance of differentially expressed positive controls.

Figure 2.3. Comparison of gene lists after upper quantile or RUV normalization. A) Upper quantile normalization identifies only 56 genes as significantly regulated by fear conditioning. Functional annotation of these genes identifies: Regulation of transcription, Embryo/blood vessel development and Vision. B) RUVneg results in 823 genes regulates by fear conditioning. Functional annotation of these genes identifies: Regulation of Transcription, Synaptic Transmission, Embryo/blood vessel Development, Metal Ion Binding, MAPK activity, Zinc fingers, Calcium homeostasis, Ear development, Cadherins, Fibronectins, Immune response, and Apoptosis.

Figure 2.4. Exon-specific regulation of *Homer1* (*Ania-3*). A) diffSplice result showing the predicted significant bin changes in red. Bins 16-18 indicate the *Ania-3* isoform. B) qPCR validation of the change in Bin18 in an independent cohort of mice. Bin 21 expression was compared as a control.

Figure 2.5. Exon-specific regulation of *Las1l*. A) diffSplice result showing the predicted significant bin changes in red. B) qPCR validation of the change in Bin 15 in an independent cohort of mice. Expression of Bin 17 was used as a control.

Figure 2.6. Exon-specific regulation of *Rbm3*. A) diffSplice result showing the predicted significant bin changes in red. B) qPCR validation of the changes in Bin 2 and Bin 22 an independent cohort of mice. Expression of Bin 7 was used as a control.

Table 2.1. Results of differential expression test after upper quantile normalization. Genes with an FDR <0.1 after upper quantile normalization are shown. 56 genes were identified. logFC is the fold change between fear conditioned and homecage in $\log_2$, logCPM is the $\log_2$ counts per million for that gene, LR is the likelihood ratio (statistical test) and FDR is the multiple testing corrected false discovery rate.

Table 2.2. Results of differential expression test after RUV normalization. Genes with an FDR <0.1 after upper quantile normalization are shown. 823 genes were identified, including all 56 identified using upper quantile normalization. logFC is the fold change between fear conditioned and homecage in $\log_2$, logCPM is the $\log_2$ counts per million for that gene, LR is the likelihood ratio (statistical test) and FDR is the multiple testing corrected false discovery rate.

Table 2.3. Results of diffSplice analysis. diffSplice tests whether the logFC of a particular bin differs from the average logFC of that gene. Ensembl Gene IDs were used for gene classification and broken into bins. 118 bins corresponding to 97 genes were identified. logFC is the fold change between fear conditioned and home cage animals in $\log_2$ format. *t* represents the t-statistic for the difference. FDR is a multiple-testing corrected false discovery rate. Only exons with an FDR <0.1 are shown.

Contributions

This chapter was written by Shane G. Poplawski with suggestions by Ted Abel, Lucia Peixoto, and Giulia Porcari. Experiments were planned by Shane Poplawski, Lucia Peixoto, and Mathieu Wimmer. Behavior and experiments were carried out by Shane Poplawski, Lucia Peixoto, Mathieu Wimmer, and Giulia Porcari. Data analysis was performed by Lucia Peixoto, Shane Poplawski and Davide Risso (Terry Speed laboratory, UC Berkeley). We thank Giulia Porcari for constructive discussions and critical reading of the chapter.

Figures

Figure 2.1

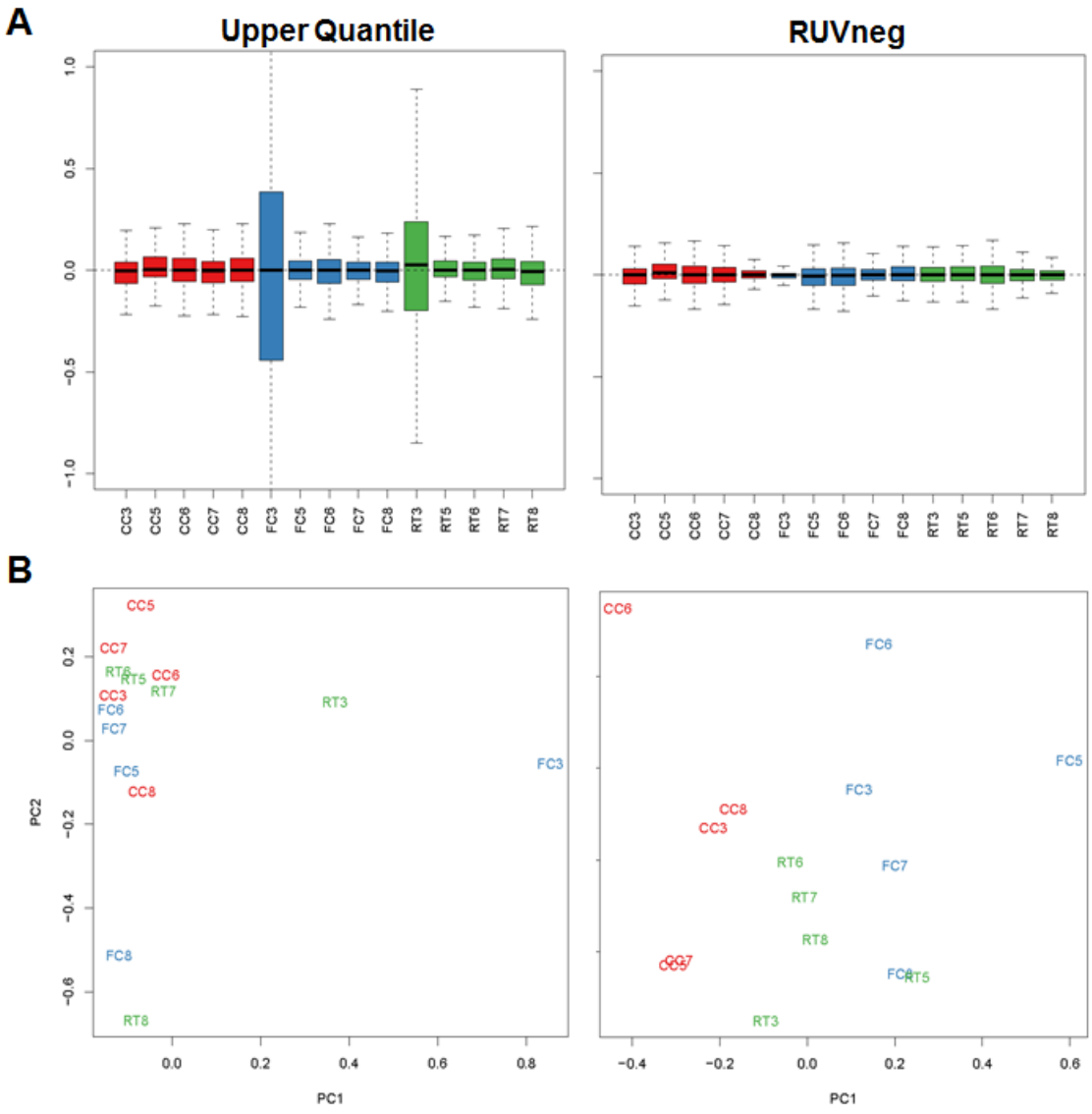
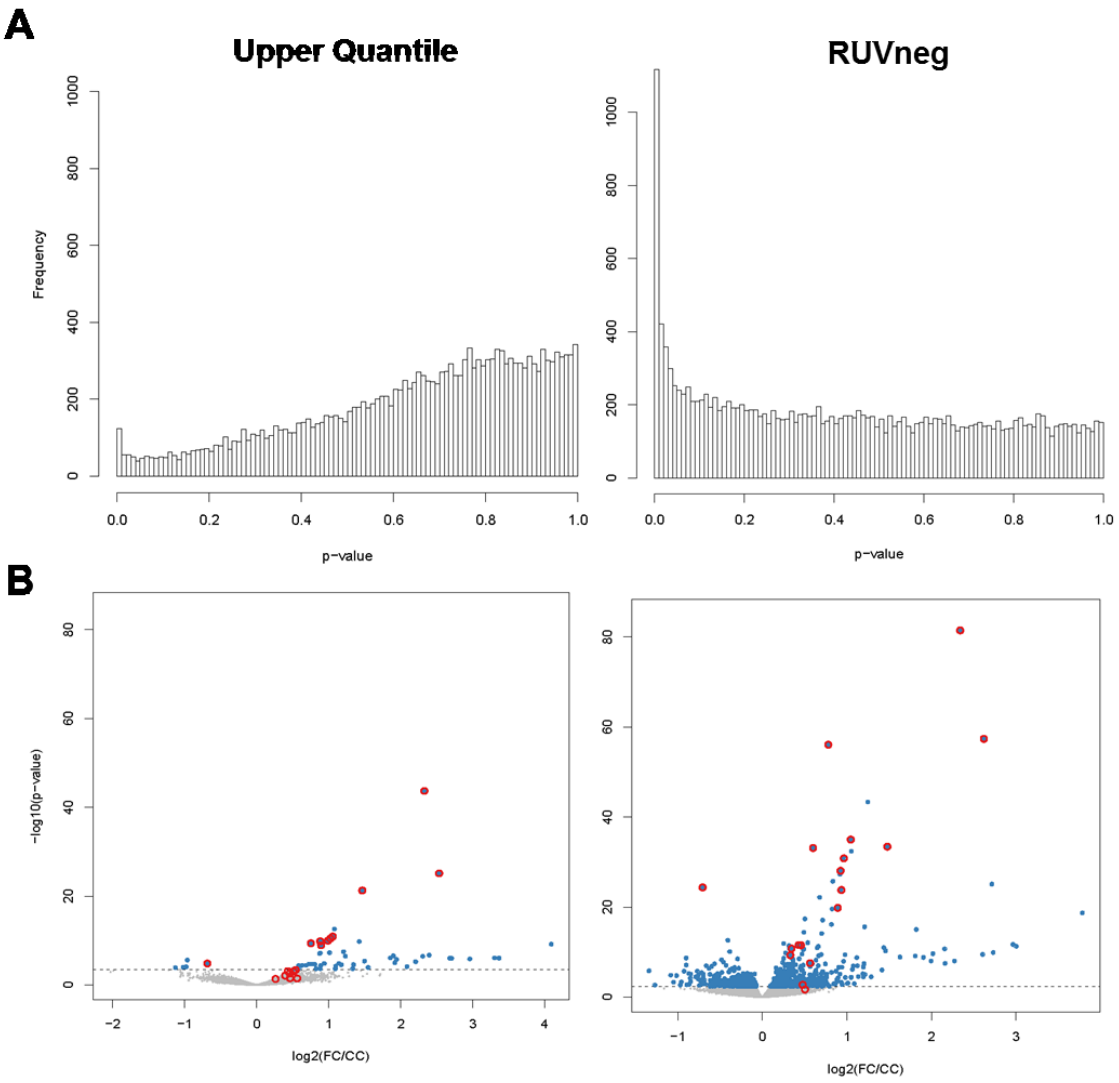


Figure 2.2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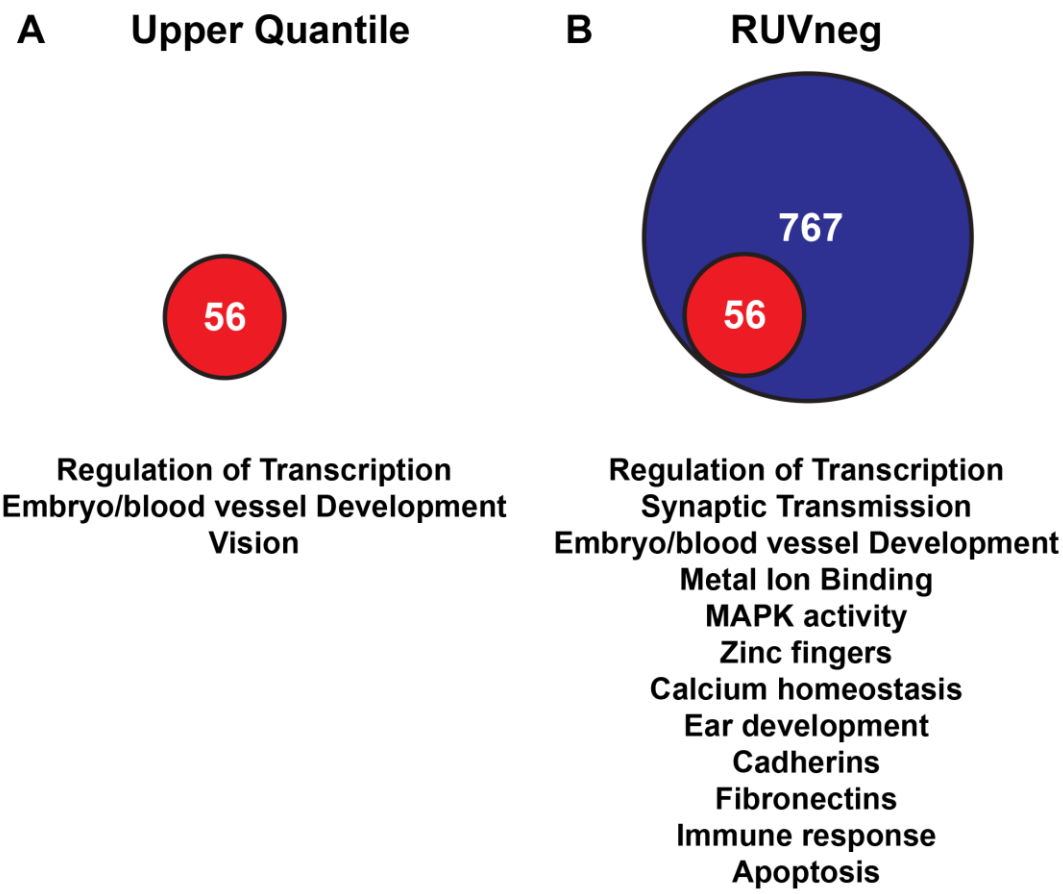
Upper Quantile
RUVneg
Frequency
p−value
B
−log10(p−value)
log2(FC/CC)

Figure 2.3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Synaptic Transmission
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Metal Ion Binding
MAPK activity
Zinc fingers
Calcium homeostasis
Ear development
Cadherins
Fibronectins
Immune response
Apoptosis

Figure 2.4

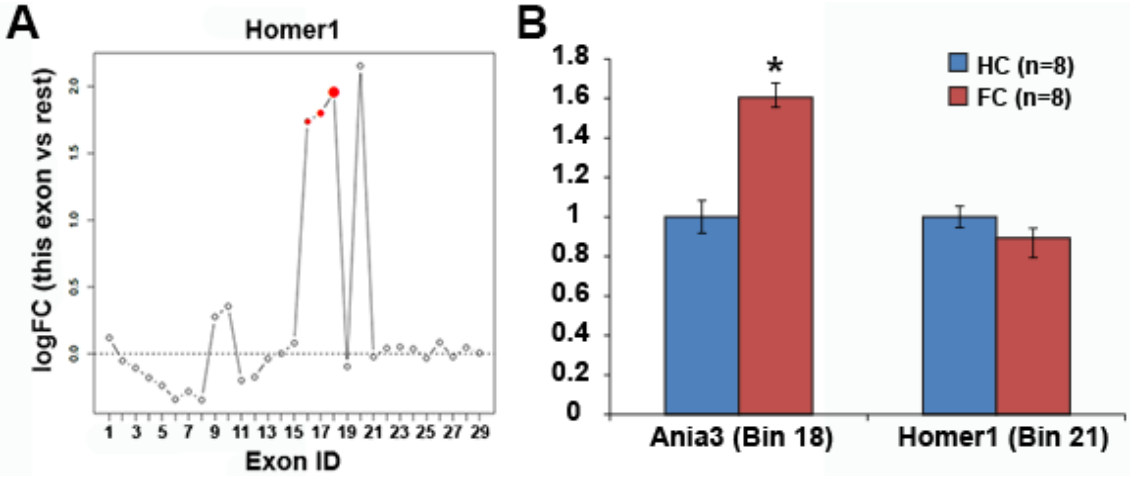

A
Homer1
logFC (this exon vs rest)
Exon ID
B
HC (n=8)
FC (n=8)
*
Ania3 (Bin 18)
Homer1 (Bin 21)

Figure 2.5

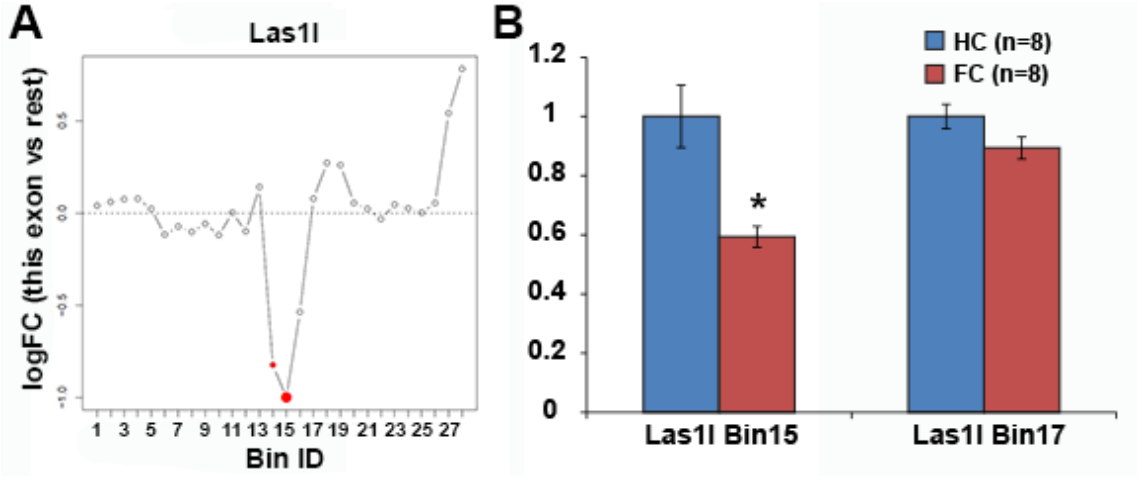
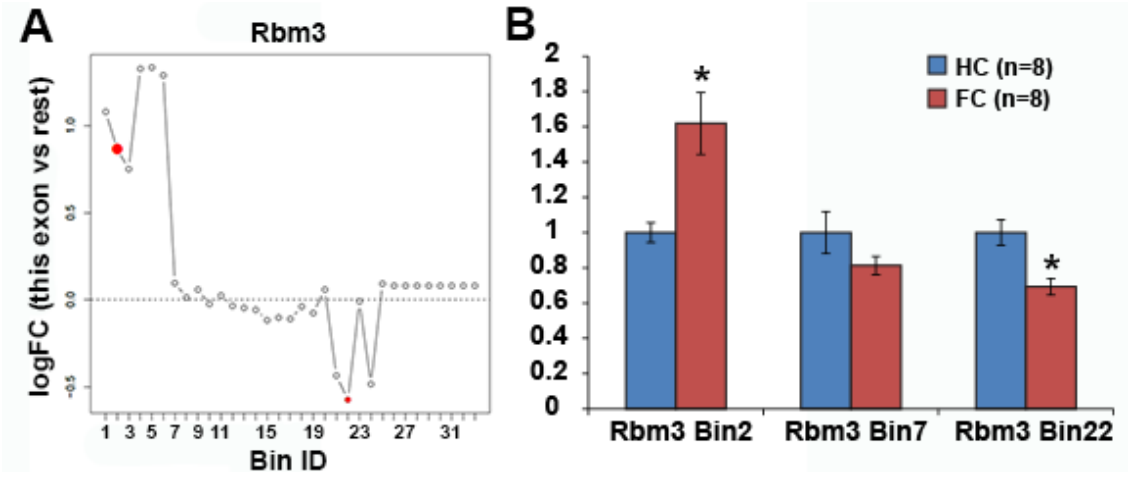

A
Las1l
logFC (this exon vs rest)
0.5
0.0
-0.5
-1.0
1 3 5 7 9 11 13 15 17 19 21 23 25 27
Bin ID
B
1.2
1
0.8
0.6
0.4
0.2
0
*
HC (n=8)
FC (n=8)
Las1l Bin15
Las1l Bin17

Figure 2.6


A
Rbm3
logFC (this exon vs rest)
1.0
0.5
0.0
-0.5
1 3 5 7 9 11 15 19 23 27 31
Bin ID
B
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
*
HC (n=8)
FC (n=8)
Rbm3 Bin2
Rbm3 Bin7
Rbm3 Bin22

Tables

Table 2.1

GeneID	Gene Name	logFC	logCPM	LR	p-value	FDR
ENSMUSG00000021250	Fos	2.33	4.13	195.21	2.3E-44	4.2E-40
ENSMUSG00000037868	Egr2	2.54	2.04	110.33	8.3E-26	7.5E-22
ENSMUSG00000022602	Arc	1.47	7.48	92.73	6.0E-22	3.6E-18
ENSMUSG00000028195	Cyr61	1.08	2.49	53.34	2.8E-13	1.3E-09
ENSMUSG00000052837	Junb	1.06	5.94	45.64	1.4E-11	5.1E-08
ENSMUSG00000023034	Nr4a1	1.02	6.43	43.57	4.1E-11	1.2E-07
ENSMUSG00000024042	Sik1	0.99	3.89	41.44	1.2E-10	3.1E-07
ENSMUSG00000024190	Dusp1	0.89	4.37	40.82	1.7E-10	3.7E-07
ENSMUSG00000085609	1700016P03Rik	1.42	2.34	40.63	1.8E-10	3.7E-07
ENSMUSG00000020423	Btg2	0.76	4.65	38.76	4.8E-10	8.6E-07
ENSMUSG00000061808	Ttr	4.09	7.15	37.94	7.3E-10	1.2E-06
ENSMUSG00000003545	Fosb	0.90	3.45	36.82	1.3E-09	2.0E-06
ENSMUSG00000065537	Mir132	1.21	0.27	30.32	3.7E-08	5.0E-05
ENSMUSG00000071341	Egr4	1.01	5.02	29.34	6.0E-08	7.8E-05
ENSMUSG00000090698	Apold1	0.88	2.39	28.99	7.3E-08	8.8E-05
ENSMUSG00000036151	Tm6sf2	0.87	2.44	28.35	1.0E-07	1.1E-04
ENSMUSG00000022949	Clic6	2.39	2.25	26.85	2.2E-07	2.3E-04
ENSMUSG00000001827	Folr1	1.90	1.14	26.28	3.0E-07	3.0E-04
ENSMUSG00000017723	Wfdc2	2.31	-1.27	25.76	3.9E-07	3.5E-04
ENSMUSG00000053560	Ier2	1.23	3.11	25.79	3.8E-07	3.5E-04
ENSMUSG00000034739	Mfrp	3.30	0.41	24.15	8.9E-07	7.7E-04
ENSMUSG00000025488	Cox8b	1.86	-1.50	24.03	9.5E-07	7.8E-04
ENSMUSG00000068323	Slc4a5	3.37	0.48	23.83	1.1E-06	8.2E-04
ENSMUSG00000079436	Kcnj13	2.68	-1.25	23.73	1.1E-06	8.3E-04
ENSMUSG00000037086	1110059M19Rik	2.71	-1.05	23.61	1.2E-06	8.5E-04
ENSMUSG00000021848	Otx2	1.94	0.42	22.48	2.1E-06	0.001
ENSMUSG00000039672	Kcne2	2.96	0.33	22.96	1.7E-06	0.001
ENSMUSG00000028348	Murc	-0.97	-0.06	21.93	2.8E-06	0.002
ENSMUSG00000026051	1500015O10Rik	1.50	1.71	20.84	5.0E-06	0.003
ENSMUSG00000004655	Aqp1	2.21	0.69	20.28	6.7E-06	0.004
ENSMUSG00000015652	Steap1	1.92	-1.11	19.21	1.2E-05	0.007
ENSMUSG00000046470	Sox18	-0.68	3.20	18.53	1.7E-05	0.009
ENSMUSG00000048489	8430408G22Rik	1.14	-0.04	18.57	1.6E-05	0.009
ENSMUSG00000055148	Klf2	0.94	3.37	18.53	1.7E-05	0.009
ENSMUSG00000034936	Arl4d	0.78	3.37	17.95	2.3E-05	0.011
ENSMUSG00000044595	Dnd1	0.73	1.20	18.06	2.1E-05	0.011
ENSMUSG00000034765	Dusp5	0.81	4.84	17.76	2.5E-05	0.012
ENSMUSG00000049382	Krt8	1.35	-0.41	17.50	2.8E-05	0.014
ENSMUSG00000020907	Rcvrn	1.18	-1.05	17.20	3.4E-05	0.016
ENSMUSG00000024793	Tnfrsf25	0.69	5.54	16.72	4.3E-05	0.020
ENSMUSG00000040287	Stac3	0.63	1.95	16.60	4.6E-05	0.020
ENSMUSG00000037447	Arid5a	0.58	3.72	16.51	4.8E-05	0.021
ENSMUSG00000079681	Zglp1	0.89	0.79	16.44	5.0E-05	0.021

ENSMUSG00000028125	Abca4	1.10	1.78	15.81	7.0E-05	0.029
ENSMUSG00000030450	Oca2	1.10	-1.09	15.61	7.8E-05	0.031
ENSMUSG00000026579	F5	2.09	1.45	15.38	8.8E-05	0.034
ENSMUSG00000084381	AA413626	-0.98	-0.97	15.26	9.3E-05	0.036
ENSMUSG00000073437	D330041H03Rik	-1.02	-1.39	14.84	1.2E-04	0.044
ENSMUSG00000023043	Krt18	1.55	0.13	14.68	1.3E-04	0.047
ENSMUSG00000000182	Fgf23	-1.13	-1.42	14.48	1.4E-04	0.051
ENSMUSG00000015467	Egfl8	1.09	-0.18	13.32	2.6E-04	0.086
ENSMUSG00000026628	Atf3	0.83	0.57	13.39	2.5E-04	0.086
ENSMUSG00000030742	Lat	0.88	0.05	13.43	2.5E-04	0.086
ENSMUSG00000048109	Rbm15	0.58	2.76	13.33	2.6E-04	0.086
ENSMUSG00000050370	Ch25h	1.33	-1.74	13.45	2.5E-04	0.086
ENSMUSG00000032265	Fam46a	0.52	2.63	13.15	2.9E-04	0.093

Table 2.2

GeneID	Gene Name	logFC	logCPM	LR	p-value	FDR
ENSMUSG00000021250	Fos	2.34	4.13	374.87	1.6E-83	2.9E-79
ENSMUSG00000037868	Egr2	2.62	2.04	266.52	6.5E-60	5.8E-56
ENSMUSG00000020423	Btg2	0.78	4.65	231.73	2.5E-52	1.5E-48
ENSMUSG00000085609	1700016P03Rik	1.24	2.34	189.85	3.4E-43	1.5E-39
ENSMUSG00000024042	Sik1	1.05	3.89	155.56	1.1E-35	3.8E-32
ENSMUSG00000022602	Arc	1.48	7.48	147.89	5.0E-34	1.5E-30
ENSMUSG00000034640	Tiparp	0.61	4.08	144.43	2.9E-33	7.4E-30
ENSMUSG00000028195	Cyr61	1.06	2.49	143.49	4.6E-33	1.0E-29
ENSMUSG00000052837	Junb	0.97	5.94	134.12	5.2E-31	1.0E-27
ENSMUSG00000003545	Fosb	0.93	3.45	127.11	1.8E-29	3.2E-26
ENSMUSG00000079436	Kcnj13	2.87	-1.25	120.87	4.1E-28	6.7E-25
ENSMUSG00000053560	Ier2	0.91	3.11	119.73	7.3E-28	1.1E-24
ENSMUSG00000046470	Sox18	-0.69	3.20	117.07	2.8E-27	3.8E-24
ENSMUSG00000036151	Tm6sf2	0.81	2.44	110.24	8.7E-26	1.1E-22
ENSMUSG00000023034	Nr4a1	0.94	6.43	104.47	1.6E-24	1.9E-21
ENSMUSG00000034936	Arl4d	0.68	3.37	87.74	7.4E-21	8.4E-18
ENSMUSG00000024190	Dusp1	0.90	4.37	86.70	1.3E-20	1.3E-17
ENSMUSG00000061808	Ttr	3.88	7.15	85.27	2.6E-20	2.6E-17
ENSMUSG00000071341	Egr4	0.83	5.02	85.12	2.8E-20	2.7E-17
ENSMUSG00000037447	Arid5a	0.50	3.72	72.76	1.5E-17	1.3E-14
ENSMUSG00000090698	Apold1	0.82	2.39	71.24	3.2E-17	2.7E-14
ENSMUSG00000024793	Tnfrsf25	0.69	5.54	69.63	7.2E-17	5.9E-14
ENSMUSG00000015652	Steap1	1.88	-1.11	68.96	1.0E-16	7.8E-14
ENSMUSG00000065537	Mir132	1.21	0.27	66.12	4.2E-16	3.2E-13
ENSMUSG00000055148	Klf2	0.71	3.37	63.78	1.4E-15	1.0E-12
ENSMUSG00000048546	Tob2	0.49	4.75	60.79	6.4E-15	4.4E-12
ENSMUSG00000034739	Mfrp	3.06	0.41	53.33	2.8E-13	1.9E-10
ENSMUSG00000068323	Slc4a5	3.09	0.48	50.60	1.1E-12	7.3E-10
ENSMUSG00000032265	Fam46a	0.53	2.63	50.19	1.4E-12	8.7E-10
ENSMUSG00000038612	Mcl1	0.25	6.24	49.78	1.7E-12	1.0E-09
ENSMUSG00000019970	Sgk1	0.35	6.04	47.48	5.6E-12	3.2E-09
ENSMUSG00000017418	Arl5b	0.32	4.59	47.36	5.9E-12	3.3E-09
ENSMUSG00000020893	Per1	0.46	6.52	47.22	6.4E-12	3.5E-09
ENSMUSG00000040287	Stac3	0.65	1.95	47.01	7.0E-12	3.7E-09
ENSMUSG00000034765	Dusp5	0.64	4.84	45.51	1.5E-11	7.8E-09
ENSMUSG00000026051	1500015O10Rik	1.47	1.71	44.61	2.4E-11	1.2E-08
ENSMUSG00000053819	Camk2d	-0.39	5.31	44.15	3.0E-11	1.5E-08
ENSMUSG00000039672	Kcne2	2.81	0.33	43.84	3.6E-11	1.7E-08
ENSMUSG00000020108	Ddit4	0.42	5.89	43.12	5.1E-11	2.4E-08
ENSMUSG00000048109	Rbm15	0.47	2.76	42.48	7.1E-11	3.2E-08
ENSMUSG00000028967	Errfi1	0.32	5.45	42.13	8.5E-11	3.7E-08
ENSMUSG00000017723	Wfdc2	2.20	-1.27	41.99	9.2E-11	3.9E-08
ENSMUSG00000049516	Spty2d1	0.28	4.40	41.74	1.0E-10	4.4E-08
ENSMUSG00000004655	Aqp1	2.09	0.69	40.85	1.6E-10	6.7E-08
ENSMUSG00000084088	Gm12941	0.99	-0.02	40.62	1.8E-10	7.4E-08
ENSMUSG00000047230	Cldn2	1.51	0.46	40.47	2.0E-10	7.8E-08
ENSMUSG00000037086	Prr32	2.66	-1.05	39.74	2.9E-10	1.1E-07
ENSMUSG00000025019	Lcor	0.65	1.14	38.76	4.8E-10	1.8E-07

ENSMUSG00000040536	Necab1	-0.37	5.34	38.66	5.0E-10	1.9E-07
ENSMUSG00000024136	Dnase1l2	0.45	3.02	37.61	8.6E-10	3.1E-07
ENSMUSG00000025488	Cox8b	1.65	-1.50	37.63	8.6E-10	3.1E-07
ENSMUSG00000061436	Hipk2	0.46	4.18	37.39	9.7E-10	3.4E-07
ENSMUSG00000001827	Folr1	1.85	1.14	37.16	1.1E-09	3.7E-07
ENSMUSG00000067833	2900097C17Rik	-0.28	4.57	37.12	1.1E-09	3.7E-07
ENSMUSG00000021848	Otx2	1.95	0.42	36.54	1.5E-09	4.9E-07
ENSMUSG00000035828	Pim3	0.26	5.10	36.07	1.9E-09	6.1E-07
ENSMUSG00000021025	Nfkbia	0.33	4.34	36.03	1.9E-09	6.1E-07
ENSMUSG00000028125	Abca4	1.15	1.78	35.92	2.1E-09	6.4E-07
ENSMUSG00000021453	Gadd45g	0.35	4.05	35.20	3.0E-09	9.1E-07
ENSMUSG00000028348	Murc	-0.90	-0.06	35.17	3.0E-09	9.1E-07
ENSMUSG00000016024	Lbp	0.72	2.51	33.70	6.4E-09	1.9E-06
ENSMUSG00000026579	F5	2.05	1.45	33.71	6.4E-09	1.9E-06
ENSMUSG00000008384	Sertad1	0.43	2.92	33.51	7.1E-09	2.0E-06
ENSMUSG00000025450	Gm9752	-0.48	1.94	32.93	9.5E-09	2.7E-06
ENSMUSG00000038268	Ovca2	0.36	3.70	32.77	1.0E-08	2.8E-06
ENSMUSG00000044595	Dnd1	0.63	1.20	32.79	1.0E-08	2.8E-06
ENSMUSG00000022949	Clic6	2.22	2.25	31.79	1.7E-08	4.6E-06
ENSMUSG00000048489	8430408G22Rik	0.95	-0.04	31.47	2.0E-08	5.4E-06
ENSMUSG00000038418	Egr1	0.57	7.19	31.07	2.5E-08	6.5E-06
ENSMUSG00000037573	Tob1	0.33	5.11	30.80	2.9E-08	7.3E-06
ENSMUSG00000056364	Six3os1	1.13	-1.11	30.80	2.9E-08	7.3E-06
ENSMUSG00000045314	Sowahb	-0.46	2.00	30.45	3.4E-08	8.4E-06
ENSMUSG00000047648	Fbxo30	0.40	2.46	30.47	3.4E-08	8.4E-06
ENSMUSG00000014747	Ankrd53	0.59	0.93	29.90	4.6E-08	1.1E-05
ENSMUSG00000049907	Rasl11b	0.24	6.01	29.77	4.9E-08	1.2E-05
ENSMUSG00000032501	Trib1	0.38	3.57	29.68	5.1E-08	1.2E-05
ENSMUSG00000058626	Capn11	2.17	1.39	29.62	5.3E-08	1.2E-05
ENSMUSG00000026628	Atf3	0.74	0.57	29.28	6.3E-08	1.4E-05
ENSMUSG00000090622	A930033H14Rik	-0.55	1.09	28.89	7.7E-08	1.7E-05
ENSMUSG00000030450	Oca2	1.14	-1.09	28.76	8.2E-08	1.8E-05
ENSMUSG00000047867	Gimap6	0.48	1.69	28.19	1.1E-07	2.4E-05
ENSMUSG00000074825	Itpripl1	0.58	1.12	28.14	1.1E-07	2.5E-05
ENSMUSG00000034342	Cbl	0.38	3.17	28.08	1.2E-07	2.5E-05
ENSMUSG00000062116	Zfp954	0.29	3.78	28.04	1.2E-07	2.5E-05
ENSMUSG00000090338	Gm17081	-0.89	-0.39	28.06	1.2E-07	2.5E-05
ENSMUSG00000066150	Slc31a1	0.27	4.50	27.92	1.3E-07	2.6E-05
ENSMUSG00000048001	Hes5	-0.43	2.32	27.87	1.3E-07	2.7E-05
ENSMUSG00000049382	Krt8	1.21	-0.41	27.76	1.4E-07	2.8E-05
ENSMUSG00000070780	Rbm47	0.98	-0.66	27.54	1.5E-07	3.1E-05
ENSMUSG00000005268	Prlr	0.94	1.79	27.49	1.6E-07	3.2E-05
ENSMUSG00000047604	Frat2	0.32	3.60	27.36	1.7E-07	3.3E-05
ENSMUSG00000015312	Gadd45b	0.47	3.49	27.32	1.7E-07	3.3E-05
ENSMUSG00000021367	Edn1	-0.65	0.85	27.32	1.7E-07	3.3E-05
ENSMUSG00000025350	Rdh5	0.67	1.60	27.28	1.8E-07	3.4E-05
ENSMUSG00000090986	Gm17275	0.45	1.79	26.99	2.0E-07	3.9E-05
ENSMUSG00000084917	Gm17477	1.19	-1.12	26.97	2.1E-07	3.9E-05
ENSMUSG00000025049	Taf5	0.36	2.89	26.88	2.2E-07	4.0E-05
ENSMUSG00000020482	Ccdc117	0.33	4.19	26.85	2.2E-07	4.0E-05
ENSMUSG00000025255	Zfhx4	0.34	3.29	26.08	3.3E-07	6.0E-05

ENSMUSG00000034538	Zfp418	0.45	1.73	26.04	3.3E-07	6.0E-05
ENSMUSG00000022893	Adamts1	0.32	4.49	25.96	3.5E-07	6.2E-05
ENSMUSG00000038775	Vill	-0.59	1.23	25.89	3.6E-07	6.4E-05
ENSMUSG00000001467	Cyp51	0.16	6.01	25.42	4.6E-07	8.1E-05
ENSMUSG00000074890	Lcmt2	-0.32	3.05	25.09	5.5E-07	9.5E-05
ENSMUSG00000086331	Gm16310	1.07	-1.14	24.79	6.4E-07	1.1E-04
ENSMUSG00000085779	Atcayos	0.44	1.99	24.71	6.7E-07	1.1E-04
ENSMUSG00000020385	Clk4	0.19	6.11	24.30	8.2E-07	1.4E-04
ENSMUSG00000091448	Gm17388	0.37	2.28	24.31	8.2E-07	1.4E-04
ENSMUSG00000020641	Rsad2	-0.62	0.49	24.28	8.4E-07	1.4E-04
ENSMUSG00000021986	Amer2	0.29	3.90	24.02	9.5E-07	1.6E-04
ENSMUSG00000050930	Map10	-0.35	2.39	24.00	9.6E-07	1.6E-04
ENSMUSG00000034295	Fhod3	-0.27	3.79	23.84	1.0E-06	1.7E-04
ENSMUSG00000024383	Map3k2	0.22	4.77	23.64	1.2E-06	1.9E-04
ENSMUSG00000032515	Csrnp1	0.48	3.40	23.60	1.2E-06	1.9E-04
ENSMUSG00000026565	Pou2f1	0.26	3.70	23.50	1.3E-06	2.0E-04
ENSMUSG00000091070	Gm3764	-0.34	3.62	23.42	1.3E-06	2.0E-04
ENSMUSG00000034161	Scx	0.63	0.50	23.30	1.4E-06	2.1E-04
ENSMUSG00000034855	Cxcl10	-1.35	-1.46	23.26	1.4E-06	2.2E-04
ENSMUSG00000018604	Tbx3	-0.49	1.56	23.12	1.5E-06	2.3E-04
ENSMUSG00000048379	Socs4	0.26	3.61	22.99	1.6E-06	2.4E-04
ENSMUSG00000030031	Kbtbd8	0.35	2.46	22.87	1.7E-06	2.6E-04
ENSMUSG00000022425	Enpp2	0.85	7.78	22.80	1.8E-06	2.6E-04
ENSMUSG00000079681	Zglp1	0.62	0.79	22.79	1.8E-06	2.6E-04
ENSMUSG00000074170	Plekhf1	0.46	1.76	22.71	1.9E-06	2.7E-04
ENSMUSG00000044177	Wfikkn2	0.91	0.78	22.62	2.0E-06	2.8E-04
ENSMUSG00000023043	Krt18	1.44	0.13	22.54	2.1E-06	2.9E-04
ENSMUSG00000041308	Sntb2	0.33	2.58	22.43	2.2E-06	3.1E-04
ENSMUSG00000010492	Uckl1os	0.71	-0.11	22.32	2.3E-06	3.2E-04
ENSMUSG00000052387	Trpm3	0.29	4.65	22.12	2.6E-06	3.6E-04
ENSMUSG00000042256	Ptchd4	0.38	2.03	22.10	2.6E-06	3.6E-04
ENSMUSG00000083929	Gm10600	-0.91	1.70	22.06	2.6E-06	3.6E-04
ENSMUSG00000091542	Gm17167	-0.77	0.76	21.64	3.3E-06	4.5E-04
ENSMUSG00000000392	Fap	0.79	-0.52	21.50	3.5E-06	4.8E-04
ENSMUSG00000037490	Slc2a12	0.51	2.03	21.31	3.9E-06	5.3E-04
ENSMUSG00000020681	Ace	0.72	3.67	21.29	3.9E-06	5.3E-04
ENSMUSG00000025981	Coq10b	0.24	3.78	21.27	4.0E-06	5.3E-04
ENSMUSG00000046962	Zbtb21	0.22	4.56	21.25	4.0E-06	5.3E-04
ENSMUSG00000034320	Slc26a2	0.30	3.15	21.22	4.1E-06	5.3E-04
ENSMUSG00000036834	Plch1	-0.38	2.85	21.21	4.1E-06	5.3E-04
ENSMUSG00000002325	Irf9	0.27	3.98	21.13	4.3E-06	5.5E-04
ENSMUSG00000026034	Clk1	0.21	6.92	21.06	4.4E-06	5.7E-04
ENSMUSG00000057716	Tmem178b	0.34	3.12	21.04	4.5E-06	5.7E-04
ENSMUSG00000024232	Bambi	0.29	3.09	20.92	4.8E-06	6.0E-04
ENSMUSG00000021670	Hmgcr	0.16	6.66	20.79	5.1E-06	6.4E-04
ENSMUSG00000022507	1810013L24Rik	0.20	5.47	20.76	5.2E-06	6.5E-04
ENSMUSG00000043953	Ccrl2	-0.71	-0.03	20.69	5.4E-06	6.7E-04
ENSMUSG00000036169	Sostdc1	0.95	2.05	20.45	6.1E-06	7.5E-04
ENSMUSG00000063354	Slc39a4	0.65	0.14	20.30	6.6E-06	8.0E-04
ENSMUSG00000055235	Wdr86	1.26	0.10	20.24	6.8E-06	8.2E-04
ENSMUSG00000056553	Ptprn2	-0.13	8.54	20.17	7.1E-06	8.5E-04

ENSMUSG00000072568	Fam84b	0.31	4.17	20.15	7.1E-06	8.5E-04
ENSMUSG00000043872	Zmym1	0.28	4.01	19.89	8.2E-06	9.7E-04
ENSMUSG00000082984	RP23-304I1.8	-0.86	-0.84	19.85	8.4E-06	9.9E-04
ENSMUSG00000039634	Zfp189	0.36	4.47	19.68	9.1E-06	0.001
ENSMUSG00000046922	Gpr6	-0.70	-0.15	19.69	9.1E-06	0.001
ENSMUSG00000048450	Msx1	0.62	2.67	19.69	9.1E-06	0.001
ENSMUSG00000032840	2410131K14Rik	0.17	5.03	19.66	9.2E-06	0.001
ENSMUSG00000027895	Kcnc4	-0.18	6.38	19.64	9.3E-06	0.001
ENSMUSG00000089857	Zfp882	0.34	2.28	19.60	9.5E-06	0.001
ENSMUSG00000025402	Nab2	0.25	4.85	19.56	9.7E-06	0.001
ENSMUSG00000056592	Zfp658	0.29	3.27	19.48	1.0E-05	0.001
ENSMUSG00000020173	Cobl	-0.38	4.47	19.33	1.1E-05	0.001
ENSMUSG00000019960	Dusp6	0.29	5.91	19.29	1.1E-05	0.001
ENSMUSG00000047777	Phf13	0.32	4.21	19.20	1.2E-05	0.001
ENSMUSG00000044641	Pard6b	0.34	2.23	19.10	1.2E-05	0.001
ENSMUSG00000079737	3110001I22Rik	0.56	0.56	19.09	1.2E-05	0.001
ENSMUSG00000049420	Tmem200a	-0.27	3.59	19.07	1.3E-05	0.001
ENSMUSG00000024298	Zfp871	0.34	3.24	19.03	1.3E-05	0.001
ENSMUSG00000078651	Aoc2	0.29	3.07	18.90	1.4E-05	0.001
ENSMUSG00000051951	Xkr4	0.29	3.06	18.89	1.4E-05	0.001
ENSMUSG00000001763	Tspan33	0.20	5.34	18.80	1.5E-05	0.002
ENSMUSG00000084381	AA413626	-0.83	-0.97	18.77	1.5E-05	0.002
ENSMUSG00000040035	Disp2	-0.09	9.15	18.73	1.5E-05	0.002
ENSMUSG00000053137	Mapk11	-0.18	5.28	18.64	1.6E-05	0.002
ENSMUSG00000067629	Syngap1	-0.16	7.52	18.63	1.6E-05	0.002
ENSMUSG00000042608	Stk40	0.20	5.21	18.57	1.6E-05	0.002
ENSMUSG00000038393	Txnip	0.32	4.14	18.52	1.7E-05	0.002
ENSMUSG00000015467	Egfl8	0.75	-0.18	18.40	1.8E-05	0.002
ENSMUSG00000033191	Tie1	-0.22	3.98	18.41	1.8E-05	0.002
ENSMUSG00000068196	Col8a1	1.24	0.44	18.41	1.8E-05	0.002
ENSMUSG00000073437	D330041H03Rik	-1.02	-1.39	18.41	1.8E-05	0.002
ENSMUSG00000030409	Dmpk	0.21	4.74	18.35	1.8E-05	0.002
ENSMUSG00000031659	Adcy7	0.31	2.81	18.36	1.8E-05	0.002
ENSMUSG00000022376	Adcy8	-0.21	4.63	18.26	1.9E-05	0.002
ENSMUSG00000087598	Zfp111	0.25	3.12	18.26	1.9E-05	0.002
ENSMUSG00000064125	BC068157	-0.18	5.72	18.14	2.1E-05	0.002
ENSMUSG00000030742	Lat	0.68	0.05	18.05	2.1E-05	0.002
ENSMUSG00000022537	Tmem44	-0.23	5.97	17.98	2.2E-05	0.002
ENSMUSG00000026686	Lmx1a	1.08	-1.58	17.91	2.3E-05	0.002
ENSMUSG00000005917	Otx1	0.36	2.03	17.84	2.4E-05	0.002
ENSMUSG00000045648	Vwc2l	-0.51	3.01	17.80	2.5E-05	0.002
ENSMUSG00000030775	Trat1	0.94	-1.45	17.78	2.5E-05	0.002
ENSMUSG00000043091	Tuba1c	0.45	1.21	17.77	2.5E-05	0.002
ENSMUSG00000030123	Plxnd1	-0.27	5.13	17.65	2.7E-05	0.002
ENSMUSG00000050370	Ch25h	1.31	-1.74	17.62	2.7E-05	0.002
ENSMUSG00000067158	Col4a4	0.73	-0.67	17.54	2.8E-05	0.003
ENSMUSG00000092492	B230208B08Rik	-0.59	0.03	17.54	2.8E-05	0.003
ENSMUSG00000048442	Smim5	0.57	0.35	17.53	2.8E-05	0.003
ENSMUSG00000037434	Slc30a1	0.18	5.20	17.45	3.0E-05	0.003
ENSMUSG00000000182	Fgf23	-0.99	-1.42	17.38	3.1E-05	0.003
ENSMUSG00000092558	Med20	0.19	4.42	17.31	3.2E-05	0.003

ENSMUSG00000056771	Gm10010	0.42	1.22	17.24	3.3E-05	0.003
ENSMUSG00000068452	Duox2	1.07	-1.66	17.20	3.4E-05	0.003
ENSMUSG00000036432	Siah2	0.24	3.99	17.18	3.4E-05	0.003
ENSMUSG00000039853	Trim14	-1.05	-1.45	17.17	3.4E-05	0.003
ENSMUSG00000053113	Socs3	-0.51	0.61	17.16	3.4E-05	0.003
ENSMUSG00000078739	CT868723.17-2	-0.73	-0.32	17.16	3.4E-05	0.003
ENSMUSG00000007812	Zfp655	0.18	4.87	17.12	3.5E-05	0.003
ENSMUSG00000040511	Pvr	0.24	3.37	17.08	3.6E-05	0.003
ENSMUSG00000083579	Gm15538	1.12	-1.58	16.98	3.8E-05	0.003
ENSMUSG00000037580	Gch1	0.46	1.01	16.86	4.0E-05	0.003
ENSMUSG00000020941	Map3k14	0.37	2.60	16.84	4.1E-05	0.003
ENSMUSG00000054893	Zfp667	0.18	4.44	16.80	4.1E-05	0.003
ENSMUSG00000055407	Map6	-0.13	6.98	16.81	4.1E-05	0.003
ENSMUSG00000065485	Mir219a-2	-0.69	-0.35	16.82	4.1E-05	0.003
ENSMUSG00000052748	Swt1	0.28	3.26	16.68	4.4E-05	0.004
ENSMUSG00000054146	Krt15	0.62	0.37	16.65	4.5E-05	0.004
ENSMUSG00000034009	Rxfp1	-0.54	1.65	16.64	4.5E-05	0.004
ENSMUSG00000040490	Lrfn2	-0.17	5.07	16.59	4.6E-05	0.004
ENSMUSG00000001506	Col1a1	-0.28	3.51	16.56	4.7E-05	0.004
ENSMUSG00000074221	Zfp568	0.42	2.08	16.51	4.8E-05	0.004
ENSMUSG00000060530	A930017M01Rik	-0.28	2.40	16.48	4.9E-05	0.004
ENSMUSG00000027204	Fbn1	0.30	3.57	16.41	5.1E-05	0.004
ENSMUSG00000027570	Col9a3	0.50	4.18	15.98	6.4E-05	0.005
ENSMUSG00000032640	Chsy1	0.30	2.33	15.98	6.4E-05	0.005
ENSMUSG00000073879	Gm5859	-0.77	0.46	15.99	6.4E-05	0.005
ENSMUSG00000054493	Gm9947	-0.86	-1.03	15.96	6.5E-05	0.005
ENSMUSG00000020907	Rcvrn	0.96	-1.05	15.91	6.6E-05	0.005
ENSMUSG00000054986	Sec14l3	-0.56	0.58	15.91	6.6E-05	0.005
ENSMUSG00000031786	Ccdc135	0.78	2.10	15.87	6.8E-05	0.005
ENSMUSG00000089896	Hsn2	0.71	-0.69	15.84	6.9E-05	0.005
ENSMUSG00000056174	Col8a2	0.77	1.97	15.78	7.1E-05	0.006
ENSMUSG00000007877	Tcap	0.67	-0.11	15.68	7.5E-05	0.006
ENSMUSG00000035692	Isg15	-0.53	1.18	15.69	7.5E-05	0.006
ENSMUSG00000024127	Prepl	-0.14	7.90	15.67	7.5E-05	0.006
ENSMUSG00000043993	2900052L18Rik	-0.31	2.15	15.66	7.6E-05	0.006
ENSMUSG00000032712	2810474O19Rik	0.22	3.50	15.64	7.7E-05	0.006
ENSMUSG00000017417	Plxdc1	-0.34	3.87	15.48	8.3E-05	0.006
ENSMUSG00000024812	Tjp2	-0.15	5.21	15.48	8.3E-05	0.006
ENSMUSG00000087064	Sap30bpos	0.55	0.17	15.42	8.6E-05	0.006
ENSMUSG00000010067	Rassf1	0.30	2.43	15.38	8.8E-05	0.007
ENSMUSG00000039252	Lgi2	-0.19	5.84	15.29	9.2E-05	0.007
ENSMUSG00000049112	Oxtr	-0.24	3.52	15.24	9.4E-05	0.007
ENSMUSG00000020522	Mfap3	0.18	4.94	15.23	9.5E-05	0.007
ENSMUSG00000000214	Th	0.60	0.00	15.20	9.7E-05	0.007
ENSMUSG00000032131	Abcg4	-0.12	6.54	15.20	9.6E-05	0.007
ENSMUSG00000037211	Spry1	0.21	3.68	15.11	1.0E-04	0.007
ENSMUSG00000028268	Gbp3	-0.35	2.22	15.08	1.0E-04	0.007
ENSMUSG00000043131	Mob1a	0.16	4.88	15.07	1.0E-04	0.007
ENSMUSG00000045954	Sdpr	0.28	2.93	15.06	1.0E-04	0.008
ENSMUSG00000035164	Zc3h12c	0.26	2.82	15.05	1.0E-04	0.008
ENSMUSG00000020638	Cmpk2	-0.23	4.26	14.99	1.1E-04	0.008

ENSMUSG00000053117	E330013P04Rik	-0.45	0.89	14.99	1.1E-04	0.008
ENSMUSG00000038587	Akap12	-0.20	4.65	14.95	1.1E-04	0.008
ENSMUSG00000046341	Gm11223	-0.19	5.88	14.96	1.1E-04	0.008
ENSMUSG00000049739	Zfp646	0.15	4.98	14.90	1.1E-04	0.008
ENSMUSG00000029718	Pcolce	0.30	3.05	14.87	1.1E-04	0.008
ENSMUSG00000026322	Htr4	0.29	3.60	14.86	1.2E-04	0.008
ENSMUSG00000037171	Nodal	-0.71	-0.52	14.84	1.2E-04	0.008
ENSMUSG00000055421	Pcdh9	-0.19	5.64	14.81	1.2E-04	0.008
ENSMUSG00000057969	Sema3b	0.50	1.83	14.78	1.2E-04	0.008
ENSMUSG00000086456	Nudt16l1	0.33	1.75	14.77	1.2E-04	0.008
ENSMUSG00000017400	Stac2	-0.14	6.66	14.75	1.2E-04	0.008
ENSMUSG00000041112	Elmo1	-0.15	5.78	14.61	1.3E-04	0.009
ENSMUSG00000092067	A230107N01Rik	-0.44	0.80	14.58	1.3E-04	0.009
ENSMUSG00000033731	3300002A11Rik	0.65	-0.34	14.52	1.4E-04	0.009
ENSMUSG00000019301	Hsd17b1	0.87	-1.04	14.50	1.4E-04	0.009
ENSMUSG00000046159	Chrm3	-0.19	4.88	14.50	1.4E-04	0.009
ENSMUSG00000051354	Samd3	1.35	-1.27	14.46	1.4E-04	0.010
ENSMUSG00000032009	Sesn3	0.22	3.58	14.43	1.5E-04	0.010
ENSMUSG00000039323	Igfbp2	0.25	4.83	14.40	1.5E-04	0.010
ENSMUSG00000026797	Stxbp1	-0.10	9.52	14.32	1.5E-04	0.010
ENSMUSG00000036052	Dnajb5	0.17	7.61	14.32	1.5E-04	0.010
ENSMUSG00000063919	Srrm4	-0.15	5.67	14.33	1.5E-04	0.010
ENSMUSG00000078202	Nrarp	0.22	4.45	14.26	1.6E-04	0.010
ENSMUSG00000067017	Gm3608	-0.27	2.26	14.19	1.7E-04	0.011
ENSMUSG00000023025	Larp4	0.19	5.05	14.18	1.7E-04	0.011
ENSMUSG00000021478	Drd1a	-0.32	2.29	14.14	1.7E-04	0.011
ENSMUSG00000030790	Adm	0.58	-0.02	14.08	1.8E-04	0.011
ENSMUSG00000051029	Serpinb1b	0.61	-0.43	14.08	1.7E-04	0.011
ENSMUSG00000033594	Spata2l	0.16	6.03	13.99	1.8E-04	0.012
ENSMUSG00000065336	Snora34	0.43	0.78	13.99	1.8E-04	0.012
ENSMUSG00000025986	Slc39a10	-0.16	6.88	13.97	1.9E-04	0.012
ENSMUSG00000032503	Arpp21	-0.20	7.41	13.98	1.9E-04	0.012
ENSMUSG00000041216	Clvs1	-0.21	3.56	13.96	1.9E-04	0.012
ENSMUSG00000087141	Plcxd2	-0.26	4.94	13.95	1.9E-04	0.012
ENSMUSG00000061894	Zscan20	0.28	2.75	13.91	1.9E-04	0.012
ENSMUSG00000056749	Nfil3	0.22	3.74	13.89	1.9E-04	0.012
ENSMUSG00000028952	Zbtb48	0.23	4.02	13.85	2.0E-04	0.012
ENSMUSG00000005220	Corin	0.93	-1.37	13.84	2.0E-04	0.012
ENSMUSG00000014158	Trpv4	0.67	1.36	13.83	2.0E-04	0.012
ENSMUSG00000046402	Rbp1	0.28	3.58	13.83	2.0E-04	0.012
ENSMUSG00000024014	Pim1	-0.44	0.70	13.80	2.0E-04	0.012
ENSMUSG00000028211	Trp53inp1	0.23	3.43	13.80	2.0E-04	0.012
ENSMUSG00000045671	Spred2	-0.14	6.70	13.80	2.0E-04	0.012
ENSMUSG00000052684	Jun	0.14	6.95	13.77	2.1E-04	0.013
ENSMUSG00000050640	Tmem150c	-0.12	6.31	13.75	2.1E-04	0.013
ENSMUSG00000050840	Cdh20	-0.23	4.24	13.74	2.1E-04	0.013
ENSMUSG00000081787	Gm13991	-0.25	2.72	13.75	2.1E-04	0.013
ENSMUSG00000066647	Gm5113	-0.20	3.59	13.73	2.1E-04	0.013
ENSMUSG00000007817	Zmiz1	-0.18	6.28	13.71	2.1E-04	0.013
ENSMUSG00000044337	Ackr3	0.23	4.10	13.69	2.2E-04	0.013
ENSMUSG00000046562	Unc119b	0.16	5.38	13.66	2.2E-04	0.013

ENSMUSG00000041193	Pla2g5	0.93	-0.61	13.65	2.2E-04	0.013
ENSMUSG00000005583	Mef2c	-0.15	6.86	13.59	2.3E-04	0.013
ENSMUSG00000034390	Cmip	-0.12	8.43	13.58	2.3E-04	0.013
ENSMUSG00000042997	Nhlrc3	-0.20	3.70	13.57	2.3E-04	0.013
ENSMUSG00000018932	Map2k3	0.20	3.85	13.56	2.3E-04	0.013
ENSMUSG00000044690	NR_002887	-0.29	2.10	13.53	2.3E-04	0.014
ENSMUSG00000049657	Zbtb5	0.19	3.73	13.53	2.3E-04	0.014
ENSMUSG00000021448	Shc3	0.22	3.07	13.49	2.4E-04	0.014
ENSMUSG00000020654	Adcy3	-0.14	5.53	13.48	2.4E-04	0.014
ENSMUSG00000029335	Bmp3	-0.43	0.81	13.46	2.4E-04	0.014
ENSMUSG00000039953	Clstn1	-0.10	10.14	13.46	2.4E-04	0.014
ENSMUSG00000050335	Lgals3	0.51	0.36	13.46	2.4E-04	0.014
ENSMUSG00000022861	Dgkg	-0.15	7.07	13.42	2.5E-04	0.014
ENSMUSG00000085667	Gm12992	-0.68	-0.62	13.42	2.5E-04	0.014
ENSMUSG00000037857	Nufip2	0.20	4.48	13.40	2.5E-04	0.014
ENSMUSG00000065608	Mirlet7c-2	-0.51	0.27	13.40	2.5E-04	0.014
ENSMUSG00000091421	Gm4202	-0.16	6.40	13.40	2.5E-04	0.014
ENSMUSG00000020846	Fam101b	-0.23	4.01	13.30	2.6E-04	0.015
ENSMUSG00000025946	Pth2r	-0.72	-0.83	13.31	2.6E-04	0.015
ENSMUSG00000085941	Gm11201	0.74	-0.93	13.30	2.7E-04	0.015
ENSMUSG00000028051	Hcn3	-0.18	3.98	13.28	2.7E-04	0.015
ENSMUSG00000028341	Nr4a3	0.18	6.65	13.25	2.7E-04	0.015
ENSMUSG00000030533	Unc45a	0.14	5.45	13.23	2.8E-04	0.015
ENSMUSG00000087424	5730405O15Rik	-0.56	-0.22	13.19	2.8E-04	0.015
ENSMUSG00000060427	Zfp868	0.19	3.69	13.17	2.9E-04	0.016
ENSMUSG00000026655	Fam107b	0.19	3.88	13.09	3.0E-04	0.016
ENSMUSG00000042216	Sgsm1	-0.15	6.60	13.07	3.0E-04	0.016
ENSMUSG00000033987	Dnah17	0.33	1.56	13.04	3.0E-04	0.017
ENSMUSG00000072663	Spef2	0.32	1.64	13.04	3.0E-04	0.017
ENSMUSG00000028354	Fmn2	-0.11	6.23	13.01	3.1E-04	0.017
ENSMUSG00000032193	Ldlr	0.24	4.84	13.01	3.1E-04	0.017
ENSMUSG00000016918	Sulf1	0.56	2.87	12.99	3.1E-04	0.017
ENSMUSG00000036452	Arhgap26	-0.13	6.32	12.95	3.2E-04	0.017
ENSMUSG00000065453	Mirlet7d	-0.60	-0.36	12.96	3.2E-04	0.017
ENSMUSG00000024033	Rsph1	0.42	2.48	12.92	3.3E-04	0.017
ENSMUSG00000091318	Gm5415	-0.38	2.33	12.92	3.2E-04	0.017
ENSMUSG00000026072	Il1r1	0.26	3.90	12.88	3.3E-04	0.018
ENSMUSG00000081989	RP23-388P16.1	-0.59	-0.35	12.89	3.3E-04	0.018
ENSMUSG00000025888	Casp1	-0.44	0.67	12.88	3.3E-04	0.018
ENSMUSG00000036687	Tmem184a	0.97	-1.91	12.87	3.3E-04	0.018
ENSMUSG00000049625	Tifab	-0.34	1.56	12.83	3.4E-04	0.018
ENSMUSG00000020190	Mknk2	0.18	5.66	12.82	3.4E-04	0.018
ENSMUSG00000029202	Pds5a	-0.14	4.86	12.81	3.4E-04	0.018
ENSMUSG00000027568	Ntsr1	-0.27	2.41	12.78	3.5E-04	0.018
ENSMUSG00000028060	2810403A07Rik	-0.16	5.82	12.79	3.5E-04	0.018
ENSMUSG00000050069	Grem2	-0.17	4.15	12.79	3.5E-04	0.018
ENSMUSG00000052558	Gm9884	-0.55	-0.07	12.78	3.5E-04	0.018
ENSMUSG00000023915	Tnfrsf21	-0.11	7.06	12.75	3.6E-04	0.018
ENSMUSG00000026312	Cdh7	-0.30	1.93	12.74	3.6E-04	0.018
ENSMUSG00000035262	Amh	0.37	1.39	12.71	3.6E-04	0.018
ENSMUSG00000047878	A4galt	-0.40	1.24	12.72	3.6E-04	0.018

ENSMUSG00000017167	Cntnap1	-0.10	8.22	12.70	3.7E-04	0.019
ENSMUSG00000025816	Sec61a2	-0.13	5.33	12.66	3.7E-04	0.019
ENSMUSG00000090444	D930048G16	-0.42	0.65	12.65	3.8E-04	0.019
ENSMUSG00000029605	Oas1b	-0.72	-0.86	12.64	3.8E-04	0.019
ENSMUSG00000028182	Lrriq3	0.70	-0.86	12.61	3.8E-04	0.019
ENSMUSG00000058488	Kl	0.59	4.57	12.58	3.9E-04	0.019
ENSMUSG00000039081	Zfp503	0.30	2.24	12.58	3.9E-04	0.019
ENSMUSG00000005533	Igf1r	0.20	3.49	12.49	4.1E-04	0.020
ENSMUSG00000015484	Fam163a	-0.17	4.10	12.51	4.0E-04	0.020
ENSMUSG00000019737	Syne4	0.27	2.28	12.51	4.1E-04	0.020
ENSMUSG00000021303	Gng4	-0.17	4.89	12.49	4.1E-04	0.020
ENSMUSG00000028172	Tacr3	-0.33	1.79	12.51	4.0E-04	0.020
ENSMUSG00000034006	Pqlc1	0.16	4.88	12.53	4.0E-04	0.020
ENSMUSG00000035969	Rusc2	-0.11	7.16	12.52	4.0E-04	0.020
ENSMUSG00000038415	Foxq1	-0.32	1.64	12.52	4.0E-04	0.020
ENSMUSG00000044548	Dact1	0.30	2.28	12.50	4.1E-04	0.020
ENSMUSG00000051495	Irf2bp2	0.18	5.22	12.53	4.0E-04	0.020
ENSMUSG00000055200	Sertad3	0.34	1.52	12.49	4.1E-04	0.020
ENSMUSG00000067279	Ppp1r3c	0.20	5.88	12.48	4.1E-04	0.020
ENSMUSG00000092526	Gm17907	-0.50	0.43	12.49	4.1E-04	0.020
ENSMUSG00000027520	Zdbf2	0.19	4.05	12.46	4.2E-04	0.020
ENSMUSG00000085527	Gm15535	-0.37	1.08	12.45	4.2E-04	0.020
ENSMUSG00000062380	Tubb3	-0.14	8.14	12.44	4.2E-04	0.020
ENSMUSG00000041483	Zfp281	0.17	5.26	12.43	4.2E-04	0.020
ENSMUSG00000038132	Rbm24	0.18	4.09	12.43	4.2E-04	0.020
ENSMUSG00000025577	Cbx2	0.33	2.03	12.42	4.3E-04	0.020
ENSMUSG00000085269	Gm15777	-0.47	0.29	12.42	4.3E-04	0.020
ENSMUSG00000072763	5430403G16Rik	0.35	1.13	12.37	4.4E-04	0.021
ENSMUSG00000085906	Gm16882	-0.23	3.36	12.37	4.4E-04	0.021
ENSMUSG00000047045	Tmem164	-0.18	4.34	12.36	4.4E-04	0.021
ENSMUSG00000007682	Dio2	0.22	5.62	12.34	4.4E-04	0.021
ENSMUSG00000072294	Klf12	0.21	3.31	12.34	4.4E-04	0.021
ENSMUSG00000092060	Bend4	0.20	3.44	12.34	4.4E-04	0.021
ENSMUSG00000036766	Dner	-0.12	7.12	12.31	4.5E-04	0.021
ENSMUSG00000030157	Clec2d	-0.34	1.85	12.30	4.5E-04	0.021
ENSMUSG00000029352	Crybb3	0.39	1.20	12.28	4.6E-04	0.021
ENSMUSG00000039298	Cdk5rap2	0.15	4.76	12.26	4.6E-04	0.021
ENSMUSG00000014301	Pam16	0.35	1.46	12.24	4.7E-04	0.021
ENSMUSG00000034275	Igsf9b	0.45	0.55	12.23	4.7E-04	0.021
ENSMUSG00000033253	Szt2	0.16	5.98	12.23	4.7E-04	0.022
ENSMUSG00000053693	Mast1	-0.14	5.91	12.22	4.7E-04	0.022
ENSMUSG00000039431	Mtmr7	-0.12	5.64	12.19	4.8E-04	0.022
ENSMUSG00000074394	Vmn2r29	-0.38	0.92	12.18	4.8E-04	0.022
ENSMUSG00000092622	Khdc3	0.49	0.48	12.17	4.8E-04	0.022
ENSMUSG00000032172	Olfm2	-0.21	5.49	12.15	4.9E-04	0.022
ENSMUSG00000090223	Pcp4	0.40	2.84	12.14	4.9E-04	0.022
ENSMUSG00000024304	Cdh2	-0.11	6.33	12.08	5.1E-04	0.023
ENSMUSG00000032135	Mcam	-0.14	5.04	12.07	5.1E-04	0.023
ENSMUSG00000046191	Pcdhb20	-0.21	3.44	12.07	5.1E-04	0.023
ENSMUSG00000026222	Sp100	-0.31	1.96	12.05	5.2E-04	0.023
ENSMUSG00000021217	Tshz3	-0.27	3.68	12.03	5.2E-04	0.023

ENSMUSG00000025902	Sox17	-0.25	2.39	12.02	5.3E-04	0.023
ENSMUSG00000081855	Rpl17-ps5	-0.48	0.43	12.03	5.2E-04	0.023
ENSMUSG00000020887	A230052G05Rik	-0.96	-1.57	12.01	5.3E-04	0.023
ENSMUSG00000091969	Pcdha3	-0.34	1.25	12.01	5.3E-04	0.023
ENSMUSG00000022377	Asap1	-0.15	5.75	11.98	5.4E-04	0.024
ENSMUSG00000032246	Calml4	0.76	0.88	11.98	5.4E-04	0.024
ENSMUSG00000031167	Rbm3	0.16	5.07	11.92	5.6E-04	0.024
ENSMUSG00000027750	Postn	0.54	1.09	11.90	5.6E-04	0.024
ENSMUSG00000026100	Mstn	-0.83	-1.58	11.89	5.7E-04	0.025
ENSMUSG00000073875	AL824709.35-2	-1.05	-1.95	11.87	5.7E-04	0.025
ENSMUSG00000033685	Ucp2	0.25	4.22	11.86	5.7E-04	0.025
ENSMUSG00000039661	Dusp26	-0.19	5.70	11.82	5.9E-04	0.025
ENSMUSG00000039813	Tbc1d2	0.32	2.60	11.76	6.0E-04	0.026
ENSMUSG00000048482	Bdnf	0.15	5.55	11.77	6.0E-04	0.026
ENSMUSG00000063239	Grm4	-0.22	3.97	11.76	6.0E-04	0.026
ENSMUSG00000018470	Kcnab3	-0.17	4.74	11.73	6.1E-04	0.026
ENSMUSG00000091793	Rian	-0.16	8.88	11.73	6.1E-04	0.026
ENSMUSG00000020032	Nuak1	-0.15	6.19	11.72	6.2E-04	0.026
ENSMUSG00000016619	Nup50	0.14	5.55	11.70	6.2E-04	0.026
ENSMUSG00000018822	Sfrp5	0.91	-1.49	11.70	6.2E-04	0.026
ENSMUSG00000075327	Zbtb2	0.17	3.83	11.68	6.3E-04	0.027
ENSMUSG00000029726	Mepce	0.15	5.71	11.65	6.4E-04	0.027
ENSMUSG00000030748	Il4ra	-0.27	2.36	11.63	6.5E-04	0.027
ENSMUSG00000043929	Klhl15	0.21	3.80	11.62	6.5E-04	0.027
ENSMUSG00000061578	Ksr2	0.35	1.98	11.62	6.5E-04	0.027
ENSMUSG00000044676	Zfp612	-0.17	3.95	11.60	6.6E-04	0.027
ENSMUSG00000055485	Soga1	0.17	4.52	11.60	6.6E-04	0.027
ENSMUSG00000087885	Gm23600	-0.50	-0.05	11.60	6.6E-04	0.027
ENSMUSG00000079362	Gbp6	-0.50	0.30	11.59	6.6E-04	0.027
ENSMUSG00000049353	Rd3	0.67	-0.76	11.58	6.6E-04	0.028
ENSMUSG00000028760	Eif4g3	-0.09	8.14	11.54	6.8E-04	0.028
ENSMUSG00000063297	Luzp2	-0.21	4.72	11.51	6.9E-04	0.029
ENSMUSG00000035711	Dok3	0.20	3.83	11.50	7.0E-04	0.029
ENSMUSG00000047330	Kcne4	0.48	0.13	11.50	7.0E-04	0.029
ENSMUSG00000022053	Ebf2	1.03	-1.85	11.49	7.0E-04	0.029
ENSMUSG00000018412	Kansl1	-0.12	6.59	11.44	7.2E-04	0.029
ENSMUSG00000027806	Tsc22d2	0.14	5.12	11.43	7.2E-04	0.029
ENSMUSG00000028645	Slc2a1	0.13	6.32	11.41	7.3E-04	0.030
ENSMUSG00000050164	Mchr1	0.18	4.38	11.41	7.3E-04	0.030
ENSMUSG00000071203	Naip5	-0.43	0.45	11.41	7.3E-04	0.030
ENSMUSG00000022111	Uchl3	-0.15	4.42	11.39	7.4E-04	0.030
ENSMUSG00000028803	Nipal3	-0.10	6.76	11.39	7.4E-04	0.030
ENSMUSG00000038194	Lhb	0.58	-0.32	11.34	7.6E-04	0.030
ENSMUSG00000025006	Sorbs1	-0.12	5.79	11.33	7.6E-04	0.030
ENSMUSG00000092099	Gm17530	0.73	-1.10	11.34	7.6E-04	0.030
ENSMUSG00000052572	Dlg2	-0.11	7.19	11.31	7.7E-04	0.031
ENSMUSG00000027079	Clp1	0.22	2.94	11.31	7.7E-04	0.031
ENSMUSG00000067578	Cbln4	-0.39	3.25	11.30	7.8E-04	0.031
ENSMUSG00000086996	4933416E14Rik	0.69	-0.96	11.30	7.8E-04	0.031
ENSMUSG00000087130	D230004N17Rik	-0.27	3.71	11.30	7.7E-04	0.031
ENSMUSG00000025404	R3hdm2	-0.12	7.61	11.28	7.8E-04	0.031

ENSMUSG00000060733	Ipmk	0.16	5.66	11.29	7.8E-04	0.031
ENSMUSG00000039834	Zfp335	-0.15	4.80	11.27	7.9E-04	0.031
ENSMUSG00000018844	Fndc8	0.29	2.37	11.26	7.9E-04	0.031
ENSMUSG00000028420	Tmem38b	0.20	3.80	11.25	8.0E-04	0.031
ENSMUSG00000035314	Gdpd5	-0.16	4.43	11.26	7.9E-04	0.031
ENSMUSG00000026384	Ptpn4	0.22	2.99	11.25	8.0E-04	0.031
ENSMUSG00000059898	Dsc3	-0.57	1.20	11.24	8.0E-04	0.031
ENSMUSG00000063179	Pstk	-0.19	3.95	11.20	8.2E-04	0.032
ENSMUSG00000038011	Dnah10	0.31	1.84	11.19	8.2E-04	0.032
ENSMUSG00000041263	Rusc1	-0.10	7.40	11.18	8.3E-04	0.032
ENSMUSG00000073752	Gm10570	-0.72	-1.05	11.16	8.3E-04	0.032
ENSMUSG00000019865	Nmbr	-0.36	0.96	11.15	8.4E-04	0.032
ENSMUSG00000041378	Cldn5	-0.27	5.80	11.14	8.4E-04	0.032
ENSMUSG00000085565	Gm15721	-0.45	0.54	11.12	8.5E-04	0.033
ENSMUSG00000082329	Gm14287	-0.44	0.59	11.11	8.6E-04	0.033
ENSMUSG00000029641	Rasl11a	0.24	3.37	11.09	8.7E-04	0.033
ENSMUSG00000087478	4930506C21Rik	0.36	0.99	11.08	8.7E-04	0.033
ENSMUSG00000051331	Cacna1c	0.15	5.63	11.05	8.9E-04	0.034
ENSMUSG00000031227	Magee1	-0.10	7.43	11.00	9.1E-04	0.035
ENSMUSG00000042372	Dmrt3	0.48	0.11	10.99	9.2E-04	0.035
ENSMUSG00000039116	Gpr126	-0.42	0.51	10.98	9.2E-04	0.035
ENSMUSG00000032122	Slc37a2	0.33	1.59	10.98	9.2E-04	0.035
ENSMUSG00000024044	Epb4.1l3	-0.10	7.34	10.95	9.4E-04	0.035
ENSMUSG00000029401	Rilpl2	0.20	3.88	10.95	9.3E-04	0.035
ENSMUSG00000038135	Crygn	0.56	-0.06	10.95	9.4E-04	0.035
ENSMUSG00000064345	mt-Nd2	-0.14	11.70	10.95	9.4E-04	0.035
ENSMUSG00000066798	Zbtb6	0.17	3.82	10.96	9.3E-04	0.035
ENSMUSG00000087674	4930447M23Rik	-0.26	2.29	10.96	9.3E-04	0.035
ENSMUSG00000047141	Zfp654	0.22	3.88	10.94	9.4E-04	0.035
ENSMUSG00000048138	Dmrt2	-0.49	0.01	10.93	9.4E-04	0.035
ENSMUSG00000013089	Etv5	-0.14	6.22	10.92	9.5E-04	0.035
ENSMUSG00000031292	Cdkl5	0.21	3.45	10.90	9.6E-04	0.035
ENSMUSG00000036197	Gxylt1	0.19	4.00	10.91	9.6E-04	0.035
ENSMUSG00000062151	Unc13c	-0.37	3.56	10.91	9.6E-04	0.035
ENSMUSG00000035493	Tgfbi	0.31	2.26	10.87	9.8E-04	0.036
ENSMUSG00000031609	Sap30	0.27	2.58	10.85	9.9E-04	0.036
ENSMUSG00000071035	Gm5499	-0.14	5.10	10.84	1.0E-03	0.036
ENSMUSG00000051413	Plagl2	0.19	3.46	10.80	0.001	0.037
ENSMUSG00000025498	Irf7	-0.48	1.26	10.79	0.001	0.037
ENSMUSG00000079164	Tlr5	-0.72	-1.12	10.79	0.001	0.037
ENSMUSG00000068966	Zbtb34	0.18	3.83	10.78	0.001	0.037
ENSMUSG00000019278	Dpep1	1.00	-1.91	10.76	0.001	0.037
ENSMUSG00000021908	Gm6768	-0.21	2.98	10.76	0.001	0.037
ENSMUSG00000005483	Dnajb1	0.18	5.78	10.75	0.001	0.038
ENSMUSG00000022307	Oxr1	-0.15	7.81	10.71	0.001	0.038
ENSMUSG00000051212	Gpr183	-0.42	0.43	10.71	0.001	0.038
ENSMUSG00000024277	Mapre2	-0.09	8.02	10.70	0.001	0.038
ENSMUSG00000050567	Maml1	0.19	3.95	10.70	0.001	0.038
ENSMUSG00000018541	Cwc25	0.16	4.22	10.70	0.001	0.038
ENSMUSG00000044211	Gm7887	-0.18	3.70	10.67	0.001	0.039
ENSMUSG00000047228	BC048546	-0.22	2.92	10.68	0.001	0.039

ENSMUSG00000085440	Sorbs2os	-0.32	1.19	10.67	0.001	0.039
ENSMUSG00000045519	Zfp560	0.22	3.05	10.66	0.001	0.039
ENSMUSG00000025921	Rdh10	0.17	3.68	10.64	0.001	0.039
ENSMUSG00000085894	Gm15832	0.30	1.75	10.63	0.001	0.039
ENSMUSG00000089872	Rps6kc1	-0.13	5.64	10.63	0.001	0.039
ENSMUSG00000063663	Brwd3	0.22	2.99	10.59	0.001	0.040
ENSMUSG00000051246	Msantd1	0.27	1.98	10.58	0.001	0.040
ENSMUSG00000050271	D8Ertd82e	-0.17	5.00	10.56	0.001	0.040
ENSMUSG00000041161	Otud3	0.23	2.95	10.47	0.001	0.042
ENSMUSG00000007655	Cav1	-0.19	3.81	10.45	0.001	0.043
ENSMUSG00000032119	Hinfp	0.19	3.18	10.45	0.001	0.043
ENSMUSG00000090061	Nwd2	-0.13	5.11	10.45	0.001	0.043
ENSMUSG00000044636	Csrnp2	0.15	5.02	10.44	0.001	0.043
ENSMUSG00000085944	1700003D09Rik	1.08	-2.24	10.43	0.001	0.043
ENSMUSG00000053153	Spag16	0.61	0.42	10.42	0.001	0.043
ENSMUSG00000086725	A630052C17Rik	-0.25	2.25	10.39	0.001	0.044
ENSMUSG00000080759	Gm15573	0.73	-1.27	10.37	0.001	0.044
ENSMUSG00000025432	Avil	0.54	-0.24	10.36	0.001	0.044
ENSMUSG00000031027	Stk33	0.37	0.85	10.36	0.001	0.044
ENSMUSG00000081605	Gm15953	0.87	-1.72	10.30	0.001	0.046
ENSMUSG00000045008	9030612E09Rik	-0.40	0.60	10.28	0.001	0.046
ENSMUSG00000039830	Olig2	-0.21	5.04	10.26	0.001	0.046
ENSMUSG00000049791	Fzd4	0.23	2.20	10.26	0.001	0.046
ENSMUSG00000090673	Gm340	0.53	-0.34	10.26	0.001	0.046
ENSMUSG00000038702	Dsel	-0.17	4.07	10.24	0.001	0.046
ENSMUSG00000060441	Trim5	-0.59	-0.74	10.23	0.001	0.047
ENSMUSG00000007888	Crlf1	0.13	5.44	10.20	0.001	0.047
ENSMUSG00000044772	Sntn	0.51	-0.11	10.16	0.001	0.048
ENSMUSG00000092300	Cdk3-ps	0.32	1.40	10.09	0.001	0.050
ENSMUSG00000069114	Zbtb10	0.23	2.71	10.07	0.002	0.050
ENSMUSG00000021196	Pfkp	-0.11	7.90	10.07	0.002	0.051
ENSMUSG00000045903	Npas4	0.49	3.94	10.05	0.002	0.051
ENSMUSG00000032579	Hemk1	0.16	4.09	10.04	0.002	0.051
ENSMUSG00000061397	Krt79	0.78	-1.23	10.01	0.002	0.052
ENSMUSG00000038331	Satb2	-0.21	3.89	10.00	0.002	0.052
ENSMUSG00000041351	Rap1gap	-0.11	7.85	10.00	0.002	0.052
ENSMUSG00000019850	Tnfaip3	0.23	2.97	9.99	0.002	0.052
ENSMUSG00000032860	P2ry2	0.47	-0.09	9.97	0.002	0.052
ENSMUSG00000061532	Zfp955b	-0.20	2.73	9.97	0.002	0.052
ENSMUSG00000024049	Myom1	-0.29	1.67	9.96	0.002	0.053
ENSMUSG00000069874	Irgm2	-0.27	2.07	9.95	0.002	0.053
ENSMUSG00000000901	Mmp11	-0.32	1.26	9.94	0.002	0.053
ENSMUSG00000027210	Meis2	-0.23	5.56	9.93	0.002	0.053
ENSMUSG00000032556	Bfsp2	-0.27	2.02	9.93	0.002	0.053
ENSMUSG00000065037	Rn7sk	-1.37	0.61	9.93	0.002	0.053
ENSMUSG00000056258	Kcnq3	0.20	3.52	9.91	0.002	0.053
ENSMUSG00000083889	E530001F21Rik	-0.38	0.78	9.91	0.002	0.053
ENSMUSG00000070639	Lrrc8b	-0.14	4.71	9.87	0.002	0.054
ENSMUSG00000078185	Chml	-0.46	0.05	9.87	0.002	0.054
ENSMUSG00000038291	Snx25	-0.14	5.73	9.86	0.002	0.055
ENSMUSG00000024300	Myo1f	-0.27	1.75	9.85	0.002	0.055

ENSMUSG00000046062	Ppp1r15b	0.13	5.87	9.84	0.002	0.055
ENSMUSG00000021483	Cdk20	0.18	3.39	9.83	0.002	0.055
ENSMUSG00000029309	Sparcl1	-0.11	10.84	9.82	0.002	0.055
ENSMUSG00000038550	Ciart	0.19	3.22	9.81	0.002	0.056
ENSMUSG00000032744	Heyl	-0.25	2.53	9.78	0.002	0.057
ENSMUSG00000022864	D16Ertd472e	0.25	2.07	9.77	0.002	0.057
ENSMUSG00000040412	5330417C22Rik	-0.14	5.45	9.77	0.002	0.057
ENSMUSG00000031748	Gnao1	-0.09	9.41	9.74	0.002	0.057
ENSMUSG00000066956	SC-144776	0.95	-2.10	9.73	0.002	0.057
ENSMUSG00000023017	Asic1	-0.13	5.35	9.72	0.002	0.058
ENSMUSG00000021071	Trim9	-0.08	8.18	9.71	0.002	0.058
ENSMUSG00000056962	Jmjd6	0.15	4.97	9.71	0.002	0.058
ENSMUSG00000057722	Lepr	0.35	0.74	9.70	0.002	0.058
ENSMUSG00000050931	Sgms2	0.29	1.79	9.68	0.002	0.059
ENSMUSG00000021696	Elovl7	0.23	3.98	9.67	0.002	0.059
ENSMUSG00000045817	Zfp36l2	-0.23	4.35	9.66	0.002	0.059
ENSMUSG00000063281	Zfp35	0.18	4.48	9.63	0.002	0.060
ENSMUSG00000042589	Cux2	-0.18	4.77	9.62	0.002	0.060
ENSMUSG00000049521	Cdc42ep1	-0.14	4.61	9.63	0.002	0.060
ENSMUSG00000030500	Slc17a6	-0.37	4.00	9.62	0.002	0.060
ENSMUSG00000020052	Ascl1	-0.23	2.90	9.61	0.002	0.060
ENSMUSG00000001128	Cfp	0.16	4.29	9.60	0.002	0.060
ENSMUSG00000017667	Zfp334	0.13	4.77	9.59	0.002	0.060
ENSMUSG00000028229	Rmdn1	-0.19	3.03	9.59	0.002	0.060
ENSMUSG00000030330	Ing4	-0.11	5.54	9.59	0.002	0.060
ENSMUSG00000036667	Fam115a	-0.09	8.26	9.60	0.002	0.060
ENSMUSG00000046572	Zfp518b	0.15	3.84	9.59	0.002	0.060
ENSMUSG00000058922	Gm10052	-0.19	5.41	9.60	0.002	0.060
ENSMUSG00000082665	Gm11470	0.56	-0.62	9.58	0.002	0.060
ENSMUSG00000028753	Vwa5b1	0.34	1.86	9.57	0.002	0.060
ENSMUSG00000031562	Dctd	-0.22	2.88	9.58	0.002	0.060
ENSMUSG00000048696	Mex3d	0.22	3.73	9.57	0.002	0.060
ENSMUSG00000050405	Tmem151b	-0.17	5.35	9.57	0.002	0.060
ENSMUSG00000020184	Mdm2	0.11	5.67	9.54	0.002	0.061
ENSMUSG00000019897	Ccdc59	-0.13	4.70	9.49	0.002	0.063
ENSMUSG00000014773	Dll1	0.21	2.86	9.49	0.002	0.063
ENSMUSG00000021962	Dcp1a	0.14	4.77	9.47	0.002	0.063
ENSMUSG00000078611	Gm5901	0.28	1.56	9.46	0.002	0.063
ENSMUSG00000038457	Tmem255b	-0.35	0.88	9.44	0.002	0.064
ENSMUSG00000052076	Gm9866	-0.14	4.61	9.44	0.002	0.064
ENSMUSG00000057387	4922502B01Rik	-0.34	0.86	9.44	0.002	0.064
ENSMUSG00000049387	Cox7b2	-0.86	-1.54	9.42	0.002	0.064
ENSMUSG00000032988	Slc16a8	0.47	-0.08	9.40	0.002	0.065
ENSMUSG00000029420	Rimbp2	-0.12	6.58	9.39	0.002	0.065
ENSMUSG00000025064	Col17a1	0.86	-1.66	9.39	0.002	0.065
ENSMUSG00000042846	Lrrtm3	-0.21	4.27	9.38	0.002	0.065
ENSMUSG00000050234	Gja4	-0.28	1.74	9.38	0.002	0.065
ENSMUSG00000041809	Efhc1	0.35	0.79	9.36	0.002	0.066
ENSMUSG00000028214	Gem	0.37	0.63	9.35	0.002	0.066
ENSMUSG00000020836	Coro6	-0.25	5.15	9.34	0.002	0.066
ENSMUSG00000028197	Col24a1	-0.48	0.07	9.34	0.002	0.066

ENSMUSG00000042707	Dnali1	0.37	1.58	9.34	0.002	0.066
ENSMUSG00000046585	Ccdc147	-0.52	-0.33	9.34	0.002	0.066
ENSMUSG00000042605	Atxn2	-0.09	6.67	9.33	0.002	0.066
ENSMUSG00000010080	Epn3	0.59	1.42	9.32	0.002	0.066
ENSMUSG00000023927	Satb1	-0.18	6.35	9.31	0.002	0.066
ENSMUSG00000036578	Fxyd7	-0.19	5.51	9.31	0.002	0.066
ENSMUSG00000038141	Tmem181a	-0.15	3.82	9.31	0.002	0.066
ENSMUSG00000075224	Lrrc55	-0.18	3.70	9.31	0.002	0.066
ENSMUSG00000085929	Gm13421	-0.65	-1.14	9.31	0.002	0.066
ENSMUSG00000091270	Gm17642	0.61	-0.98	9.31	0.002	0.066
ENSMUSG00000042115	Klhdc8a	-0.26	3.25	9.29	0.002	0.067
ENSMUSG00000041439	Mfsd6	-0.12	6.71	9.28	0.002	0.067
ENSMUSG00000032422	Snx14	-0.13	5.41	9.27	0.002	0.067
ENSMUSG00000050587	Lrrc4c	-0.14	6.33	9.27	0.002	0.067
ENSMUSG00000052229	Gpr17	-0.15	5.70	9.27	0.002	0.067
ENSMUSG00000071533	Pcnp	-0.12	5.93	9.26	0.002	0.067
ENSMUSG00000025855	Prkar1b	-0.09	9.28	9.24	0.002	0.068
ENSMUSG00000028758	Kif17	-0.14	4.95	9.23	0.002	0.068
ENSMUSG00000003418	St8sia6	0.24	1.96	9.23	0.002	0.068
ENSMUSG00000035356	Nfkbiz	0.18	3.27	9.22	0.002	0.069
ENSMUSG00000038623	Tm6sf1	-0.20	2.95	9.22	0.002	0.069
ENSMUSG00000035033	Tbr1	-0.17	5.53	9.21	0.002	0.069
ENSMUSG00000053025	Sv2b	-0.07	8.86	9.21	0.002	0.069
ENSMUSG00000038806	Sde2	0.14	4.62	9.20	0.002	0.069
ENSMUSG00000045322	Tlr9	-0.36	0.58	9.19	0.002	0.069
ENSMUSG00000036362	P2ry13	-0.19	3.67	9.19	0.002	0.069
ENSMUSG00000050945	Zfp438	0.23	2.55	9.19	0.002	0.069
ENSMUSG00000040016	Ptger3	-0.34	0.77	9.17	0.002	0.069
ENSMUSG00000030235	Slco1c1	0.13	5.88	9.16	0.002	0.070
ENSMUSG00000048416	Mlf1	0.41	0.43	9.15	0.002	0.070
ENSMUSG00000021177	Tdp1	-0.15	4.21	9.14	0.003	0.070
ENSMUSG00000063415	Cyp26b1	-0.20	4.03	9.13	0.003	0.071
ENSMUSG00000055980	Irs1	0.19	3.00	9.13	0.003	0.071
ENSMUSG00000028337	Coro2a	-0.14	4.43	9.11	0.003	0.071
ENSMUSG00000026383	Epb4.1l5	0.19	2.90	9.10	0.003	0.071
ENSMUSG00000070601	Vmn2r84	-0.43	0.14	9.10	0.003	0.071
ENSMUSG00000089832	Shkbp1	0.17	4.66	9.09	0.003	0.072
ENSMUSG00000034227	Foxj1	0.29	3.63	9.08	0.003	0.072
ENSMUSG00000026725	Tnn	-0.97	-2.10	9.07	0.003	0.072
ENSMUSG00000068696	Gpr88	-0.51	2.46	9.07	0.003	0.072
ENSMUSG00000081382	Rpl18-ps1	0.47	0.20	9.06	0.003	0.072
ENSMUSG00000028522	Mier1	0.13	4.98	9.04	0.003	0.073
ENSMUSG00000078493	A930039A15Rik	0.36	0.50	9.04	0.003	0.073
ENSMUSG00000046207	Pik3r6	-0.29	1.49	9.02	0.003	0.073
ENSMUSG00000082160	Gm11578	-0.33	0.82	9.02	0.003	0.073
ENSMUSG00000047657	Crry-ps	-0.61	-0.96	9.02	0.003	0.074
ENSMUSG00000087018	2900072N19Rik	-0.29	1.18	9.01	0.003	0.074
ENSMUSG00000020363	Gfpt2	0.23	2.68	9.00	0.003	0.074
ENSMUSG00000050671	Ism2	0.73	-1.30	8.99	0.003	0.074
ENSMUSG00000032715	Trib3	-0.54	-0.57	8.98	0.003	0.075
ENSMUSG00000043015	Tmem194b	-0.22	2.94	8.97	0.003	0.075

ENSMUSG00000086782	E130102H24Rik	-0.25	1.97	8.97	0.003	0.075
ENSMUSG00000026167	Wnt10a	-0.26	2.01	8.96	0.003	0.075
ENSMUSG00000035248	Zcchc6	-0.11	5.35	8.96	0.003	0.075
ENSMUSG00000039725	2810408M09Rik	0.14	4.14	8.96	0.003	0.075
ENSMUSG00000066189	Cacng3	-0.13	5.92	8.96	0.003	0.075
ENSMUSG00000092210	A930009A15Rik	-0.62	-0.86	8.96	0.003	0.075
ENSMUSG00000028943	Espn	0.22	3.02	8.94	0.003	0.075
ENSMUSG00000028030	Tbck	-0.16	3.57	8.92	0.003	0.076
ENSMUSG00000054945	Gm9958	-0.41	0.20	8.91	0.003	0.076
ENSMUSG00000045034	Ankrd34b	-0.17	3.92	8.91	0.003	0.076
ENSMUSG00000021913	Ogdhl	-0.11	6.64	8.90	0.003	0.076
ENSMUSG00000031627	Irf2	-0.13	4.67	8.90	0.003	0.076
ENSMUSG00000078923	Ube2v1	0.70	-1.34	8.90	0.003	0.076
ENSMUSG00000042045	Sln	0.58	-0.34	8.89	0.003	0.076
ENSMUSG00000071192	Wfikkn1	0.58	-0.64	8.89	0.003	0.076
ENSMUSG00000062691	Cebpzos	0.27	1.76	8.87	0.003	0.077
ENSMUSG00000038195	Rilp	0.36	0.76	8.87	0.003	0.077
ENSMUSG00000046441	Cmtr2	0.19	2.92	8.84	0.003	0.078
ENSMUSG00000070565	Rasal2	0.17	6.24	8.84	0.003	0.078
ENSMUSG00000032334	Loxl1	0.22	2.52	8.83	0.003	0.078
ENSMUSG00000023882	Zfp54	0.42	0.12	8.83	0.003	0.078
ENSMUSG00000081885	Gm13231	0.67	-1.46	8.83	0.003	0.078
ENSMUSG00000001494	Sost	0.84	-1.65	8.81	0.003	0.079
ENSMUSG00000033715	Akr1c14	-0.37	0.43	8.80	0.003	0.079
ENSMUSG00000021966	Prss52	0.66	-1.17	8.79	0.003	0.080
ENSMUSG00000050295	Foxc1	-0.24	2.42	8.78	0.003	0.080
ENSMUSG00000068566	Myadm	-0.11	6.25	8.79	0.003	0.080
ENSMUSG00000022816	Fstl1	-0.15	5.29	8.78	0.003	0.080
ENSMUSG00000044795	Cyb5d1	-0.17	3.69	8.77	0.003	0.080
ENSMUSG00000047227	Gm527	0.27	1.67	8.77	0.003	0.080
ENSMUSG00000028840	Zfp593	0.54	-0.34	8.76	0.003	0.080
ENSMUSG00000038893	Fam117a	0.22	3.15	8.76	0.003	0.080
ENSMUSG00000037096	Gm9762	-0.14	4.06	8.76	0.003	0.080
ENSMUSG00000046182	Gsg1l	-0.23	5.36	8.75	0.003	0.080
ENSMUSG00000030987	Stim1	-0.09	6.32	8.75	0.003	0.080
ENSMUSG00000034751	Mast4	0.16	5.84	8.74	0.003	0.080
ENSMUSG00000019890	Nts	-0.33	2.43	8.74	0.003	0.081
ENSMUSG00000019861	Gopc	-0.10	5.73	8.73	0.003	0.081
ENSMUSG00000030111	A2m	0.31	2.22	8.72	0.003	0.081
ENSMUSG00000030674	Qprt	0.57	-0.82	8.71	0.003	0.081
ENSMUSG00000047643	Gm5454	-0.22	3.34	8.70	0.003	0.082
ENSMUSG00000008153	Clstn3	-0.08	8.66	8.69	0.003	0.082
ENSMUSG00000027547	Sall4	0.54	-0.71	8.69	0.003	0.082
ENSMUSG00000020420	Zfp607	0.46	-0.25	8.66	0.003	0.083
ENSMUSG00000021260	Hhipl1	-0.29	1.26	8.66	0.003	0.083
ENSMUSG00000025407	Gli1	-0.38	1.37	8.65	0.003	0.083
ENSMUSG00000029636	Wasf3	-0.14	6.60	8.65	0.003	0.083
ENSMUSG00000035852	Misp	0.59	-0.61	8.65	0.003	0.083
ENSMUSG00000022211	Lrrc16b	-0.11	6.38	8.65	0.003	0.083
ENSMUSG00000028804	Csmd2	0.14	5.94	8.64	0.003	0.084
ENSMUSG00000042182	Bend6	-0.15	6.48	8.62	0.003	0.084

ENSMUSG00000002885	Cd97	-0.17	3.33	8.61	0.003	0.084
ENSMUSG00000020014	4930485B16Rik	0.26	2.67	8.62	0.003	0.084
ENSMUSG00000026185	Igfbp5	-0.15	7.79	8.61	0.003	0.084
ENSMUSG00000027177	Hipk3	0.10	5.94	8.61	0.003	0.084
ENSMUSG00000031750	Il34	-0.14	5.57	8.61	0.003	0.084
ENSMUSG00000045775	Slc16a5	-0.44	0.13	8.61	0.003	0.084
ENSMUSG00000019359	Gdpd2	-0.20	2.67	8.58	0.003	0.085
ENSMUSG00000036560	Lgi4	-0.15	3.78	8.58	0.003	0.085
ENSMUSG00000059839	Zfp874b	0.16	4.03	8.58	0.003	0.085
ENSMUSG00000072494	Ppp1r3e	-0.18	3.20	8.59	0.003	0.085
ENSMUSG00000093149	Gm23374	-0.60	-0.97	8.58	0.003	0.085
ENSMUSG00000046192	Iqub	0.32	1.11	8.56	0.003	0.085
ENSMUSG00000030666	Calcb	-0.85	-1.76	8.56	0.003	0.085
ENSMUSG00000059040	Eno1b	-0.18	3.94	8.56	0.003	0.086
ENSMUSG00000040495	Chrm4	0.20	4.65	8.55	0.003	0.086
ENSMUSG00000022607	Ptk2	-0.11	6.93	8.54	0.003	0.086
ENSMUSG00000034917	Tjp3	0.35	0.71	8.54	0.003	0.086
ENSMUSG00000038605	Samd10	-0.16	4.13	8.52	0.004	0.086
ENSMUSG00000048004	Tmem196	-0.30	1.17	8.52	0.004	0.086
ENSMUSG00000074406	Zfp628	-0.17	3.85	8.52	0.004	0.086
ENSMUSG00000091641	Gm17593	-0.49	-0.44	8.52	0.004	0.086
ENSMUSG00000039737	Prkrip1	0.15	4.64	8.47	0.004	0.088
ENSMUSG00000026377	Nifk	0.12	4.98	8.47	0.004	0.089
ENSMUSG00000004105	Angptl2	0.28	1.78	8.46	0.004	0.089
ENSMUSG00000005148	Klf5	-0.26	1.72	8.46	0.004	0.089
ENSMUSG00000026141	Col19a1	0.19	2.95	8.44	0.004	0.089
ENSMUSG00000030098	Grip2	-0.15	4.07	8.45	0.004	0.089
ENSMUSG00000033871	Ppargc1b	0.23	2.05	8.44	0.004	0.089
ENSMUSG00000038072	Galnt11	-0.13	5.23	8.45	0.004	0.089
ENSMUSG00000041911	Dlx1	0.13	5.16	8.45	0.004	0.089
ENSMUSG00000050157	Gm867	0.38	0.43	8.46	0.004	0.089
ENSMUSG00000052861	Dnah6	0.29	2.06	8.45	0.004	0.089
ENSMUSG00000073176	Zfp449	0.22	2.37	8.45	0.004	0.089
ENSMUSG00000018599	Mief2	-0.13	4.43	8.44	0.004	0.089
ENSMUSG00000030446	Zfp273	-0.19	2.69	8.44	0.004	0.089
ENSMUSG00000041515	Irf8	-0.22	2.45	8.43	0.004	0.089
ENSMUSG00000074671	Tspyl3	-0.13	4.93	8.43	0.004	0.089
ENSMUSG00000042364	Snx18	0.12	4.71	8.41	0.004	0.090
ENSMUSG00000037253	Mex3c	0.13	5.26	8.40	0.004	0.090
ENSMUSG00000031971	Ccsap	-0.14	5.68	8.39	0.004	0.091
ENSMUSG00000026437	Cdk18	-0.12	5.05	8.38	0.004	0.091
ENSMUSG00000091890	A830073O21Rik	-0.36	0.38	8.37	0.004	0.091
ENSMUSG00000023852	Chd1	0.12	4.48	8.37	0.004	0.091
ENSMUSG00000054044	Gm9933	-0.72	-1.57	8.37	0.004	0.091
ENSMUSG00000033055	Ankrd54	0.14	4.12	8.36	0.004	0.091
ENSMUSG00000024176	Sox8	0.17	5.54	8.36	0.004	0.091
ENSMUSG00000022099	Dmtn	-0.07	8.53	8.35	0.004	0.091
ENSMUSG00000046010	Zfp830	0.16	3.47	8.35	0.004	0.091
ENSMUSG00000044252	Osbpl1a	-0.12	7.17	8.33	0.004	0.092
ENSMUSG00000065968	Ifitm7	0.76	-1.72	8.33	0.004	0.092
ENSMUSG00000030201	Lrp6	0.12	5.57	8.33	0.004	0.092

ENSMUSG00000024501	Dpysl3	-0.10	6.08	8.31	0.004	0.093
ENSMUSG00000036904	Fzd8	-0.20	2.49	8.30	0.004	0.093
ENSMUSG00000045294	Insig1	0.13	6.03	8.29	0.004	0.094
ENSMUSG00000021891	Mettl6	0.18	4.04	8.28	0.004	0.094
ENSMUSG00000028744	Pqlc2	0.16	3.72	8.28	0.004	0.094
ENSMUSG00000024043	Arhgap28	0.49	-0.50	8.27	0.004	0.094
ENSMUSG00000033454	Zbtb1	0.14	4.27	8.26	0.004	0.094
ENSMUSG00000044835	Ankrd45	-0.14	4.75	8.26	0.004	0.094
ENSMUSG00000084098	Gm13422	0.22	1.98	8.27	0.004	0.094
ENSMUSG00000007440	Pcdha10	-0.08	7.26	8.24	0.004	0.095
ENSMUSG00000021573	Tppp	-0.07	9.04	8.24	0.004	0.095
ENSMUSG00000027399	Il1a	-0.59	-0.97	8.25	0.004	0.095
ENSMUSG00000028149	Rap1gds1	-0.09	8.27	8.24	0.004	0.095
ENSMUSG00000055831	Gm9982	0.51	-0.59	8.25	0.004	0.095
ENSMUSG00000060510	Zfp266	0.13	5.03	8.26	0.004	0.095
ENSMUSG00000087081	6430590A07Rik	-0.57	-0.83	8.25	0.004	0.095
ENSMUSG00000090194	Gm16161	0.66	-1.24	8.25	0.004	0.095
ENSMUSG00000091166	Tstd1	0.40	0.29	8.25	0.004	0.095
ENSMUSG00000060240	Cend1	-0.09	8.12	8.24	0.004	0.095
ENSMUSG00000030699	Tbx6	0.35	0.80	8.23	0.004	0.095
ENSMUSG00000017740	Slc12a5	-0.09	9.15	8.21	0.004	0.096
ENSMUSG00000020134	Peli1	0.12	5.31	8.20	0.004	0.096
ENSMUSG00000020377	Ltc4s	0.55	-0.49	8.20	0.004	0.096
ENSMUSG00000030898	Cckbr	-0.24	3.45	8.20	0.004	0.096
ENSMUSG00000036882	Arhgap33	-0.14	7.28	8.20	0.004	0.096
ENSMUSG00000042106	Fam212a	0.22	2.45	8.19	0.004	0.096
ENSMUSG00000026301	Iqca	0.35	1.02	8.18	0.004	0.096
ENSMUSG00000031351	Zfp185	0.30	1.79	8.19	0.004	0.096
ENSMUSG00000031431	Tsc22d3	0.23	5.84	8.18	0.004	0.096
ENSMUSG00000074657	Kif5a	-0.08	11.29	8.17	0.004	0.097
ENSMUSG00000025196	Cpn1	0.71	-1.44	8.16	0.004	0.097
ENSMUSG00000021457	Syk	-0.21	2.29	8.13	0.004	0.098
ENSMUSG00000021763	BC067074	0.46	1.15	8.15	0.004	0.098
ENSMUSG00000029343	Crybb1	-0.24	2.12	8.14	0.004	0.098
ENSMUSG00000037709	Fam13a	0.16	4.76	8.13	0.004	0.098
ENSMUSG00000042508	Dmtf1	-0.11	5.81	8.13	0.004	0.098
ENSMUSG00000052142	Rasal3	-0.24	1.87	8.15	0.004	0.098
ENSMUSG00000053004	Hrh1	-0.34	0.74	8.14	0.004	0.098
ENSMUSG00000054604	Cggbp1	0.12	5.89	8.14	0.004	0.098
ENSMUSG00000058013	Sept11	-0.10	6.59	8.14	0.004	0.098
ENSMUSG00000064293	Cntn4	-0.18	3.99	8.13	0.004	0.098
ENSMUSG00000005718	Tfap4	-0.24	2.04	8.12	0.004	0.098
ENSMUSG00000001034	Mapk7	0.15	5.23	8.12	0.004	0.098
ENSMUSG00000001441	Npepps	-0.11	7.41	8.11	0.004	0.098
ENSMUSG00000024855	Pacs1	-0.10	6.64	8.11	0.004	0.098
ENSMUSG00000028955	Vamp3	0.13	4.91	8.10	0.004	0.098
ENSMUSG00000029189	Sel1l3	-0.16	5.84	8.11	0.004	0.098
ENSMUSG00000031391	L1cam	-0.13	7.20	8.10	0.004	0.098
ENSMUSG00000074227	Spint2	0.19	4.38	8.10	0.004	0.098
ENSMUSG00000082585	Gm15387	-0.34	0.88	8.10	0.004	0.098
ENSMUSG00000024897	Apba1	-0.11	7.62	8.09	0.004	0.098

ENSMUSG00000030019	Fbxl14	0.15	4.93	8.09	0.004	0.098
ENSMUSG00000031520	Vegfc	-0.26	1.99	8.09	0.004	0.098
ENSMUSG00000032637	Atxn2l	-0.08	7.82	8.09	0.004	0.098
ENSMUSG00000035621	Midn	0.14	5.76	8.09	0.004	0.098
ENSMUSG00000029861	Fam131b	-0.10	6.87	8.07	0.004	0.099
ENSMUSG00000032908	Sgpp2	-0.23	3.25	8.07	0.004	0.099
ENSMUSG00000044352	Sowaha	0.09	7.28	8.07	0.005	0.099
ENSMUSG00000031077	Fadd	-0.29	1.34	8.06	0.005	0.099
ENSMUSG00000027811	4930579G24Rik	0.34	0.65	8.05	0.005	0.100
ENSMUSG00000049892	Rasd1	0.20	5.33	8.05	0.005	0.100

Table 2.3

GeneID	Gene Name	BinID	logFC	*t*	p-value	FDR
ENSMUSG00000007617	Homer1	18	2.0	13.3	5.0E-30	1.3E-24
ENSMUSG00000029657	Hsph1	12	1.0	9.2	1.7E-17	2.3E-12
ENSMUSG00000039801	2410089E03Rik	53	-1.1	-8.5	2.2E-16	2.0E-11
ENSMUSG00000007617	Homer1	17	1.8	8.0	9.3E-14	6.2E-09
ENSMUSG00000031167	Rbm3	2	0.9	7.8	1.8E-13	9.8E-09
ENSMUSG00000034083	Ccdc174	9	1.0	7.2	1.7E-11	7.7E-07
ENSMUSG00000025372	Baiap2	27	0.5	7.0	2.9E-11	1.1E-06
ENSMUSG00000008153	Clstn3	4	1.2	6.6	2.4E-10	7.7E-06
ENSMUSG00000057421	Las1l	15	-1.0	-6.6	2.6E-10	7.7E-06
ENSMUSG00000020287	Mpg	10	-0.6	-6.9	4.9E-10	1.3E-05
ENSMUSG00000063077	Kif1b	41	0.5	6.3	7.3E-10	1.8E-05
ENSMUSG00000043872	Zmym1	1	1.0	6.5	1.1E-09	2.4E-05
ENSMUSG00000035206	Sppl2b	16	0.4	6.1	7.6E-09	1.6E-04
ENSMUSG00000024576	Csnk1a1	27	0.6	5.9	1.4E-08	2.5E-04
ENSMUSG00000025372	Baiap2	26	0.6	5.9	1.4E-08	2.5E-04
ENSMUSG00000041879	Ipo9	36	-0.8	-5.8	1.6E-08	2.7E-04
ENSMUSG00000024576	Csnk1a1	28	0.5	5.7	3.1E-08	4.7E-04
ENSMUSG00000063160 ENSMUSG00000003762	Numbl/Adck4	37	0.4	5.7	3.1E-08	4.7E-04
ENSMUSG00000028782	Bai2	46	0.3	5.6	3.9E-08	5.4E-04
ENSMUSG00000023952	Gtpbp2	44	0.5	5.6	5.2E-08	7.0E-04
ENSMUSG00000028826	Tmem57	2	-0.5	-5.6	7.7E-08	9.8E-04
ENSMUSG00000022710	Usp7	20	0.7	5.4	8.4E-08	0.001
ENSMUSG00000034171	Faah	14	0.4	5.5	1.1E-07	0.001
ENSMUSG00000024826	Dpf2	11	0.5	5.5	1.3E-07	0.001
ENSMUSG00000038383	Pigu	4	0.4	5.4	2.6E-07	0.003
ENSMUSG00000060216	Arrb2	11	0.8	5.3	2.7E-07	0.003
ENSMUSG00000023952	Gtpbp2	35	0.9	5.2	3.1E-07	0.003
ENSMUSG00000027569	Mrgbp	10	0.5	5.4	3.3E-07	0.003
ENSMUSG00000036052	Dnajb5	11	0.4	5.4	3.4E-07	0.003
ENSMUSG00000014873	Surf2	9	0.4	5.3	3.5E-07	0.003
ENSMUSG00000024777	Ppp2r5b	6	0.5	5.3	4.3E-07	0.004
ENSMUSG00000071984	Fndc1	1	0.4	5.2	4.2E-07	0.004
ENSMUSG00000035202	Lars2	21	-0.8	-5.2	5.3E-07	0.004
ENSMUSG00000039219	Arid4b	44	-0.6	-5.1	5.4E-07	0.004
ENSMUSG00000031878	Nae1	15	1.0	5.0	8.3E-07	0.006
ENSMUSG00000044308	Ubr3	54	-0.7	-5.0	8.1E-07	0.006
ENSMUSG00000006676	Usp19	27	0.3	5.1	9.5E-07	0.007
ENSMUSG00000092679 ENSMUSG00000026872	Mir5129/Zeb2	1	-0.2	-5.0	9.4E-07	0.007
ENSMUSG00000075003 ENSMUSG00000037876	Jmjd1c	41	0.7	5.0	9.8E-07	0.007
ENSMUSG00000038664	Herc1	95	-0.6	-4.9	1.1E-06	0.007
ENSMUSG00000038538	Ubn2	38	0.3	5.0	1.1E-06	0.007
ENSMUSG00000040479	Dgkz	11	0.6	4.9	1.2E-06	0.008
ENSMUSG00000063160 ENSMUSG00000003762	Numbl	35	0.5	4.9	1.4E-06	0.008
ENSMUSG00000084896	Gm11632/Fbxl20	14	-0.7	-4.9	1.4E-06	0.009

ENSMUSG00000020883						
ENSMUSG00000050357	Rltpr	40	0.4	4.9	1.8E-06	0.011
ENSMUSG00000075876 ENSMUSG00000064791 ENSMUSG00000075924 ENSMUSG00000015656	Hspa8/Snord14e	38	1.4	4.9	1.8E-06	0.011
ENSMUSG00000020258	Glyctk	3	-1.8	-5.0	2.1E-06	0.012
ENSMUSG00000034739 ENSMUSG00000079592	Mfrp/C1qtnf5	30	-1.2	-4.8	2.1E-06	0.012
ENSMUSG00000034656	Cacna1a	68	1.0	4.7	2.8E-06	0.015
ENSMUSG00000074247	Dda1	13	0.8	4.9	3.4E-06	0.018
ENSMUSG00000027429	Sec23b	30	0.8	4.7	3.7E-06	0.019
ENSMUSG00000042605	Atxn2	51	0.6	4.7	3.8E-06	0.019
ENSMUSG00000007617	Homer1	16	1.7	4.7	3.9E-06	0.020
ENSMUSG00000075876 ENSMUSG00000064791 ENSMUSG00000075924 ENSMUSG00000015656	Hspa8/Snord14e	37	1.3	4.7	4.0E-06	0.020
ENSMUSG00000078789 ENSMUSG00000038268	Dph1/Ovca2	1	0.3	4.7	4.2E-06	0.020
ENSMUSG00000023952	Gtpbp2	31	0.4	4.6	4.8E-06	0.023
ENSMUSG00000031167	Rbm3	22	-0.6	-4.7	4.9E-06	0.023
ENSMUSG00000039515	Ppp2r4	26	0.6	4.7	5.2E-06	0.024
ENSMUSG00000027185	Nat10	27	0.9	4.6	5.3E-06	0.024
ENSMUSG00000035640	Dos	14	1.7	4.6	5.7E-06	0.025
ENSMUSG00000071646	Mta2	3	1.0	4.7	5.8E-06	0.025
ENSMUSG00000038664	Herc1	93	-0.5	-4.6	6.2E-06	0.027
ENSMUSG00000019790	Stxbp5	17	-0.7	-4.6	6.4E-06	0.027
ENSMUSG00000027893	Ahcyl1	7	0.8	4.6	7.2E-06	0.030
ENSMUSG00000040479	Dgkz	10	0.6	4.5	7.2E-06	0.030
ENSMUSG00000026918	Brd3	13	-1.2	-4.6	7.5E-06	0.030
ENSMUSG00000009073	Nf2	14	0.8	4.5	1.1E-05	0.041
ENSMUSG00000079737 ENSMUSG00000022684	3110001I22Rik/Bfar	15	-0.8	-4.6	1.0E-05	0.041
ENSMUSG00000046709	Mapk10	14	-0.4	-4.5	1.1E-05	0.042
ENSMUSG00000052423	B4galt3	14	-0.8	-4.5	1.1E-05	0.042
ENSMUSG00000039219	Arid4b	42	-0.7	-4.5	1.1E-05	0.042
ENSMUSG00000074247	Dda1	12	0.7	4.6	1.1E-05	0.042
ENSMUSG00000035027	Map2k2	9	-0.9	-4.5	1.2E-05	0.045
ENSMUSG00000033365	Ipo13	8	0.4	4.4	1.3E-05	0.047
ENSMUSG00000006024	Napa	16	0.3	4.5	1.4E-05	0.048
ENSMUSG00000019189	Rnf145	12	-0.6	-4.5	1.4E-05	0.048
ENSMUSG00000005378	Wbscr22	30	0.9	4.4	1.4E-05	0.048
ENSMUSG00000020978	Klhdc2	2	0.8	4.5	1.4E-05	0.048
ENSMUSG00000023952	Gtpbp2	36	0.6	4.4	1.4E-05	0.048
ENSMUSG00000040268	Plekha1	15	-1.1	-4.4	1.4E-05	0.048
ENSMUSG00000042726	Trafd1	10	-0.4	-4.4	1.5E-05	0.048
ENSMUSG00000038406	Scaf1	10	0.7	4.4	1.5E-05	0.050
ENSMUSG00000063160 ENSMUSG00000003762	Numbl/Adck4	34	0.7	4.4	1.6E-05	0.050
ENSMUSG00000022099	Dmtn	6	0.5	4.4	1.6E-05	0.052
ENSMUSG00000008153	Clstn3	5	1.3	4.4	1.7E-05	0.053

ENSMUSG00000050875	A730017C20Rik	12	-0.7	-4.5	1.7E-05	0.053
ENSMUSG00000001018	Snapin	7	-0.5	-4.5	1.8E-05	0.054
ENSMUSG00000007850	Hnrnph1	46	0.9	4.3	1.9E-05	0.056
ENSMUSG00000021327	Zkscan3	9	0.3	4.4	1.9E-05	0.056
ENSMUSG00000084708 ENSMUSG00000065862 ENSMUSG00000059796	Gm22988/Gm2402 9/Eif4a1	38	0.5	4.3	1.8E-05	0.056
ENSMUSG00000029050	Ski	20	0.5	4.4	2.0E-05	0.059
ENSMUSG00000024576	Csnk1a1	25	0.4	4.3	2.1E-05	0.062
ENSMUSG00000003269	Cyth2	23	0.4	4.3	2.4E-05	0.065
ENSMUSG00000020612	Prkar1a	11	0.4	4.4	2.3E-05	0.065
ENSMUSG00000020882	Cacnb1	3	0.6	4.3	2.3E-05	0.065
ENSMUSG00000024807	Syvn1	34	0.6	4.3	2.4E-05	0.065
ENSMUSG00000025575	Cant1	5	0.8	4.4	2.4E-05	0.065
ENSMUSG00000040447	Spns2	7	0.5	4.3	2.4E-05	0.065
ENSMUSG00000052387	Trpm3	15	2.6	4.3	2.4E-05	0.065
ENSMUSG00000031367	Ap1s2	9	0.5	4.4	2.5E-05	0.067
ENSMUSG00000040029	Ipo8	20	1.5	4.3	2.5E-05	0.067
ENSMUSG00000057522	Spop	19	-1.2	-4.3	2.6E-05	0.068
ENSMUSG00000038696	Mapkap1	7	0.5	4.3	2.7E-05	0.071
ENSMUSG00000002280	Narfl	18	-0.6	-4.3	2.8E-05	0.072
ENSMUSG00000057421	Las1l	14	-0.8	-4.3	3.0E-05	0.077
ENSMUSG00000038611	Phrf1	21	1.8	4.2	3.2E-05	0.079
ENSMUSG00000034739 ENSMUSG00000079592	Mfrp/C1qtnf5	10	4.5	4.2	3.3E-05	0.083
ENSMUSG00000031167	Rbm3	3	0.8	4.2	3.5E-05	0.085
ENSMUSG00000005417	Mprip	15	0.5	4.2	3.5E-05	0.086
ENSMUSG00000020894	Vamp2	13	0.5	4.3	3.6E-05	0.088
ENSMUSG00000060261	Gtf2i	47	-1.6	-4.2	3.7E-05	0.088
ENSMUSG00000084708 ENSMUSG00000065862 ENSMUSG00000059796	Gm22988/Gm2402 9/Eif4a1	37	0.5	4.2	3.7E-05	0.088
ENSMUSG00000025571	Tnrc6c	16	-0.5	-4.2	3.8E-05	0.090
ENSMUSG00000052593	Adam17	7	2.4	4.2	3.9E-05	0.092
ENSMUSG00000020918	Kat2a	8	0.5	4.2	4.0E-05	0.094
ENSMUSG00000025860	Xiap	24	0.2	4.2	4.1E-05	0.095
ENSMUSG00000034675	Dbn1	11	0.3	4.2	4.4E-05	0.099
ENSMUSG00000071646	Mta2	2	1.5	4.2	4.4E-05	0.099

CHAPTER 3: Object-Location Training Elicits An Overlapping But Temporally Distinct Transcriptional Profile From Contextual Fear Conditioning

Abstract

Hippocampus-dependent learning is known to induce changes in gene expression, but information on gene expression differences between different learning paradigms that require the hippocampus is limited. The bulk of studies investigating RNA expression after learning use the contextual fear conditioning task, which couples a novel environment with a footshock. Although contextual fear conditioning has been useful in discovering gene targets, gene expression after spatial memory tasks has received less attention. In this study, we used the object-location memory task and studied gene expression at two time points after learning in a high-throughput manner using a microfluidic qPCR approach. We found that expression of the classic immediate-early genes changes after object-location training in a fashion similar to that observed after contextual fear conditioning. However, the temporal dynamics of gene expression are different between the two tasks, with object-location memory producing gene expression changes that last at least 2 hours. Our findings indicate that different training paradigms may give rise to distinct temporal dynamics of gene expression after learning.

Introduction

Long-term memory is critical to our lives, yet the molecular mechanisms that create and stabilize memories are still poorly understood. The hippocampus, which encodes contextual information, has been heavily studied in an effort to better understand these mechanisms. Transcription is required to convert labile short-term memories into stable long-term memories during the period of memory consolidation [11, 108]. The expression of many genes is regulated within the first hour after learning in the hippocampus [61, 70-72, 138, 139]. Epigenetic mechanisms, such as histone acetylation, can modulate this transcription to enhance or dampen long-term memory formation [23, 24, 26, 27, 51, 55, 58, 63].

Most research into transcriptional regulation in the hippocampus has used contextual fear conditioning as the paradigm to test learning and memory [70, 71, 111, 113]. This is primarily because contextual fear conditioning produces a robust memory that has a well-defined time of acquisition due to the requirement of only a single training session [2]. Although this task has proven useful for dissecting the phases of memory and mapping the transcriptional landscape after learning, it also introduces a footshock that can be stressful to the animal. It is therefore important to study gene expression in other memory tasks that are more similar to the learning events that occur in daily life.

Spatial learning requires the hippocampus and can be measured using the Morris water maze, Barnes maze, or object-location memory (OLM) tasks that do not require a footshock. These spatial tasks are also known to regulate transcription in the hippocampus, including many of the same genes and processes required for contextual fear memory [56, 76, 77, 110, 112, 140-144]. There is evidence that contextual and spatial learning in the hippocampus can utilize different molecular pathways [130], so gene expression may also differ after these two tasks. Like contextual fear memory,

OLM is a hippocampus-dependent task [43]. However, the targets and temporal resolution of the gene expression changes after OLM have not been thoroughly studied. The goal of this study was to investigate the transcriptional profile that occurs within the first transcriptional wave after OLM learning [16, 108] and compare this transcriptional profile to that of contextual fear conditioning. Gene expression changes within this window after fear conditioning are typically highest 30 minutes after training and return to baseline by 2 hours [61, 70, 109]. Using a Fluidigm HD microfluidic high-throughput qPCR system, we examined expression of 96 different candidate genes at both 30 minutes and 2 hours after OLM training in a single experiment. We found that the most commonly studied genes after fear conditioning show a similar profile after OLM. However, OLM produces long-lasting expression changes in a number of genes that are not observed after fear conditioning.

Materials and Methods

Subjects

Forty-two C57BL/6J mice were maintained under standard conditions with food and water available *ad libitum*. Adult male mice 3 months of age were kept on a 12-hr light/12-hr dark cycle with lights on at 7AM. All behavioral and biochemical experiments were performed during the light cycle with training starting at approximately 7AM (ZT0). All procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Behavior

Object-location memory (OLM) was carried out as previously described [43, 56]. Briefly, naïve three month old male C57Bl/6J mice were singly housed for a week and handled for 2 min/day for five consecutive days prior to tissue collection. One animal per behavioral group was trained and dissected each day for 10 total days to allow all animals to be dissected at the same circadian time. Exploration was normal in all mice used in this experiment (data not shown). One animal per training session was tested in a 24hr retrieval test the following day to ensure the training proceeded correctly. Half of the handled animals received OLM training, and half of the animals were left undisturbed on training day and were sacrificed at the same circadian time points as trained animals. On the day of training, OLM mice were given a single block of four 6 min trials with an inter-trial interval of 3 min. The animals were habituated to an empty arena with a black and white striped spatial cue on one wall in the first trial, followed by three trials of object exposure. Each mouse was exposed to three distinct objects: a rectangular metal tower, a glass bottle, and a white plastic cylinder that were arranged in a V-shaped spatial pattern in the arena. Objects were positioned in the arena with at least two inches of spacing around each object to allow free exploration of all objects. During the inter-training interval (ITI), animals were gently removed from the arenas, and the arenas and objects were cleaned with 70% ethanol. Objects were not moved during the ITI. Immediately following the final trial, animals were gently placed in their home cage, and returned to the colony room until tissue collection.

Fear conditioning was performed as previously described [55, 61] with handling for 3 days prior to conditioning. Briefly, the conditioning protocol entailed a single 2-sec, 1.5mA footshock terminating at 2.5 minutes after placement of the mouse in the novel

chamber. Mice were left in the chamber for an additional 30 seconds and then returned to their homecage.

RNA isolation

Hippocampi were dissected 30 minutes and 2 hours after the last training session into RNAlater (Qiagen, Valencia, CA) and frozen on dry ice. Tissue was homogenized using a TissueLyser system and RNA was extracted using the miRNeasy kit (Qiagen) according to the manufacturer's instructions.

cDNA synthesis and high-throughput qPCR

RNA concentration was determined using a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA) and 1µg RNA was used in each RETROscript (Ambion, Austin, TX) cDNA synthesis reaction with random decamers, 10x RT Buffer and no heat denaturation according to the manufacturer's protocol. Concentrated cDNA was used in a specific target reaction following the manufacturer's recommendations (Fluidigm Corp. South San Francisco, CA). Briefly, Taqman assays for all 96 probes were pooled to a concentration of 0.2X (1:100) and 1.25ul of the pooled assay mix was combined with 2.5ul 2X Taqman Preamp Master Mix (Life technologies, Carlsbad, CA) and 1.25µl cDNA. Taqman probe IDs can be found in **Table S1**. The preamplification reaction was cycled using the following protocol in a 7500 Fast Real-Time PCR system: 10 min at 95C, then 14 cycles of 95C for 15s followed by 60C for 4min. Preamplified samples were diluted 1:5 using 1X TE. Samples were then delivered to the Molecular Profiling Core at the University of Pennsylvania, where they were run on a 96.96 Dynamic Array IFC on the Biomark HD machine (Fluidigm Corp).

For validation and the 120 minute fear conditioning experiment, cDNA reactions were diluted to 2 ng/ul in water, and real-time RT-PCR reactions were prepared in 384-well optical reaction plates with optical adhesive covers (Life technologies). Each reaction was composed of 2.25µl cDNA (2 ng/ul), 2.5µl 2x Taqman Fast Universal Master Mix (Life Technologies), and 0.25µl of Taqman probe. Reactions were performed in triplicate on the Viia7 Real-Time PCR system (Life Technologies, Carlsbad, CA).

Data analysis

High-throughput qPCR was analyzed using the Fluidigm Real Time PCR Analysis program and Microsoft Excel. Genes with at least one sample having an average Ct ≥20 were discarded as being non-expressed or failed reactions. This included *Dnmt3b*, *Erbb2*, *Esrrg*, *Fosb*, *Hdac1*, *Hdac4*, *Jun*, *Nr6a1*, *Pparg*, and *Trdmt1*, which brought the total number of genes tested to 86. Relative quantification of gene expression between groups was performed using the ΔΔCt method as described previously [55]. The difference between each Ct and the average Ct for that gene was subtracted from the average of three housekeeper genes treated in the same manner. A p-value of 0.01 was used for significance to control for the number of t-tests performed. This p-value cutoff was chosen because we selected genes for analysis that we expected to change, and thus Bonferroni correction is too strict. This 1% chance of a type I error corresponds to one false positive per 100 t-tests. Because 86 t-tests were performed, this p-value would suggest less than 1 false positive in the data, limiting the amount of type I errors introduced by multiple testing.

Results

Immediate Early Genes Are Regulated 30 minutes after OLM training

We chose sixteen representative genes that have been studied 30 minutes after fear conditioning to examine expression profiles 30 minutes after OLM training. The genes were chosen for well-studied expression changes (*Arc*, *Bdnf4, Egr1, Fos, Homer1*), genes our lab has previously studied (*Fosl2 [61], Gadd45* family [73]), or from microarray data (*Btg2*, *Cpeb3*, *Histh2hab*, *Sik1*, *Sox18*, *Tob1*, *Tob2 [109]*). cDNA samples underwent specific target amplification and were run on a 96.96 Fluidigm Biomark HD plate in triplicate (96 genes, 32 samples). The full list of Taqman assays is available in **Table 3.1**. Ten genes were excluded due to too low expression or a failed reaction, bringing the total number of genes tested to 86 (See **Methods** for genes). In all cases, immediate early gene (IEG) expression after OLM mirrored expression after fear conditioning (**Figure 3.1**). Previously studied genes including *Arc*, *Bdnf4*, *Egr1*, *Fos* and *Homer1* were upregulated as anticipated (*Arc* 272% $p=4.7x10^{-8}$; *Bdnf4* 53% $p=3.3x10^{-7}$; *Egr1* 225% $p=4.4x10^{-8}$; *Fos* 410% $p=1.2x10^{-10}$; *Homer1* 31% $p=1.4x10^{-4}$) [70, 121, 139, 145]. The probe against *Homer1* recognizes both *Homer1a* and *Homer1c*, but research from our lab and others suggests that this effect is primarily due to *Homer1a* [121]. Further investigation is required to investigate specific *Homer1* isoforms regulated by OLM. Genes that our lab discovered to be regulated after contextual fear conditioning using microarrays [109], including *Btg2* (27% $p=8.9x10^{-6}$), *Hist2h2ab* (-26% $p=8.8x10^{-4}$), *Sik1* (70% $p=1.3x10^{-5}$), *Sox18* (-21% p=0.002), and *Tob2* (30% p=0.004) showed similar changes after OLM. The genes *Gadd45b* and *Gadd45g* showed increased expression (32% p=0.004, 32% p=0.001) while *Gadd45a* did not (p=0.20), as has been reported previously by our lab and others [73, 146]. This observation suggests that the most

commonly studied genes after contextual fear conditioning are similarly regulated after spatial behavioral tasks such as object-location memory.

Nuclear Hormone Receptors Display a Limited Response to OLM

A subset of nuclear hormone receptors are known to be regulated 30 minutes after fear conditioning, including the *Nr4a* family of orphan nuclear receptors [61]. We tested all 37 nuclear hormone receptors that are expressed in the hippocampus for changes after OLM training (**Figure 3.2**). The Nr4a family of nuclear receptors (*Nr4a1*, *Nr4a2*, *Nr4a3*), which are known to be necessary for long-term fear memory [58, 61, 144], all displayed increased expression at 30 minutes after OLM. Rev-ErbA (*NR1D1*), COUP-TFII (*NR2F2*), and retinoid X receptor gamma (*Rxrg*) all showed decreased expression at 30 minutes. No other nuclear receptors were observed to respond to spatial learning at this time point or at 120 minutes after OLM (data not shown). This contrasts with the large number of nuclear receptors that our lab observed to change after fear conditioning training in our previous study [61], which included increased expression of 13 nuclear receptor genes between 30 and 120 minutes after training. These results may indicate transcriptional regulation of this class of genes depends on the training paradigm.

Regulators of Transcription Show Limited Changes in Response to OLM

Histone acetylation is known to be a crucial regulator of transcription during memory consolidation [23, 24, 26-28, 51, 55, 58, 143]. To test whether expression levels of histone acetylation modifying enzymes are regulated by OLM, we tested all histone deacetylases (HDACs, **Figure 3.3A**) and 16 histone acetyltransferases (HATs, **Figure 3.3B**) representing each class of enzyme, including the HATs CBP and p300 that have been shown to be essential for memory formation. The probes against *Hdac1* and

Hdac4 did not amplify and were discarded. None of the HATs tested showed a gene expression change, in contrast to previous reports showing changes in expression of CBP, p300 and PCAF after the Morris Water Maze [77]. However, *Hdac7* displayed reduced expression after OLM. HDAC7 is a class IIa HDAC that has not been previously linked to memory formation. This may suggest a novel role for HDAC7 in hippocampus-dependent memory formation. In addition to the regulators of histone acetylation, we chose ten genes that are known to regulate transcription in other ways. None of these genes showed any changes in transcription at 30 minutes after OLM training (**Figure 3.4**).

OLM Induces Longer Lasting Gene Expression Changes than Fear Conditioning

In addition to the 30 minute timepoint that has shown such robust changes after fear conditioning, we also tested hippocampal samples taken 2 hours after OLM training to investigate the persistence of these transcriptional changes. Interestingly, a number of genes that are upregulated at 30 minutes remain elevated 2 hours after OLM training. This includes highly induced genes that appear to be slowly returning to baseline, such as *Egr1* and *Fos*, but also genes that maintain a similar level of induction as observed at 30 minutes such as *Bdnf4*, *Fosl2*, *Homer1*, *Nr4a2* and *Nr4a3* (**Figure 3.5A**). *Sin3a* was not changed at 30 minutes, but shows a selective change at 2 hours. The gene expression profiles at 30 minutes and 2 hours for *Arc*, *Egr1*, *Fos*, *Nr4a1*, and *Nr4a2* were confirmed by standard 384-well qPCR (data not shown). To test whether these same genes show transcriptional changes after fear conditioning, we prepared cDNA from samples that were collected 2 hours after fear conditioning. None of the genes determined to change 2 hours after OLM showed a significant change 2 hours after fear

conditioning (**Figure 3.5B**), indicating a long-lasting gene expression response specific to OLM.

Discussion

In this study, we investigated the transcriptional changes that occur in response to OLM training using powerful high-throughput qPCR technology and compared these changes to fear conditioning. In a single run, we were able to study 96 different genes in 2 different time points after OLM training with n=8 mice per group using microfluidic high-throughput qPCR. This type of throughput, flexibility, and consistency is not possible with any other qPCR technology. In addition to requiring more pipetting steps, standard qPCR would have required the same housekeepers to be run on each individual plate and limited the number of targets that could be tested. Using a high-throughput approach allowed us to reliably determine that gene expression changes after OLM last longer than similar expression changes after contextual fear.

Our study discovered that commonly studied IEGs, such as *Fos* and *Arc*, show similar expression differences after fear conditioning and after OLM, indicating overlap between contextual and spatial learning. In a previous study from our lab [61], we found that a number of nuclear receptors exhibit increased expression after contextual fear conditioning. Our current findings suggest a more limited regulation of this class of genes after OLM. It is unclear whether the wider regulation after fear conditioning is in response to the footshock or whether the timecourse of expression after OLM is different. As seen after fear conditioning, all 3 members of the *Nr4a* family of orphan nuclear receptors were upregulated after OLM. However, while *Nr4a1* returned to baseline by 2 hours, *Nr4a2* and *Nr4a3* did not, suggesting that different processes may

regulate *Nr4a1* than the other two family members. Future studies will aim to determine how expression increases of *Nr4a2* and *Nr4a3* are maintained after OLM training.

It is interesting to note that *Hdac7* and *Sin3a* are regulated by OLM while HATs are not. This may suggest that relieving the negative repression of histone acetylation is a crucial step for long-term memory formation. Although class I HDACs have been heavily implicated in learning and memory [56, 58, 63, 101], class IIa HDACs have received less attention. A study by Agis-Balboa et al. demonstrated that loss of the class IIa member HDAC5 impairs spatial memory [104], but those experiments used a complete knockout mouse line that has the potential for developmental or extrahippocampal effects. Our study is the first to observe changes in *Hdac7* in response to learning in the hippocampus.

The most intriguing finding of this study was the long-lasting regulation of gene expression 2 hours after OLM, something that is not seen after fear conditioning. It might be expected that the fear of a footshock would produce a stronger transcriptional response in the hippocampus than would the spatial rearrangement of objects. There are a number of potential causes for this disparity, although the most likely explanation is that the multiple training sessions required for OLM induce a stronger response than the single shock training used by our lab for fear conditioning. It would be interesting to test whether a multiple shock fear conditioning protocol induces longer lasting gene expression changes. Also, there could be an association between the novel context and the novel objects formed during OLM training that is not present in fear conditioning. Testing mice in the context only, introducing novel objects, or altering the number of training trials could determine whether these changes are sufficient to elicit gene expression changes. Further, different molecular mechanisms may regulate contextual

and spatial learning [130]. Future studies can test for changes at the protein level, although mRNA and protein levels generally agree after learning [15, 147]. Additional investigation into later time points after OLM training will be required to see if gene expression changes that occur well after fear conditioning [145] also exist after OLM. It is interesting that not all genes with increased expression at the 30 minute timepoint remain elevated for 2 hours after OLM. Future studies will determine whether specific epigenetic modifications regulate this longer term maintenance of gene expression at particular genes.

Figure Legends

Figure 3.1. Classic IEGs Show Expected Expression Changes after OLM Training. 16 genes that are known to be induced 30 minutes after contextual fear conditioning were studied 30 minutes after OLM training. Each gene tested displayed the expression change that would be expected after contextual fear conditioning, indicating these genes may represent a common transcriptional response to learning. All error bars denote s.e.m. and * indicates $p<0.01$.

Figure 3.2. Limited Expression Changes of Nuclear Receptors after OLM training. Because of the known involvement of the *Nr4a* nuclear receptor family in memory, we tested expression of all nuclear receptors expressed in the hippocampus 30 minutes after OLM training. The *Nr4a* family displayed increased expression after OLM, while *NR1D1*, *NR2F2*, and *RXRg* had reduced expression. All error bars denote s.e.m. and * indicates $p<0.01$.

Figure 3.3. Modifiers of Histone Acetylation Display Limited Regulation after OLM Training. Histone modifying enzymes were tested for expression changes 30 minutes after OLM training. (A) *Hdac7*, a class IIa HDAC, was the only family member found to change expression after OLM. (B) No HATs were observed to change expression after OLM. All error bars denote s.e.m. and * indicates $p<0.01$.

Figure 3.4. No Changes in Other Transcriptional Regulators after OLM Training. Other genes that can regulate gene expression, including DNMTs, were tested 30

minutes after OLM training. No differences in any gene were observed. All error bars denote s.e.m. and * indicates $p<0.01$.

Figure 3.5. OLM Training Induces Long-Lasting Changes in Gene Expression Not Seen after Fear Conditioning. (A) Every gene was also tested 2 hours after OLM training to observe the maintenance of transcription. Genes shown in this figure are those that were changed at 2 hours after OLM, all other genes were unchanged. *Sin3a* was the only gene uniquely regulated at the 2 hour time point. (B) These same genes do not show gene expression changes 2 hours after contextual fear conditioning. All error bars denote s.e.m. and * indicates $p<0.01$.

Table 3.1. Taqman Assays Used In This Experiment.

Contributions

This chapter was written by Shane G. Poplawski with suggestions by Ted Abel, Hannah Schoch and Karl Peter Giese. Experiments were planned by Shane Poplawski, Hannah Schoch and Mathieu Wimmer. Experiments were carried out by Shane Poplawski, Hannah Schoch, and Joshua Hawk. Behavioral scoring was performed by Jennifer Walsh. We thank Morgan Bridi, Giulia Porcari, and Robbert Havekes for constructive discussions and critical reading of the chapter.

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Figures

Figure 3.1

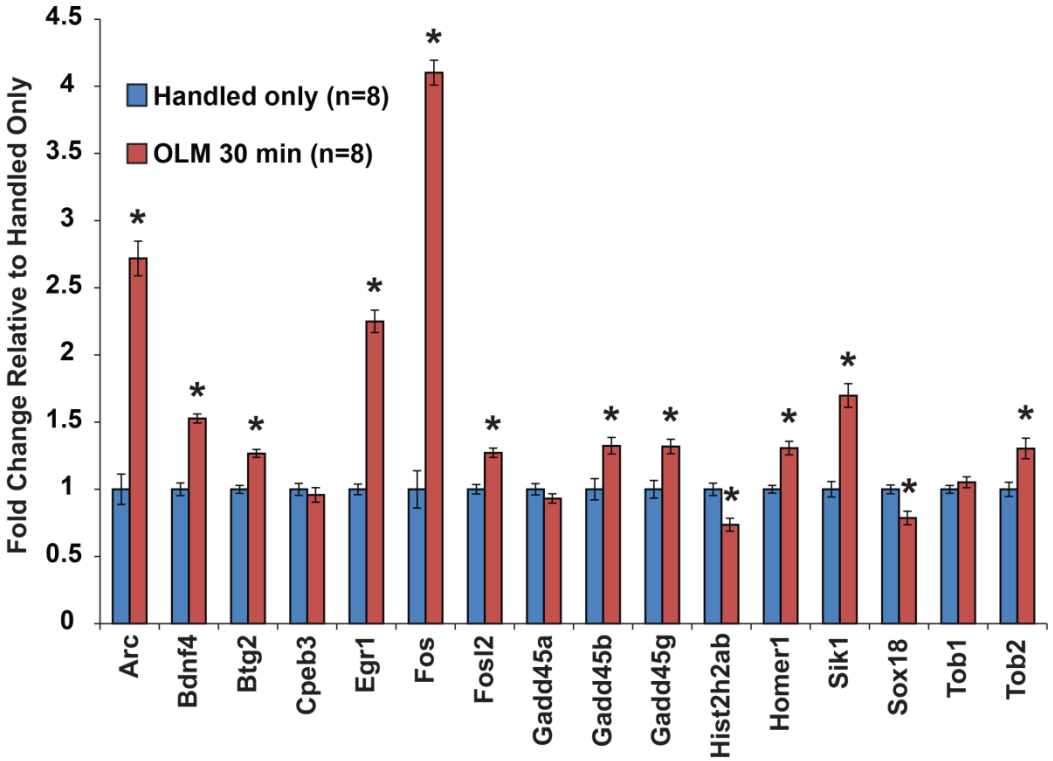
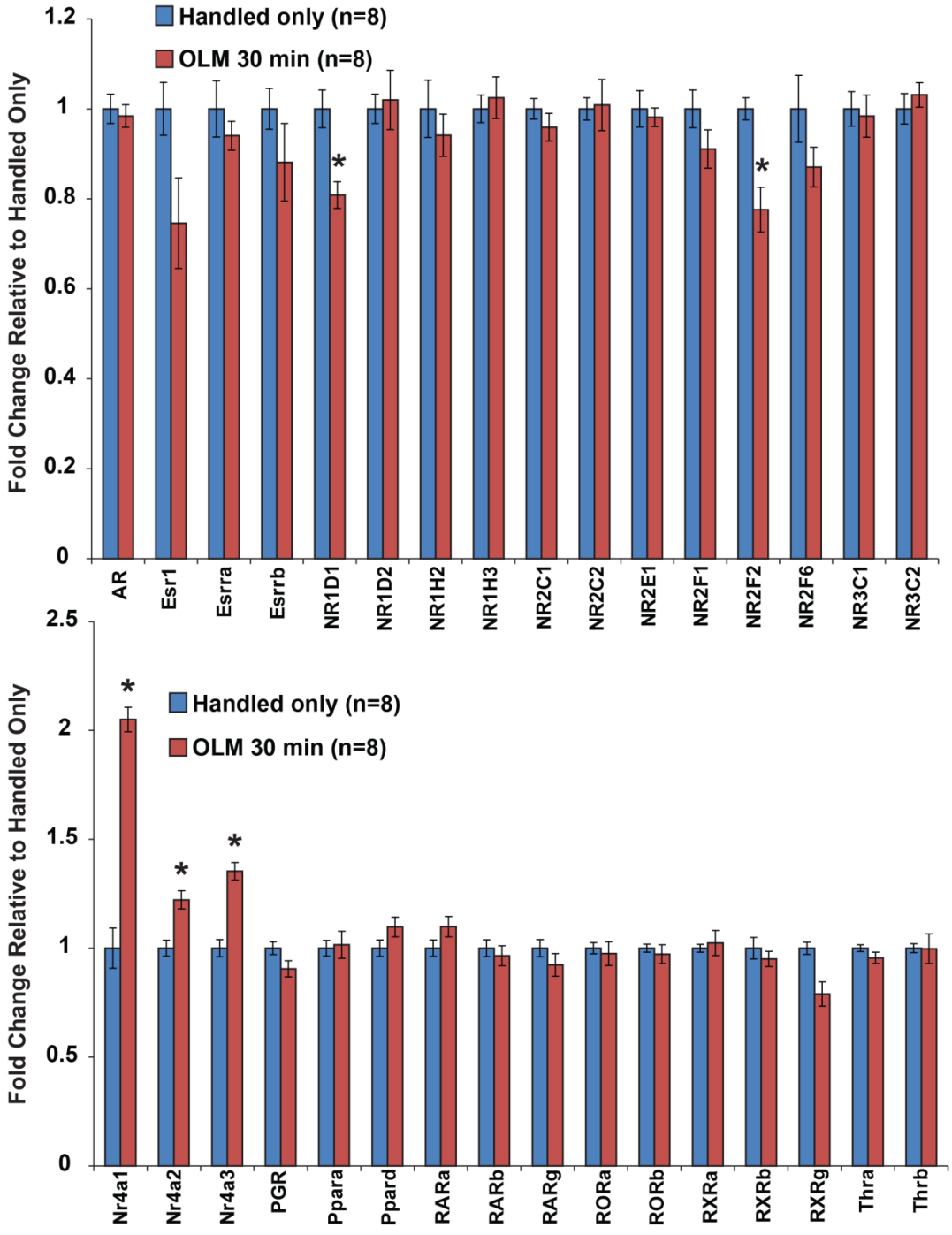


Figure 3.2


Handled only (n=8)
OLM 30 min (n=8)
Fold Change Relative to Handled Only
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1
0.8
0.6
0.4
0.2
0
AR
Esr1
Esrra
Esrrb
NR1D1
NR1D2
NR1H2
NR1H3
NR2C1
NR2C2
NR2E1
NR2F1
NR2F2
NR2F6
NR3C1
NR3C2
Handled only (n=8)
OLM 30 min (n=8)
Fold Change Relative to Handled Only
2.5
2
1.5
1
0.5
0
Nr4a1
Nr4a2
Nr4a3
PGR
Ppara
Ppard
RARa
RARb
RARg
RORa
RORb
RXRa
RXRb
RXRg
Thra
Thrb

Figure 3.3

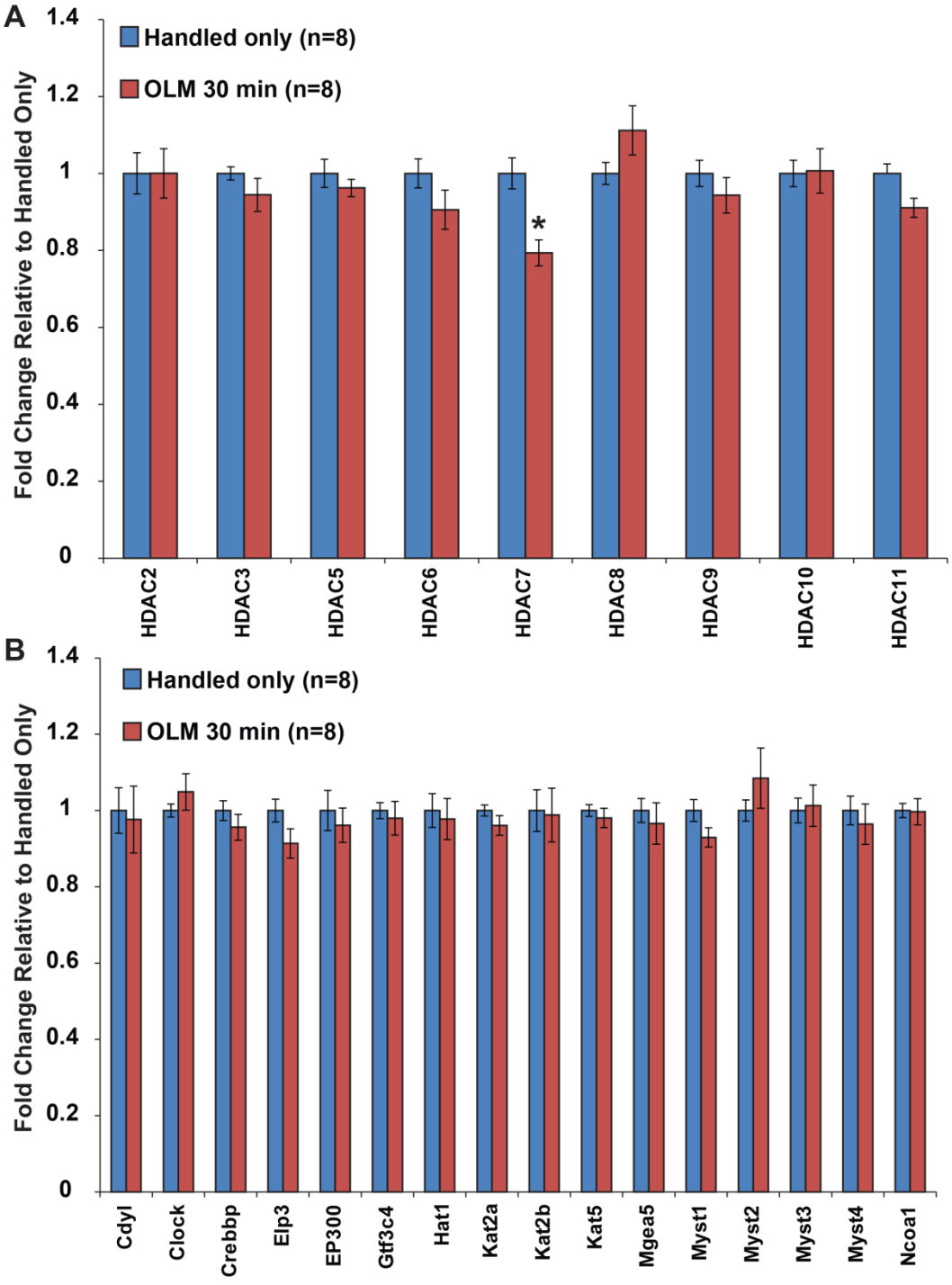

A
Handled only (n=8)
OLM 30 min (n=8)
Fold Change Relative to Handled Only
1.4
1.2
1
0.8
0.6
0.4
0.2
0
*
HDAC2
HDAC3
HDAC5
HDAC6
HDAC7
HDAC8
HDAC9
HDAC10
HDAC11
B
Handled only (n=8)
OLM 30 min (n=8)
Fold Change Relative to Handled Only
Cdyl
Clock
Crebbp
Elp3
EP300
Gtf3c4
Hat1
Kat2a
Kat2b
Kat5
Mgea5
Myst1
Myst2
Myst3
Myst4
Ncoa1

Figure 3.4

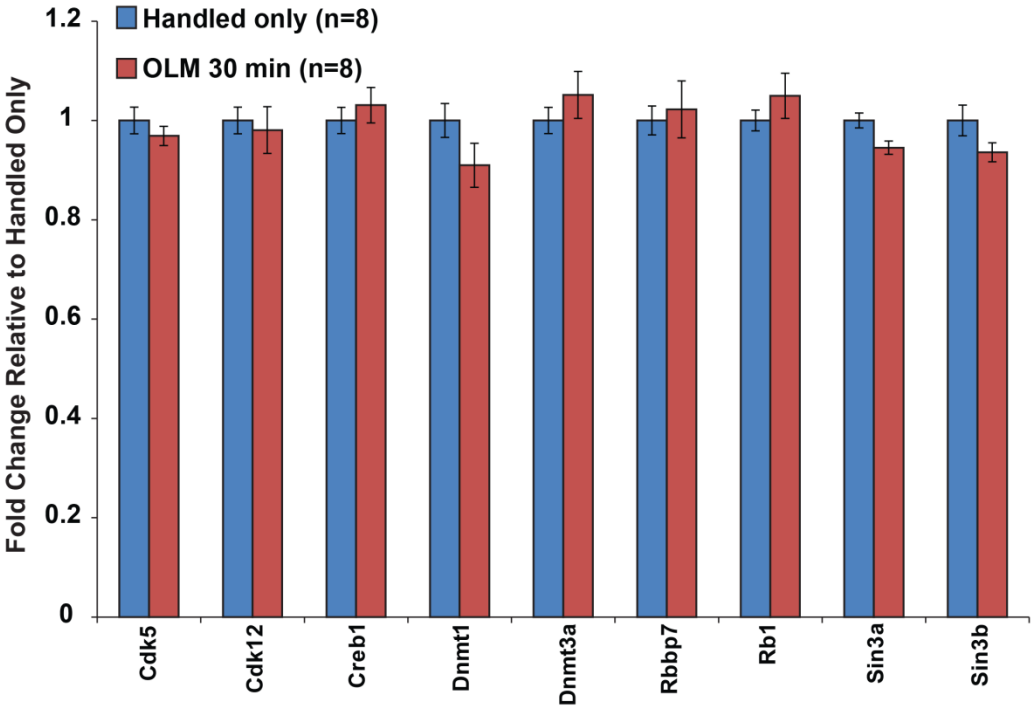
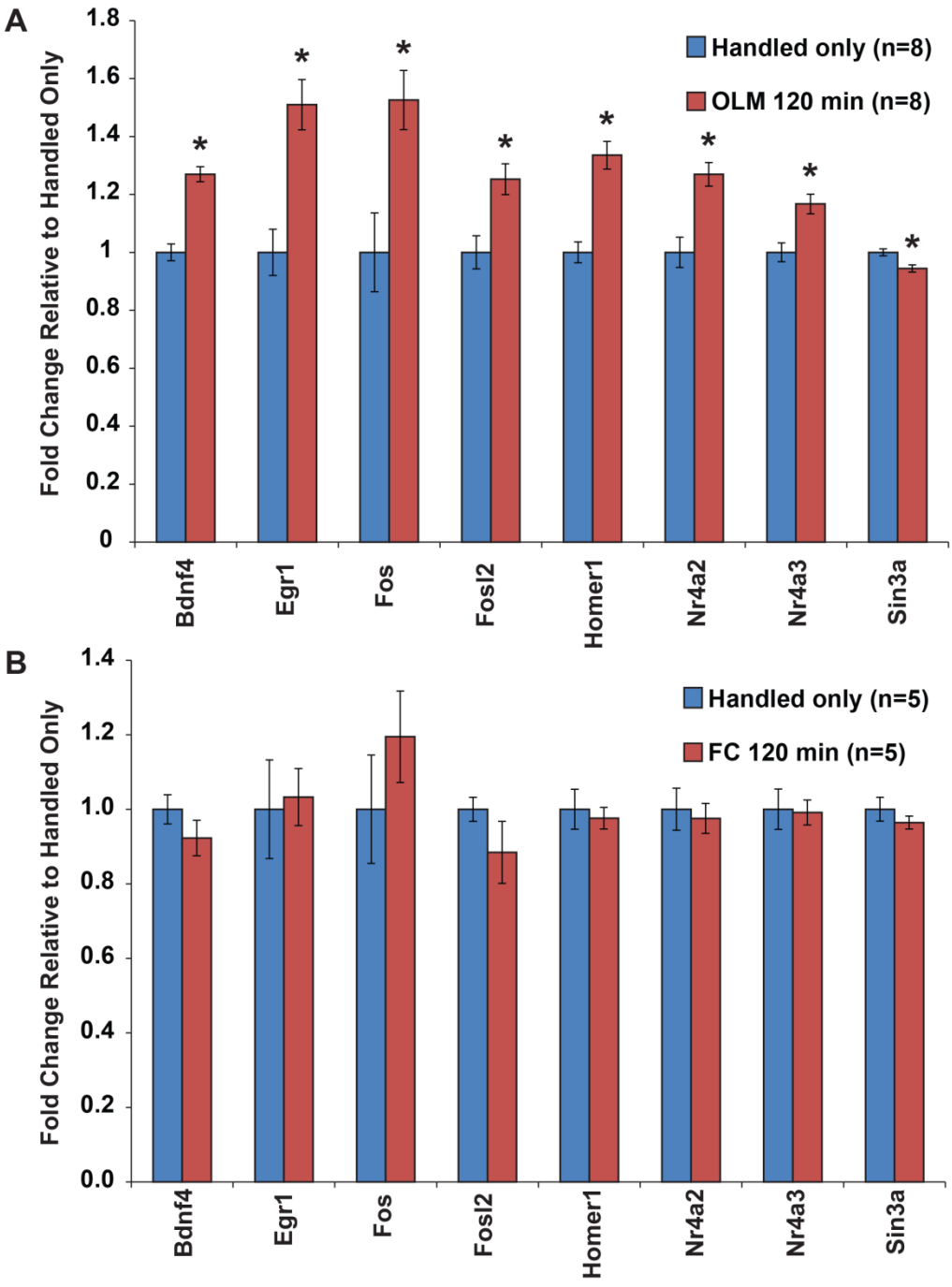

Handled only (n=8)
OLM 30 min (n=8)
Fold Change Relative to Handled Only
1.2
1
0.8
0.6
0.4
0.2
0
Cdk5
Cdk12
Creb1
Dnmt1
Dnmt3a
Rbbp7
Rb1
Sin3a
Sin3b

Figure 3.5


A
Fold Change Relative to Handled Only
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Handled only (n=8)
OLM 120 min (n=8)
*
Bdnf4
Egr1
Fos
Fosl2
Homer1
Nr4a2
Nr4a3
Sin3a
B
Fold Change Relative to Handled Only
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Handled only (n=5)
FC 120 min (n=5)
Bdnf4
Egr1
Fos
Fosl2
Homer1
Nr4a2
Nr4a3
Sin3a

Tables

Table 3.1

Gene	Taqman ID
AR	Mm00442688_m1
Arc	Mm01204954_g1
Bdnf4	Mm00432069_m1
Btg2	Mm00476162_m1
Cdk12	Mm00660704_m1
Cdk5	Mm01164910_m1
Cdyl	Mm00515473_g1
Clock	Mm00455950_m1
COUP-Tfa (NR2f1)	Mm01354342_m1
COUP-TFb (NR2f2)	Mm00772789_m1
COUP-Tfy (NR2f6)	Mm01340321_m1
Cpeb3	Mm01204299_m1
Creb1	Mm01254160_m1
Crebbp	Mm01342452_m1
Dnmt1	Mm01151062_g1
Dnmt2 (Trdmt1)	Mm00438511_m1
Dnmt3a	Mm00432881_m1
Dnmt3b	Mm01240113_m1
Egr1/Zif268	Mm00656724_m1
Elp3	Mm00804536_m1
Ep300	Mm00625535_m1
ERa (NR3a1, ESR1)	Mm00433149_m1
Erbb2	Mm01306793_g1

Esrra (ERRa)	Mm00433143_m1
Esrrb (ERRb)	Mm00442411_m1
Esrrg (ERRg)	Mm00516267_m1
Fos	Mm00487425_m1
FosB	Mm00500401_m1
Fosl2	Mm00484442_m1
Gadd45a	Mm00432802_m1
Gadd45b	Mm00435123_m1
Gadd45g	Mm00442225_m1
Gapdh	Mm99999915_g1
GCNF (NR6a1)	Mm00599848_m1
GR, Nr3c1	Mm00433832_m1
Gtf3c4 (TFIIIC90)	Mm00557022_m1
Hat1	Mm00509140_m1
Hdac1	Mm01351187_m1
Hdac10	Mm01308119_g1
Hdac11	Mm00523422_m1
Hdac2	Mm00515108_m1
Hdac3	Mm00515916_m1
Hdac4	Mm01304741_m1
Hdac5	Mm00515917_m1
Hdac6	Mm00515945_m1
Hdac7	Mm00469520_m1
Hdac8	Mm01224980_m1
Hdac9	Mm00458454_m1
Hist2h2ab	Mm01613463_s1

Homer1	Mm00516275_m1
Hprt	Mm01545399_m1
Jun	Mm00495062_s1
Kat2a (GCN5L2)	Mm00517402_m1
Kat2B (PCAF)	Mm00451387_m1
Kat5 (TIP60)	Mm01231512_m1
LXRb (NR1h2)	Mm00437265_g1
Mgea5 (NCOAT)	Mm00452409_m1
MR (NR3C2)	Mm01241596_m1
Myst1 (MOF)	Mm00458911_m1
Myst2 (HBO1/HBOA)	Mm00624391_m1
Myst3 (MOZ)	Mm01211941_m1
Myst4 (MOZ2)	Mm00450564_m1
Ncoa1 (SRC1)	Mm00447958_m1
Nr1h2 (LXRb)	Mm00437265_g1
Nr1h3 (LXRa)	Mm00443451_m1
Nr4a1	Mm00439358_m1
Nr4a2	Mm00443056_m1
Nr4a3	Mm00450074_m1
PPARa	Mm00440939_m1
PPARd	Mm00803184_m1
PPARg	Mm01184322_m1
PR (NR3C3, PGR)	Mm00435628_m1
RARa	Mm01296312_m1
RARb	Mm01319677_m1
RARg	Mm00441091_m1

Rb1	Mm00485586_m1
Rbbp7	Mm01702744_mH
REV-ERBa (Nr1d1)	Mm00520708_m1
REV-ERBb (Nr1d2)	Mm01310356_g1
RORa	Mm01173766_m1
RORb	Mm01204855_m1
RXRa	Mm01332431_m1
RXRb	Mm00441193_m1
RXRg	Mm00436410_m1
Sik1	Mm00440317_m1
Sin3a	Mm00488256_m1
Sin3b	Mm00550123_m1
Sox18	Mm00656049_gH
TLX (NR2e1)	Mm00455855_m1
Tob1	Mm01204299_m1
Tob2	Mm00451524_s1
TR2 (NR2C1)	Mm00449123_m1
TR4 (NR2c2)	Mm01182440_m1
TRa (NR1a1, Thra)	Mm00579691_m1
TRb (NR1a2, Thrb)	Mm00437044_m1
Tuba4a	Mm00849767_s1

CHAPTER 4: Chromatin Accessibility Is Increased After Learning

Abstract

Transcription is a tightly regulated process that can be controlled by changes to the epigenome, including histone modifications and chromatin accessibility. Although it is well established that transcription during memory consolidation is crucial for long-term memory, little is known about how this transcription is regulated. Histone acetylation is known to increase in bulk extracts after contextual fear conditioning and pharmacologically increasing histone acetylation enhances memory formation. Increasing histone acetylation would be expected to "loosen" the interaction between DNA and histones and increase accessibility. Using high-throughput sequencing methods, we investigated the genome-wide changes in histone acetylation and chromatin accessibility that occur after learning. We discovered a genome-wide increase in chromatin accessibility after learning that has not been previously reported. Surprisingly, this increase in accessibility was not accompanied by a corresponding increase in H3K9/14 acetylation or change in nucleosome positioning. We propose that a combination of histone and DNA modifications act in concert to regulate chromatin accessibility after learning.

Introduction

Despite 50 years of evidence that transcription and protein synthesis are required for long-term memory formation [11, 148], little is understood about the regulation of this gene expression. CREB and CREB-binding protein (CBP) are transcriptional regulators that are known to be crucial for this process [23-28, 149]. Besides being a CREB coactivator [150], CBP is also a histone acetyltransferase (HAT) that acetylates the N-terminal tails of histone proteins [24]. This has led to numerous studies linking histone acetylation to long-term memory, including the finding that memory can be enhanced by blocking histone deacetylase (HDAC) proteins that remove acetyl groups [51, 55, 143]. We therefore hypothesized that histone acetylation is the critical epigenetic modification that regulates the gene transcription necessary for memory.

One way in which histone acetylation is thought to increase transcription is by reducing the positive charge of lysine residues, thereby weakening the interaction between histone proteins and the negatively charged DNA [35]. This weakening would be expected to increase the accessibility of chromatin to transcription factors and basal transcriptional machinery, allowing gene expression to occur [36]. Histone acetylation works in concert with a number of other histone and DNA modifications to form a “code” that can regulate gene expression [30]. Histone acetylation can also be “read” by proteins containing a bromodomain, including nucleosome remodeler Brg1 [151], further increasing the accessibility of chromatin. Because of the known association with Brg1, histone acetylation can lead to nucleosome repositioning. Nucleosome repositioning can include shifting or displacement of nucleosomes that alter chromatin accessibility [152].

Therefore, the increase in histone acetylation after learning [51, 53] would be expected to result in increased chromatin accessibility to facilitate gene expression.

In this study, we used high-throughput sequencing techniques to study genome-wide histone acetylation after learning using ChIP-seq. Specifically, we investigated H3K9/14ac, a mark that our lab has previously seen to increase at the *Nr4a2* promoter after contextual fear conditioning [55]. Increases in H3K9ac and H3K14ac after learning have also been noted previously in bulk histone extracts [51, 53]. We also used Sono-seq [82] to study chromatin accessibility and MNase-seq [153] to study nucleosome positioning, two potential downstream effects of histone modifications. We found that H3K9/14ac shows a small increase in response to fear conditioning, but that this change is not large enough to be statistically significant at any individual gene. However, chromatin accessibility shows a large increase in response to learning that is not due to nucleosome repositioning. The regions showing increases after learning are enriched within gene bodies and show overlap with genes that are alternatively spliced after learning and those that regulate autism, a disorder associated with cognitive deficits. Publicly available H3K4me3 and H3K27me3 data sets also show high overlap with differential Sono-seq signal. This may suggest that other histone modifications may play a larger role in transcriptional regulation than H3K9/14ac, or that small changes in a number of modifications may cause a large change in accessibility.

Materials and Methods

Subjects

C57BL/6J mice were maintained under standard conditions with food and water available *ad libitum*. Adult male mice 3 months of age were kept on a 12-hr light/12-hr

dark cycle with lights on at 7AM. All behavioral and biochemical experiments were performed during the light cycle with training starting at approximately 10AM (ZT3). All procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Fear conditioning

Fear conditioning was performed as previously described [55, 61]. Briefly, mice were handled for 3 days prior to training, placed in a novel context within a soundproof chamber and allowed to explore for 148 seconds. A 2 second 1.5 mA footshock was delivered and mice were kept in the novel context for an additional 30 seconds. Mice were removed to their homecage after training. One mouse was trained and dissected per day to allow for dissection to occur at the same circadian time every day.

Histone acetylation ChIP-seq pilot study

ChIP was performed following the Covaris truChIP Tissue Chromatin Shearing Kit (Covaris Woburn, MA) with minor modifications. Hippocampi were rapidly dissected 30 minutes after fear conditioning, finely chopped, and placed in 500 µl 1% formaldehyde for 5 minutes. 28 µl Covaris quenching buffer was added and samples were rocked for an additional 5 minutes. After two washes in PBS containing protease inhibitor, crosslinked tissue was frozen on dry ice. Each sample was homogenized in 1 ml Covaris lysis buffer containing protease inhibitors, washed in PBS, and resuspended in 280 µl Covaris SDS buffer. Samples were split into two MICROtubes, sonicated in a Covaris S220 ultrasonicator, combined and precleared with 50 µl 50% protein G slurry (Life Technologies Grand Island, NY). The supernatant was then split into immunoprecipitations for H3K9/14ac (Millipore #06-599), H3K18ac (Cell Signaling

#9675), or H3K27ac (Abcam #ab4729) (2 µg antibody per IP) or set aside as an input sample. After rocking overnight, 80 µl 50% protein G slurry was added to each IP and samples were rocked for 2 hours at 4°C. Beads were washed for 5 min at RT with the following chilled buffers: Low Salt (0.1% SDS, 1% TX-100, 2 mM EDTA, 20 mM Tris-HCl pH8, 150 mM NaCl) High Salt (0.1% SDS, 1% TX-100, 2 mM EDTA, 20 mM Tris-HCl pH8, 500 mM NaCl), LiCl (1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH8, 250 mM LiCl), 1x TE, 1x TE. Samples were then eluted twice in 100 µl ChIP Elution Buffer (1% SDS, 0.1M $NaHCO_3$), 8 µl 5M NaCl was added, and samples were incubated at 65°C overnight. The next day, 1 µl Proteinase K (Roche Diagnostics Indianapolis, IN) was added and samples were incubated an additional hour at 65°C, then 1 µl RNaseA was added and samples were incubated for 30 min at 37°C. Samples were purified using a standard phenol:chloroform extraction and ethanol precipitation.

Sono-seq and H3K9/14ac ChIP-seq DNA preparation

ChIP was performed as previously described [55], and Sono-seq analysis was performed using the input samples of this experiment. Thirty minutes after fear conditioning, mice were sacrificed and the hippocampus was rapidly dissected. The hippocampus was finely chopped and placed into 500 µl 2% PFA for 10 minutes to crosslink tissue. Crosslinking was stopped by the addition of 100 µl 1M glycine, crosslinked tissue was washed 3x in ice cold PBS containing protease inhibitor cocktail (Sigma-Aldrich St. Louis, MO), and crosslinked tissue was frozen at -80°C. Chromatin was prepared by dounce homogenizing the tissue in 1 ml ChIP cell lysis buffer (10 mM Tris HCl pH 8.1, 10 mM NaCl, 3 mM MgCl2, 0.5% NP-40), centrifuging at 5500g for 5 minutes at 4°C, removing the supernatant, and resuspending the pellet in 300 µl ChIP nuclear lysis buffer (50 mM Tris pH 8.1, 5 mM EDTA, 1% SDS).

Chromatin was sonicated using a Bioruptor sonicator with the following conditions: 30 sec on, 1 min off for 15 minutes for two bouts with ice addition between bouts. Chromatin was centrifuged for 5 minutes at 14000g at 4°C and the supernatant was removed. Chromatin size was tested by removing an aliquot, reversing crosslinks, cleaning DNA using the Qiagen MinElute PCR purification kit (Qiagen Valencia, CA), quantification by NanoDrop (Thermo Scientific Whatham, MA), and running the DNA on a 1% agarose gel. Sono-seq DNA was prepared by removing 1 µg sonicated chromatin. H3K9/14ac ChIP-seq DNA was prepared by removing 10 µg chromatin, adding 7.5 µg of H3K9/14ac antibody (Millipore #06-599), and ChIP dilution buffer (16.7 mM Tris-Hcl pH 8.1, 1.1% TX-100, 0.01%SDS, 167 mM NaCl, 1.2 mM EDTA) to 1 mL. IPs were rocked at 4°C overnight. Protein G incubation and washes were performed as described for the histone acetylation pilot experiment. The next morning, 4 µl 0.5M EDTA, 8 µl 1M Tris-HCl pH 7.5, and 1 µl Proteinase K (Roche Diagnostics) were added and samples were incubated an additional hour at 65°C. Samples were purified using the Qiagen MinElute PCR purification kit.

MNase-seq DNA preparation

MNase-seq [153] was performed by dissecting mice 30 minutes after contextual fear conditioning, rapidly dissecting the hippocampus, and immediately dounce homogenizing in 500 µl Douncing buffer (10 mM Tris, 4 mM MgCl2, 1 mM CaCl2 adjust pH of solution to 7.5) containing protease inhibitor cocktail. 2 µl micrococcal nuclease (NEB Ipswich, MA) was added to each sample and they were incubated at 37°C for 16 min. 125 µl 0.5M EDTA was added to stop the reaction, and 375 µl water was added to bring the volume to 1ml. Samples were rocked at 4°C for 1 hour, precleared with 50 µl

protein G agarose beads for 30 minutes, and the supernatant was removed. An aliquot from each sample was proteinase K and RNase A (NEB) treated, quantified by NanoDrop, and run on a 2% agarose gel to ensure mononucleosomes were selectively isolated.

Library Preparation

Library preparation for ChIP-seq pilot study was performed using the KAPA library preparation kit (KAPA Biosystems Boston, MA) according to the manufacturer's instructions with 3 μM adapters to account for low concentration samples. Size selection was performed using AMPure XP beads (Beckman Coulter Brea, CA) by adding 40 μl beads to 50 μl of sample, discarding the beads, adding 9.5 μL beads to the supernatant and eluting. Ideal PCR cycles were determined for each set of samples and 18 cycles was used for H3K9/14ac, 15 cycles for H3K18ac, and 13 cycles for H3K27ac.

Library preparation for Sono-seq and H3K9/14ac ChIP-seq samples was performed using a combination of the ChIP-seq DNA sample prep kit and the Multiplexing Sample Preparation Oligonucleotide Kit (Illumina San Diego, CA). End repair and adenylation were performed as described in the ChIP protocol, while adapter ligation was performed as described in the Multiplexing protocol with the modification that adapters were diluted 1:10 for H3K9/14ac samples to account for reduced input. A gel slice 300bp +/- 25bp was cut from the gel for size selection, the Qiagen Gel Extraction kit was used to purify DNA and 23 PCR cycles were used following the PCR reaction setup described in the Multiplexing kit. The final product was run on a gel and purified by the Qiagen Gel Extraction kit again.

Library preparation for MNase-seq libraries was performed using the KAPA library preparation kit. 2 µg of each sample was used according to the manufacturer's instructions. The mononucleosome band was gel extracted and 7 cycles of PCR were determined sufficient to obtain a suitable library concentration.

Sequencing

Libraries were quantified using the KAPA Library Quantification kit, normalized to 10 nM, and submitted to the PGFI sequencing core (Sono-seq and H3K9/14ac) or Next-Generation Sequencing Core (ChIP-seq pilot study, MNase-seq) at the University of Pennsylvania for 100bp single-end sequencing on an Illumina HiSeq2000. The histone acetylation pilot experiment produced an average of 57 million input reads, 57 million H3K9/14ac reads, 57 million H3K18ac reads, and 56 million H3K27ac reads. Sono-seq samples were run in their own lane producing an average of 148 million reads per sample. H3K9/14ac ChIP-seq samples were multiplexed 3 per lane producing an average of 45 million reads per sample. MNase-seq samples were multiplexed 2 per lane producing an average of 99 million reads per sample.

Analysis

The histone acetylation pilot experiment was analyzed using uniquely aligned reads +/- 1kb around each promoter. After upper quantile normalization [125], an arbitrary 2-fold cutoff was applied and the resulting differences were reported.

Sono-seq and H3K9/14ac samples were analyzed using a custom algorithm. Aligned reads cover ~25% of the genome, showing that only ¼ of the chromatin is accessible in the hippocampus. Aligned reads with alignment score <40 as well as isolated duplicate

reads were removed for subsequent analysis. No published algorithm for the analysis of epigenetic HTS data exists that will incorporate biological replicates and detect statistically significant differences between treatments, such as learning and controls. We developed a novel approach to determine differences in Sono-seq peaks between learning and control mice in collaboration with Dr. Nancy Zhang (Wharton School of the University of Pennsylvania), which we named DEScan. **Figure 4.1** depicts the series of computational steps taken to obtain differential peaks between learning and controls using DEScan. First, peaks were called separately on each sample using a variable window scan with a Poisson model and the surrounding 10kb as background. Second, peaks from all samples were aligned and only peaks present in at least 30%-50% of the samples were considered for further analysis. Third, aligned peaks were sampled to determine the dispersion parameter for a negative binomial model and subsequently a variable window scan was used within the aligned peaks to test for differential enrichment in FC vs HC using EdgeR [125] and the previously determined dispersion parameter. P-values were transformed to z-scores to ensure normality and only regions with a z-score >1.9 were reported (2 or more standard deviations from the average).

MNase-seq samples were analyzed using HTSeq [124] to produce a TSS plot, which was compared to the Sono-seq TSS plot.

Results

Histone acetylation is a critical component regulating the transcription necessary for long-term memory formation [23, 24, 27, 51, 55, 58, 63, 143]. Therefore, we used ChIP-seq to test genome-wide histone acetylation occupancy throughout the genome 30

minutes after fear conditioning. We chose 3 common acetylation marks: H3K9/14ac and H3K18ac and H3K27ac. H3K9/14ac has been previously observed to increase at *Nr4a* genes at this timepoint by our lab [55] and both H3K9ac and H3K14ac have been demonstrated to change in bulk histone extracts after learning [51, 53]. H3K18ac and H3K27ac are two marks that have been shown to be dependent on CBP/p300 [54]. Although these marks are found primarily at promoters of expressed genes, H3K27ac is also found at active enhancers [154].

Our pilot ChIP-seq study (n=1) with these marks found that H3K9/14ac showed the largest number of promoters with >2 fold enrichment in either homecage or fear conditioned animals (**Figure 4.2**). Therefore, we followed up H3K9/14ac with ChIP-seq of n=9 samples to determine whether these changes at promoters were statistically significant. Genome-wide occupancy of H3K9/14ac at transcription start sites (TSSs) throughout the genome showed only a modest increase after fear conditioning (**Figure 4.3**). 38,211 regions in the genome were found to contain H3K9/14ac peaks with an average length of 2.3kb. The small increase seen when averaging over all TSSs was not observed to be statistically significant at any individual gene, indicating that the changes in H3K9/14ac after learning are too small to be studied using ChIP-seq on whole hippocampus.

H3K9/14ac is not the only histone acetylation mark that could be regulating the gene expression changes that occur after learning [51, 53]. Histone methylation, phosphorylation, ubiquitination and other marks work in concert to regulate transcriptional status [30, 155]. Indeed, histone phosphorylation [52] and methylation [156] have also been implicated in memory formation. However, studying every possible modification occurring during memory consolidation would be too costly and time-consuming. Therefore, we focused our attention on the common target of these histone

modifications, which is increased chromatin accessibility. To study chromatin accessibility, we used Sono-seq [82]. Sono-seq combines weak sonication with low basepair size selection. The DNA that accumulates fastest at the low basepair size is the chromatin that was easiest to sonicate, which would be expected to be the most accessible chromatin. In our experiment, weak sonication of our ChIP-seq samples produced Sono-seq signal in our "input" samples. Although this is a potential confounding factor in the H3K9/14ac data, we found that H3K9/14ac signal does not correlate better with its paired input data as it does with input data from another sample (data not shown), suggesting the immunoprecipitation overcomes any bias introduced by small peaks in the input.

Sono-seq signal was found to correlate with DNase hypersensitivity and RNA polymerase II binding, two hallmarks of accessible chromatin, but not have a perfect overlap with either as previously reported [82]. Using Sono-seq, we saw a striking increase in accessibility at transcription start sites throughout the genome (**Figure 4.4**). Of the 36,186 regions in the genome that show Sono-seq peaks (800bp average length), 3064 regions (8.5%) were determined to be significantly regulated (p-value <0.05) after fear conditioning with an average length of 560 bp (**Table 4.1**). To determine whether these differential regions represent an important class of genes, we investigated where the signal was occurring. Differentially enriched regions were more likely to occur within gene bodies than at TSSs (**Figure 4.5**). Differences in the gene body suggest that mRNA elongation or splicing may be a target of chromatin accessibility. Promoter regions (upstream and overlapStart) also show an enrichment in differential regions (26% of all signal, 35% of differential signal).

This increase in chromatin accessibility may regulate the gene expression necessary for long-term memory formation. Therefore, we first investigated whether

genes enriched in this data set correlated with genes identified in our previous RNA-seq study (**Chapter 2**). Although these regions do not show significant enrichment of genes regulated by contextual fear, these differential regions do exhibit significant enrichment for genes that showed differential alternative splicing in our previous study (**Table 4.2**). This finding agrees with the previous result that the differential Sono-seq peaks were enriched within gene bodies. Enrichment for alternatively spliced genes suggests that the Sono-seq changes that occur within gene bodies may be regulating splicing, but total levels of gene expression may or may not be affected.

Because these differential regions include many more genes in addition to those showing gene expression changes after learning, we also investigated whether they exhibit overlap with other gene sets related to cognitive functioning. We discovered that genes associated with autism (from the SFARI database) showed enrichment in the differential Sono-seq gene list (**Table 4.2**). Autism is a disorder with known cognitive dysfunction and often shows comorbidity with intellectual disability [157]. Although many of these genes don't show gene expression changes at 30 minutes after fear conditioning, increasing accessibility at these genes may still be affecting cognitive processing through regulation at other times. Genes associated with a brain disorder without cognitive dysfunction, epilepsy (from GenEpi), were not observed to have any enrichment in the differential Sono-seq signal.

We next sought to determine how this increased chromatin accessibility was being regulated. The increase in chromatin accessibility after fear conditioning could be due to many factors, but the most obvious explanation is that it represents a change in nucleosome positioning. To test this, we performed MNase-seq [153]. Micrococcal nuclease (MNase) is an enzyme that digests DNA. When incubated under optimized conditions, MNase can be used to preferentially digest linker chromatin between

nucleosomes, leaving behind only mononucleosome-bound DNA. The mononucleosome-bound DNA can then be sequenced to map genome-wide nucleosome occupancy. However, we found no difference in MNase-seq after learning (**Figure 4.6**). Therefore, nucleosome repositioning is not the major driver in changes in chromatin accessibility after learning.

Another possibility is that other histone modifications besides H3K9/14ac are regulating this accessibility. The histone code hypothesis [30] states that histone modifications will act in a combinatorial manner to form a code that can be read to cause downstream effects. Thus, a small change in a number of histone modifications, such as H3K9/14ac, could have large consequences for downstream effects such as chromatin accessibility. To test whether differential Sono-seq regions overlap with other histone modifications, we decided to look at other marks computationally. We compared publicly available ENCODE ChIP-seq data to our differential Sono-seq regions (**Table 4.3**). As anticipated, we found good overlap between differential Sono-seq regions and our H3K9/14ac ChIP-seq peaks (5.1% of H3K9/14ac peaks overlap with differential Sono-seq peaks for a total of 64% of differential Sono-seq peaks). We also found overlap with both H3K4me3, an activating mark, and H3K27me3, a repressive mark, in the ENCODE data. Interestingly, these marks can be found in combination at “bivalent domains” – regions that are repressed but poised for activation during development [158]. This may suggest a role for memory in regulating chromatin accessibility at poised genes. Fear conditioning may cause changes in a multitude of histone and DNA modifications that work cooperatively to regulate chromatin accessibility throughout the genome.

Discussion

Although the regulation of gene expression after learning is not well understood, epigenetic mechanisms are beginning to be appreciated as an important player in the process. In this study, we sought to investigate the genes regulated by histone acetylation and chromatin accessibility. Our data indicate that the changes in H3K9/14ac are too small to be detected significantly when using whole hippocampal tissue. To our surprise, chromatin accessibility, which would be the expected effect of histone acetylation changes, was significantly changed after learning. The differential regions were enriched for gene bodies and promoter regions. Genes that show differential alternative splicing and those known to be implicated in autism spectrum disorders were enriched in differential Sono-seq regions, implicating the importance of this regulation.

Our data suggest that the increase in H3K9/14ac at promoters is small and not significant when applied on a gene-by-gene basis. This could be due to the cellular dilution of our sample. The brain is a very complex organ and the hippocampus contains excitatory neurons, inhibitory neurons and glia. Although neurons account for roughly 50% of cells in the brain [159], these include both excitatory and inhibitory cells. Only 18-35% of excitatory neurons are activated by any particular memory trace in the hippocampus [17]. Therefore, changes in this subset of cells will be highly diluted by signal from surrounding cells, making changes look small and insignificant. We therefore suggest that sorting may be necessary to study epigenetic changes in the brain, especially when dealing with small responses such as that to contextual learning. Despite these limitations, we were able to detect a significant difference in chromatin accessibility by using Sono-seq after learning.

There are a number of explanations for the increase chromatin accessibility without a corresponding increase in H3K9/14ac, including nucleosome repositioning,

other epigenetic marks, transcription factor occupancy, or histone variant incorporation. We used MNase-seq to rule out nucleosome repositioning as the mechanism regulating this increase in chromatin accessibility. We believe the most likely explanation for the large increase in chromatin accessibility is that a number of histone and DNA modifications are working in concert to promote opening [30]. In this case, the change of any one modification (such as H3K9/14ac) may be small throughout the genome, but the effect of the combinatorial combinations of marks could be large. Based on computational comparisons of our data with publicly available ChIP-seq data, H3K4me3 and H3K27me3 appear to have a role in this process. Future studies will be needed to demonstrate overlap between regions of chromatin accessibility and histone modifications occurring at these sites after learning. However, we cannot rule out other possibilities such as transcription factor binding as the cause of this increase in accessibility.

Although the function of an increase in chromatin accessibility at such a large number of genes is uncertain, it is feasible that chromatin accessibility is acting as a gate to transcriptional regulation. Contextual conditioning may open this gate, and allow transcription or epigenetic factors to bind to their respective targets to activate or poise transcription. An alternative hypothesis would be that there are a larger number of gene expression changes than we currently appreciate, which may be more easily observed after cell sorting. Finally, it is also possible that these changes occurring within cell bodies are affecting processing of RNA such as splicing. Because we know that genes showing differential exon usage (**Chapter 2**) are enriched in differential Sono-seq regions, this is an exciting avenue for future research.

Figure Legends

Figure 4.1. DEScan flowthrough. Our custom computational approach to detect differential peaks between treatment and controls using replicates is shown.

Figure 4.2. H3K9/14ac shows the most promoters with >2 fold differences. H3K9/14ac displayed 485 promoters with higher acetylation after fear conditioning and 265 with lower acetylation after fear conditioning. H3K18ac had 128 and 175 promoters respectively. H3K27ac showed fewer changes at promoters (4 and 9) in part because H3K27ac is found at both promoter and enhancer regions.

Figure 4.3. H3K9/14ac signal is slightly increased around the TSS after learning. Normalized read counts of H3K9/14ac data are plotted +/- 2kb of the TSS. The dip is seen directly over the TSS because the nucleosome at that position is ejected during transcription. The read counts in the 3' peak appear to be slightly increased after fear conditioning.

Figure 4.4. Sono-seq signal has a dramatic increase around the TSS after learning. Normalized read counts of Sono-seq data are plotted +/- 2kb of the TSS. There is a large increase surrounding the TSS in the fear conditioned group.

Figure 4.5. Differences in Sono-seq signal are primarily located within gene bodies. Pie chart shows the distribution of A) total Sono-seq regions and B) differential Sono-seq regions with regards to gene location. Intergenic (orange) represents regions

with no genes within 5kb. Differential regions are more likely to occur near genes and specifically within gene bodies (green).

Figure 4.6. No difference in nucleosome positioning around the TSS after learning. Normalized read counts of MNase-seq data are plotted +/- 2kb of the TSS. There is a no difference seen at any point around the TSS. The large dip just before the TSS and a well-positioned nucleosome just after the TSS are typical of this type of data.

Table 4.1. List of significantly regulated Sono-seq peaks after learning. Differential Sono-seq peaks with a Z-score greater than 1.9 (p-value <0.05) are shown. Start and End are the respective genomic start at end sites of each peak. Z denotes the Z-score. Distance measures the distance to the nearest TSS. Feature displays what this peak was called for the chart in Figure 4.6.

Table 4.2. Comparison of differential Sono-seq regions to gene lists that regulate memory. The differential Sono-seq peaks were compared to other gene lists in a 2x2 contingency table using Chi-square tests with Yates correction. Significant enrichment is shown in red. Number of expressed genes is how many genes from that gene list are expressed and Expressed genes near differential Sono-seq region show how many of those expressed genes were near a differential Sono-seq peak. Overlap represents the number of expressed genes near differential Sono-seq region/number of differential Sono-seq regions (1694). Expected overlap represents the number of expressed genes in that list divided by the number of expressed genes in the hippocampus (18008). p-values were found to be 0.40 (RNA-seq), 0.02 (diffSplice), 0.01 (autism), and 0.43 (GenEpi).

Table 4.3. Comparison of publicly available ChIP-seq data to our differential Sono-seq regions. Differential regions compose 8.5% of all Sono-seq regions. Our histone acetylation peaks show 5.1% overlap, which cover 64% of differential Sono-seq peaks. As previously shown, Sono-seq signal has overlap with both RNA polymerase II binding and DNase hypersensivity, but not perfect overlap with either. H3K4me3 (7.8% and 8.7%, 48% and 56% of differential peaks) and H3K27me3 (8.7%, 34% of differential peaks) appear to have large overlap with Sono-seq differential signals (red), implicating these marks in regulating chromatin accessibility after learning.

Contributions

This chapter was written by Shane G. Poplawski with suggestions by Ted Abel. Lucia Peixoto co-wrote the Sono-seq and H3K9/14ac methods section, helped to design figures, and was instrumental in the computational work performed in this chapter. Experiments were planned and carried out by Lucia Peixoto, Shane Poplawski, and Mathieu Wimmer. Jieun Jeong (UPENN) assisted with analysis of the ChIP-seq pilot study and Nancy Zhang (Wharton) provided tremendous help with the analysis of the H3K9/14ac, Sono-seq and MNase-seq samples. Members of both the PGFI sequencing core as well as the NGSC core at UPENN provided assistance with library preparation, pooling and sequencing. We thank Giulia Porcari for constructive discussions and critical reading of the chapter.

Figures

Figure 4.1

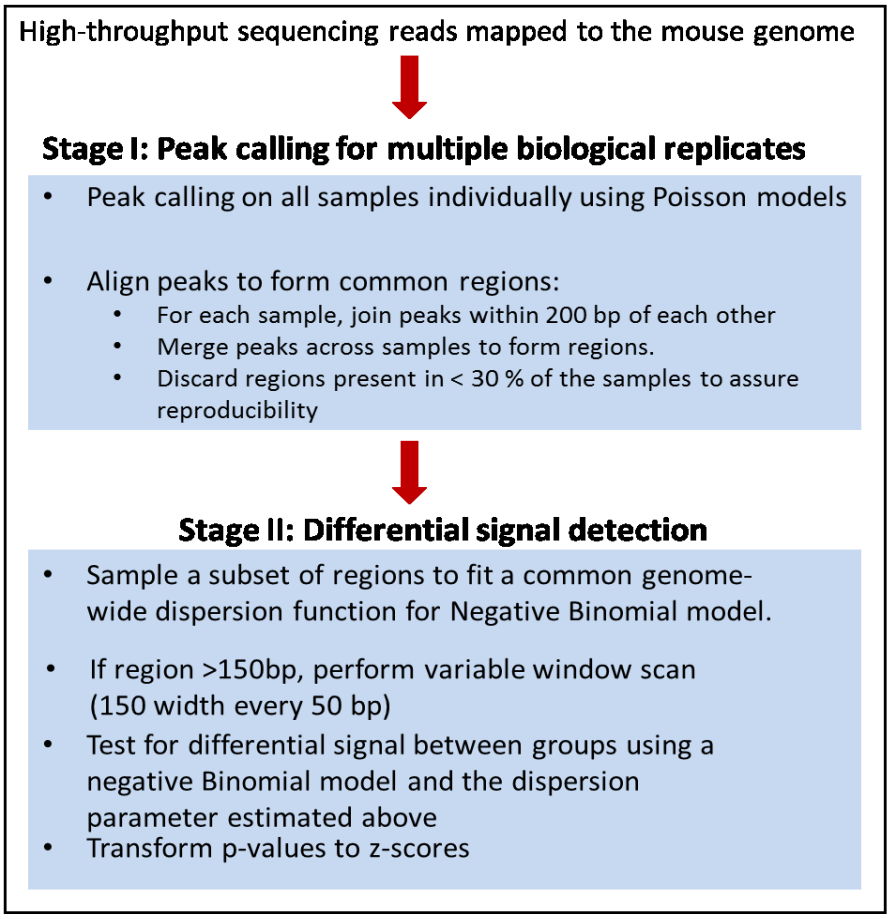


Figure 4.2

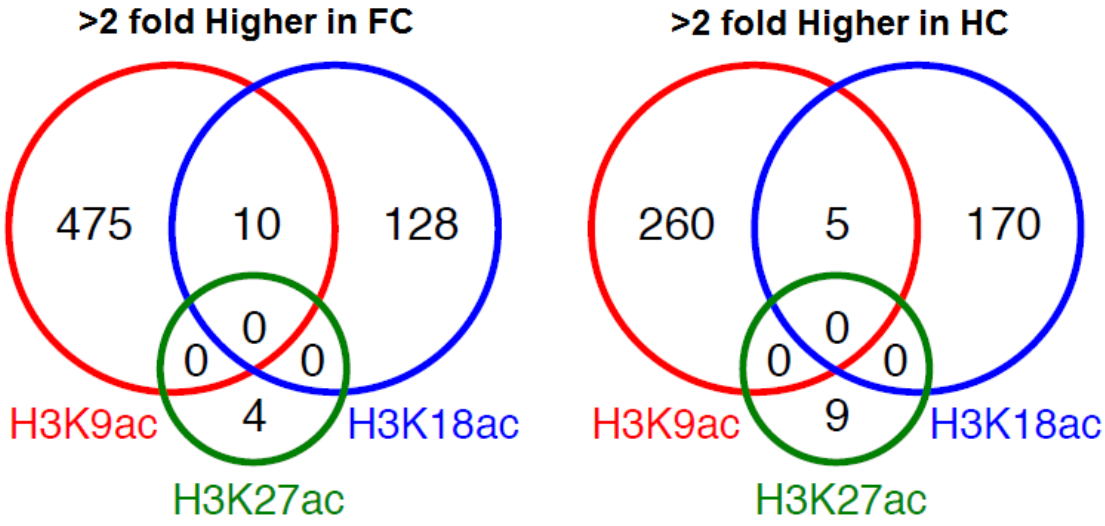

>2 fold Higher in FC
475
10
128
0
0
0
4
H3K9ac
H3K18ac
H3K27ac
>2 fold Higher in HC
260
5
170
0
0
0
9
H3K9ac
H3K18ac
H3K27ac

Figure 4.3

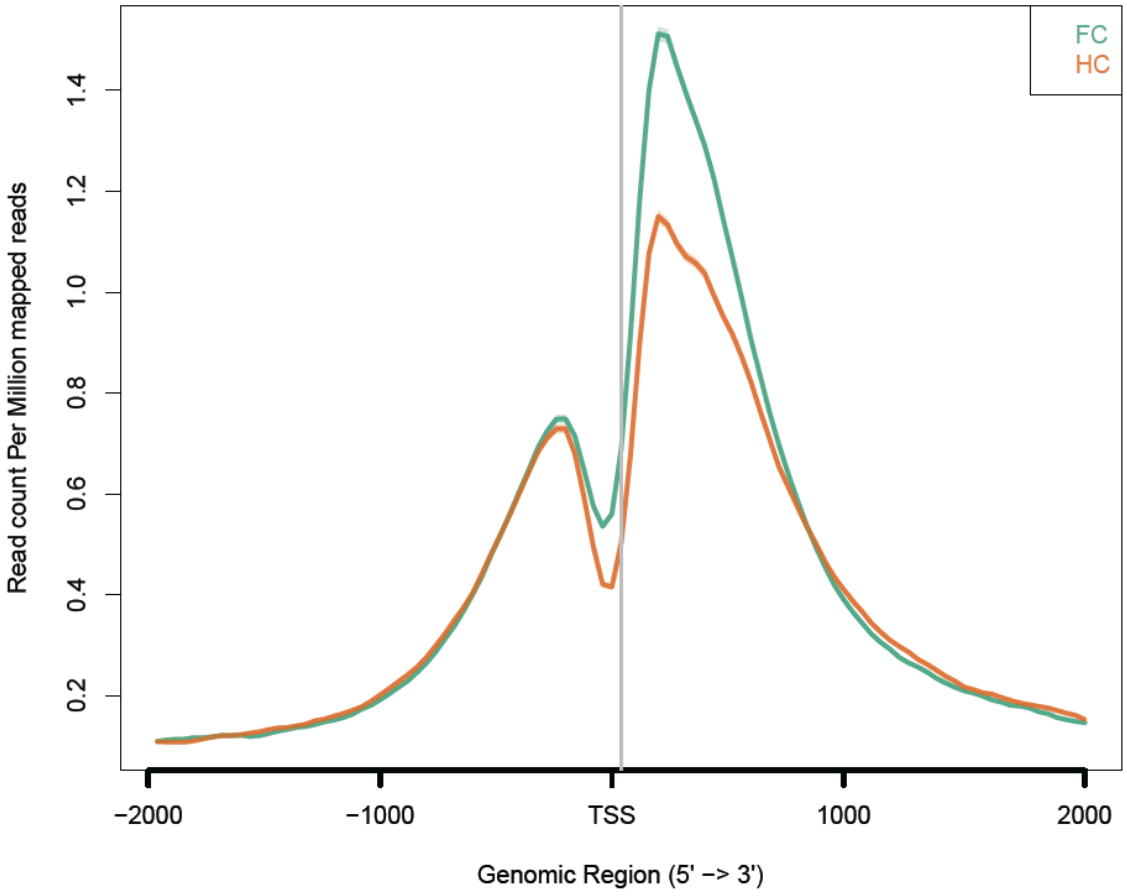

FC
HC
Read count Per Million mapped reads
0.2
0.4
0.6
0.8
1.0
1.2
1.4
−2000
−1000
TSS
1000
2000
Genomic Region (5' −> 3')

Figure 4.4

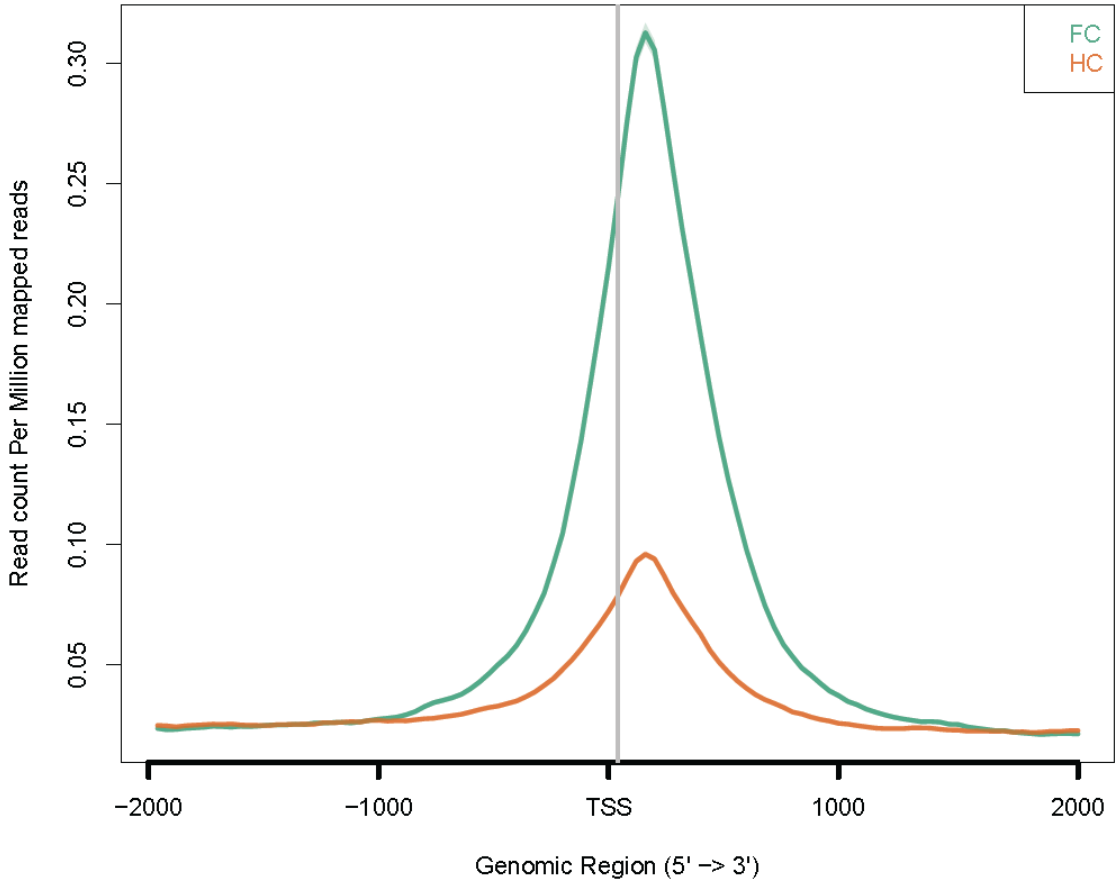

FC
HC
Read count Per Million mapped reads
0.05
0.10
0.15
0.20
0.25
0.30
−2000
−1000
TSS
1000
2000
Genomic Region (5' −> 3')

Figure 4.5

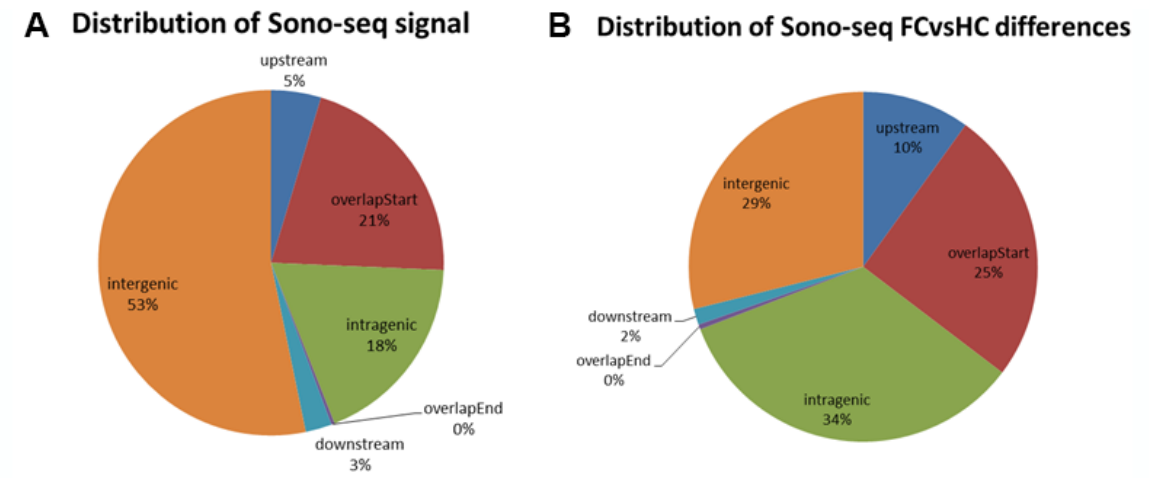
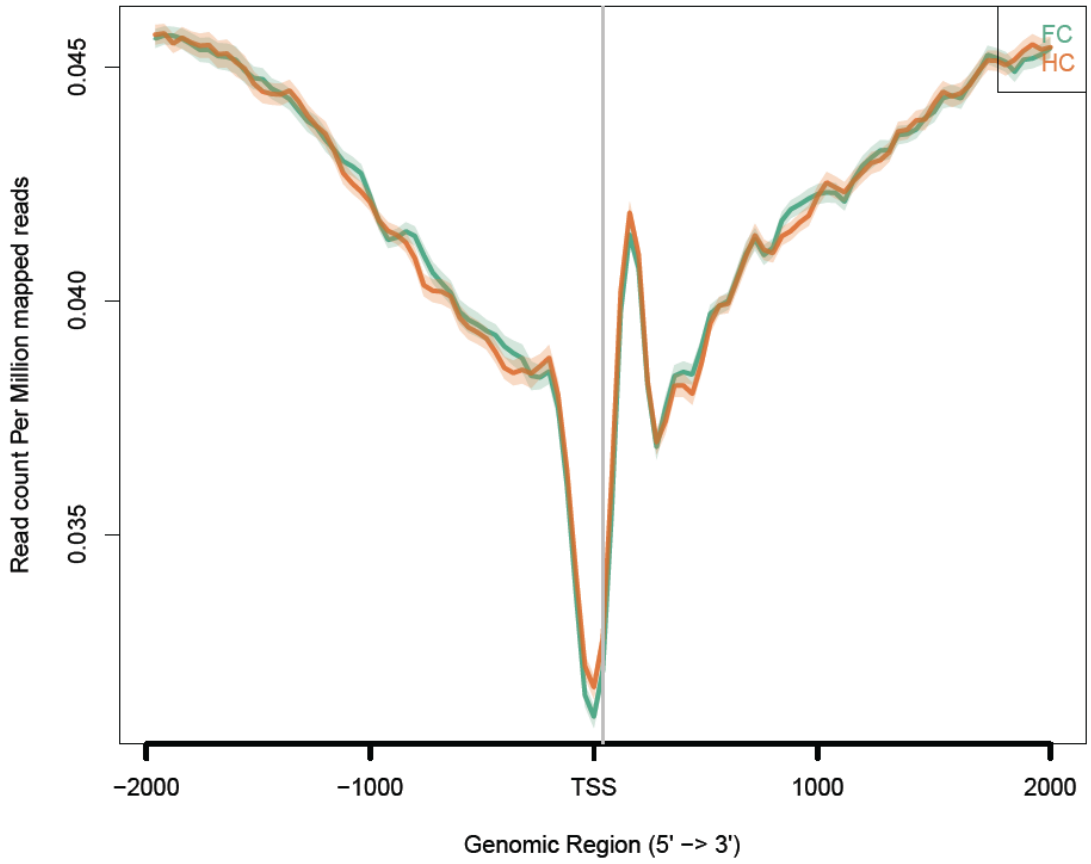

A Distribution of Sono-seq signal
upstream 5%
overlapStart 21%
intragenic 18%
overlapEnd 0%
downstream 3%
intergenic 53%
B Distribution of Sono-seq FCvsHC differences
upstream 10%
overlapStart 25%
intragenic 34%
overlapEnd 0%
downstream 2%
intergenic 29%

Figure 4.6


FC
HC
Read count Per Million mapped reads
0.045
0.040
0.035
−2000
−1000
TSS
1000
2000
Genomic Region (5' −> 3')

Tables

Table 4.1

GeneID	Start	End	Z	Distance	Feature
ENSMUSG00000038545	46650358	46650808	2.38	21	inside
ENSMUSG00000091175	91126296	91127646	2.35	-35491	intergenic
ENSMUSG00000058975	46397243	46397993	2.35	746	inside
ENSMUSG00000021061	76710090	76710690	2.32	457	overlap
ENSMUSG00000092763	99656981	99657381	2.32	-761	upstream
ENSMUSG00000043372	103132165	103132565	2.31	-264	overlap
ENSMUSG00000027618	156144135	156144535	2.31	51	overlap
ENSMUSG00000058537	58224812	58225312	2.30	894	inside
ENSMUSG00000041650	122525555	122526055	2.30	-8769	intergenic
ENSMUSG00000022419	55112141	55112641	2.30	-176	overlap
ENSMUSG00000021549	85289235	85289685	2.29	-206	upstream
ENSMUSG00000062087	70479907	70480307	2.29	-97522	intergenic
ENSMUSG00000079657	24533112	24533762	2.29	1098	inside
ENSMUSG00000095972	67133377	67133927	2.29	41614	intergenic
ENSMUSG00000018661	113649274	113649674	2.28	105	inside
ENSMUSG00000096863	52240748	52242098	2.28	-86141	intergenic
ENSMUSG00000087993	80834863	80835613	2.27	6066	intergenic
ENSMUSG00000035486	80356556	80357106	2.27	97	inside
ENSMUSG00000021278	111275016	111275416	2.27	3905	inside
ENSMUSG00000088128	76177521	76178071	2.27	36684	intergenic
ENSMUSG00000004892	87999317	88000017	2.27	1039	inside
ENSMUSG00000038156	113766062	113766712	2.27	64	inside
ENSMUSG00000078670	73739960	73740410	2.27	-347	overlap
ENSMUSG00000055053	81430265	81430815	2.26	740	inside
ENSMUSG00000098341	55471155	55471755	2.26	6621	intergenic
ENSMUSG00000048915	62881908	62882658	2.26	-591	upstream
ENSMUSG00000096065	147595873	147596723	2.26	29012	intergenic
ENSMUSG00000097960	120227930	120228480	2.26	300	overlap
ENSMUSG00000041012	114843817	114844317	2.26	335	overlap
ENSMUSG00000026207	75375444	75375944	2.25	147	inside
ENSMUSG00000019897	105840552	105841252	2.25	-515	overlap
ENSMUSG00000037904	110978217	110978767	2.25	823	inside
ENSMUSG00000035451	57542311	57543011	2.25	3810	inside
ENSMUSG00000052727	99515235	99516485	2.25	1367	inside
ENSMUSG00000033960	4635339	4635739	2.25	410	inside
ENSMUSG00000023391	71546078	71546628	2.25	676	inside
ENSMUSG00000083780	105798487	105799137	2.25	8618	downstream
ENSMUSG00000099130	111276966	111277465	2.25	-1872	upstream
ENSMUSG00000046658	143254598	143255048	2.25	15424	inside
ENSMUSG00000015957	98835066	98835566	2.25	-46	overlap
ENSMUSG00000039428	89404046	89404446	2.25	176	overlap
ENSMUSG00000043794	25597000	25597550	2.25	-12	overlap
ENSMUSG00000025161	120959067	120959517	2.24	10587	inside

ENSMUSG00000041730	32597844	32598244	2.24	-2083	upstream
ENSMUSG00000050846	75941039	75941489	2.24	87	inside
ENSMUSG00000073600	35654077	35655127	2.24	1110	inside
ENSMUSG00000055044	40270954	40271354	2.24	888	inside
ENSMUSG00000050592	32083919	32084519	2.24	938	inside
ENSMUSG00000063600	29082090	29082790	2.24	67	inside
ENSMUSG00000064254	24585394	24585894	2.24	-2149	upstream
ENSMUSG00000034799	71671159	71671659	2.24	598	inside
ENSMUSG00000021273	63164339	63164839	2.23	13454	inside
ENSMUSG00000039456	93831900	93832300	2.23	-221	overlap
ENSMUSG00000046138	29805113	29805563	2.23	896	inside
ENSMUSG00000085438	119594235	119594785	2.23	-62	overlap
ENSMUSG00000027070	69585014	69585914	2.23	1051	inside
ENSMUSG00000090116	99467139	99467539	2.23	39631	inside
ENSMUSG00000031775	94695839	94696489	2.23	403	overlap
ENSMUSG00000026608	189007610	189008010	2.22	230	overlap
ENSMUSG00000059406	80888106	80888656	2.22	8386	inside
ENSMUSG00000020152	20112478	20112978	2.22	435	overlap
ENSMUSG00000051864	86620266	86620716	2.22	405807	intergenic
ENSMUSG00000034730	74517866	74518516	2.22	1670	inside
ENSMUSG00000016624	84854221	84855071	2.22	1828	inside
ENSMUSG00000071033	91088370	91088920	2.22	-123	overlap
ENSMUSG00000045817	84184777	84185877	2.22	3170	inside
ENSMUSG00000054057	18039358	18039908	2.22	-1621	upstream
ENSMUSG00000086657	14073608	14074658	2.22	326	overlap
ENSMUSG00000034185	115897537	115898587	2.22	6871	inside
ENSMUSG00000099149	19529593	19530393	2.22	-25749	intergenic
ENSMUSG00000015112	6216654	6217154	2.22	464	overlap
ENSMUSG00000007946	101818499	101818949	2.22	186	inside
ENSMUSG00000086604	5056480	5057030	2.22	631	inside
ENSMUSG00000040725	25754494	25754894	2.22	236	overlap
ENSMUSG00000032518	120127867	120128267	2.22	101	inside
ENSMUSG00000056724	110653331	110653881	2.22	830	inside
ENSMUSG00000056031	85843978	85844378	2.22	-693	upstream
ENSMUSG00000041040	59912450	59913050	2.21	-556	overlap
ENSMUSG00000041889	135374906	135375356	2.21	331	overlap
ENSMUSG00000039239	186705304	186705854	2.21	688	inside
ENSMUSG00000020178	75316584	75317184	2.21	-359	overlap
ENSMUSG00000035873	108331873	108332423	2.21	-316	overlap
ENSMUSG00000033697	76817021	76817921	2.21	1149	inside
ENSMUSG00000033581	22161341	22161791	2.21	1958	inside
ENSMUSG00000040852	84511909	84512309	2.21	14	inside
ENSMUSG00000049112	112488739	112489339	2.21	1069	inside
ENSMUSG00000046295	71406140	71406590	2.21	130	inside
ENSMUSG00000097928	32009200	32010000	2.21	696	inside
ENSMUSG00000097120	84201042	84201492	2.21	-3375	upstream
ENSMUSG00000019066	21918091	21918491	2.21	101	overlap
ENSMUSG00000037419	14380891	14381291	2.21	351	overlap
ENSMUSG00000026494	178527813	178528463	2.20	-1312	upstream
ENSMUSG00000026238	86525614	86526564	2.20	-1122	upstream
ENSMUSG00000090142	20432188	20433188	2.20	12372	intergenic

ENSMUSG00000019817	13060623	13061173	2.20	-30068	intergenic
ENSMUSG00000087036	118568665	118569165	2.20	-89207	intergenic
ENSMUSG00000018537	97699765	97700215	2.20	732	inside
ENSMUSG00000020867	94491168	94491568	2.20	-4989	upstream
ENSMUSG00000044052	101173515	101174015	2.20	1928	inside
ENSMUSG00000053263	3331826	3332226	2.20	-648	upstream
ENSMUSG00000097917	99627535	99627935	2.20	63694	intergenic
ENSMUSG00000034168	86881877	86883077	2.20	2937	inside
ENSMUSG00000062691	78916567	78917017	2.20	68	inside
ENSMUSG00000052026	61013638	61014688	2.20	561	overlap
ENSMUSG00000062661	31246001	31246401	2.20	178	inside
ENSMUSG00000027490	154569629	154570029	2.20	263	overlap
ENSMUSG00000027994	129969399	129969849	2.20	807	inside
ENSMUSG00000072872	100125297	100125697	2.20	162144	intergenic
ENSMUSG00000035314	99381366	99381866	2.20	-183	overlap
ENSMUSG00000030544	79792560	79793110	2.20	1228	inside
ENSMUSG00000014837	105289122	105289622	2.20	406	overlap
ENSMUSG00000097736	122568617	122569017	2.20	-3880	upstream
ENSMUSG00000031993	30426650	30427050	2.20	-679	upstream
ENSMUSG00000087514	69106814	69107214	2.19	-74	upstream
ENSMUSG00000084332	69376959	69378309	2.19	-245660	intergenic
ENSMUSG00000062075	80917613	80918113	2.19	632	inside
ENSMUSG00000017776	75679327	75679777	2.19	68	inside
ENSMUSG00000063564	51289580	51290030	2.19	-340	overlap
ENSMUSG00000061111	120549636	120550036	2.19	91	overlap
ENSMUSG00000045201	15509735	15510185	2.19	-70748	intergenic
ENSMUSG00000047921	73060523	73060973	2.19	681	inside
ENSMUSG00000058392	32036472	32036872	2.19	372	inside
ENSMUSG00000044646	75819509	75819909	2.19	-669	upstream
ENSMUSG00000024483	36560377	36560877	2.19	390	inside
ENSMUSG00000097593	80322124	80322624	2.19	-1211	upstream
ENSMUSG00000024423	12973578	12974078	2.19	1326	inside
ENSMUSG00000038555	34840309	34840709	2.19	-280	overlap
ENSMUSG00000025213	45076425	45076925	2.19	1184	inside
ENSMUSG00000035372	3708265	3708815	2.19	-68	overlap
ENSMUSG00000068290	130664223	130664623	2.19	436	inside
ENSMUSG00000041912	94413090	94413490	2.19	-206	overlap
ENSMUSG00000085909	12402901	12403301	2.19	131502	intergenic
ENSMUSG00000028610	107683763	107684213	2.19	467	inside
ENSMUSG00000053839	31093184	31093634	2.19	-1800	upstream
ENSMUSG00000037541	143978027	143979327	2.19	-23901	intergenic
ENSMUSG00000071064	79027931	79029281	2.19	-506	overlap
ENSMUSG00000047897	87015500	87016100	2.19	-37	overlap
ENSMUSG00000097642	22532850	22533600	2.18	-207914	intergenic
ENSMUSG00000048402	119053261	119054061	2.18	358	overlap
ENSMUSG00000020020	93834084	93834484	2.18	2529	inside
ENSMUSG00000020325	79779656	79780156	2.18	2384	inside
ENSMUSG00000020346	49250136	49250586	2.18	5945	inside
ENSMUSG00000070407	63924927	63925527	2.18	-2637	upstream
ENSMUSG00000037275	58167936	58168336	2.18	603	inside
ENSMUSG00000046605	121673194	121673644	2.18	-41	upstream

ENSMUSG00000035910	25055687	25056087	2.18	-317	overlap
ENSMUSG00000023938	45506657	45507057	2.18	-184	overlap
ENSMUSG00000096847	45545258	45545658	2.18	4419	inside
ENSMUSG00000097330	37762959	37763659	2.18	5283	inside
ENSMUSG00000083282	4855116	4855516	2.18	-13	overlap
ENSMUSG00000037771	158610903	158611553	2.18	136	inside
ENSMUSG00000037211	37639840	37640540	2.18	-107	overlap
ENSMUSG00000037661	30855845	30856245	2.18	-105	overlap
ENSMUSG00000077161	62832648	62833198	2.18	20220	intergenic
ENSMUSG00000094832	22745761	22746461	2.18	-29869	intergenic
ENSMUSG00000037428	137032787	137033237	2.18	2492	inside
ENSMUSG00000055204	90365326	90365826	2.18	859	inside
ENSMUSG00000044341	119744332	119744782	2.18	4406	downstream
ENSMUSG00000030760	98309116	98309666	2.18	12092	inside
ENSMUSG00000032504	113708067	113708517	2.18	192	overlap
ENSMUSG00000088730	15634811	15635461	2.17	650141	intergenic
ENSMUSG00000018334	79146377	79146777	2.17	30	overlap
ENSMUSG00000020340	46311636	46312036	2.17	1223	inside
ENSMUSG00000044847	45944727	45945327	2.17	208	overlap
ENSMUSG00000063129	55538457	55538907	2.17	-26782	intergenic
ENSMUSG00000021670	96670585	96671135	2.17	351	overlap
ENSMUSG00000036606	89167239	89168589	2.17	3881	inside
ENSMUSG00000047428	46297844	46298294	2.17	423	inside
ENSMUSG00000001870	75005270	75005770	2.17	-298	overlap
ENSMUSG00000045817	84186427	84186877	2.17	1520	inside
ENSMUSG00000092569	75342959	75343509	2.17	24129	intergenic
ENSMUSG00000018822	42201626	42202026	2.17	626	inside
ENSMUSG00000053896	68599286	68600386	2.17	16873	inside
ENSMUSG00000068154	146222835	146223235	2.17	914	inside
ENSMUSG00000026755	39008201	39008651	2.17	125	inside
ENSMUSG00000050896	84886482	84886932	2.17	228	overlap
ENSMUSG00000017817	163375349	163375749	2.17	22644	inside
ENSMUSG00000008999	172940058	172940508	2.17	263	overlap
ENSMUSG00000028262	144828325	144829625	2.17	-8831	intergenic
ENSMUSG00000082279	107322737	107323687	2.17	5762	downstream
ENSMUSG00000029366	88765076	88765576	2.17	80	inside
ENSMUSG00000030435	5061761	5062561	2.17	-382	overlap
ENSMUSG00000038497	13287130	13287580	2.17	-883	upstream
ENSMUSG00000079070	32884700	32885200	2.17	65326	downstream
ENSMUSG00000033106	106210389	106210889	2.17	544	inside
ENSMUSG00000019777	36974595	36975195	2.16	51	inside
ENSMUSG00000034707	121365245	121365695	2.16	155	inside
ENSMUSG00000085707	53463327	53463877	2.16	286	overlap
ENSMUSG00000010086	61449227	61449627	2.16	4904	inside
ENSMUSG00000021265	108835844	108836244	2.16	39	overlap
ENSMUSG00000040867	109033584	109033984	2.16	34633	inside
ENSMUSG00000091105	21783788	21784188	2.16	-7081	intergenic
ENSMUSG00000040640	27621727	27622377	2.16	-715	upstream
ENSMUSG00000054863	87664816	87665266	2.16	39586	inside
ENSMUSG00000088128	76180771	76182071	2.16	33434	intergenic
ENSMUSG00000006740	6241365	6241765	2.16	809	inside

ENSMUSG00000024360	34931277	34931727	2.16	730	inside
ENSMUSG00000042834	33384477	33385177	2.16	79552	intergenic
ENSMUSG00000040913	45659976	45660376	2.16	336	overlap
ENSMUSG00000050174	37481153	37481553	2.16	-60942	intergenic
ENSMUSG00000035069	103313637	103314237	2.16	-175	overlap
ENSMUSG00000046079	105700288	105700688	2.16	319	inside
ENSMUSG00000084950	87980339	87980839	2.16	-544	upstream
ENSMUSG00000092318	12010383	12010783	2.16	5317	downstream
ENSMUSG00000006315	30729057	30729507	2.16	477	inside
ENSMUSG00000031627	46740654	46741254	2.16	909	inside
ENSMUSG00000031986	124898054	124898554	2.16	168	inside
ENSMUSG00000034518	74994040	74994540	2.16	684	inside
ENSMUSG00000086225	100643416	100644266	2.16	-17451	intergenic
ENSMUSG00000032440	116174667	116175067	2.16	696	inside
ENSMUSG00000039349	184882810	184883310	2.15	408	overlap
ENSMUSG00000088582	148301958	148302508	2.15	25150	intergenic
ENSMUSG00000036478	96617194	96617644	2.15	193	inside
ENSMUSG00000055670	72796377	72796777	2.15	151	inside
ENSMUSG00000048616	89301568	89301968	2.15	991	inside
ENSMUSG00000020831	70237638	70238038	2.15	276	overlap
ENSMUSG00000045440	55598811	55599561	2.15	-106	overlap
ENSMUSG00000097801	80518826	80519326	2.15	90	overlap
ENSMUSG00000046314	45074158	45074608	2.15	335	overlap
ENSMUSG00000038175	45389047	45389747	2.15	-695	overlap
ENSMUSG00000084651	109903058	109903558	2.15	-751	upstream
ENSMUSG00000042622	79347421	79348171	2.15	-120	overlap
ENSMUSG00000022360	58134546	58134946	2.15	536	inside
ENSMUSG00000022450	82354071	82354621	2.15	220	overlap
ENSMUSG00000041205	20240641	20241041	2.15	717	inside
ENSMUSG00000073411	35262722	35263172	2.15	-8	overlap
ENSMUSG00000024421	12333854	12334304	2.15	-170	overlap
ENSMUSG00000097430	37143459	37143909	2.15	35063	intergenic
ENSMUSG00000069833	8988876	8989326	2.15	-408	overlap
ENSMUSG00000037902	129593423	129593923	2.15	588	inside
ENSMUSG00000059540	181680271	181680771	2.15	-39	overlap
ENSMUSG00000065454	180893616	180894116	2.15	-415	overlap
ENSMUSG00000025314	90579764	90580364	2.15	883	inside
ENSMUSG00000037197	11553444	11553844	2.15	50709	intergenic
ENSMUSG00000087100	172007471	172008121	2.15	-3476	upstream
ENSMUSG00000037625	31149717	31150267	2.15	-229	overlap
ENSMUSG00000033721	109340750	109341300	2.15	97	inside
ENSMUSG00000029003	148130033	148130783	2.15	-351	overlap
ENSMUSG00000052520	96650989	96651589	2.15	13165	inside
ENSMUSG00000033365	117914567	117915167	2.15	432	overlap
ENSMUSG00000015942	134184157	134184557	2.15	138	inside
ENSMUSG00000035266	100798174	100798574	2.15	424	inside
ENSMUSG00000043614	135077707	135078107	2.15	559	inside
ENSMUSG00000030087	90462724	90463274	2.15	148	inside
ENSMUSG00000003423	45154156	45154556	2.15	-147	overlap
ENSMUSG00000021217	36698154	36698554	2.15	36	inside
ENSMUSG00000009545	143106808	143107358	2.15	-446	overlap

ENSMUSG00000041775	138846143	138846793	2.15	130	overlap
ENSMUSG00000066180	126291859	126292409	2.15	8057	intergenic
ENSMUSG00000093241	108956072	108956622	2.15	19212	intergenic
ENSMUSG00000031995	31131700	31132150	2.15	153	overlap
ENSMUSG00000074345	54068078	54068478	2.15	333	overlap
ENSMUSG00000026563	166379202	166379602	2.14	105	inside
ENSMUSG00000048960	10993107	10993707	2.14	-358	overlap
ENSMUSG00000026080	38897388	38897938	2.14	772	inside
ENSMUSG00000019997	24595473	24596073	2.14	31	inside
ENSMUSG00000020218	121033695	121034395	2.14	-265	overlap
ENSMUSG00000000282	74837627	74838577	2.14	6707	inside
ENSMUSG00000040610	33202438	33203038	2.14	1150	inside
ENSMUSG00000000804	85138630	85139930	2.14	1531	inside
ENSMUSG00000001504	72631468	72632068	2.14	2648	inside
ENSMUSG00000021991	29720727	29721727	2.14	1137	inside
ENSMUSG00000036158	93519120	93519570	2.14	76771	inside
ENSMUSG00000068284	44173560	44174010	2.14	314	inside
ENSMUSG00000089774	92058700	92059100	2.14	378	inside
ENSMUSG00000094726	82000307	82001007	2.14	-542922	intergenic
ENSMUSG00000034786	34587301	34587701	2.14	-2505	upstream
ENSMUSG00000043991	36287327	36288027	2.14	6230	inside
ENSMUSG00000053441	58836727	58837677	2.14	-37	overlap
ENSMUSG00000089290	23803630	23804030	2.14	13741	intergenic
ENSMUSG00000075227	6061665	6062065	2.14	473	inside
ENSMUSG00000025171	41981219	41981719	2.14	-544	upstream
ENSMUSG00000097787	32388425	32389225	2.14	-791	overlap
ENSMUSG00000074793	131127090	131127540	2.14	-190	overlap
ENSMUSG00000026849	30967501	30968201	2.14	432	overlap
ENSMUSG00000042272	77279969	77280369	2.14	623	inside
ENSMUSG00000085388	19445944	19446744	2.14	730	overlap
ENSMUSG00000008604	88553767	88554167	2.14	51	inside
ENSMUSG00000013622	31048284	31049184	2.14	-28	overlap
ENSMUSG00000035187	101663738	101664288	2.14	1488	inside
ENSMUSG00000068328	83054299	83054849	2.14	-354	overlap
ENSMUSG00000001761	29735443	29735893	2.14	-251	overlap
ENSMUSG00000042087	117907354	117907854	2.14	406	overlap
ENSMUSG00000086414	105761512	105762712	2.14	-809	overlap
ENSMUSG00000098708	63757577	63758177	2.14	-138956	intergenic
ENSMUSG00000052301	126847659	126848809	2.14	243	inside
ENSMUSG00000048583	142667608	142668108	2.14	-792	upstream
ENSMUSG00000056043	35584394	35584844	2.14	1188	inside
ENSMUSG00000062944	128238249	128238649	2.14	-218	upstream
ENSMUSG00000070526	62463727	62464377	2.14	783	inside
ENSMUSG00000055148	72318873	72319523	2.14	-160	overlap
ENSMUSG00000031715	80739142	80739642	2.14	355	overlap
ENSMUSG00000033021	75422513	75423013	2.13	-13417	intergenic
ENSMUSG00000019467	127188754	127189154	2.13	1300	inside
ENSMUSG00000097586	22272945	22273445	2.13	8791	intergenic
ENSMUSG00000034427	115859118	115859568	2.13	-2494	upstream
ENSMUSG00000099101	78178677	78179077	2.13	-150	includeFeature
ENSMUSG00000043099	75165277	75166427	2.13	4242	inside

ENSMUSG00000087842	113229316	113230066	2.13	15167	intergenic
ENSMUSG00000057469	16811092	16811542	2.13	127	inside
ENSMUSG00000042286	31176549	31177249	2.13	-7908	intergenic
ENSMUSG00000087836	30447641	30448041	2.13	-142646	intergenic
ENSMUSG00000039568	4879753	4880403	2.13	562	overlap
ENSMUSG00000022983	90283608	90284058	2.13	817	inside
ENSMUSG00000059791	35861211	35862461	2.13	-107	overlap
ENSMUSG00000046711	27555015	27555415	2.13	-1605	upstream
ENSMUSG00000024014	29490072	29490522	2.13	-740	upstream
ENSMUSG00000024163	24936272	24936922	2.13	705	inside
ENSMUSG00000033717	54046025	54046825	2.13	843	inside
ENSMUSG00000084837	144331049	144331449	2.13	-410	upstream
ENSMUSG00000026764	49619473	49619973	2.13	175	inside
ENSMUSG00000086308	116066799	116067249	2.13	-426	overlap
ENSMUSG00000016386	106693252	106693652	2.13	-17	overlap
ENSMUSG00000026791	32981351	32981801	2.13	732	inside
ENSMUSG00000035513	29252551	29252951	2.13	454	inside
ENSMUSG00000027184	103797382	103797782	2.13	267	overlap
ENSMUSG00000026754	39065319	39065719	2.13	222	overlap
ENSMUSG00000027794	55181696	55182096	2.13	-332	overlap
ENSMUSG00000033147	101924302	101924802	2.13	151	overlap
ENSMUSG00000070867	114406157	114406557	2.13	-567	upstream
ENSMUSG00000001089	136470077	136470577	2.13	316	inside
ENSMUSG00000070717	132074657	132075207	2.13	-40	overlap
ENSMUSG00000034645	108217293	108217743	2.13	629	inside
ENSMUSG00000087516	119680292	119680992	2.13	-8927	intergenic
ENSMUSG00000033706	85431828	85432228	2.13	-161	overlap
ENSMUSG00000030270	113283074	113283474	2.13	767	inside
ENSMUSG00000030199	134035765	134036415	2.13	65	inside
ENSMUSG00000034203	91472960	91473410	2.13	463	inside
ENSMUSG00000005362	106799549	106800049	2.13	525	inside
ENSMUSG00000030287	146501921	146502321	2.13	302	overlap
ENSMUSG00000031074	144838651	144839551	2.13	568	inside
ENSMUSG00000047371	127345482	127346032	2.13	-168	upstream
ENSMUSG00000031480	22227236	22227636	2.13	-77	overlap
ENSMUSG00000031737	92360390	92360790	2.13	2594	inside
ENSMUSG00000034472	75212973	75213573	2.13	-971	upstream
ENSMUSG00000034796	123117391	123117791	2.13	17	inside
ENSMUSG00000036611	25481500	25481900	2.13	-47	overlap
ENSMUSG00000026504	176813896	176814296	2.12	-764	upstream
ENSMUSG00000026686	167688396	167689446	2.12	-843	overlap
ENSMUSG00000038702	111864600	111865050	2.12	309	overlap
ENSMUSG00000038160	44268237	44268687	2.12	-121	overlap
ENSMUSG00000020102	125388987	125389487	2.12	-60024	intergenic
ENSMUSG00000020770	116030436	116030836	2.12	114	inside
ENSMUSG00000001552	100397515	100398015	2.12	248	overlap
ENSMUSG00000077590	65356158	65357208	2.12	49812	intergenic
ENSMUSG00000015002	65787125	65787525	2.12	91	inside
ENSMUSG00000040605	97356407	97356807	2.12	-321	overlap
ENSMUSG00000085783	62664497	62664897	2.12	-52456	intergenic
ENSMUSG00000038168	26105517	26105917	2.12	267	overlap

ENSMUSG00000089883	13844100	13844650	2.12	18703	intergenic
ENSMUSG00000024974	53599025	53600225	2.12	-1373	upstream
ENSMUSG00000052188	6531165	6531565	2.12	846	inside
ENSMUSG00000027301	130576473	130576873	2.12	300	inside
ENSMUSG00000074582	166805748	166806148	2.12	160	inside
ENSMUSG00000027525	178142221	178142721	2.12	23246	inside
ENSMUSG00000027315	119237535	119237985	2.12	173	inside
ENSMUSG00000087455	59347928	59348328	2.12	39543	intergenic
ENSMUSG00000027967	127633149	127633849	2.12	13	inside
ENSMUSG00000028032	131564411	131564811	2.12	-357	overlap
ENSMUSG00000077253	110682444	110683244	2.12	-117903	intergenic
ENSMUSG00000028351	68832764	68833214	2.12	121633	inside
ENSMUSG00000080412	120302020	120302770	2.12	14294	intergenic
ENSMUSG00000060621	19519914	19520314	2.12	1183	inside
ENSMUSG00000054716	127249099	127249499	2.12	4573	inside
ENSMUSG00000012889	84130823	84131273	2.12	4834	inside
ENSMUSG00000014786	105349108	105349508	2.12	850	inside
ENSMUSG00000085795	26978567	26979217	2.12	1231	inside
ENSMUSG00000002393	71380681	71381181	2.12	1279	inside
ENSMUSG00000032178	21367891	21368741	2.12	-128	overlap
ENSMUSG00000046997	97018016	97018716	2.12	827	inside
ENSMUSG00000071072	128058322	128058872	2.11	-632	upstream
ENSMUSG00000044199	81499056	81499556	2.11	1076	inside
ENSMUSG00000020097	61146431	61147181	2.11	1272	inside
ENSMUSG00000063760	31608461	31608861	2.11	723	inside
ENSMUSG00000038594	53379037	53379437	2.11	814	inside
ENSMUSG00000000976	83753027	83753577	2.11	-669	upstream
ENSMUSG00000086058	82930286	82930786	2.11	3096	inside
ENSMUSG00000077270	65881168	65881768	2.11	315078	intergenic
ENSMUSG00000052632	21112037	21112587	2.11	289	inside
ENSMUSG00000088158	80380290	80380890	2.11	11461	intergenic
ENSMUSG00000051111	96132085	96132635	2.11	492	overlap
ENSMUSG00000021359	40731200	40731600	2.11	2623	inside
ENSMUSG00000036422	79770219	79770769	2.11	1093	inside
ENSMUSG00000072294	100049861	100050411	2.11	99903	inside
ENSMUSG00000023484	99055615	99056215	2.11	441	inside
ENSMUSG00000062901	20097772	20098322	2.11	218	inside
ENSMUSG00000040785	94370116	94370516	2.11	-502	upstream
ENSMUSG00000060475	12991380	12991880	2.11	1166	inside
ENSMUSG00000016498	29424626	29425026	2.11	13707	inside
ENSMUSG00000024758	7482963	7483363	2.11	326	overlap
ENSMUSG00000027007	79635502	79635952	2.11	150	inside
ENSMUSG00000042821	167538171	167539021	2.11	-24	overlap
ENSMUSG00000003662	127247785	127248285	2.11	31	overlap
ENSMUSG00000027171	104849469	104849919	2.11	407	overlap
ENSMUSG00000098383	38351095	38351495	2.11	-2779	upstream
ENSMUSG00000027597	155073673	155074173	2.11	824	inside
ENSMUSG00000061809	24123489	24124039	2.11	-26894	intergenic
ENSMUSG00000048001	154960889	154961289	2.11	-34	overlap
ENSMUSG00000028919	141247827	141248277	2.11	8328	inside
ENSMUSG00000042380	127243736	127244136	2.11	-48	overlap

ENSMUSG00000052135	120286457	120287157	2.11	892	inside
ENSMUSG00000029231	75152732	75153332	2.11	441	inside
ENSMUSG00000029135	32136270	32136670	2.11	-202	overlap
ENSMUSG00000070473	134986137	134986887	2.11	-77	overlap
ENSMUSG00000015053	88198189	88198589	2.11	4298	inside
ENSMUSG00000068303	85154610	85155310	2.11	-395	overlap
ENSMUSG00000030002	85961460	85961960	2.11	207	overlap
ENSMUSG00000003099	17027624	17028024	2.11	300	overlap
ENSMUSG00000045969	11555008	11555408	2.11	-1058	upstream
ENSMUSG00000087761	77906623	77907873	2.11	99088	intergenic
ENSMUSG00000050912	7764185	7764585	2.11	144	inside
ENSMUSG00000031963	23222191	23222591	2.11	-885	upstream
ENSMUSG00000032340	59036778	59037178	2.11	-337	upstream
ENSMUSG00000006005	150368597	150368997	2.10	-24241	intergenic
ENSMUSG00000097316	192136562	192137012	2.10	-336	overlap
ENSMUSG00000033007	75461064	75461814	2.10	10628	inside
ENSMUSG00000089866	105026211	105027411	2.10	-113878	intergenic
ENSMUSG00000097487	101425165	101425565	2.10	168	overlap
ENSMUSG00000001444	97114974	97115574	2.10	357	overlap
ENSMUSG00000034271	85598548	85599098	2.10	-557	upstream
ENSMUSG00000021264	108757917	108758867	2.10	-35056	intergenic
ENSMUSG00000051166	98900535	98901035	2.10	949	inside
ENSMUSG00000042622	79357139	79357639	2.10	9598	inside
ENSMUSG00000035828	88863189	88863639	2.10	1003	inside
ENSMUSG00000000532	101174188	101174638	2.10	84	inside
ENSMUSG00000025076	56397120	56397520	2.10	-9	overlap
ENSMUSG00000043531	50677725	50678575	2.10	921	inside
ENSMUSG00000059326	61225363	61225763	2.10	3055	inside
ENSMUSG00000085322	168765785	168766285	2.10	-358	overlap
ENSMUSG00000076312	155628953	155629803	2.10	6073	intergenic
ENSMUSG00000055612	72476364	72476814	2.10	205	inside
ENSMUSG00000027217	93333878	93334378	2.10	627	inside
ENSMUSG00000062319	6996347	6997597	2.10	-44667	intergenic
ENSMUSG00000053819	126596544	126596944	2.10	242	inside
ENSMUSG00000099169	140304904	140305504	2.10	276655	intergenic
ENSMUSG00000070687	136424557	136424957	2.10	1033	inside
ENSMUSG00000029326	100039782	100040182	2.10	-212	overlap
ENSMUSG00000029504	110653516	110653966	2.10	65	inside
ENSMUSG00000089415	77994776	77995326	2.10	-324930	intergenic
ENSMUSG00000028995	24029861	24030261	2.10	829	inside
ENSMUSG00000052751	48596394	48597594	2.10	2511	inside
ENSMUSG00000032652	134830073	134830523	2.10	-81	overlap
ENSMUSG00000043131	83325739	83326189	2.10	-277	overlap
ENSMUSG00000047710	13869258	13869758	2.10	-383	overlap
ENSMUSG00000042195	53771628	53772128	2.10	93	inside
ENSMUSG00000048752	110858081	110858481	2.10	114	inside
ENSMUSG00000048000	87327364	87327864	2.09	366	inside
ENSMUSG00000026234	86358308	86358808	2.09	1147	inside
ENSMUSG00000025938	12992046	12992496	2.09	-869	upstream
ENSMUSG00000065096	12274860	12275960	2.09	-88550	intergenic
ENSMUSG00000000686	77515080	77515530	2.09	-41	overlap

ENSMUSG00000085609	75172177	75172977	2.09	-383	overlap
ENSMUSG00000017376	78697027	78697477	2.09	346	overlap
ENSMUSG00000017631	76623080	76623480	2.09	-766	upstream
ENSMUSG00000048616	89301968	89302418	2.09	591	inside
ENSMUSG00000021051	75595111	75595561	2.09	1089	inside
ENSMUSG00000077473	4982260	4982810	2.09	207674	intergenic
ENSMUSG00000021943	33923476	33923876	2.09	-111	overlap
ENSMUSG00000022552	76350423	76351623	2.09	688	overlap
ENSMUSG00000022964	91597019	91597419	2.09	781	inside
ENSMUSG00000024664	10041490	10042090	2.09	-58	overlap
ENSMUSG00000085596	167565476	167565876	2.09	12162	inside
ENSMUSG00000068735	93187064	93187564	2.09	-484	overlap
ENSMUSG00000026959	25318689	25319089	2.09	498	inside
ENSMUSG00000027016	77816928	77817328	2.09	2711	inside
ENSMUSG00000078578	135437953	135438653	2.09	-344	overlap
ENSMUSG00000087440	51275945	51276595	2.09	-1626	upstream
ENSMUSG00000027985	131110100	131111250	2.09	-371	overlap
ENSMUSG00000053965	122729221	122729921	2.09	63	inside
ENSMUSG00000027715	36571145	36571845	2.09	1005	inside
ENSMUSG00000037325	36613090	36613540	2.09	387	overlap
ENSMUSG00000028840	134249397	134249997	2.09	-3805	upstream
ENSMUSG00000025858	139252439	139252939	2.09	115	inside
ENSMUSG00000029152	73292562	73293012	2.09	-232	overlap
ENSMUSG00000023353	24485175	24485575	2.09	32998	inside
ENSMUSG00000002633	28593395	28594245	2.09	-126294	intergenic
ENSMUSG00000077474	83715318	83715768	2.09	8271	intergenic
ENSMUSG00000029575	114444048	114444498	2.09	11	overlap
ENSMUSG00000092928	48024611	48025011	2.09	-24092	intergenic
ENSMUSG00000076327	128034875	128035725	2.09	60151	intergenic
ENSMUSG00000030527	80688027	80688677	2.09	850	inside
ENSMUSG00000082281	103966768	103967218	2.09	10339	intergenic
ENSMUSG00000087530	128010009	128010459	2.09	-4819	upstream
ENSMUSG00000034330	117498430	117498880	2.09	139	inside
ENSMUSG00000019464	83667842	83668242	2.09	1009	inside
ENSMUSG00000031609	57486992	57487392	2.09	868	inside
ENSMUSG00000036466	64385979	64386379	2.09	353	inside
ENSMUSG00000086158	72985328	72985728	2.09	54	overlap
ENSMUSG00000026176	74391945	74392345	2.08	436	inside
ENSMUSG00000038473	170589852	170590252	2.08	9	overlap
ENSMUSG00000015961	177795558	177796408	2.08	953	inside
ENSMUSG00000035873	108333173	108333573	2.08	984	inside
ENSMUSG00000034994	81176756	81177256	2.08	125	inside
ENSMUSG00000019979	91082263	91082763	2.08	507	inside
ENSMUSG00000045912	79613865	79614265	2.08	160	overlap
ENSMUSG00000090266	116842068	116843018	2.08	-1210	upstream
ENSMUSG00000044950	43682427	43682827	2.08	429	inside
ENSMUSG00000085516	24164050	24164500	2.08	8774	inside
ENSMUSG00000039976	119227965	119228365	2.08	534	inside
ENSMUSG00000097061	69196776	69197376	2.08	-435	overlap
ENSMUSG00000006356	113139987	113140387	2.08	-249	overlap
ENSMUSG00000048251	107916795	107917295	2.08	86807	inside

ENSMUSG00000091105	21898703	21899303	2.08	-121996	intergenic
ENSMUSG00000021118	78906761	78907161	2.08	203	overlap
ENSMUSG00000091387	96924401	96924851	2.08	-288	overlap
ENSMUSG00000021391	49652421	49652871	2.08	325	overlap
ENSMUSG00000021676	95891735	95892435	2.08	187	overlap
ENSMUSG00000025544	122107177	122107577	2.08	139	inside
ENSMUSG00000021838	46881905	46882955	2.08	-949	overlap
ENSMUSG00000022012	77156456	77156856	2.08	-307	overlap
ENSMUSG00000065760	49600055	49600455	2.08	61860	intergenic
ENSMUSG00000050761	18621117	18621717	2.08	1286	inside
ENSMUSG00000023143	5203502	5204052	2.08	510	overlap
ENSMUSG00000071054	56584773	56585173	2.08	-52	overlap
ENSMUSG00000096361	47410008	47410458	2.08	-355	overlap
ENSMUSG00000040140	43629844	43630294	2.08	455	inside
ENSMUSG00000024608	60748677	60749077	2.08	-25833	intergenic
ENSMUSG00000024565	80986048	80986498	2.08	530	inside
ENSMUSG00000024487	40219159	40219559	2.08	240	overlap
ENSMUSG00000024378	33330398	33330998	2.08	-116536	intergenic
ENSMUSG00000067199	41829640	41830040	2.08	-528	upstream
ENSMUSG00000027678	165992971	165993421	2.08	335	inside
ENSMUSG00000042448	74763202	74763652	2.08	222	inside
ENSMUSG00000035403	37786701	37787101	2.08	10452	inside
ENSMUSG00000002100	91115269	91115719	2.08	-2875	upstream
ENSMUSG00000059173	79936936	79937386	2.08	192522	inside
ENSMUSG00000074912	119337835	119338335	2.08	-11641	intergenic
ENSMUSG00000044320	30801215	30801765	2.08	2446	inside
ENSMUSG00000082678	112979593	112979993	2.08	220	overlap
ENSMUSG00000066224	41730760	41731360	2.08	382	overlap
ENSMUSG00000029334	99037160	99037660	2.08	191	overlap
ENSMUSG00000039474	36988768	36989168	2.08	214	overlap
ENSMUSG00000028995	24030411	24030811	2.08	279	overlap
ENSMUSG00000084934	141383175	141383925	2.08	-2249	upstream
ENSMUSG00000079511	83102089	83102489	2.08	472	inside
ENSMUSG00000063870	125095975	125096525	2.08	-6	overlap
ENSMUSG00000002222	71440339	71441039	2.08	298	overlap
ENSMUSG00000049583	87584213	87584713	2.08	-18331	intergenic
ENSMUSG00000030731	44352027	44352477	2.08	-32075	intergenic
ENSMUSG00000099103	143445141	143445591	2.08	-2464	upstream
ENSMUSG00000048617	84944990	84945990	2.08	-2001	upstream
ENSMUSG00000074136	95806789	95807539	2.08	-41	overlap
ENSMUSG00000096943	83896990	83897390	2.08	2511	inside
ENSMUSG00000084128	106136358	106136808	2.08	616	inside
ENSMUSG00000032591	108079005	108079505	2.08	-1431	upstream
ENSMUSG00000026308	91344850	91345350	2.07	-6166	intergenic
ENSMUSG00000067028	99593862	99594912	2.07	-178903	intergenic
ENSMUSG00000026028	58973094	58973544	2.07	336	overlap
ENSMUSG00000025982	55027389	55027889	2.07	89	overlap
ENSMUSG00000074785	94943633	94944033	2.07	945	inside
ENSMUSG00000048756	42276473	42276873	2.07	282	overlap
ENSMUSG00000042650	60536877	60537327	2.07	496	inside
ENSMUSG00000005417	59662030	59662430	2.07	725	inside

ENSMUSG00000049336	37236177	37236577	2.07	-213	upstream
ENSMUSG00000020598	44328790	44329490	2.07	-95	overlap
ENSMUSG00000063632	27342279	27343229	2.07	430	overlap
ENSMUSG00000050671	87299913	87300313	2.07	-208	upstream
ENSMUSG00000037169	12941321	12941821	2.07	593	inside
ENSMUSG00000056770	108177495	108178795	2.07	1819	inside
ENSMUSG00000021318	15463923	15464373	2.07	688	inside
ENSMUSG00000034928	54693955	54694455	2.07	-48	upstream
ENSMUSG00000038009	99093665	99094115	2.07	495	inside
ENSMUSG00000055745	84557116	84557566	2.07	707	inside
ENSMUSG00000001076	99088224	99088674	2.07	-407	upstream
ENSMUSG00000058600	34442991	34443491	2.07	649	inside
ENSMUSG00000041935	3995253	3995653	2.07	499	inside
ENSMUSG00000071636	17208379	17208779	2.07	244	inside
ENSMUSG00000047953	30308317	30308767	2.07	2462	inside
ENSMUSG00000023067	29094398	29094798	2.07	3419	inside
ENSMUSG00000037089	45563247	45564047	2.07	-717	overlap
ENSMUSG00000024242	80727217	80727617	2.07	876	inside
ENSMUSG00000024335	34118212	34118912	2.07	4422	inside
ENSMUSG00000047466	77712892	77713342	2.07	1118	inside
ENSMUSG00000054874	5688066	5688466	2.07	842	inside
ENSMUSG00000077351	43113751	43114501	2.07	304716	intergenic
ENSMUSG00000027015	71117764	71119114	2.07	-159	overlap
ENSMUSG00000005803	122764985	122765385	2.07	-252	overlap
ENSMUSG00000081878	71786808	71787408	2.07	-24041	intergenic
ENSMUSG00000084339	19599812	19601162	2.07	15021	intergenic
ENSMUSG00000074517	83127795	83128195	2.07	1451	inside
ENSMUSG00000070990	46343376	46344026	2.07	-233	overlap
ENSMUSG00000028409	40757559	40758009	2.07	364	overlap
ENSMUSG00000039813	46649550	46650150	2.07	659	inside
ENSMUSG00000056596	133498257	133498707	2.07	293	overlap
ENSMUSG00000059991	144545366	144545816	2.07	-521	upstream
ENSMUSG00000018001	143622574	143623074	2.07	127	inside
ENSMUSG00000075551	145681225	145681725	2.07	38911	intergenic
ENSMUSG00000029147	31219712	31220212	2.07	833	inside
ENSMUSG00000062960	75977852	75978352	2.07	606	inside
ENSMUSG00000029173	52669504	52669904	2.07	225	overlap
ENSMUSG00000073144	39118444	39118944	2.07	36	inside
ENSMUSG00000049661	45361603	45362003	2.07	-4560	upstream
ENSMUSG00000055652	75995927	75996377	2.07	147589	intergenic
ENSMUSG00000042797	97737522	97738072	2.07	725	inside
ENSMUSG00000046792	6155821	6156271	2.07	150	overlap
ENSMUSG00000066697	71328546	71329796	2.07	4375	downstream
ENSMUSG00000052837	84977905	84978305	2.07	843	inside
ENSMUSG00000031667	91133609	91134009	2.07	856	inside
ENSMUSG00000053399	113848330	113848830	2.07	285	overlap
ENSMUSG00000004319	60982807	60983407	2.07	493	overlap
ENSMUSG00000066687	48835031	48835481	2.07	914	inside
ENSMUSG00000052331	54925444	54925944	2.06	943	inside
ENSMUSG00000018417	51914294	51915244	2.06	1777	inside
ENSMUSG00000020009	19591852	19592302	2.06	-97	overlap

ENSMUSG00000019916	59323503	59324003	2.06	207	inside
ENSMUSG00000071369	19933595	19934145	2.06	-877	upstream
ENSMUSG00000019992	20347502	20348002	2.06	-317	overlap
ENSMUSG00000020225	120201195	120201995	2.06	-395	overlap
ENSMUSG00000023068	52417854	52418354	2.06	307	inside
ENSMUSG00000056316	29147423	29147873	2.06	-3229	upstream
ENSMUSG00000020261	55204227	55204727	2.06	-123	overlap
ENSMUSG00000020883	98149715	98150365	2.06	688	inside
ENSMUSG00000021051	75596411	75596861	2.06	-211	upstream
ENSMUSG00000034928	54687070	54687470	2.06	6837	inside
ENSMUSG00000041078	34820427	34820827	2.06	602	inside
ENSMUSG00000041408	34673944	34674594	2.06	16	inside
ENSMUSG00000046204	66910543	66911093	2.06	-627	upstream
ENSMUSG00000068697	20660908	20661458	2.06	-4368	upstream
ENSMUSG00000022568	76074171	76074571	2.06	-4387	upstream
ENSMUSG00000047565	20551946	20552546	2.06	114804	intergenic
ENSMUSG00000094605	43142810	43143260	2.06	16577	intergenic
ENSMUSG00000045038	86166520	86166920	2.06	-1265	upstream
ENSMUSG00000042644	27056765	27057465	2.06	-539	overlap
ENSMUSG00000041168	56626737	56627137	2.06	166	overlap
ENSMUSG00000054723	56716287	56716687	2.06	1412	inside
ENSMUSG00000052397	6783207	6783607	2.06	-423	upstream
ENSMUSG00000063239	27512001	27512401	2.06	1340	inside
ENSMUSG00000034484	53176427	53176877	2.06	62	inside
ENSMUSG00000025885	74442727	74443177	2.06	1791	inside
ENSMUSG00000024927	5637015	5637515	2.06	-468	overlap
ENSMUSG00000027624	156421085	156422035	2.06	176	inside
ENSMUSG00000075304	70476402	70476802	2.06	1479	inside
ENSMUSG00000027035	68861964	68862664	2.06	523	inside
ENSMUSG00000053615	125505835	125506235	2.06	746	inside
ENSMUSG00000027593	154790749	154791249	2.06	-347	overlap
ENSMUSG00000027326	119047203	119047653	2.06	84	inside
ENSMUSG00000089458	148167785	148168785	2.06	16635	intergenic
ENSMUSG00000027544	168570693	168571343	2.06	30964	inside
ENSMUSG00000075012	102451014	102451414	2.06	778	inside
ENSMUSG00000074796	130697135	130697635	2.06	384	overlap
ENSMUSG00000027777	68166030	68166930	2.06	101228	inside
ENSMUSG00000040389	108590907	108591357	2.06	-372	overlap
ENSMUSG00000084163	27534515	27535215	2.06	-8663	intergenic
ENSMUSG00000052135	120268707	120269157	2.06	18642	inside
ENSMUSG00000028909	131836665	131837065	2.06	1623	inside
ENSMUSG00000040928	129189478	129190078	2.06	249	overlap
ENSMUSG00000039137	63494698	63495098	2.06	1293	inside
ENSMUSG00000049907	74195132	74195982	2.06	-156	overlap
ENSMUSG00000054252	33721908	33722358	2.06	184	inside
ENSMUSG00000088751	57108318	57108718	2.06	-2506	upstream
ENSMUSG00000029192	36581294	36581744	2.06	11982	inside
ENSMUSG00000030094	91515110	91515860	2.06	774	inside
ENSMUSG00000071341	85512497	85513097	2.06	1092	inside
ENSMUSG00000055407	99268366	99268816	2.06	919	inside
ENSMUSG00000038244	112225462	112225862	2.06	-394	overlap

ENSMUSG00000025586	81453460	81454010	2.06	2005	inside
ENSMUSG00000030849	133121930	133122330	2.06	1420	inside
ENSMUSG00000096938	11006580	11007180	2.06	-1270	upstream
ENSMUSG00000019261	70913905	70914405	2.06	7931	inside
ENSMUSG00000097702	120598272	120598722	2.06	-280	overlap
ENSMUSG00000098322	15028008	15028458	2.06	-9101	intergenic
ENSMUSG00000025237	59617278	59617878	2.06	-6	overlap
ENSMUSG00000096786	109366971	109367521	2.06	-8636	intergenic
ENSMUSG00000045087	21244241	21244691	2.06	4202	inside
ENSMUSG00000032396	64340428	64340978	2.06	860	inside
ENSMUSG00000025945	65979370	65979970	2.05	52848	intergenic
ENSMUSG00000097347	180777438	180777888	2.05	-25130	intergenic
ENSMUSG00000090031	6214346	6214746	2.05	244	overlap
ENSMUSG00000026620	184956290	184956790	2.05	43259	inside
ENSMUSG00000020037	84754806	84755206	2.05	-1256	upstream
ENSMUSG00000035529	85916706	85917106	2.05	239	overlap
ENSMUSG00000065395	79711577	79712027	2.05	-392	overlap
ENSMUSG00000046215	103649124	103649524	2.05	-385	overlap
ENSMUSG00000005951	73199398	73199798	2.05	-62	overlap
ENSMUSG00000018217	63132938	63133388	2.05	3956	inside
ENSMUSG00000046719	95513468	95513868	2.05	1102	inside
ENSMUSG00000020902	68399836	68400336	2.05	987	inside
ENSMUSG00000021140	81860657	81861157	2.05	627	inside
ENSMUSG00000056553	116757082	116757982	2.05	271362	inside
ENSMUSG00000048982	75379040	75379540	2.05	37741	intergenic
ENSMUSG00000095777	78329195	78329795	2.05	-31902	intergenic
ENSMUSG00000016477	29985221	29985671	2.05	447	overlap
ENSMUSG00000022176	54479226	54479626	2.05	3126	inside
ENSMUSG00000090534	118235258	118235658	2.05	-974	upstream
ENSMUSG00000021767	21500141	21501041	2.05	18707	inside
ENSMUSG00000040123	56811426	56811826	2.05	290	overlap
ENSMUSG00000044819	120137640	120138140	2.05	-95205	intergenic
ENSMUSG00000034731	78731806	78732206	2.05	-6717	intergenic
ENSMUSG00000050310	6708119	6708519	2.05	-262	overlap
ENSMUSG00000036800	71169473	71170773	2.05	558365	intergenic
ENSMUSG00000022840	35156174	35156574	2.05	1297	inside
ENSMUSG00000071475	83499757	83500207	2.05	-128024	intergenic
ENSMUSG00000024188	26252911	26253461	2.05	1	inside
ENSMUSG00000001228	56304144	56304594	2.05	823	inside
ENSMUSG00000040356	34849851	34850401	2.05	359	overlap
ENSMUSG00000027381	128125785	128126185	2.05	-253	overlap
ENSMUSG00000086436	80638819	80639269	2.05	-9251	intergenic
ENSMUSG00000013465	25210939	25211439	2.05	550	inside
ENSMUSG00000034075	84714708	84715258	2.05	472	overlap
ENSMUSG00000026932	26091401	26092051	2.05	31819	inside
ENSMUSG00000036591	20968594	20969444	2.05	287	overlap
ENSMUSG00000092345	59003352	59003802	2.05	142	inside
ENSMUSG00000028341	48045220	48045620	2.05	67	inside
ENSMUSG00000073684	155249683	155250083	2.05	-119	overlap
ENSMUSG00000070803	120667093	120667493	2.05	530	inside
ENSMUSG00000028456	43058840	43059290	2.05	-113	overlap

ENSMUSG00000008305	72199608	72200158	2.05	1311	inside
ENSMUSG00000014030	44703176	44704076	2.05	8270	inside
ENSMUSG00000055235	24730033	24730533	2.05	694	inside
ENSMUSG00000000568	99978610	99979460	2.05	328	overlap
ENSMUSG00000056755	110645539	110646039	2.05	-42	overlap
ENSMUSG00000053297	48619842	48620492	2.05	-6922	intergenic
ENSMUSG00000029833	37871183	37871683	2.05	372	inside
ENSMUSG00000032667	5297853	5298303	2.05	602	inside
ENSMUSG00000063757	5030604	5031054	2.05	-3514	upstream
ENSMUSG00000030541	80115137	80115587	2.05	255	overlap
ENSMUSG00000048583	142663718	142664168	2.05	3098	inside
ENSMUSG00000002396	71371473	71371873	2.05	175	inside
ENSMUSG00000031737	92359190	92360540	2.05	1394	inside
ENSMUSG00000056267	99243266	99243666	2.05	-176	overlap
ENSMUSG00000049742	7836585	7837135	2.05	479	includeFeature
ENSMUSG00000059237	66805828	66806228	2.05	110	overlap
ENSMUSG00000026638	193152945	193153495	2.04	-167	overlap
ENSMUSG00000026113	37299584	37300184	2.04	-281	overlap
ENSMUSG00000026277	93634867	93635267	2.04	855	inside
ENSMUSG00000026482	152765847	152766497	2.04	504	overlap
ENSMUSG00000026121	36557499	36558049	2.04	850	inside
ENSMUSG00000014329	71159145	71159545	2.04	555	inside
ENSMUSG00000041164	6389629	6390029	2.04	555	inside
ENSMUSG00000052915	98795115	98795515	2.04	-401	upstream
ENSMUSG00000038351	74896927	74897527	2.04	133	overlap
ENSMUSG00000003934	69559736	69560186	2.04	469	inside
ENSMUSG00000020868	94670465	94670865	2.04	7050	inside
ENSMUSG00000018537	97700115	97700515	2.04	382	overlap
ENSMUSG00000045440	55600511	55600911	2.04	1594	inside
ENSMUSG00000021264	108793888	108794288	2.04	915	inside
ENSMUSG00000020973	69197826	69198326	2.04	603	inside
ENSMUSG00000064138	77857495	77858245	2.04	148805	inside
ENSMUSG00000021314	18948237	18948687	2.04	-134	overlap
ENSMUSG00000015396	43784671	43785071	2.04	-436	upstream
ENSMUSG00000035248	59822608	59823008	2.04	539	inside
ENSMUSG00000016477	29983771	29984171	2.04	1897	inside
ENSMUSG00000021506	55825929	55826329	2.04	10263	inside
ENSMUSG00000025872	54468704	54469304	2.04	145	overlap
ENSMUSG00000033885	8097599	8098199	2.04	-614	upstream
ENSMUSG00000097136	25607094	25607944	2.04	-52725	intergenic
ENSMUSG00000058655	102073870	102074370	2.04	97	inside
ENSMUSG00000022427	79669373	79670523	2.04	-1488	upstream
ENSMUSG00000036678	102355465	102355865	2.04	-4706	upstream
ENSMUSG00000097536	96285170	96285570	2.04	-675	upstream
ENSMUSG00000022483	98004415	98004915	2.04	280	overlap
ENSMUSG00000022797	32608709	32609159	2.04	-211	overlap
ENSMUSG00000022503	10411644	10412144	2.04	-304	overlap
ENSMUSG00000024155	24803915	24804815	2.04	-467	overlap
ENSMUSG00000095687	8147528	8148028	2.04	569	inside
ENSMUSG00000050138	87796233	87797083	2.04	1761	inside
ENSMUSG00000088493	37732833	37733333	2.04	26854	intergenic

ENSMUSG00000069378	53463548	53464098	2.04	-998	upstream
ENSMUSG00000052928	75696433	75696833	2.04	1263	inside
ENSMUSG00000024759	7494063	7494513	2.04	23	inside
ENSMUSG00000033768	6428466	6428916	2.04	9735	inside
ENSMUSG00000025010	40842325	40842725	2.04	11046	inside
ENSMUSG00000040565	36925613	36926163	2.04	-466	overlap
ENSMUSG00000053080	7417326	7418276	2.04	-299	overlap
ENSMUSG00000024869	4000210	4000760	2.04	-370	overlap
ENSMUSG00000024833	5963739	5964289	2.04	467	overlap
ENSMUSG00000059326	61226663	61227413	2.04	1755	inside
ENSMUSG00000082971	55631010	55631410	2.04	-144538	intergenic
ENSMUSG00000053166	165233376	165234326	2.04	1477	inside
ENSMUSG00000026888	65022082	65022582	2.04	2905	inside
ENSMUSG00000029419	25577889	25578289	2.04	2210	inside
ENSMUSG00000027765	61002617	61003367	2.04	-178	overlap
ENSMUSG00000077441	147780003	147780553	2.04	574215	intergenic
ENSMUSG00000028654	122995481	122996031	2.04	-171	overlap
ENSMUSG00000073700	152008713	152009113	2.04	-90	overlap
ENSMUSG00000028341	48043276	48043676	2.04	-1877	upstream
ENSMUSG00000028736	139821577	139822027	2.04	11951	inside
ENSMUSG00000071019	4048633	4049433	2.04	28889	intergenic
ENSMUSG00000041530	126468498	126468948	2.04	85	overlap
ENSMUSG00000029287	107289066	107289466	2.04	563	inside
ENSMUSG00000002297	8422143	8422593	2.04	573	inside
ENSMUSG00000048450	37823918	37824368	2.04	665	inside
ENSMUSG00000063935	72580562	72581062	2.04	522	inside
ENSMUSG00000037235	34187428	34187878	2.04	292	overlap
ENSMUSG00000059518	136987373	136987973	2.04	648	inside
ENSMUSG00000015053	88189749	88190249	2.04	-4142	upstream
ENSMUSG00000049093	67508047	67508597	2.04	-16192	intergenic
ENSMUSG00000010797	18030043	18030493	2.04	542	inside
ENSMUSG00000080562	27364344	27364744	2.04	1030	downstream
ENSMUSG00000007946	101821409	101821809	2.04	3096	inside
ENSMUSG00000046591	79660227	79660877	2.04	31	inside
ENSMUSG00000002635	34196528	34196978	2.04	133	overlap
ENSMUSG00000098839	45007103	45007803	2.04	5384	downstream
ENSMUSG00000001472	123373139	123373539	2.04	-685	upstream
ENSMUSG00000049946	58966461	58967311	2.04	54706	intergenic
ENSMUSG00000025809	128684639	128685739	2.04	-1015	overlap
ENSMUSG00000098560	121085008	121085408	2.04	-302	upstream
ENSMUSG00000041440	96118966	96119516	2.04	-396	overlap
ENSMUSG00000032403	63398728	63399128	2.04	516	inside
ENSMUSG00000084701	96536916	96537816	2.04	12793	intergenic
ENSMUSG00000039224	186967160	186967660	2.03	-256	overlap
ENSMUSG00000026502	178187813	178188213	2.03	396	inside
ENSMUSG00000009907	106796300	106796700	2.03	428	inside
ENSMUSG00000050069	174938158	174938958	2.03	-16339	intergenic
ENSMUSG00000026466	156036264	156036714	2.03	216	overlap
ENSMUSG00000055493	11342812	11343362	2.03	-592	upstream
ENSMUSG00000038602	52690704	52691454	2.03	171	inside
ENSMUSG00000048756	42275273	42275673	2.03	1482	inside

ENSMUSG00000025366	128525295	128525745	2.03	564	inside
ENSMUSG00000020456	6291626	6292076	2.03	-7	overlap
ENSMUSG00000018899	53769536	53770186	2.03	-478	overlap
ENSMUSG00000017774	75654077	75654477	2.03	3573	inside
ENSMUSG00000020907	67678186	67678886	2.03	-17140	intergenic
ENSMUSG00000037243	58307377	58307777	2.03	308	inside
ENSMUSG00000004040	100939168	100939568	2.03	372	overlap
ENSMUSG00000071234	84698677	84699077	2.03	130	overlap
ENSMUSG00000021381	48664587	48665137	2.03	1589	inside
ENSMUSG00000041112	20090197	20090647	2.03	-310	overlap
ENSMUSG00000059877	106632386	106633036	2.03	-85326	intergenic
ENSMUSG00000046908	55767843	55768243	2.03	1881	inside
ENSMUSG00000025555	121035941	121036491	2.03	741	inside
ENSMUSG00000021939	63122126	63122826	2.03	-336	overlap
ENSMUSG00000054423	12821348	12821948	2.03	1731	inside
ENSMUSG00000047347	82516066	82516666	2.03	-245	overlap
ENSMUSG00000089804	37233850	37234300	2.03	-541	upstream
ENSMUSG00000033707	76722371	76722771	2.03	-198	upstream
ENSMUSG00000071637	15887160	15887610	2.03	-126	overlap
ENSMUSG00000046119	90514800	90515250	2.03	-39364	intergenic
ENSMUSG00000014074	32277436	32277886	2.03	-23	overlap
ENSMUSG00000024301	26916962	26917362	2.03	-129	overlap
ENSMUSG00000024462	37050704	37051104	2.03	4738	inside
ENSMUSG00000024014	29490972	29491372	2.03	160	inside
ENSMUSG00000097566	15374909	15375809	2.03	-91	overlap
ENSMUSG00000072082	24251165	24251665	2.03	244	overlap
ENSMUSG00000093508	67143770	67144670	2.03	-117535	intergenic
ENSMUSG00000039615	25832251	25832801	2.03	1110	inside
ENSMUSG00000055795	14291054	14292104	2.03	-133814	intergenic
ENSMUSG00000024304	16808204	16808904	2.03	1042	inside
ENSMUSG00000025218	45541663	45542213	2.03	18868	intergenic
ENSMUSG00000050530	3118643	3119143	2.03	4419	inside
ENSMUSG00000062175	156840735	156841235	2.03	658	inside
ENSMUSG00000068859	73273364	73273764	2.03	1439	inside
ENSMUSG00000042662	152950812	152951512	2.03	886	inside
ENSMUSG00000090625	174327820	174328570	2.03	18892	downstream
ENSMUSG00000077761	168912009	168912559	2.03	-29841	intergenic
ENSMUSG00000082460	83547169	83547669	2.03	-8494	intergenic
ENSMUSG00000025782	10048062	10048712	2.03	534	overlap
ENSMUSG00000028221	14864101	14864501	2.03	25	inside
ENSMUSG00000028747	139074565	139075165	2.03	1005	inside
ENSMUSG00000029154	73405840	73406240	2.03	-236	overlap
ENSMUSG00000039000	29568745	29569145	2.03	-497	upstream
ENSMUSG00000060708	36748418	36749018	2.03	261	overlap
ENSMUSG00000029128	41707704	41708254	2.03	451	overlap
ENSMUSG00000055923	76857682	76858182	2.03	47832	intergenic
ENSMUSG00000037822	65537162	65537562	2.03	22	overlap
ENSMUSG00000025821	47877284	47877684	2.03	80	inside
ENSMUSG00000029754	6863543	6863943	2.03	209	inside
ENSMUSG00000030279	143099123	143099623	2.03	984	inside
ENSMUSG00000030512	66060687	66061087	2.03	388	inside

ENSMUSG00000041037	24533237	24533987	2.03	2589	inside
ENSMUSG00000030731	44352877	44353427	2.03	-31225	intergenic
ENSMUSG00000038296	111780032	111780432	2.03	-55	upstream
ENSMUSG00000058886	141327411	141327911	2.03	314	overlap
ENSMUSG00000031910	106870389	106870839	2.03	147	inside
ENSMUSG00000079070	32883250	32883850	2.03	66776	downstream
ENSMUSG00000079157	9770530	9771730	2.03	488	overlap
ENSMUSG00000031654	87472473	87472873	2.03	119	overlap
ENSMUSG00000032477	109875831	109876331	2.03	252	inside
ENSMUSG00000032612	108347454	108348054	2.03	-377	overlap
ENSMUSG00000041064	65587628	65588078	2.03	468	inside
ENSMUSG00000052428	167308296	167308796	2.02	-374	overlap
ENSMUSG00000026123	34849584	34850084	2.02	-392	overlap
ENSMUSG00000026238	86527564	86528064	2.02	828	inside
ENSMUSG00000023150	151344489	151345039	2.02	-9	overlap
ENSMUSG00000026255	87264352	87264752	2.02	-11	overlap
ENSMUSG00000091476	177983107	177983507	2.02	-8328	intergenic
ENSMUSG00000097934	20890388	20890788	2.02	85	overlap
ENSMUSG00000073530	159021758	159022208	2.02	-41268	intergenic
ENSMUSG00000098876	47420735	47421935	2.02	46390	intergenic
ENSMUSG00000026478	153332539	153332939	2.02	247	overlap
ENSMUSG00000019943	98913195	98914345	2.02	-1957	upstream
ENSMUSG00000006342	75667698	75668148	2.02	-23690	intergenic
ENSMUSG00000020133	80323506	80324106	2.02	-2969	upstream
ENSMUSG00000087833	17586626	17587026	2.02	38340	intergenic
ENSMUSG00000017548	79992927	79993377	2.02	-179	overlap
ENSMUSG00000000384	6625576	6626126	2.02	491	overlap
ENSMUSG00000018446	70982780	70983180	2.02	246	overlap
ENSMUSG00000020176	12037037	12037787	2.02	1646	inside
ENSMUSG00000020660	3929905	3930405	2.02	-25046	intergenic
ENSMUSG00000020646	24830890	24831340	2.02	-709	upstream
ENSMUSG00000048982	75371768	75372968	2.02	45013	intergenic
ENSMUSG00000021248	85374277	85374827	2.02	440	overlap
ENSMUSG00000064972	94714945	94715945	2.02	664006	intergenic
ENSMUSG00000005583	82859441	82860441	2.02	-644593	intergenic
ENSMUSG00000077473	4983210	4983810	2.02	206724	intergenic
ENSMUSG00000022269	26309041	26309441	2.02	-7	overlap
ENSMUSG00000047166	75909716	75910116	2.02	-44	overlap
ENSMUSG00000022629	91048815	91049315	2.02	1133	inside
ENSMUSG00000037458	38518098	38518548	2.02	1168	inside
ENSMUSG00000022840	35154774	35155174	2.02	-103	overlap
ENSMUSG00000022883	72663108	72663508	2.02	-41	overlap
ENSMUSG00000097139	92697500	92697950	2.02	84686	intergenic
ENSMUSG00000040732	95585157	95585657	2.02	1436	inside
ENSMUSG00000038037	10784560	10785260	2.02	976	inside
ENSMUSG00000095407	68840677	68841227	2.02	3541	inside
ENSMUSG00000024130	24351311	24352361	2.02	-639	overlap
ENSMUSG00000073394	44735324	44736074	2.02	-521	overlap
ENSMUSG00000045215	22344412	22344962	2.02	-471	overlap
ENSMUSG00000087960	19312554	19313754	2.02	-394539	intergenic
ENSMUSG00000035342	45028463	45029013	2.02	13287	downstream

ENSMUSG00000056829	16872425	16872825	2.02	1405	inside
ENSMUSG00000042401	42432263	42432663	2.02	-480	upstream
ENSMUSG00000024870	5106853	5107303	2.02	143	overlap
ENSMUSG00000043342	74698564	74698964	2.02	884	inside
ENSMUSG00000026790	29889915	29890315	2.02	694	inside
ENSMUSG00000027207	125858535	125858985	2.02	-574	upstream
ENSMUSG00000035877	160872949	160873399	2.02	49	overlap
ENSMUSG00000063275	14055532	14056282	2.02	603	overlap
ENSMUSG00000046470	181670280	181670830	2.02	1360	inside
ENSMUSG00000051817	152397890	152398540	2.02	173	overlap
ENSMUSG00000027544	168601326	168601876	2.02	331	overlap
ENSMUSG00000039059	180103230	180103780	2.02	1258	inside
ENSMUSG00000026779	23155615	23156065	2.02	409	overlap
ENSMUSG00000093230	87617067	87617917	2.02	-332	includeFeature
ENSMUSG00000070227	24599267	24600267	2.02	37193	intergenic
ENSMUSG00000039617	20955180	20955780	2.02	-94265	intergenic
ENSMUSG00000061143	52104467	52104967	2.02	424	overlap
ENSMUSG00000047281	133599865	133600265	2.02	2303	downstream
ENSMUSG00000028612	107846017	107846417	2.02	-15173	intergenic
ENSMUSG00000098364	138324747	138325997	2.02	1342	overlapEnd
ENSMUSG00000097904	110386132	110386632	2.02	297	inside
ENSMUSG00000063146	134551659	134552109	2.02	775	inside
ENSMUSG00000029128	41566018	41566518	2.02	142137	intergenic
ENSMUSG00000029705	136565937	136566337	2.02	1553	inside
ENSMUSG00000043059	31200304	31200704	2.02	1999	inside
ENSMUSG00000033726	85187810	85188410	2.02	372	inside
ENSMUSG00000073155	35252343	35252743	2.02	-311	overlap
ENSMUSG00000052751	48594044	48594444	2.02	161	inside
ENSMUSG00000038759	35177404	35177804	2.02	-26	overlap
ENSMUSG00000030243	142386823	142387223	2.02	264	overlap
ENSMUSG00000055633	5052730	5053430	2.02	1198	inside
ENSMUSG00000041420	16158497	16159047	2.02	-16593	intergenic
ENSMUSG00000001918	16781964	16782364	2.02	618	inside
ENSMUSG00000030725	100159186	100159586	2.02	-91	overlap
ENSMUSG00000093405	65862446	65862946	2.02	-454	upstream
ENSMUSG00000030590	29161394	29161794	2.02	-5160	intergenic
ENSMUSG00000045777	142371818	142372418	2.02	1935	inside
ENSMUSG00000005575	13038007	13038457	2.02	268	overlap
ENSMUSG00000003863	45366506	45366906	2.02	513	inside
ENSMUSG00000097424	121553822	121554322	2.02	9440	intergenic
ENSMUSG00000031749	110919230	110919980	2.02	-692	overlap
ENSMUSG00000014907	66860440	66860840	2.02	223	inside
ENSMUSG00000007950	71463209	71463609	2.02	448	inside
ENSMUSG00000001911	84798973	84799373	2.02	1371	inside
ENSMUSG00000044006	69881023	69881523	2.02	6664	inside
ENSMUSG00000035606	102506005	102506405	2.02	-133	overlap
ENSMUSG00000049932	44334902	44335502	2.02	187	inside
ENSMUSG00000032504	113590217	113590717	2.02	118042	intergenic
ENSMUSG00000032128	37459850	37460400	2.02	-26687	intergenic
ENSMUSG00000026167	74791615	74792015	2.01	99	inside
ENSMUSG00000044340	106172362	106172762	2.01	610	inside

ENSMUSG00000026283	93804117	93804517	2.01	152	inside
ENSMUSG00000026167	74803215	74803615	2.01	11699	inside
ENSMUSG00000026356	128416917	128417317	2.01	499	inside
ENSMUSG00000038608	80758614	80759014	2.01	-61	upstream
ENSMUSG00000045005	64736414	64737464	2.01	1336	inside
ENSMUSG00000035262	80806956	80807356	2.01	1708	inside
ENSMUSG00000019996	20149345	20149745	2.01	874	inside
ENSMUSG00000040054	128096737	128097137	2.01	3954	inside
ENSMUSG00000061904	91123165	91123665	2.01	894	inside
ENSMUSG00000054450	53478677	53479077	2.01	-1489	upstream
ENSMUSG00000020458	29693529	29694029	2.01	582	inside
ENSMUSG00000020471	5878076	5878476	2.01	216	overlap
ENSMUSG00000046474	99851065	99851465	2.01	543	inside
ENSMUSG00000017291	77607677	77608077	2.01	138	overlap
ENSMUSG00000021124	79171961	79172361	2.01	706	inside
ENSMUSG00000017756	73763768	73764168	2.01	71	inside
ENSMUSG00000021493	55513158	55513558	2.01	518	inside
ENSMUSG00000021572	74061718	74062168	2.01	567	inside
ENSMUSG00000038372	32338197	32338697	2.01	515	inside
ENSMUSG00000090907	92426661	92427061	2.01	563	inside
ENSMUSG00000040717	27038794	27039194	2.01	-207	overlap
ENSMUSG00000099315	69502128	69502628	2.01	-728	upstream
ENSMUSG00000065238	99721058	99721508	2.01	52750	intergenic
ENSMUSG00000090691	6889421	6889971	2.01	541	overlap
ENSMUSG00000033004	103345996	103346446	2.01	818	inside
ENSMUSG00000064959	82340771	82341371	2.01	-1882	upstream
ENSMUSG00000036661	73512573	73512973	2.01	13	inside
ENSMUSG00000089837	79833021	79833471	2.01	1216	inside
ENSMUSG00000022999	98917665	98918315	2.01	566	overlap
ENSMUSG00000022994	98609315	98609715	2.01	-1682	upstream
ENSMUSG00000043683	56257334	56257784	2.01	541	inside
ENSMUSG00000024327	34031578	34031978	2.01	112	overlap
ENSMUSG00000024304	16808804	16809454	2.01	442	overlap
ENSMUSG00000025081	56825725	56826225	2.01	-484	overlap
ENSMUSG00000039126	16955725	16956175	2.01	-393	overlap
ENSMUSG00000075044	8251775	8252275	2.01	-32875	intergenic
ENSMUSG00000040929	28155220	28155620	2.01	-144054	intergenic
ENSMUSG00000035576	162943099	162943849	2.01	-373	overlap
ENSMUSG00000017740	164968285	164968935	2.01	7469	inside
ENSMUSG00000039849	164879735	164880235	2.01	431	inside
ENSMUSG00000029419	25578989	25579589	2.01	1110	inside
ENSMUSG00000055897	173659388	173659838	2.01	252	overlap
ENSMUSG00000026836	58566674	58567324	2.01	483	overlap
ENSMUSG00000074637	34648467	34648917	2.01	-1538	upstream
ENSMUSG00000073752	130308774	130309624	2.01	-100	upstream
ENSMUSG00000028744	139310397	139310797	2.01	311	overlap
ENSMUSG00000037962	125028637	125029187	2.01	25190	intergenic
ENSMUSG00000037962	125003373	125003773	2.01	-74	overlap
ENSMUSG00000029229	75043618	75044218	2.01	1156	inside
ENSMUSG00000034040	131306937	131307437	2.01	1141	inside
ENSMUSG00000029673	132543437	132543887	2.01	-93	upstream

ENSMUSG00000047881	63968868	63969268	2.01	37	overlap
ENSMUSG00000007415	3647107	3647657	2.01	827	inside
ENSMUSG00000009471	46348706	46349106	2.01	-27768	intergenic
ENSMUSG00000060260	139247133	139247683	2.01	-1349	upstream
ENSMUSG00000037772	142532741	142533191	2.01	-58	overlap
ENSMUSG00000002083	16313797	16314347	2.01	4181	inside
ENSMUSG00000041769	138830099	138830699	2.01	-15980	intergenic
ENSMUSG00000059263	112023549	112024049	2.01	43	inside
ENSMUSG00000030402	19279274	19279774	2.01	775	inside
ENSMUSG00000084882	45632443	45632943	2.01	634	inside
ENSMUSG00000052566	84990359	84990959	2.01	-236	overlap
ENSMUSG00000074357	47674935	47675585	2.01	-456	overlap
ENSMUSG00000031849	70374181	70374831	2.01	633	inside
ENSMUSG00000006276	72421073	72421473	2.01	401	inside
ENSMUSG00000032024	40685452	40686102	2.01	-510	overlap
ENSMUSG00000057626	105568005	105569355	2.01	-7243	intergenic
ENSMUSG00000032065	50560930	50561330	2.01	338	overlap
ENSMUSG00000032515	119983217	119983767	2.01	-5967	intergenic
ENSMUSG00000066877	43446194	43446594	2.00	443	inside
ENSMUSG00000073586	123857012	123857412	2.00	-187811	intergenic
ENSMUSG00000077846	134799262	134799862	2.00	-10013	intergenic
ENSMUSG00000026604	189728110	189728510	2.00	-158	overlap
ENSMUSG00000073486	179802658	179803208	2.00	-718	upstream
ENSMUSG00000006301	74304065	74304465	2.00	1557	inside
ENSMUSG00000026107	51433135	51433985	2.00	45264	intergenic
ENSMUSG00000026566	165642396	165643146	2.00	-7855	intergenic
ENSMUSG00000060935	85102863	85103263	2.00	236	inside
ENSMUSG00000039697	30802195	30802895	2.00	912	inside
ENSMUSG00000069814	74619630	74620030	2.00	25	inside
ENSMUSG00000039230	121451786	121452186	2.00	-163	overlap
ENSMUSG00000087042	97534415	97534965	2.00	-12520	intergenic
ENSMUSG00000086058	82931186	82931586	2.00	2196	inside
ENSMUSG00000040548	106612315	106612765	2.00	1108	inside
ENSMUSG00000048562	118836654	118837204	2.00	-9675	intergenic
ENSMUSG00000021385	49399247	49399647	2.00	-22064	intergenic
ENSMUSG00000021338	24281121	24281521	2.00	-326	upstream
ENSMUSG00000021569	73937192	73937642	2.00	575	inside
ENSMUSG00000048904	56252005	56252455	2.00	158	overlap
ENSMUSG00000035953	50930342	50930792	2.00	514	inside
ENSMUSG00000023034	101266370	101267220	2.00	-476	overlap
ENSMUSG00000056605	101785770	101786220	2.00	688	inside
ENSMUSG00000096883	82207016	82207416	2.00	5799	inside
ENSMUSG00000022971	91372827	91373227	2.00	44	inside
ENSMUSG00000022808	33251078	33251978	2.00	-378	overlap
ENSMUSG00000046962	97962107	97962707	2.00	514	overlap
ENSMUSG00000008393	8671530	8671980	2.00	623	inside
ENSMUSG00000002844	38452115	38452765	2.00	574	overlap
ENSMUSG00000093026	61398409	61398809	2.00	596	downstream
ENSMUSG00000054072	60339759	60340809	2.00	-36270	intergenic
ENSMUSG00000060534	72576509	72577009	2.00	-225440	intergenic
ENSMUSG00000024268	25678601	25679101	2.00	75382	inside

ENSMUSG00000025049	47068175	47068575	2.00	427	inside
ENSMUSG00000007338	6057360	6057960	2.00	391	overlap
ENSMUSG00000051984	44545354	44545754	2.00	510	inside
ENSMUSG00000032802	152093635	152094085	2.00	-11881	intergenic
ENSMUSG00000074876	122630407	122630807	2.00	-218	overlap
ENSMUSG00000027569	180582334	180582734	2.00	1030	inside
ENSMUSG00000038831	33371265	33371665	2.00	221	overlap
ENSMUSG00000083325	150500299	150500799	2.00	546	inside
ENSMUSG00000027434	147193923	147194323	2.00	320	overlap
ENSMUSG00000056476	59005845	59006245	2.00	-1133	upstream
ENSMUSG00000027793	55054703	55055153	2.00	352	overlap
ENSMUSG00000059857	110142450	110142850	2.00	778	inside
ENSMUSG00000040809	106171599	106172099	2.00	-4035	upstream
ENSMUSG00000041263	89090403	89090803	2.00	2960	inside
ENSMUSG00000043572	106638737	106639237	2.00	-12332	intergenic
ENSMUSG00000028243	6191115	6191515	2.00	17	inside
ENSMUSG00000077260	102494477	102494877	2.00	-242335	intergenic
ENSMUSG00000025743	130792507	130792907	2.00	-30	overlap
ENSMUSG00000029076	155993071	155993471	2.00	199	inside
ENSMUSG00000054679	33248107	33248857	2.00	39116	intergenic
ENSMUSG00000028521	103214967	103215567	2.00	197	overlap
ENSMUSG00000060862	137048415	137048815	2.00	386	overlap
ENSMUSG00000039682	45493412	45493862	2.00	38	inside
ENSMUSG00000061882	123930420	123931020	2.00	3002	inside
ENSMUSG00000043059	31201856	31202256	2.00	447	inside
ENSMUSG00000037822	65492604	65493004	2.00	44580	inside
ENSMUSG00000051391	135933837	135934787	2.00	779	overlap
ENSMUSG00000097626	19226267	19226667	2.00	288	overlap
ENSMUSG00000072641	121869420	121869820	2.00	-10452	intergenic
ENSMUSG00000029860	42349692	42350142	2.00	-136	overlap
ENSMUSG00000015053	88193839	88194239	2.00	-52	overlap
ENSMUSG00000029687	47594119	47594669	2.00	1222	inside
ENSMUSG00000030732	100042053	100042553	2.00	35649	intergenic
ENSMUSG00000031068	137437680	137438280	2.00	32	inside
ENSMUSG00000036862	105786768	105787318	2.00	886	inside
ENSMUSG00000000131	126200312	126200712	2.00	189	overlap
ENSMUSG00000043866	105743699	105744099	2.00	662	inside
ENSMUSG00000077409	142082427	142082977	2.00	-3990	upstream
ENSMUSG00000003824	84871959	84872359	2.00	-152	overlap
ENSMUSG00000031887	105264089	105264589	2.00	520	inside
ENSMUSG00000031673	102784522	102784972	2.00	589	inside
ENSMUSG00000031681	79398681	79399131	2.00	837	inside
ENSMUSG00000032309	55208578	55208978	2.00	-347	overlap
ENSMUSG00000032303	55026178	55026578	2.00	381	overlap
ENSMUSG00000032368	91381248	91381798	2.00	-15438	intergenic
ENSMUSG00000093745	42520503	42520953	2.00	328	includeFeature
ENSMUSG00000032462	99140266	99140666	2.00	355	overlap
ENSMUSG00000004936	64253329	64253779	2.00	302	overlap
ENSMUSG00000048960	10993707	10994107	1.99	242	inside
ENSMUSG00000079330	132190212	132190962	1.99	-1224	upstream
ENSMUSG00000036206	89070252	89070752	1.99	-163	overlap

ENSMUSG00000052062	61639059	61639509	1.99	235	inside
ENSMUSG00000025925	15805546	15805996	1.99	-112	overlap
ENSMUSG00000026494	178530313	178530763	1.99	1188	inside
ENSMUSG00000098815	117587011	117587611	1.99	25781	intergenic
ENSMUSG00000041075	59481944	59482344	1.99	-203	overlap
ENSMUSG00000033722	155244308	155244708	1.99	-11584	intergenic
ENSMUSG00000081623	65979920	65980370	1.99	53005	intergenic
ENSMUSG00000035206	80855398	80855898	1.99	123	inside
ENSMUSG00000097007	11280531	11281131	1.99	690	inside
ENSMUSG00000020232	81350083	81350483	1.99	397	overlap
ENSMUSG00000048217	102145265	102145665	1.99	-248	overlap
ENSMUSG00000018765	69633148	69633648	1.99	158	inside
ENSMUSG00000036264	52764127	52764677	1.99	-507	overlap
ENSMUSG00000017774	75655580	75656080	1.99	5076	inside
ENSMUSG00000023764	3180626	3181026	1.99	12837	inside
ENSMUSG00000086851	53567086	53567536	1.99	227	overlap
ENSMUSG00000049755	58323127	58323527	1.99	219	overlap
ENSMUSG00000037957	110738217	110738617	1.99	268	inside
ENSMUSG00000033731	99392667	99393217	1.99	51513	intergenic
ENSMUSG00000042029	116405172	116405672	1.99	-230	overlap
ENSMUSG00000043398	72070857	72071257	1.99	134	overlap
ENSMUSG00000020672	30372812	30373262	1.99	563	inside
ENSMUSG00000091387	96925001	96925601	1.99	312	inside
ENSMUSG00000021423	37406847	37407997	1.99	61502	inside
ENSMUSG00000038246	34746847	34747247	1.99	11997	inside
ENSMUSG00000021596	76353358	76353758	1.99	-31177	intergenic
ENSMUSG00000021685	94873435	94873885	1.99	-2167	upstream
ENSMUSG00000055137	17694632	17695782	1.99	100	overlap
ENSMUSG00000089827	80885654	80886054	1.99	-130	upstream
ENSMUSG00000087286	59878926	59880176	1.99	161565	intergenic
ENSMUSG00000021983	60176975	60177375	1.99	20204	inside
ENSMUSG00000002320	55643298	55643798	1.99	508	inside
ENSMUSG00000097286	54952296	54952696	1.99	-652	upstream
ENSMUSG00000034161	76457825	76458225	1.99	387	inside
ENSMUSG00000016541	85336571	85337021	1.99	190	inside
ENSMUSG00000097259	37007498	37007998	1.99	-25	overlap
ENSMUSG00000000555	103366015	103366665	1.99	748	inside
ENSMUSG00000002524	76079973	76080573	1.99	973	inside
ENSMUSG00000088128	76178571	76179771	1.99	35634	intergenic
ENSMUSG00000022568	76068716	76069266	1.99	1068	inside
ENSMUSG00000053774	32332109	32332859	1.99	-143	overlap
ENSMUSG00000039427	5233571	5234021	1.99	-50	overlap
ENSMUSG00000040681	96126916	96127516	1.99	813	inside
ENSMUSG00000041130	24669965	24670365	1.99	213	inside
ENSMUSG00000024220	28176678	28177228	1.99	-529	overlap
ENSMUSG00000040276	27655172	27655672	1.99	-337	overlap
ENSMUSG00000045999	39848628	39849078	1.99	199	overlap
ENSMUSG00000024112	25433115	25433515	1.99	668	inside
ENSMUSG00000023828	12506941	12507391	1.99	763	inside
ENSMUSG00000023951	46031268	46031818	1.99	1109	inside
ENSMUSG00000024530	67464959	67465359	1.99	110	inside

ENSMUSG00000056481	5068253	5068653	1.99	175	inside
ENSMUSG00000024776	34192320	34192770	1.99	91	inside
ENSMUSG00000074923	118676635	118677135	1.99	13332	inside
ENSMUSG00000068154	146221335	146221735	1.99	-586	upstream
ENSMUSG00000027455	151494529	151494979	1.99	347	inside
ENSMUSG00000045319	6100275	6100875	1.99	29936	inside
ENSMUSG00000000823	181592716	181593116	1.99	87	overlap
ENSMUSG00000050896	84871614	84872014	1.99	15096	overlapEnd
ENSMUSG00000026885	35979665	35980065	1.99	248	overlap
ENSMUSG00000027660	31095496	31095896	1.99	438	inside
ENSMUSG00000002233	104788871	104789321	1.99	-140	overlap
ENSMUSG00000027985	131111150	131111600	1.99	679	inside
ENSMUSG00000028102	96635529	96635929	1.99	153	inside
ENSMUSG00000027894	107516950	107517800	1.99	1068	inside
ENSMUSG00000028710	115784925	115785325	1.99	113	inside
ENSMUSG00000028811	129223298	129223948	1.99	33538	downstream
ENSMUSG00000082195	146066779	146067579	1.99	-745	overlap
ENSMUSG00000028542	117835537	117835937	1.99	1031	inside
ENSMUSG00000043753	89688551	89688951	1.99	9115	inside
ENSMUSG00000028756	138326097	138326497	1.99	210	overlap
ENSMUSG00000039809	46990800	46991200	1.99	1073	inside
ENSMUSG00000028546	110186793	110187193	1.99	165116	intergenic
ENSMUSG00000084179	23657525	23657975	1.99	2819	downstream
ENSMUSG00000049686	123014576	123014976	1.99	-498	upstream
ENSMUSG00000016503	146948775	146949225	1.99	118	inside
ENSMUSG00000029359	118026692	118027992	1.99	-1051	overlap
ENSMUSG00000085376	116005684	116006584	1.99	-3632	upstream
ENSMUSG00000001566	29467637	29468387	1.99	10882	downstream
ENSMUSG00000028959	24444436	24445086	1.99	851	inside
ENSMUSG00000029363	117388948	117389398	1.99	99	overlap
ENSMUSG00000030166	119902673	119903123	1.99	-25	overlap
ENSMUSG00000096282	74530066	74530516	1.99	-24902	intergenic
ENSMUSG00000053907	72438774	72439224	1.99	784	inside
ENSMUSG00000050917	144861608	144862008	1.99	222	inside
ENSMUSG00000035354	99140363	99140763	1.99	781	inside
ENSMUSG00000010476	137309063	137309463	1.99	5382	inside
ENSMUSG00000097842	16874747	16875647	1.99	139	overlap
ENSMUSG00000074004	98193413	98193863	1.99	6068	inside
ENSMUSG00000064450	123102508	123102908	1.99	-530	upstream
ENSMUSG00000088312	42904835	42905335	1.99	162028	intergenic
ENSMUSG00000002396	71375509	71375909	1.99	4211	inside
ENSMUSG00000037300	124721289	124721789	1.99	694	inside
ENSMUSG00000031487	27121267	27121867	1.99	7365	downstream
ENSMUSG00000032872	87021600	87022050	1.99	-414	overlap
ENSMUSG00000010607	22164641	22165091	1.99	7795	intergenic
ENSMUSG00000037808	13827441	13827891	1.99	-275	overlap
ENSMUSG00000097820	121759168	121759818	1.99	620	overlap
ENSMUSG00000032366	67043428	67043828	1.99	5978	inside
ENSMUSG00000067028	99592112	99592912	1.98	-180653	intergenic
ENSMUSG00000090071	74855559	74856309	1.98	530	inside
ENSMUSG00000079330	132190862	132191262	1.98	-574	upstream

ENSMUSG00000067081	89992913	89993313	1.98	21753	inside
ENSMUSG00000067071	91414067	91414467	1.98	-29	upstream
ENSMUSG00000026209	75317319	75317869	1.98	318	overlap
ENSMUSG00000046980	73099403	73100053	1.98	-51	includeFeature
ENSMUSG00000019907	108161973	108162623	1.98	-427	overlap
ENSMUSG00000084658	80381184	80381784	1.98	-1118	upstream
ENSMUSG00000097086	21992995	21994095	1.98	53990	intergenic
ENSMUSG00000059901	61272753	61273253	1.98	685	inside
ENSMUSG00000020788	72960977	72961377	1.98	-192	overlap
ENSMUSG00000050288	102604265	102605315	1.98	-166	overlap
ENSMUSG00000070345	87616827	87617877	1.98	-337	overlap
ENSMUSG00000047284	69901830	69902230	1.98	758	inside
ENSMUSG00000019312	98458015	98458465	1.98	11621	downstream
ENSMUSG00000040610	33201382	33201782	1.98	2206	inside
ENSMUSG00000020176	12025576	12025976	1.98	13107	inside
ENSMUSG00000041771	102128917	102129317	1.98	184	inside
ENSMUSG00000089603	60385011	60386361	1.98	-350487	intergenic
ENSMUSG00000072949	84009077	84009627	1.98	-425	overlap
ENSMUSG00000041669	103242117	103242517	1.98	33	overlap
ENSMUSG00000063200	43398447	43398847	1.98	71	inside
ENSMUSG00000021423	37404887	37405437	1.98	59542	inside
ENSMUSG00000051627	23622212	23622612	1.98	290	overlap
ENSMUSG00000021451	51792597	51793197	1.98	1150	inside
ENSMUSG00000021991	29722077	29722627	1.98	-213	upstream
ENSMUSG00000022255	34082696	34083346	1.98	2	inside
ENSMUSG00000033170	78802171	78802571	1.98	871	inside
ENSMUSG00000095440	101078765	101079265	1.98	-24366	intergenic
ENSMUSG00000046546	30599817	30600567	1.98	94	inside
ENSMUSG00000039903	90830708	90831258	1.98	3989	inside
ENSMUSG00000022760	17530480	17530880	1.98	656	inside
ENSMUSG00000063952	28801362	28801762	1.98	272	inside
ENSMUSG00000091705	35342278	35342678	1.98	36	inside
ENSMUSG00000044279	57059194	57059644	1.98	89	inside
ENSMUSG00000095325	32859222	32859622	1.98	26861	intergenic
ENSMUSG00000024165	24960512	24960912	1.98	124	overlap
ENSMUSG00000037013	14682972	14683422	1.98	-58	upstream
ENSMUSG00000040385	4191566	4192166	1.98	-592	overlap
ENSMUSG00000085196	6276560	6277010	1.98	45	overlap
ENSMUSG00000038467	154645090	154645940	1.98	-6615	intergenic
ENSMUSG00000060227	121867149	121867549	1.98	179	inside
ENSMUSG00000027652	158409999	158410499	1.98	151	inside
ENSMUSG00000068079	152142485	152143585	1.98	-1076	overlap
ENSMUSG00000026740	18391908	18392558	1.98	922	inside
ENSMUSG00000086537	174299634	174300034	1.98	-4198	upstream
ENSMUSG00000086555	35581901	35582501	1.98	-23199	intergenic
ENSMUSG00000045493	180775871	180776921	1.98	1029	overlap
ENSMUSG00000028161	136671388	136671838	1.98	1264	inside
ENSMUSG00000041220	129533349	129533849	1.98	963	inside
ENSMUSG00000049119	5643795	5644395	1.98	-295	overlap
ENSMUSG00000036856	137278047	137278447	1.98	558	inside
ENSMUSG00000091921	155694803	155695203	1.98	461	inside

ENSMUSG00000094253	3546801	3547451	1.98	9711	intergenic
ENSMUSG00000028405	40142989	40143439	1.98	-92	overlap
ENSMUSG00000034042	116557393	116557893	1.98	-265	overlap
ENSMUSG00000085482	74251844	74252244	1.98	-130	upstream
ENSMUSG00000055761	20777951	20778351	1.98	915	inside
ENSMUSG00000040659	141874207	141874707	1.98	713	inside
ENSMUSG00000046671	134534909	134535309	1.98	478	inside
ENSMUSG00000029430	129020510	129020910	1.98	441	inside
ENSMUSG00000029310	104046416	104046916	1.98	110	inside
ENSMUSG00000085639	148928831	148929681	1.98	20035	intergenic
ENSMUSG00000060261	134313909	134314559	1.98	851	inside
ENSMUSG00000029603	120711242	120711742	1.98	685	inside
ENSMUSG00000086040	54429589	54430389	1.98	-14	overlap
ENSMUSG00000029999	86195449	86195899	1.98	198	inside
ENSMUSG00000030019	119479765	119481065	1.98	97	inside
ENSMUSG00000038058	54972049	54972749	1.98	563	overlap
ENSMUSG00000030852	130646109	130646559	1.98	68625	inside
ENSMUSG00000030664	116031509	116032009	1.98	-470	overlap
ENSMUSG00000040940	24902987	24903387	1.98	75	inside
ENSMUSG00000042462	127253682	127254132	1.98	7027	downstream
ENSMUSG00000095115	118490959	118491509	1.98	1016	inside
ENSMUSG00000037664	143459568	143460118	1.98	1482	inside
ENSMUSG00000004637	114439558	114440058	1.98	-97	overlap
ENSMUSG00000031902	106059389	106059789	1.98	-214	overlap
ENSMUSG00000056724	110653931	110654381	1.98	230	overlap
ENSMUSG00000041729	62536278	62536728	1.98	766	inside
ENSMUSG00000000168	50698730	50699330	1.98	-38950	intergenic
ENSMUSG00000074505	16501541	16501991	1.98	-123310	intergenic
ENSMUSG00000026511	182124763	182125163	1.97	26	inside
ENSMUSG00000093782	82838952	82839452	1.97	-494	overlap
ENSMUSG00000090252	85254669	85255069	1.97	7818	inside
ENSMUSG00000040596	166409752	166410152	1.97	111	overlap
ENSMUSG00000093064	190651000	190651500	1.97	-167843	intergenic
ENSMUSG00000084849	81464383	81464783	1.97	3224	downstream
ENSMUSG00000025795	121475673	121476323	1.97	577	overlap
ENSMUSG00000043670	81030834	81031334	1.97	-5172	intergenic
ENSMUSG00000039232	12964195	12964595	1.97	64	overlap
ENSMUSG00000043822	80348372	80348822	1.97	40	overlap
ENSMUSG00000020329	79735606	79736006	1.97	10975	downstream
ENSMUSG00000013858	79977306	79977906	1.97	7024	inside
ENSMUSG00000020277	78009634	78010134	1.97	449	overlap
ENSMUSG00000083844	5761978	5762378	1.97	-151	overlap
ENSMUSG00000049800	20542985	20543435	1.97	-268	overlap
ENSMUSG00000080152	58961780	58962180	1.97	-32	overlap
ENSMUSG00000001510	95120065	95120615	1.97	-24	overlap
ENSMUSG00000000093	85838229	85838679	1.97	5678	inside
ENSMUSG00000038255	98327818	98328268	1.97	1830	inside
ENSMUSG00000020773	116109674	116110324	1.97	17536	inside
ENSMUSG00000072963	53456736	53457236	1.97	374	overlap
ENSMUSG00000025582	119546868	119547368	1.97	885	inside
ENSMUSG00000018363	106919574	106920224	1.97	1141	inside

ENSMUSG00000053113	117949667	117950117	1.97	20380	intergenic
ENSMUSG00000085207	119942118	119943368	1.97	580	overlap
ENSMUSG00000009079	5099128	5099878	1.97	138	overlap
ENSMUSG00000043448	59176630	59177030	1.97	6583	inside
ENSMUSG00000020722	107686515	107686965	1.97	30007	intergenic
ENSMUSG00000064616	48887518	48887918	1.97	-122365	intergenic
ENSMUSG00000021108	73584111	73584611	1.97	-686	upstream
ENSMUSG00000021025	55492058	55492458	1.97	589	inside
ENSMUSG00000034168	86883327	86883877	1.97	1487	inside
ENSMUSG00000096107	3358394	3359094	1.97	-2635	upstream
ENSMUSG00000021763	113293348	113293748	1.97	-23736	intergenic
ENSMUSG00000098266	31626071	31627021	1.97	472	includeFeature
ENSMUSG00000088201	36210491	36210891	1.97	5231	intergenic
ENSMUSG00000099013	66057284	66057734	1.97	-17268	intergenic
ENSMUSG00000038879	34678396	34678796	1.97	310	overlap
ENSMUSG00000023169	96641220	96641770	1.97	1693	inside
ENSMUSG00000044442	87353978	87354428	1.97	-207	overlap
ENSMUSG00000022702	18877321	18878071	1.97	284	inside
ENSMUSG00000009097	18582510	18582910	1.97	4459	inside
ENSMUSG00000024036	31295578	31296478	1.97	95	inside
ENSMUSG00000024462	37045715	37046465	1.97	-251	overlap
ENSMUSG00000002249	28340762	28341262	1.97	10043	inside
ENSMUSG00000092564	33909078	33909778	1.97	239	overlap
ENSMUSG00000024218	27909201	27909601	1.97	41	overlap
ENSMUSG00000032688	65430677	65431177	1.97	-286	overlap
ENSMUSG00000024535	53245409	53246209	1.97	-253	overlap
ENSMUSG00000024276	23954336	23954736	1.97	-352	overlap
ENSMUSG00000025429	77794013	77794463	1.97	-532	upstream
ENSMUSG00000038299	32836659	32837409	1.97	-566	overlap
ENSMUSG00000025184	42518320	42518820	1.97	-439	overlap
ENSMUSG00000027605	155517673	155518173	1.97	-275	overlap
ENSMUSG00000089762	30472545	30473045	1.97	1654	overlapEnd
ENSMUSG00000075415	31141945	31142345	1.97	63	overlap
ENSMUSG00000098944	65659367	65659817	1.97	79	overlapEnd
ENSMUSG00000004894	88022545	88023245	1.97	5038	inside
ENSMUSG00000086556	96219944	96220744	1.97	224	overlap
ENSMUSG00000027599	19073417	19074067	1.97	89648	intergenic
ENSMUSG00000082368	70117114	70118164	1.97	69957	intergenic
ENSMUSG00000045751	24496115	24496615	1.97	-336	overlap
ENSMUSG00000092812	134468647	134469097	1.97	283	downstream
ENSMUSG00000028879	132884165	132884565	1.97	344	overlap
ENSMUSG00000028367	57955826	57956226	1.97	585	inside
ENSMUSG00000003644	133887859	133888259	1.97	-62	upstream
ENSMUSG00000059816	108383037	108383587	1.97	312	overlap
ENSMUSG00000029233	76140382	76140782	1.97	111	inside
ENSMUSG00000029474	122849976	122850626	1.97	-212	overlap
ENSMUSG00000034462	104460032	104460432	1.97	582	inside
ENSMUSG00000048988	139907897	139908597	1.97	-46	overlap
ENSMUSG00000035187	101664388	101664838	1.97	838	inside
ENSMUSG00000097191	149183709	149184259	1.97	354	overlap
ENSMUSG00000048578	115158220	115158620	1.97	-41	upstream

ENSMUSG00000097114	125493975	125494475	1.97	-449	overlap
ENSMUSG00000005893	92091999	92092449	1.97	609	inside
ENSMUSG00000078169	53819610	53820210	1.97	-365	includeFeature
ENSMUSG00000087136	82161399	82161899	1.97	-108818	intergenic
ENSMUSG00000040797	121413175	121413675	1.97	60503	inside
ENSMUSG00000038784	35133591	35134091	1.97	146	overlap
ENSMUSG00000048022	24955633	24956083	1.97	492	inside
ENSMUSG00000030057	87850689	87851089	1.97	417	inside
ENSMUSG00000030471	48744412	48745012	1.97	-44591	intergenic
ENSMUSG00000043671	35753663	35754163	1.97	791	inside
ENSMUSG00000031783	94857430	94857930	1.97	-20	overlap
ENSMUSG00000031860	69832659	69833059	1.97	35	inside
ENSMUSG00000096943	83875181	83875581	1.97	24320	intergenic
ENSMUSG00000098976	71629286	71629686	1.97	1820	downstream
ENSMUSG00000031880	104630658	104631108	1.97	663	inside
ENSMUSG00000069633	3451604	3452054	1.97	16076	intergenic
ENSMUSG00000071793	20424102	20424552	1.97	712	inside
ENSMUSG00000055435	115706439	115707189	1.97	1355	inside
ENSMUSG00000025647	109036271	109037171	1.97	-2294	upstream
ENSMUSG00000041268	54500278	54501478	1.97	1482	inside
ENSMUSG00000045414	94537566	94537966	1.97	515	inside
ENSMUSG00000033688	103304805	103305355	1.97	277	overlap
ENSMUSG00000045087	21244591	21245191	1.97	3852	inside
ENSMUSG00000032405	62980129	62980579	1.97	750	inside
ENSMUSG00000026585	163779608	163780158	1.96	25	inside
ENSMUSG00000089358	13896958	13897408	1.96	-35420	intergenic
ENSMUSG00000046404	130717267	130717767	1.96	-60	overlap
ENSMUSG00000016528	131096811	131097261	1.96	732	inside
ENSMUSG00000025980	55088175	55088675	1.96	68	overlap
ENSMUSG00000025956	64617614	64618014	1.96	-372	upstream
ENSMUSG00000025774	18058013	18058463	1.96	87889	intergenic
ENSMUSG00000098811	81387634	81388034	1.96	1287	downstream
ENSMUSG00000019820	12869303	12869703	1.96	-7568	intergenic
ENSMUSG00000020052	87492598	87492998	1.96	1062	inside
ENSMUSG00000035781	79921163	79921713	1.96	-4157	upstream
ENSMUSG00000074734	116113402	116113852	1.96	515	inside
ENSMUSG00000075410	116653274	116653924	1.96	-260	overlap
ENSMUSG00000020788	72961277	72961677	1.96	108	inside
ENSMUSG00000038976	94991218	94991618	1.96	183	inside
ENSMUSG00000059248	117199317	117199967	1.96	-344	overlap
ENSMUSG00000063109	89060186	89060836	1.96	-4543	upstream
ENSMUSG00000083555	20631829	20632229	1.96	-56640	intergenic
ENSMUSG00000078154	48837777	48838177	1.96	-11122	intergenic
ENSMUSG00000093164	74610336	74610886	1.96	8177	intergenic
ENSMUSG00000025576	118908465	118908865	1.96	3132	inside
ENSMUSG00000018931	60912980	60913630	1.96	1770	inside
ENSMUSG00000001440	97187867	97188367	1.96	14	overlap
ENSMUSG00000070407	63921127	63921527	1.96	1163	inside
ENSMUSG00000054204	30884362	30884762	1.96	40	inside
ENSMUSG00000035451	57542861	57543261	1.96	3260	inside
ENSMUSG00000021279	111377687	111378137	1.96	31	overlap

ENSMUSG00000020949	65073811	65074311	1.96	133	overlap
ENSMUSG00000021120	79089358	79089758	1.96	312	overlap
ENSMUSG00000050545	4768549	4768999	1.96	718	inside
ENSMUSG00000021701	110394258	110394658	1.96	-786	upstream
ENSMUSG00000087143	78163205	78163805	1.96	-34812	intergenic
ENSMUSG00000021552	58273405	58273855	1.96	783	inside
ENSMUSG00000041417	101767226	101768526	1.96	991	overlap
ENSMUSG00000017485	16364513	16365363	1.96	-666	overlap
ENSMUSG00000063458	22019355	22019755	1.96	-357	overlap
ENSMUSG00000021771	21830805	21831255	1.96	-464	upstream
ENSMUSG00000095311	122406690	122407140	1.96	-18530	intergenic
ENSMUSG00000022456	82274966	82275716	1.96	31	inside
ENSMUSG00000022622	89548019	89548669	1.96	-20307	intergenic
ENSMUSG00000064210	95790715	95791115	1.96	-128	overlap
ENSMUSG00000091198	81523039	81523439	1.96	-23491	intergenic
ENSMUSG00000038965	17200610	17201060	1.96	2039	inside
ENSMUSG00000069729	4908157	4908557	1.96	-86175	intergenic
ENSMUSG00000024027	30901322	30901772	1.96	-545	upstream
ENSMUSG00000002372	56673073	56673573	1.96	-152	overlap
ENSMUSG00000034868	70990183	70990583	1.96	604	inside
ENSMUSG00000024805	36347826	36348276	1.96	-31241	intergenic
ENSMUSG00000065454	180893966	180894766	1.96	-65	includeFeature
ENSMUSG00000026781	23102751	23103151	1.96	34583	inside
ENSMUSG00000027273	136678723	136679173	1.96	-34730	intergenic
ENSMUSG00000027422	144011162	144011562	1.96	101	overlap
ENSMUSG00000027566	180041834	180042284	1.96	599	inside
ENSMUSG00000027634	156991499	156991899	1.96	557	inside
ENSMUSG00000028152	138741575	138742025	1.96	-620	upstream
ENSMUSG00000028086	84814945	84815345	1.96	-323	overlap
ENSMUSG00000028060	88685803	88686703	1.96	9	inside
ENSMUSG00000042579	89772896	89773946	1.96	344	overlap
ENSMUSG00000045031	37312590	37312990	1.96	118	overlap
ENSMUSG00000027793	55055203	55055653	1.96	-148	upstream
ENSMUSG00000008763	100685316	100685716	1.96	187	overlap
ENSMUSG00000005813	138488603	138489053	1.96	779	inside
ENSMUSG00000028709	115827887	115828287	1.96	-205	overlap
ENSMUSG00000034210	115737217	115737867	1.96	-527	overlap
ENSMUSG00000081501	88757126	88757626	1.96	-718	upstream
ENSMUSG00000083154	24289745	24290345	1.96	51645	intergenic
ENSMUSG00000029050	155221489	155221889	1.96	1103	inside
ENSMUSG00000083743	84545094	84545544	1.96	-115970	intergenic
ENSMUSG00000028826	134853177	134853777	1.96	168	overlap
ENSMUSG00000028337	46601958	46602458	1.96	244	overlap
ENSMUSG00000028849	126237030	126237430	1.96	19313	inside
ENSMUSG00000048988	139912625	139913075	1.96	4682	inside
ENSMUSG00000043833	25100825	25101225	1.96	-158	overlap
ENSMUSG00000029359	118028442	118028842	1.96	699	inside
ENSMUSG00000044134	121852220	121852720	1.96	3192	inside
ENSMUSG00000072754	110363174	110363574	1.96	9078	intergenic
ENSMUSG00000036377	76657782	76658182	1.96	99	inside
ENSMUSG00000048988	139908597	139909147	1.96	654	inside

ENSMUSG00000029405	92082875	92083275	1.96	860	inside
ENSMUSG00000001566	29477189	29477789	1.96	1330	inside
ENSMUSG00000029823	38550993	38551543	1.96	-341	overlap
ENSMUSG00000068551	48437905	48438305	1.96	7920	inside
ENSMUSG00000050654	116578665	116579065	1.96	4366	downstream
ENSMUSG00000093052	90716289	90716789	1.96	-34769	intergenic
ENSMUSG00000038077	126740215	126740765	1.96	447	overlap
ENSMUSG00000053442	66896639	66897089	1.96	-118	upstream
ENSMUSG00000055409	50175093	50176443	1.96	200229	inside
ENSMUSG00000093238	79534987	79535587	1.96	29723	intergenic
ENSMUSG00000092381	19714614	19715114	1.96	-402	overlap
ENSMUSG00000002083	16312314	16312714	1.96	2698	inside
ENSMUSG00000046058	28267394	28267894	1.96	-487	overlap
ENSMUSG00000025584	81213624	81214024	1.96	28	inside
ENSMUSG00000047085	44444612	44445112	1.96	15594	inside
ENSMUSG00000032875	100930849	100931249	1.96	1312	inside
ENSMUSG00000003872	45369803	45370403	1.96	780	inside
ENSMUSG00000034656	84414409	84415009	1.96	25969	inside
ENSMUSG00000036442	105854958	105855458	1.96	-145	overlap
ENSMUSG00000025521	64947286	64947736	1.96	101	inside
ENSMUSG00000049717	9977472	9977872	1.96	214	overlap
ENSMUSG00000061313	25753667	25754117	1.96	613	inside
ENSMUSG00000025044	39905168	39905768	1.96	-262490	intergenic
ENSMUSG00000032012	43744252	43744702	1.96	-324	overlap
ENSMUSG00000036867	64020278	64020678	1.96	1781	inside
ENSMUSG00000097083	39650274	39650674	1.95	98	inside
ENSMUSG00000026455	134455492	134455942	1.95	-39	overlap
ENSMUSG00000034353	91179962	91180462	1.95	140	inside
ENSMUSG00000005886	13374096	13374746	1.95	-13	upstream
ENSMUSG00000037503	34801682	34802132	1.95	41383	intergenic
ENSMUSG00000026670	170174596	170174996	1.95	361	overlap
ENSMUSG00000040675	3973047	3973447	1.95	-71	overlap
ENSMUSG00000009092	75893113	75893513	1.95	-285	overlap
ENSMUSG00000020183	117629095	117629495	1.95	-405	upstream
ENSMUSG00000020185	110745254	110745704	1.95	-185	overlap
ENSMUSG00000020114	119239361	119239761	1.95	694	inside
ENSMUSG00000062866	13402145	13402695	1.95	72251	inside
ENSMUSG00000020402	52361480	52361880	1.95	620	inside
ENSMUSG00000055805	103170574	103171324	1.95	-533	overlap
ENSMUSG00000050830	11113526	11114176	1.95	-697	upstream
ENSMUSG00000020869	94629776	94630176	1.95	9	inside
ENSMUSG00000020721	107547676	107548276	1.95	-254	overlap
ENSMUSG00000034449	84828527	84829027	1.95	467	overlap
ENSMUSG00000048732	100471924	100472624	1.95	817	inside
ENSMUSG00000000120	95587286	95587686	1.95	449	inside
ENSMUSG00000054517	116130115	116130665	1.95	1013	inside
ENSMUSG00000045546	84416898	84417298	1.95	-296	overlap
ENSMUSG00000071379	17691098	17691498	1.95	284	inside
ENSMUSG00000085622	71015740	71016340	1.95	92	overlap
ENSMUSG00000021240	84616927	84617327	1.95	539	inside
ENSMUSG00000002997	32060630	32061030	1.95	666	inside

ENSMUSG00000099100	67221557	67222157	1.95	-308	upstream
ENSMUSG00000005320	55152679	55153479	1.95	39	inside
ENSMUSG00000077380	95335811	95336361	1.95	3193	downstream
ENSMUSG00000034575	69534937	69535337	1.95	-1098	upstream
ENSMUSG00000021838	46883455	46883855	1.95	601	inside
ENSMUSG00000048279	61173175	61173575	1.95	34718	inside
ENSMUSG00000048582	57057193	57057593	1.95	837	inside
ENSMUSG00000022203	54925643	54926043	1.95	1145	inside
ENSMUSG00000022994	98609565	98610165	1.95	-1932	upstream
ENSMUSG00000037465	38301050	38301500	1.95	-343	upstream
ENSMUSG00000033653	21423350	21423750	1.95	232	inside
ENSMUSG00000042644	27057315	27057765	1.95	11	inside
ENSMUSG00000086534	29841898	29842298	1.95	-48186	intergenic
ENSMUSG00000005370	87974856	87975256	1.95	-194	overlap
ENSMUSG00000024335	34120462	34121112	1.95	2172	inside
ENSMUSG00000024241	80480303	80480703	1.95	150	overlap
ENSMUSG00000035765	75018433	75019033	1.95	-339	overlap
ENSMUSG00000024576	61555033	61555483	1.95	-241	overlap
ENSMUSG00000049173	60596288	60596738	1.95	-4500	upstream
ENSMUSG00000033382	20895612	20896062	1.95	466	inside
ENSMUSG00000033768	6418710	6419110	1.95	-21	overlap
ENSMUSG00000025231	46397020	46397470	1.95	124	inside
ENSMUSG00000004085	72286014	72286414	1.95	377	inside
ENSMUSG00000068267	131179390	131179840	1.95	622	inside
ENSMUSG00000038860	33129715	33130415	1.95	1939	inside
ENSMUSG00000040016	157566804	157567404	1.95	-88	overlap
ENSMUSG00000086181	39099567	39100917	1.95	-211308	intergenic
ENSMUSG00000090817	98428089	98428589	1.95	28942	intergenic
ENSMUSG00000061175	79567740	79568140	1.95	56	overlap
ENSMUSG00000028081	86142317	86143317	1.95	385	overlap
ENSMUSG00000080771	30069472	30070322	1.95	-124175	intergenic
ENSMUSG00000038024	86748270	86748670	1.95	-285	overlap
ENSMUSG00000028876	124885530	124885980	1.95	4631	inside
ENSMUSG00000034926	106560543	106560993	1.95	-495	upstream
ENSMUSG00000084869	132048259	132048659	1.95	81	inside
ENSMUSG00000028635	120148337	120148837	1.95	-12869	intergenic
ENSMUSG00000078582	119814575	119815075	1.95	80779	intergenic
ENSMUSG00000029056	154964263	154964813	1.95	140	inside
ENSMUSG00000051279	9843625	9844125	1.95	-747	upstream
ENSMUSG00000040761	141537509	141537909	1.95	1088	inside
ENSMUSG00000036087	72913812	72914262	1.95	-492	upstream
ENSMUSG00000029578	142629597	142630047	1.95	55	inside
ENSMUSG00000015880	45669568	45670518	1.95	-354	overlap
ENSMUSG00000089809	99728588	99728988	1.95	477	inside
ENSMUSG00000061898	143180522	143180922	1.95	253	overlap
ENSMUSG00000029467	122502520	122502920	1.95	-295	upstream
ENSMUSG00000052751	48601534	48601984	1.95	7651	downstream
ENSMUSG00000030059	97178499	97178949	1.95	625	inside
ENSMUSG00000052512	49246056	49247006	1.95	337340	inside
ENSMUSG00000060621	19523433	19524083	1.95	4702	inside
ENSMUSG00000040177	131362009	131362459	1.95	689	inside

ENSMUSG00000036459	34133213	34133613	1.95	55	overlap
ENSMUSG00000054808	28961644	28962194	1.95	696	inside
ENSMUSG00000030850	130519382	130519782	1.95	579	inside
ENSMUSG00000030660	116443349	116443749	1.95	109	overlap
ENSMUSG00000031004	135715990	135716390	1.95	389	overlap
ENSMUSG00000051550	4993961	4994511	1.95	2197	inside
ENSMUSG00000038502	44868803	44869303	1.95	985	inside
ENSMUSG00000076144	84084609	84085059	1.95	-1490	upstream
ENSMUSG00000074247	71468673	71469123	1.95	-526	upstream
ENSMUSG00000087408	70329981	70331331	1.95	14194	inside
ENSMUSG00000031511	11758058	11758608	1.95	29953	inside
ENSMUSG00000031447	13159480	13159880	1.95	345	inside
ENSMUSG00000046413	91800423	91801173	1.95	8	inside
ENSMUSG00000031799	72135190	72135590	1.95	215	inside
ENSMUSG00000031486	27085917	27086417	1.95	334	inside
ENSMUSG00000086067	84559259	84559959	1.95	57925	intergenic
ENSMUSG00000000743	123212422	123212922	1.95	341	overlap
ENSMUSG00000038542	13104530	13104980	1.95	929	inside
ENSMUSG00000053716	106368254	106368854	1.95	-378	overlap
ENSMUSG00000032435	114688767	114689167	1.95	-23	overlap
ENSMUSG00000054693	70678728	70679128	1.95	-269	overlap
ENSMUSG00000045414	94538766	94539266	1.95	-685	upstream
ENSMUSG00000066456	83145628	83146028	1.95	979	inside
ENSMUSG00000032018	42264002	42264552	1.95	298	overlap
ENSMUSG00000032265	85327628	85328178	1.95	-504	upstream
ENSMUSG00000031935	15279241	15279741	1.95	626	inside
ENSMUSG00000053286	151428489	151429089	1.94	-159	overlap
ENSMUSG00000041642	136131556	136131956	1.94	167	inside
ENSMUSG00000004768	33719388	33719938	1.94	-494	overlap
ENSMUSG00000089418	141599311	141599711	1.94	284956	intergenic
ENSMUSG00000098273	118837742	118838242	1.94	-116478	intergenic
ENSMUSG00000026584	163929863	163930263	1.94	763	inside
ENSMUSG00000077237	108327312	108327912	1.94	434338	intergenic
ENSMUSG00000034066	93512111	93512561	1.94	32	inside
ENSMUSG00000041570	136345461	136345861	1.94	643	inside
ENSMUSG00000018199	143776764	143777614	1.94	304	overlap
ENSMUSG00000077608	163049358	163050458	1.94	5593	downstream
ENSMUSG00000035754	79960265	79960665	1.94	113	inside
ENSMUSG00000020042	85387534	85388034	1.94	720	inside
ENSMUSG00000025422	127090895	127091345	1.94	11988	inside
ENSMUSG00000071359	22731745	22732145	1.94	193	overlap
ENSMUSG00000001120	76960648	76961948	1.94	1239	overlap
ENSMUSG00000047712	8518303	8518703	1.94	522	inside
ENSMUSG00000051043	114851526	114852226	1.94	374	inside
ENSMUSG00000020849	75733030	75733530	1.94	161	inside
ENSMUSG00000038976	94992018	94992418	1.94	983	inside
ENSMUSG00000020700	106084986	106085386	1.94	373	inside
ENSMUSG00000087013	85831730	85832230	1.94	493	overlap
ENSMUSG00000043099	75168127	75168577	1.94	1392	inside
ENSMUSG00000041654	113649874	113650274	1.94	205	overlap
ENSMUSG00000034520	102818215	102818715	1.94	1485	inside

ENSMUSG00000049659	20741376	20741926	1.94	213	overlap
ENSMUSG00000048562	118848704	118849104	1.94	2375	inside
ENSMUSG00000020647	4476770	4477220	1.94	412	overlap
ENSMUSG00000021054	75735457	75736007	1.94	272	overlap
ENSMUSG00000038683	41000147	41001347	1.94	-876	overlap
ENSMUSG00000052293	100651514	100651964	1.94	171	inside
ENSMUSG00000021719	105053786	105054236	1.94	1144	inside
ENSMUSG00000038991	38527747	38528147	1.94	1077	inside
ENSMUSG00000036282	49172705	49173155	1.94	479	inside
ENSMUSG00000021994	28505727	28506127	1.94	977	inside
ENSMUSG00000021775	18238849	18239249	1.94	278	overlap
ENSMUSG00000047888	80711121	80711571	1.94	-198	overlap
ENSMUSG00000097938	54147141	54147641	1.94	-111159	intergenic
ENSMUSG00000001281	102224222	102224672	1.94	7713	inside
ENSMUSG00000097875	38989755	38990405	1.94	16430	inside
ENSMUSG00000077468	31053996	31054496	1.94	140342	intergenic
ENSMUSG00000071632	18836329	18836879	1.94	-249	overlap
ENSMUSG00000041215	20141249	20141649	1.94	186	inside
ENSMUSG00000040605	97356757	97357157	1.94	29	inside
ENSMUSG00000022811	33381178	33381578	1.94	403	inside
ENSMUSG00000022799	38712740	38713340	1.94	534	overlap
ENSMUSG00000022974	91043878	91044278	1.94	665	inside
ENSMUSG00000005580	4418945	4419995	1.94	1553	inside
ENSMUSG00000079507	35320115	35320965	1.94	-290	overlap
ENSMUSG00000024317	21002141	21002541	1.94	800	inside
ENSMUSG00000088493	37722209	37722659	1.94	16230	intergenic
ENSMUSG00000048799	53744409	53744809	1.94	138	overlap
ENSMUSG00000024892	4509560	4510460	1.94	-912	upstream
ENSMUSG00000006463	4878660	4879210	1.94	-8	overlap
ENSMUSG00000052188	6498003	6498903	1.94	34008	intergenic
ENSMUSG00000045045	4615689	4616089	1.94	-22	upstream
ENSMUSG00000087182	18693988	18694438	1.94	-44	overlap
ENSMUSG00000057914	14559332	14560132	1.94	-44974	intergenic
ENSMUSG00000085946	152048690	152049090	1.94	28118	intergenic
ENSMUSG00000027238	121806762	121807162	1.94	325	overlap
ENSMUSG00000027404	130178685	130179085	1.94	718	inside
ENSMUSG00000065485	29845769	29846219	1.94	-42	upstream
ENSMUSG00000078137	118703029	118703829	1.94	934	inside
ENSMUSG00000026915	37703489	37703939	1.94	370	overlap
ENSMUSG00000081056	119872699	119873199	1.94	5704	downstream
ENSMUSG00000015335	30093365	30093765	1.94	283	overlap
ENSMUSG00000026805	28916119	28916619	1.94	549	inside
ENSMUSG00000089444	81597196	81598046	1.94	-283531	intergenic
ENSMUSG00000028271	142764804	142765304	1.94	-422	overlap
ENSMUSG00000098515	129213499	129214199	1.94	3493	downstream
ENSMUSG00000004891	87971095	87971545	1.94	17	inside
ENSMUSG00000089149	19335575	19336175	1.94	4802	downstream
ENSMUSG00000014599	107759744	107760294	1.94	725	inside
ENSMUSG00000089027	120740771	120742071	1.94	-130678	intergenic
ENSMUSG00000028070	88058367	88058767	1.94	128	overlap
ENSMUSG00000027947	89913045	89913445	1.94	117	overlap

ENSMUSG00000027840	104961021	104961471	1.94	688	inside
ENSMUSG00000068696	116251871	116252271	1.94	1613	inside
ENSMUSG00000061143	52101867	52102517	1.94	3024	inside
ENSMUSG00000028488	85204894	85205344	1.94	-232	overlap
ENSMUSG00000006445	141300877	141301377	1.94	-363	overlap
ENSMUSG00000039852	150281833	150282383	1.94	187	inside
ENSMUSG00000024793	152131413	152132063	1.94	15479	intergenic
ENSMUSG00000029049	155150589	155151089	1.94	64012	intergenic
ENSMUSG00000028476	43874996	43875546	1.94	-534	overlap
ENSMUSG00000028664	136836383	136836983	1.94	-395	upstream
ENSMUSG00000028943	152152113	152152563	1.94	258	overlap
ENSMUSG00000028842	126429618	126430018	1.94	-62	upstream
ENSMUSG00000028551	109666375	109666975	1.94	814	inside
ENSMUSG00000042500	126532804	126533204	1.94	668	inside
ENSMUSG00000029345	112307687	112308087	1.94	-18671	intergenic
ENSMUSG00000075703	30231995	30232595	1.94	-586	overlap
ENSMUSG00000037373	33274228	33274628	1.94	776	inside
ENSMUSG00000036285	77309232	77309782	1.94	852	inside
ENSMUSG00000053121	29735767	29736167	1.94	169	overlap
ENSMUSG00000029832	51421834	51422384	1.94	-10836	intergenic
ENSMUSG00000059187	95951847	95952347	1.94	-161307	intergenic
ENSMUSG00000000182	127080242	127080692	1.94	7340	inside
ENSMUSG00000045095	94283049	94283599	1.94	273	overlap
ENSMUSG00000030086	89595010	89595710	1.94	642	overlap
ENSMUSG00000030093	91410739	91411189	1.94	624	inside
ENSMUSG00000035585	3685604	3686054	1.94	-7771	intergenic
ENSMUSG00000033967	12927833	12928233	1.94	417	inside
ENSMUSG00000053877	127512212	127512912	1.94	185	inside
ENSMUSG00000030374	16809977	16811027	1.94	-5912	upstream
ENSMUSG00000033917	118705299	118705849	1.94	479	overlap
ENSMUSG00000003873	45466643	45467043	1.94	255	overlap
ENSMUSG00000031548	23411286	23411686	1.94	-216	overlap
ENSMUSG00000031782	94838139	94838539	1.94	-182	overlap
ENSMUSG00000055707	70476459	70476959	1.94	484	overlap
ENSMUSG00000063049	47675635	47676035	1.94	-79	upstream
ENSMUSG00000081316	37554054	37554454	1.94	234638	intergenic
ENSMUSG00000032185	21546141	21546591	1.94	-753	upstream
ENSMUSG00000042138	37489300	37489850	1.94	-21	overlap
ENSMUSG00000098171	93594197	93594647	1.94	-201827	intergenic
ENSMUSG00000032120	44319452	44319952	1.94	830	inside
ENSMUSG00000032244	62811678	62812078	1.94	-30	upstream
ENSMUSG00000032112	44407402	44408052	1.94	146	overlap
ENSMUSG00000032026	48479881	48480731	1.94	730	overlap
ENSMUSG00000097334	57076828	57077278	1.94	-815	upstream
ENSMUSG00000076999	105567011	105568311	1.93	-7676	intergenic
ENSMUSG00000034220	92831106	92831806	1.93	-539	overlap
ENSMUSG00000026514	181352708	181353108	1.93	80	inside
ENSMUSG00000026239	86581869	86582419	1.93	760	inside
ENSMUSG00000055676	180332302	180332702	1.93	-1752	upstream
ENSMUSG00000066842	150993314	150993714	1.93	121	overlap
ENSMUSG00000010609	180255646	180256146	1.93	7792	inside

ENSMUSG00000035011	81143913	81144413	1.93	8693	inside
ENSMUSG00000058297	60106653	60107053	1.93	434	inside
ENSMUSG00000058806	61978930	61979380	1.93	178	overlap
ENSMUSG00000007850	50378379	50378829	1.93	1389	inside
ENSMUSG00000056687	109363065	109363665	1.93	589	overlap
ENSMUSG00000046719	95514318	95514718	1.93	252	overlap
ENSMUSG00000015837	50210486	50210886	1.93	341	overlap
ENSMUSG00000044066	20249326	20249776	1.93	103	overlap
ENSMUSG00000020941	103267265	103267665	1.93	207	overlap
ENSMUSG00000044548	71309211	71309711	1.93	-673	upstream
ENSMUSG00000020654	4132954	4133354	1.93	-149	overlap
ENSMUSG00000021277	111166733	111167133	1.93	363	inside
ENSMUSG00000021136	81027018	81027418	1.93	210	inside
ENSMUSG00000086022	11455689	11456189	1.93	-390	overlap
ENSMUSG00000091264	41249521	41249921	1.93	-339	overlap
ENSMUSG00000000253	45507021	45507621	1.93	-423	overlap
ENSMUSG00000021488	55210005	55210455	1.93	223	inside
ENSMUSG00000076431	28951997	28952397	1.93	1716	inside
ENSMUSG00000097775	62759175	62760025	1.93	-1326	upstream
ENSMUSG00000040760	26970499	26970899	1.93	733	inside
ENSMUSG00000006289	50924351	50924751	1.93	542	inside
ENSMUSG00000036046	84987166	84987716	1.93	1385	inside
ENSMUSG00000032988	79252175	79252575	1.93	2586	inside
ENSMUSG00000056258	66285589	66286539	1.93	635	overlap
ENSMUSG00000055065	79545839	79546739	1.93	902	inside
ENSMUSG00000039830	91227277	91227677	1.93	1727	inside
ENSMUSG00000062713	94084927	94085327	1.93	-333	overlap
ENSMUSG00000039345	8470610	8471110	1.93	-178	overlap
ENSMUSG00000022858	22265250	22265650	1.93	755	inside
ENSMUSG00000022898	94526816	94527216	1.93	14	overlap
ENSMUSG00000022892	85173519	85173919	1.93	188	overlap
ENSMUSG00000043445	24469811	24470411	1.93	-662	upstream
ENSMUSG00000002365	5840157	5841057	1.93	-1171	upstream
ENSMUSG00000061232	33999572	34000122	1.93	761	inside
ENSMUSG00000059409	46704308	46704758	1.93	694	inside
ENSMUSG00000001525	35836765	35837215	1.93	1541	inside
ENSMUSG00000024563	76241277	76241677	1.93	-303	overlap
ENSMUSG00000034006	80256283	80256683	1.93	2991	inside
ENSMUSG00000057506	44146126	44146526	1.93	320	overlap
ENSMUSG00000085444	110157052	110157452	1.93	-39837	intergenic
ENSMUSG00000081070	3713649	3714049	1.93	27287	intergenic
ENSMUSG00000087294	38286565	38287065	1.93	310	inside
ENSMUSG00000087524	131545673	131546223	1.93	-15941	intergenic
ENSMUSG00000017817	163339673	163340423	1.93	58320	inside
ENSMUSG00000027297	119758223	119758723	1.93	2208	inside
ENSMUSG00000087679	26140306	26140706	1.93	215	overlap
ENSMUSG00000089251	44191367	44192117	1.93	265798	intergenic
ENSMUSG00000027722	37419495	37419895	1.93	-401	upstream
ENSMUSG00000037610	31948830	31949680	1.93	46323	inside
ENSMUSG00000074513	85887317	85887817	1.93	199	overlap
ENSMUSG00000091329	28781003	28781803	1.93	105	overlap

ENSMUSG00000039701	122984144	122984694	1.93	303	overlap
ENSMUSG00000027895	107458944	107459494	1.93	-46	upstream
ENSMUSG00000028527	101419425	101419925	1.93	148	inside
ENSMUSG00000092724	9668775	9669175	1.93	-504	upstream
ENSMUSG00000042198	3938527	3938977	1.93	-361	overlap
ENSMUSG00000039911	149954573	149954973	1.93	470	inside
ENSMUSG00000086774	28815062	28815462	1.93	14	overlap
ENSMUSG00000098563	91371151	91371551	1.93	2212	downstream
ENSMUSG00000040860	141040309	141041009	1.93	20241	inside
ENSMUSG00000054920	65107762	65108162	1.93	194	inside
ENSMUSG00000010825	143372878	143373378	1.93	15540	inside
ENSMUSG00000041870	114772570	114773020	1.93	-2107	upstream
ENSMUSG00000040274	3340657	3341557	1.93	-3236	upstream
ENSMUSG00000058291	138619365	138619815	1.93	396	overlap
ENSMUSG00000029408	124095442	124095842	1.93	356	overlap
ENSMUSG00000029207	66618368	66618868	1.93	460	overlap
ENSMUSG00000016128	151233376	151233776	1.93	460	inside
ENSMUSG00000093910	143293150	143293700	1.93	-794	upstream
ENSMUSG00000077474	83783752	83784302	1.93	-60163	intergenic
ENSMUSG00000042599	39205255	39205655	1.93	1534	inside
ENSMUSG00000084772	17064633	17065133	1.93	363	overlap
ENSMUSG00000034832	83441099	83441599	1.93	579	inside
ENSMUSG00000051184	5015561	5016011	1.93	60	inside
ENSMUSG00000037337	29018913	29019713	1.93	36059	intergenic
ENSMUSG00000039176	79464340	79465340	1.93	2022	inside
ENSMUSG00000098839	45007653	45008053	1.93	4834	downstream
ENSMUSG00000031700	85492131	85492631	1.93	-445	overlap
ENSMUSG00000054823	25601517	25602067	1.93	-84	overlap
ENSMUSG00000071138	27318967	27319617	1.93	-25427	intergenic
ENSMUSG00000036990	79640031	79640531	1.93	413	inside
ENSMUSG00000083285	23057900	23058900	1.93	-69556	intergenic
ENSMUSG00000038872	108791989	108792689	1.93	77345	inside
ENSMUSG00000031458	13889522	13890022	1.93	749	inside
ENSMUSG00000004383	73353323	73353773	1.93	217	overlap
ENSMUSG00000051495	126593089	126593489	1.93	897	inside
ENSMUSG00000001911	84799273	84799723	1.93	1071	inside
ENSMUSG00000032009	14276641	14277041	1.93	340	inside
ENSMUSG00000025887	5475435	5476085	1.93	130005	intergenic
ENSMUSG00000032411	96195816	96196516	1.93	-459	overlap
ENSMUSG00000032498	111271131	111271531	1.93	477	inside
ENSMUSG00000019471	21149791	21150441	1.93	115	overlap
ENSMUSG00000032218	70421228	70421728	1.93	326	overlap
ENSMUSG00000026062	40681194	40681594	1.92	-518	upstream
ENSMUSG00000026229	86064808	86065208	1.92	421	inside
ENSMUSG00000026208	75360019	75360869	1.92	-310	overlap
ENSMUSG00000026049	44102382	44102782	1.92	59	overlap
ENSMUSG00000026393	138619512	138620062	1.92	184	overlap
ENSMUSG00000041859	20819838	20820288	1.92	440	overlap
ENSMUSG00000025404	127380004	127380404	1.92	-323	overlap
ENSMUSG00000049764	76032583	76032983	1.92	-71	overlap
ENSMUSG00000020190	80676463	80676863	1.92	-4487	upstream

ENSMUSG00000074785	94944033	94944433	1.92	545	inside
ENSMUSG00000097086	21994345	21994845	1.92	52640	intergenic
ENSMUSG00000015202	7211582	7211982	1.92	655	inside
ENSMUSG00000020057	88356656	88357106	1.92	22424	inside
ENSMUSG00000037533	54522880	54523280	1.92	33	inside
ENSMUSG00000000617	50851429	50851829	1.92	744	inside
ENSMUSG00000037926	77215577	77216077	1.92	-710	upstream
ENSMUSG00000086459	23497658	23498158	1.92	-95	overlap
ENSMUSG00000019368	4017126	4017576	1.92	-14336	intergenic
ENSMUSG00000000282	74831248	74831648	1.92	328	inside
ENSMUSG00000020937	103101074	103101524	1.92	584	inside
ENSMUSG00000034621	102555567	102556567	1.92	825	overlap
ENSMUSG00000086058	82929086	82930286	1.92	4296	inside
ENSMUSG00000063251	99627615	99628065	1.92	624	inside
ENSMUSG00000089247	8058135	8059485	1.92	-53119	intergenic
ENSMUSG00000021259	108334367	108334917	1.92	-10	overlap
ENSMUSG00000020650	31634630	31635130	1.92	28	overlap
ENSMUSG00000052593	21373316	21373716	1.92	316	overlap
ENSMUSG00000051367	73045040	73045840	1.92	8847	inside
ENSMUSG00000021287	111813843	111814243	1.92	30	overlap
ENSMUSG00000021385	49399997	49400547	1.92	-21314	intergenic
ENSMUSG00000019726	13590489	13591239	1.92	80	inside
ENSMUSG00000021696	108214521	108215021	1.92	117	inside
ENSMUSG00000001504	72628371	72628821	1.92	-449	overlap
ENSMUSG00000032846	107890371	107890771	1.92	-307	upstream
ENSMUSG00000034522	65343712	65344162	1.92	-14964	intergenic
ENSMUSG00000063895	60251243	60251643	1.92	264	overlap
ENSMUSG00000021944	63240326	63240726	1.92	31366	inside
ENSMUSG00000022201	12118398	12118798	1.92	567	inside
ENSMUSG00000022367	56692891	56693291	1.92	1648	inside
ENSMUSG00000036800	71650071	71650971	1.92	77767	inside
ENSMUSG00000039100	31531141	31531641	1.92	-104	upstream
ENSMUSG00000068167	64450347	64450747	1.92	28801	intergenic
ENSMUSG00000022537	30550467	30550867	1.92	375	overlap
ENSMUSG00000073411	35263422	35264222	1.92	692	inside
ENSMUSG00000032855	24549728	24550128	1.92	-222	overlap
ENSMUSG00000084880	47672111	47672611	1.92	-15499	intergenic
ENSMUSG00000048915	62880708	62881458	1.92	609	overlap
ENSMUSG00000079553	33890222	33890672	1.92	439	overlap
ENSMUSG00000024456	37934827	37935377	1.92	649	inside
ENSMUSG00000025231	46441925	46442425	1.92	45029	inside
ENSMUSG00000024639	16133375	16133775	1.92	544	inside
ENSMUSG00000024978	55098790	55099190	1.92	661	inside
ENSMUSG00000027314	119322373	119322773	1.92	-3411	upstream
ENSMUSG00000027134	112238952	112239402	1.92	-516	upstream
ENSMUSG00000027349	117249835	117250235	1.92	96	inside
ENSMUSG00000037843	157944049	157944499	1.92	29396	inside
ENSMUSG00000016458	105127008	105127458	1.92	479	inside
ENSMUSG00000082930	40917819	40918669	1.92	-40572	intergenic
ENSMUSG00000087264	70562264	70562714	1.92	1093	inside
ENSMUSG00000075270	76338664	76339064	1.92	110	overlap

ENSMUSG00000004113	24762228	24762878	1.92	924	inside
ENSMUSG00000046470	181671330	181671730	1.92	310	overlap
ENSMUSG00000041997	70824678	70825378	1.92	1050	inside
ENSMUSG00000074796	130697535	130697985	1.92	-16	upstream
ENSMUSG00000074682	152414349	152414999	1.92	695	inside
ENSMUSG00000046688	127789834	127790234	1.92	-38	overlap
ENSMUSG00000027555	14641567	14642067	1.92	-160	overlap
ENSMUSG00000029056	154984113	154984563	1.92	19990	downstream
ENSMUSG00000085863	101291675	101292325	1.92	7454	intergenic
ENSMUSG00000028522	103113807	103114257	1.92	-583	upstream
ENSMUSG00000028790	129742074	129742474	1.92	229	overlap
ENSMUSG00000089051	108031837	108032237	1.92	9963	intergenic
ENSMUSG00000028741	139352007	139352457	1.92	569	inside
ENSMUSG00000029466	122422492	122422942	1.92	799	inside
ENSMUSG00000058558	107900338	107900738	1.92	-164	overlap
ENSMUSG00000000916	135369731	135370131	1.92	-222	overlap
ENSMUSG00000029345	112326109	112326509	1.92	-249	overlap
ENSMUSG00000034981	91517381	91517881	1.92	-234	overlap
ENSMUSG00000028949	24604569	24604969	1.92	-2557	upstream
ENSMUSG00000043614	135089673	135090123	1.92	-11407	intergenic
ENSMUSG00000055204	90366125	90366525	1.92	60	overlap
ENSMUSG00000043323	110431785	110432185	1.92	16718	inside
ENSMUSG00000029863	42264792	42265392	1.92	-193	overlap
ENSMUSG00000030256	145865883	145866333	1.92	-325	upstream
ENSMUSG00000099103	143445491	143446041	1.92	-2114	upstream
ENSMUSG00000002083	16309133	16309883	1.92	-483	overlap
ENSMUSG00000030562	87292166	87292716	1.92	46070	inside
ENSMUSG00000033676	57590426	57590876	1.92	-92	overlap
ENSMUSG00000025505	141328061	141328461	1.92	322	inside
ENSMUSG00000092071	89632453	89633153	1.92	671	overlap
ENSMUSG00000030583	29518504	29519054	1.92	137	overlap
ENSMUSG00000030678	127025268	127025718	1.92	1211	inside
ENSMUSG00000031570	25719708	25720108	1.92	-353	overlap
ENSMUSG00000019139	70598581	70598981	1.92	4100	downstream
ENSMUSG00000039067	107587630	107588230	1.92	852	inside
ENSMUSG00000000792	70892540	70892940	1.92	217	overlap
ENSMUSG00000045636	41133168	41133668	1.92	558	inside
ENSMUSG00000096188	104394789	104395239	1.92	1018	inside
ENSMUSG00000032410	95954616	95955066	1.92	-144	overlap
ENSMUSG00000032468	99568116	99568516	1.92	783	inside
ENSMUSG00000099275	110638231	110638831	1.92	-2116	upstream
ENSMUSG00000036867	64020828	64021278	1.92	1231	inside
ENSMUSG00000049307	14751491	14751891	1.92	631	inside
ENSMUSG00000038412	121857817	121858217	1.92	183	overlap
ENSMUSG00000032582	107872554	107873054	1.92	683	inside
ENSMUSG00000067336	59763150	59763600	1.91	-1129	upstream
ENSMUSG00000026072	40266543	40266993	1.91	41463	inside
ENSMUSG00000070644	133363556	133364256	1.91	-16	overlap
ENSMUSG00000089534	17511257	17511657	1.91	-8744	intergenic
ENSMUSG00000050069	175015157	175016107	1.91	-93338	intergenic
ENSMUSG00000099119	171064458	171064958	1.91	194	includeFeature

ENSMUSG00000057173	39756549	39757149	1.91	-35552	intergenic
ENSMUSG00000085184	172562007	172563057	1.91	-3878	upstream
ENSMUSG00000042772	152901889	152902589	1.91	757	inside
ENSMUSG00000026360	144003839	144004239	1.91	322	overlap
ENSMUSG00000026509	182517446	182517896	1.91	49	overlap
ENSMUSG00000026121	36558049	36558499	1.91	300	overlap
ENSMUSG00000055197	74882209	74882659	1.91	3210	inside
ENSMUSG00000035027	81106083	81106483	1.91	168	inside
ENSMUSG00000019952	99107299	99107849	1.91	263	inside
ENSMUSG00000069539	89685513	89685913	1.91	772	inside
ENSMUSG00000019803	42582554	42582954	1.91	1078	inside
ENSMUSG00000039497	34207223	34207673	1.91	328	overlap
ENSMUSG00000056758	120474945	120475545	1.91	1524	inside
ENSMUSG00000004934	81154234	81154634	1.91	13689	inside
ENSMUSG00000033416	75516783	75517283	1.91	1189	inside
ENSMUSG00000020709	80153827	80154277	1.91	-278	overlap
ENSMUSG00000020359	51584627	51585027	1.91	-130	overlap
ENSMUSG00000087111	3146026	3146476	1.91	3978	downstream
ENSMUSG00000018849	35979588	35980138	1.91	939	inside
ENSMUSG00000085564	50602580	50603230	1.91	-132	upstream
ENSMUSG00000020902	68385327	68385777	1.91	15496	inside
ENSMUSG00000020435	3863626	3864076	1.91	277	overlap
ENSMUSG00000020925	102697565	102698065	1.91	217	overlap
ENSMUSG00000096279	24251540	24252190	1.91	641	includeFeature
ENSMUSG00000051367	73045940	73046490	1.91	7947	inside
ENSMUSG00000034168	86884063	86884563	1.91	751	inside
ENSMUSG00000091105	21899203	21899603	1.91	-122496	intergenic
ENSMUSG00000038175	45389697	45390197	1.91	-45	overlap
ENSMUSG00000039242	13954549	13954999	1.91	-125	overlap
ENSMUSG00000021326	21180421	21180821	1.91	976	inside
ENSMUSG00000042167	93219485	93220285	1.91	-27202	intergenic
ENSMUSG00000069272	23570671	23571071	1.91	549	inside
ENSMUSG00000021466	63572358	63573158	1.91	-6838	intergenic
ENSMUSG00000041014	39473325	39473775	1.91	-237	upstream
ENSMUSG00000021978	65097848	65098248	1.91	258	overlap
ENSMUSG00000021944	63240926	63241326	1.91	30766	inside
ENSMUSG00000036218	92397220	92397620	1.91	305	inside
ENSMUSG00000063704	75998421	75998821	1.91	4652	inside
ENSMUSG00000022472	82015971	82016421	1.91	17891	inside
ENSMUSG00000022476	81925966	81926466	1.91	247	overlap
ENSMUSG00000022451	94589738	94590138	1.91	151	overlap
ENSMUSG00000089979	18066130	18066530	1.91	-3084	upstream
ENSMUSG00000022792	16302560	16303010	1.91	-405	overlap
ENSMUSG00000090882	60948288	60948738	1.91	-110473	intergenic
ENSMUSG00000022663	45158901	45159351	1.91	116	inside
ENSMUSG00000014039	97851480	97851930	1.91	370	overlap
ENSMUSG00000036304	43978959	43979509	1.91	134	overlap
ENSMUSG00000047434	31081278	31081728	1.91	154	overlap
ENSMUSG00000022894	85899858	85900458	1.91	1267	inside
ENSMUSG00000024172	55445345	55445745	1.91	-37	overlap
ENSMUSG00000092612	36230348	36231298	1.91	1422	downstream

ENSMUSG00000067235	35470078	35470478	1.91	-11	overlap
ENSMUSG00000089487	72464770	72465170	1.91	34648	intergenic
ENSMUSG00000024070	79020853	79021253	1.91	-37	upstream
ENSMUSG00000024077	78736606	78737006	1.91	590	inside
ENSMUSG00000023845	17623793	17624293	1.91	696	inside
ENSMUSG00000046668	35847889	35848289	1.91	18071	inside
ENSMUSG00000098276	76145609	76146009	1.91	-24943	intergenic
ENSMUSG00000042705	46958848	46959248	1.91	-14	overlap
ENSMUSG00000032656	56925183	56925633	1.91	365	overlap
ENSMUSG00000024598	58209377	58209777	1.91	549	inside
ENSMUSG00000003228	60906863	60907463	1.91	17114	inside
ENSMUSG00000071657	8837320	8837770	1.91	-147	overlap
ENSMUSG00000064105	46761475	46761875	1.91	-121	overlap
ENSMUSG00000023307	37206770	37207170	1.91	-773	upstream
ENSMUSG00000024900	3325260	3325710	1.91	1959	inside
ENSMUSG00000049401	180589166	180589666	1.91	-79	overlap
ENSMUSG00000086449	35719689	35720139	1.91	-99501	intergenic
ENSMUSG00000027259	121139907	121140557	1.91	-521	overlap
ENSMUSG00000027018	71388982	71389582	1.91	24	inside
ENSMUSG00000059842	154613135	154613535	1.91	-162	overlap
ENSMUSG00000015647	180225730	180226230	1.91	129	overlap
ENSMUSG00000028180	157533775	157534225	1.91	-385	overlap
ENSMUSG00000027562	14886425	14886875	1.91	-1	overlap
ENSMUSG00000037814	40949490	40949890	1.91	-1141	upstream
ENSMUSG00000025757	40744567	40745067	1.91	72	inside
ENSMUSG00000027799	56135895	56136395	1.91	47806	inside
ENSMUSG00000033882	82875930	82876430	1.91	553	inside
ENSMUSG00000028745	139192177	139192577	1.91	-722	upstream
ENSMUSG00000042380	127214354	127214754	1.91	-29430	intergenic
ENSMUSG00000050212	126149398	126149798	1.91	1654	inside
ENSMUSG00000046637	154855503	154855903	1.91	-697	upstream
ENSMUSG00000036052	42949826	42950376	1.91	12	inside
ENSMUSG00000082388	98246831	98247481	1.91	-11541	intergenic
ENSMUSG00000041135	16163483	16163883	1.91	164	overlap
ENSMUSG00000054659	33189317	33189717	1.91	420	inside
ENSMUSG00000070576	111416073	111416823	1.91	-1289	upstream
ENSMUSG00000070639	105415682	105416082	1.91	-93	overlap
ENSMUSG00000034118	130079487	130079987	1.91	6161	inside
ENSMUSG00000029095	35757486	35757886	1.91	-394	overlap
ENSMUSG00000029122	37336312	37336912	1.91	582	overlap
ENSMUSG00000058153	112577065	112577465	1.91	133	overlap
ENSMUSG00000044221	88674882	88675682	1.91	1289	inside
ENSMUSG00000029505	110770016	110770666	1.91	701	inside
ENSMUSG00000001632	113306449	113306849	1.91	-732	upstream
ENSMUSG00000044927	87981089	87981939	1.91	393	overlap
ENSMUSG00000030376	16144648	16145398	1.91	14348	inside
ENSMUSG00000002068	38106854	38107304	1.91	680	inside
ENSMUSG00000037606	143739927	143740327	1.91	17058	inside
ENSMUSG00000070462	83884053	83884453	1.91	288	overlap
ENSMUSG00000055323	126776532	126776932	1.91	286	overlap
ENSMUSG00000031962	122870289	122870689	1.91	21915	downstream

ENSMUSG00000080348	43869718	43870118	1.91	-169532	intergenic
ENSMUSG00000031511	11727472	11727872	1.91	-633	upstream
ENSMUSG00000001472	123373389	123373839	1.91	-435	overlap
ENSMUSG00000040028	4325022	4325622	1.91	78	overlap
ENSMUSG00000092842	65108023	65108423	1.91	-14109	intergenic
ENSMUSG00000069867	122630289	122630889	1.91	-7550	intergenic
ENSMUSG00000003316	111258472	111258872	1.91	744	inside
ENSMUSG00000003657	110167939	110168489	1.91	267	overlap
ENSMUSG00000003575	70451605	70452055	1.91	-12026	intergenic
ENSMUSG00000006676	108488304	108488854	1.91	-2372	upstream
ENSMUSG00000032336	58582178	58582778	1.91	-62	overlap
ENSMUSG00000057895	122922767	122923167	1.91	-305	overlap
ENSMUSG00000087817	112981341	112981741	1.91	-422134	intergenic
ENSMUSG00000038119	35420900	35421300	1.91	-228	overlap
ENSMUSG00000032459	98601366	98601816	1.91	313	overlap
ENSMUSG00000092963	107231354	107231804	1.91	354	includeFeature
ENSMUSG00000079559	51278580	51278980	1.91	-26	upstream
ENSMUSG00000042207	134556617	134557017	1.90	-3554	upstream
ENSMUSG00000035595	80171406	80171906	1.90	-1538	upstream
ENSMUSG00000019943	98915545	98915945	1.90	393	inside
ENSMUSG00000020308	79669106	79669806	1.90	-304	overlap
ENSMUSG00000020124	123196637	123197137	1.90	298	overlap
ENSMUSG00000058537	58225162	58225562	1.90	544	inside
ENSMUSG00000043999	30885279	30885729	1.90	-79	overlap
ENSMUSG00000062115	60105636	60106536	1.90	623	inside
ENSMUSG00000082587	38164179	38165329	1.90	-54890	intergenic
ENSMUSG00000009073	4848876	4849526	1.90	660	inside
ENSMUSG00000056962	116843568	116843968	1.90	-119	upstream
ENSMUSG00000072825	112721316	112722016	1.90	-858	upstream
ENSMUSG00000020653	24651440	24651890	1.90	69	inside
ENSMUSG00000097758	110187484	110187934	1.90	90584	intergenic
ENSMUSG00000021488	55211705	55212455	1.90	1923	inside
ENSMUSG00000051335	43303077	43303577	1.90	1095	inside
ENSMUSG00000005148	99299193	99299593	1.90	502	inside
ENSMUSG00000046160	91270242	91270742	1.90	473	inside
ENSMUSG00000063239	27512251	27512751	1.90	1090	inside
ENSMUSG00000049090	84087321	84087721	1.90	-837	upstream
ENSMUSG00000024529	52528927	52529377	1.90	940	inside
ENSMUSG00000078201	25255456	25255856	1.90	17	inside
ENSMUSG00000038467	154657073	154657573	1.90	5368	inside
ENSMUSG00000027439	148680990	148681390	1.90	-33	overlap
ENSMUSG00000065083	120730049	120730849	1.90	-89214	intergenic
ENSMUSG00000048647	119547329	119547729	1.90	298	overlap
ENSMUSG00000026878	35200715	35201265	1.90	405	overlap
ENSMUSG00000004897	87906095	87906495	1.90	-226	overlap
ENSMUSG00000046743	38885846	38886246	1.90	-1094	upstream
ENSMUSG00000028078	86920330	86920930	1.90	554	overlap
ENSMUSG00000028478	44012476	44012976	1.90	8024	inside
ENSMUSG00000015247	53011808	53012258	1.90	-72	overlap
ENSMUSG00000055761	20778251	20778751	1.90	615	inside
ENSMUSG00000087383	46345100	46345550	1.90	415	overlap

ENSMUSG00000047221	21424911	21425411	1.90	-47	overlap
ENSMUSG00000037736	66745718	66746318	1.90	-117	overlap
ENSMUSG00000029120	36867912	36868462	1.90	-601	upstream
ENSMUSG00000029725	137779223	137779623	1.90	374	inside
ENSMUSG00000041609	115732342	115732792	1.90	-721	upstream
ENSMUSG00000029467	122500920	122501420	1.90	1305	inside
ENSMUSG00000015882	45857140	45857540	1.90	475	inside
ENSMUSG00000049694	90309647	90310047	1.90	8428	downstream
ENSMUSG00000001521	128355665	128356215	1.90	186	overlap
ENSMUSG00000030091	91116799	91117199	1.90	30	overlap
ENSMUSG00000044030	19004657	19005157	1.90	592	inside
ENSMUSG00000002083	16299377	16299877	1.90	-10239	intergenic
ENSMUSG00000076051	44852606	44853056	1.90	2907	downstream
ENSMUSG00000031812	121590691	121591091	1.90	330	inside
ENSMUSG00000037070	78508631	78509931	1.90	297	overlap
ENSMUSG00000001300	8660830	8661330	1.90	-57	upstream
ENSMUSG00000003410	22050191	22050641	1.90	1832	inside

Table 4.2

Gene list	Number of expressed genes	Expressed genes near DE Sono-seq peak	Overlap	Expected Overlap
Differential RNA-seq (Chapter 2)	823	86	5.1%	4.6%
diffSplice (Chapter 2)	118	20	1.2%	0.7%
Autism (SFARI)	420	59	3.5%	2.3%
Epilepsy (GenEpi)	83	5	0.3%	0.5%

Table 4.3

Data set	Number of regions in the genome	Percent overlap with differential Sono-seq regions
Sono-seq Hippocampus (Abel lab)	**36186**	**8.5%**
H3K9/14ac Hippocampus (Abel lab)	**38211**	**5.1%**
H3K27ac Adult Cortex	35684	3.2%
H3K27ac E14 Whole brain	36505	3.1%
H3K4me1 Adult Cortex	90067	0.5%
H3K4me1 E14 Whole brain	131408	1.3%
H3K4me3 Adult Cortex	18981	7.8%
H3K4me3 E14 Whole brain	19939	8.7%
H3K27me3 E14 Whole brain	12020	8.7%
H3K36me3 E14 Whole brain	168449	0.1%
Pol2 Adult Cortex	19448	4.6%
DNASE I Cerebrum	147444	0.6%

CHAPTER 5: Conclusions And Future Directions

This work focuses on the regulation of gene expression during memory consolidation, the process that converts labile short-term memories to stable long-term memories. Memory consolidation has been known to involve transcription for decades, yet the targets and regulation of this transcription have remained unclear. The advancement of high-throughput sequencing technology in recent years has greatly influenced our ability to study these changes on a genome-wide scale. This work aimed to define the transcriptional profile that occurs in response to contextual and spatial learning and determine how this profile is regulated. In **Chapter 1**, I introduced what is known about the regulation of gene expression by histone acetylation during memory consolidation. In **Chapter 2**, I used RNA-seq with a newly designed normalization method to discover the genes regulated during memory consolidation. We also used this data to probe for exon-level changes and discovered large-scale regulation of alternative splicing following training. In **Chapter 3**, I compared the gene expression profile during memory consolidation after contextual learning to that after spatial learning in the hippocampus. I found that differentially expressed genes are similar between training paradigms but the temporal dynamics of these changes differ. In **Chapter 4**, I used ChIP-seq, Sono-seq, and MNase-seq to determine how genes were being regulated during memory consolidation. We found that chromatin accessibility changes without large changes in H3K9/14ac or nucleosome positioning. In **Chapter 5**, I will review my findings and suggest future directions for research on this topic. Additionally, I will propose a model for how gene regulation could occur during memory consolidation.

5.1 Gene expression changes during memory consolidation

Studies of genome-wide gene expression changes after hippocampal learning have been previously attempted using microarrays [70, 71, 110-112], but there have been few genes discovered by this method and little overlap between labs. Two recent studies have used RNA-seq to study gene expression in mutant mice after learning [76, 160]. The advent of next-generation sequencing technology to study gene expression using RNA-seq provides a number of benefits over these previous studies. RNA-seq produces better resolution than microarrays, the ability to detect novel transcripts, and the ability to quantify alternative splicing. In addition, the variance between sequencing runs could prove to be less substantial than between microarray runs that rely on hybridization. Therefore, normalizing RNA-seq data in a standard way should produce reproducible results between labs and between training paradigms.

In **Chapter 2**, we used RNA-seq to study gene expression at 30 minutes after contextual fear conditioning. This is a time point at which our lab has observed maximum gene expression differences after learning [61, 70]. We discovered that standard RNA-seq normalization procedures are unable to capture the difference between untrained and trained groups. This leads to a small list of differentially regulated genes that may or may not be caused by the learning event. Therefore, we applied the recently published remove unwanted variation (RUV) normalization [114] that is an improved normalization method for noisy data sets such as the whole hippocampal samples used in our study. Briefly, this method of normalization includes an additional factor to account for unwanted variation by using negative control genes that are known not to be altered by training. RUV allowed us to differentiate between trained and untrained groups, meaning that any differentially expressed genes were likely the result of the contextual fear training. We discovered that this normalization method greatly improved the number of

novel genes detected as different after learning. Importantly, this analysis also increased the proportion of positive control genes discovered, suggesting that it was functioning as expected. Because RUV normalization makes fear conditioning the major source of variation between samples, the list of genes differentially expressed 30 minutes after learning using RUV normalization will provide a reproducible set of genes showing changes in response to learning. This method of normalization can be applied to all RNA-seq studies and will greatly improve detection power and reliability of results in future studies of brain function.

The regulation of gene expression is a highly complex process that includes transcription of a primary transcript, 5' capping, polyadenylation, and splicing into a mature mRNA. Alternative splicing is a coordinated process by which different transcripts can be produced from the same gene. Regulation of alternative splicing has been recognized in circadian function [161], addiction [162], and neurodegeneration [163]. There are also multiple individual examples of alternative splicing regulating learning and memory [134, 164-169], indicating this process may be an important regulatory step in the nervous system. However, no genome-wide studies have been used to investigate the regulation of alternative splicing during memory consolidation. Because RNA-seq also provides the ability to study exon-specific events such as those occurring by differential splicing, we applied RUV normalization to exon-specific analysis and demonstrated numerous exon-specific expression changes occurring during memory consolidation. We validated a number of these changes, including *Ania-3* (a poorly studied isoform of *Homer1*), translational regulator *Las1l* and RNA-binding protein *Rbm3*. We believe that this analysis provides the first description of large-scale differential exon usage in response to learning. Although transcription of splicing factors is regulated by fear conditioning [134], it is unclear whether these changes would be

translated quickly enough to cause the splicing changes observed. It is possible that histone modification changes could be altering exon usage [135, 137], but more work is necessary to show these marks change in response to training. Future studies can be conducted to see if transcripts containing or excluding the identified differential exon lead to changes in localization or function of the protein.

The major question that remained about the genes discovered by our RNA-seq analysis was whether these changes depend on the training paradigm used. In other words, are the genes regulated by contextual fear conditioning the same as those regulated by other hippocampus-dependent learning tasks? To answer this question, we used a high-throughput qPCR approach in **Chapter 3**. The goal of this study was to compare the targets and temporal profile of gene expression after training for object-location memory (OLM), a spatial learning task, to that of fear conditioning, a contextual learning task. We discovered that while gene targets are regulated in a similar manner after OLM, the temporal dynamics of these gene expression changes differs from that observed after fear conditioning. A subset of genes regulated 30 minutes after OLM remain elevated 2 hours after training, while these same genes return to baseline by 2 hours after fear conditioning. Although the stress of a footshock during contextual fear training may be expected to produce a larger transcriptional response, it appears that the three training trials used for OLM result in longer lasting transcriptional changes. Therefore, we hypothesize that a common set of targets are regulated by all forms of hippocampal learning, but the timing of these changes can differ based on the paradigm being tested.

5.2 Chromatin accessibility during memory and the histone code hypothesis

Although the mechanism of highly coordinated regulation of specific transcripts during memory consolidation remains a mystery, epigenetic mechanisms are beginning to be implicated in this process. Histone modifications [51, 52, 156], histone variants [160], DNA methylation [170], miRNA regulation [107], and nucleosome positioning [76] have all been implicated in regulation of hippocampus-dependent learning. Histone acetylation, an activating histone modification, is the best studied epigenetic modification during memory consolidation. Work from our lab and others has implicated the histone acetyltransferase CBP as a positive regulator of learning [26, 27] and the class I histone deacetylase (HDAC) proteins as negative regulators [55, 58, 63]. Thus, it appears that more histone acetylation during memory consolidation leads to enhanced long-term memory and less histone acetylation leads to impaired long-term memory. Histone acetylation is thought to decrease the interaction between the positively charged lysine residue of the histone and negatively charged DNA backbone, thereby increasing accessibility of chromatin in the surrounding region [35]. In **Chapter 4**, we used high-throughput sequencing to study both the histone acetylation and chromatin accessibility changes that occur 30 minutes after contextual fear memory.

A pilot ChIP-seq experiment investigating three histone acetylation marks found that H3K9/14ac, a mark we have previously studied [55], displayed the largest number of changes at promoters. We followed up this result by studying H3K9/14ac genome-wide in a large cohort of mice. We also investigated whether changes in chromatin accessibility, which would be the anticipated result of histone acetylation, occur at the same time. To our surprise, we found only a modest increase in H3K9/14ac surrounding the transcription start site of genes after fear conditioning. This small increase was not large enough to be significant at any one particular gene but could be seen when

averaging across all genes. However, we found a large increase in chromatin accessibility surrounding the transcription start site of genes using Sono-seq [82]. This increase was significant at 3064 regions in the genome. These regions are often found within gene bodies and are enriched in genes that show alternative splicing after learning and those implicated in autism, a known cognitive disorder. We believe that these sites of increased chromatin accessibility represent sites in the genome with active chromatin reorganization occurring during memory consolidation.

There are a number of potential causes for this increase in chromatin accessibility after learning. The most obvious explanation would be a shift in nucleosome position. Less nucleosomes in a region would be expected to increase accessibility to that region. Therefore, we used MNase-seq to map nucleosome positioning throughout the genome. We found no difference in nucleosome positioning in response to learning, indicating that this is not the primary force driving the increase in chromatin accessibility. Changes in transcription factor occupancy, including CREB, could also be driving this change in accessibility. However, it is unclear whether increased transcription factor occupancy would result in increases or decreases in Sono-seq signal. Also, there are many more differential peaks than genes showing altered gene expression, so the transcription factor would have to be selectively active at a subset of genes.

Finally, histone modifications besides H3K9/14ac may be responsible for this increase in accessibility. The histone code hypothesis, put forward in 2000 by Strahl and Allis [30], states that “distinct histone modifications, on one or more tails, act sequentially or in combination to form a 'histone code' that is, read by other proteins to bring about distinct downstream events.” This hypothesis has since been updated to acknowledge that combinatorial modifications probably do not create a specific “code” but rather a “language” that is dependent on surrounding context [155]. In either case, small changes

in any particular histone modification could lead to large changes in downstream function through combinatorial interactions with other marks, which would match our results. H3K9/14ac alone may not be significant enough to regulate the accessibility of chromatin, but instead may act in concert with a large number of other histone modifications to regulate this accessibility. Hippocampal learning may therefore be changing histone modifications in such a manner as to increase accessibility and prepare for transcription to occur.

We propose that the increase in chromatin accessibility 30 minutes after learning may be a set of combinatorial histone modifications removing a gate that allows transcription to occur. In this model (**Figure 5.1**), a number of sites would be "opened" after learning but only a subset of those sites would be bound by the factors necessary to drive transcription, regulate alternative splicing, or maintain that "open" state for later transcription. This leads to the intriguing question of whether there is a specific histone "memory code" that hippocampal neurons use to regulate activity after a learning event. This "memory code" could be a storage mechanism for long-term memory, with neurons exhibiting a particular epigenomic code ready to be rapidly activated during memory recall. This would be a large departure from the classic view of memory being stored at particular synapses through strengthening or weakening of connections, which has a limited number of possibilities at any individual synapse. Given the tremendous array of possible histone modifications, it seems likely that each neuron will have an individual code that could regulate the ability of that cell to participate in memory traces.

Although there is still a lot of work needed to test this possibility, there are technological advances that are going to make this possible in the near future. First, the cost of sequencing is getting cheaper every year and numerous histone modifications could be tested and overlaid to look for patterns in response to learning. Second, there

has been a major advancement in mass spectrometry-based quantification of histone modifications. This technology will allow users to test the relative abundance of all histone modifications throughout the genome in response to learning [171]. In addition, new techniques can quantify combinatorial modifications occurring on the same histone molecule, truly testing the histone code hypothesis [172, 173]. Future studies will use these novel technologies to test whether a specific “memory code” exists in response to a learning event. Novel genome-targeting technologies [174] can then be used to test whether disrupting this code at particular genes changes the response of these genes to a learning event.

5.3 Future Directions: The need for sorting technologies for brain epigenomic research

The study of epigenomics in the brain is a difficult, but promising, avenue for future research in to learning and memory, addiction, depression, neurodevelopmental and neurodegenerative disease. The brain is a complex organ that contains numerous cell types functioning in coordinated fashion to elicit specific responses. Our data make it clear that the changes in response to a behavior such as contextual or spatial learning are small. We believe this is due to a dilution of signal caused by the small number of cells responding to any particular memory trace. Transcription is an amplification step, where one copy of DNA can lead to multiple copies of RNA. This allows mRNA studies to identify reliable changes throughout the genome. However, epigenomic changes such as H3K9/14ac do not involve an amplification step and are much more susceptible to being lost by cellular dilution. Therefore, future studies of the epigenome in the brain will need to rely on sorting technologies to study the cells of interest for that particular

experiment. Efforts are being made by a number of labs to produce a reliable method of sorting for this type of research. Flourescence-activated cell sorting has been using in combination with ChIP-qPCR [175] or ChIP-seq [176] to study the epigenome of cells of interest, and biotin tagging of the nuclear envelope can immunoprecipitate whole nuclei [177].

Combining technologies will lead to a greater understanding of the role of the epigenome in regulating long-term memory. RNA-seq could be performed on the same sorted cells used by the technology above. Technologies to sort cells for chromatin analysis could be combined with translating ribosome affinity purification (TRAP), which is a technique that purifies actively translating mRNA [178, 179]. When combined with the sorting strategies above, information on the timing of epigenomic changes could be compared to mRNA levels and translation in a cell-type specific manner. This would provide the first timeline of gene regulation, transcription, and translation during memory consolidation and would be a powerful method for determining the crucial steps regulating the transcription and translation necessary for long-term memory formation.

Figure Legends

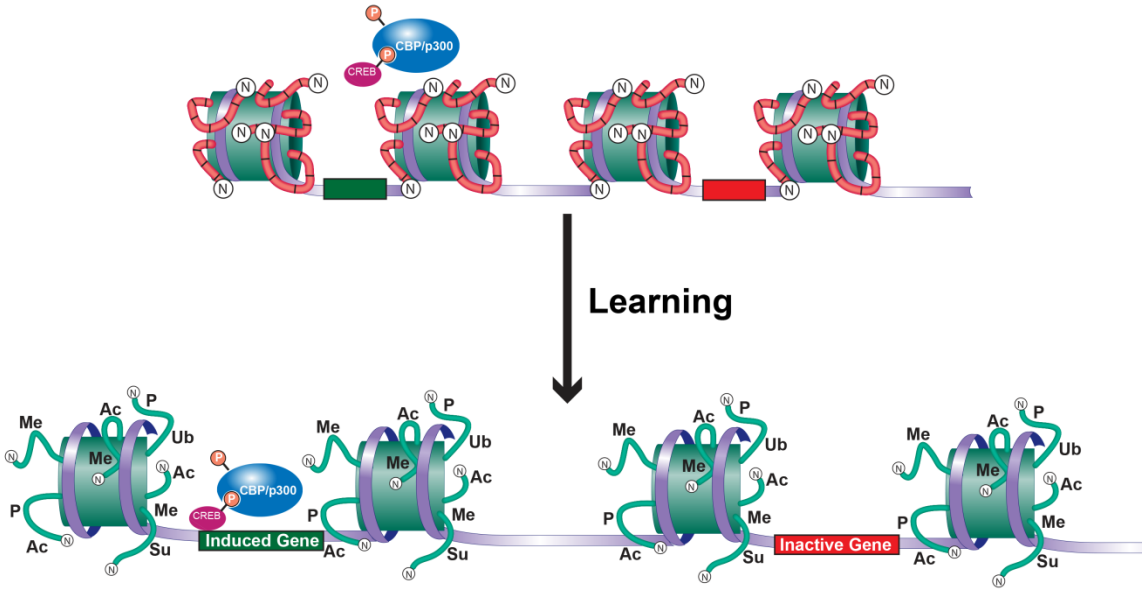
Figure 5.1. Model for the regulation of chromatin accessibility after learning. Learning causes changes in many histone modifications including H3K9/14ac, H3K4me3 and H3K27me3. These modifications act in concert to increase in chromatin accessibility in a large number of genes throughout the genome, opening the “gate” of repression. Transcription factors including CREB and CBP, splicing factors, and basal transcriptional machinery can then bind to their intended targets, leading to increased transcription of only a subset of “open” regions. Green indicates an induced gene and red indicated an inactive gene.

Contributions

This chapter was written by Shane G. Poplawski with suggestions by Ted Abel.

Figures

Figure 5.1



APPENDIX: Cell-type Specific Epigenomics In The Brain

Abstract

Epigenetics, the modification of gene expression without altering the underlying DNA sequence, plays a crucial role in regulating brain function including memory, drug addiction, and neurodegenerative disease. Despite important breakthroughs in epigenetics in the brain, the tools to study epigenetic regulation in specific cellular subpopulations do not currently exist. This is due to the complex heterogeneity of the brain, which can obscure important signals that occur in specific subsets of cells. To solve this problem, we are using a tetO-regulated, HA-tagged histone H3.3. Histone H3.3 incorporates preferentially into chromatin in actively transcribed regions independently of DNA replication, while H3.1 and H3.2 incorporate into silenced regions – a phenomenon known as histone barcoding [180]. The tetracycline transactivator (tTA), which allows expression of tetO-regulated transgenes, can be controlled in a cell-specific manner using cell-type specific promoters and temporally regulated by administration of doxycycline. Therefore, the tagged histone H3.3 will be a marker of active chromatin specifically in cells of interest. We are using the CaMKIIα-tTA driver line to express this tagged histone in excitatory forebrain neurons. ChIP for the HA tag will isolate nucleosomes bound to active regions of the excitatory neuron genome for further investigation. Our work initially focuses on excitatory neurons because epigenetic changes in these cells have been linked to memory storage, but future studies will extend this approach to interneurons, glia and cells recently activated by experience. Understanding the histone modifications that occur during memory consolidation may uncover novel therapeutic targets for diseases in which cognitive deficits occur, including schizophrenia and Alzheimer's disease. In addition to addressing the important question

of which combinations of histone modifications change after memory, this approach promises to provide tools that can be used by researchers in all fields that struggle with cellular heterogeneity.

Introduction

The formation and storage of long-term memory depends on the hippocampus. This occurs through a process involving distinct stages of acquisition, consolidation, maintenance and retrieval [2]. Through years of research, it has been demonstrated that memory consolidation requires transcription and translation to convert labile short-term memories to stable long-term memories [11, 16]. Although transcription has been known to be involved in memory formation for many years, the processes involved in regulating this transcription have only recently been uncovered in the field of epigenetics. Epigenetics refers to the set of mechanisms that control the regulation of gene expression without altering the underlying DNA sequence. Epigenetic regulation guides the binding of transcriptional machinery to the proper genetic loci. The best-studied epigenetic process in the brain is the post-translational modification of histone proteins within the nucleosome [50-52, 156]. Histones can be modified by the addition of acetylation, phosphorylation, methylation and other marks to achieve a combinatorial "histone code" that regulates transcription [30]. Many of these modifications demonstrate positive or negative correlations with transcriptional level [181]. Histone acetylation, an activating mark, plays a critical role in long-term memory. Decreasing histone acetylation by genetic deletion of the histone acetyltransferase CREB-binding protein (CBP) in the hippocampus reduces long-term memory [23, 24, 26-28, 149], whereas increasing histone acetylation by pharmacological inhibition of histone deacetylases (HDACs), which remove acetyl groups, enhances long-term memory [51, 55, 56, 63]. These published studies all used extracts prepared from the hippocampus which contain excitatory neurons, inhibitory neurons and glia.

A major problem for the field of epigenetics in the brain and other organs is the complex cellular heterogeneity of the tissue. The hippocampus contains excitatory neurons, inhibitory neurons, and glia, each of which can be divided into smaller subpopulations. We propose using histone barcoding to isolate chromatin of interest from specific cellular subpopulations. Prior attempts to study chromatin dynamics in particular cell types in mice have relied on fluorescence-activated cell sorting (FACS), which is an arduous procedure that has the potential to alter chromatin dynamics and gene expression [175]. Alternatively, immunopanning (using antibodies to purify cells based on external receptors) has been used to isolate specific cells from heterogeneous tissue to study RNA expression [182]. This technique could be used to study epigenetic regulation, but the time and dissociation steps required could themselves alter both histone modifications and gene expression [183]. Therefore, use of a single, simple approach adaptable to multiple cell types is necessary to avoid technique-driven biases.

Canonical histone proteins can be replaced by variant forms. Histone H3 has three variants in the mouse, H3.1 and H3.2, which differ by only one amino acid, and H3.3, which differs by four amino acids from H3.2. Despite this high sequence similarity, histones H3.1 and H3.2 incorporate into chromatin only during DNA replication whereas H3.3 incorporates independently of replication. Further, H3.3 has been shown to preferentially incorporate into actively expressed regions of chromatin, whereas H3.1 and H3.2 are found in non-transcribed regions [184, 185]. This phenomenon is termed the “H3 barcode hypothesis,” which states that genomic regions are “barcoded” with H3 histone variants to serve as a method for long-term cellular memory of transcriptional states [180]. We have developed a mouse model expressing a tagged histone H3.3 in specific cell populations to isolate actively transcribed regions of the genome selectively

from cells of interest. This mouse model uses a tetO-driven tagged H3.3 in combination with a tTA driver line of choice [186]. This system has the advantage of being temporally controlled by administration of doxycycline and provides modularity so that this mouse line can be used to isolate any cell population of interest.

Memory is thought to depend critically on transcriptional changes in excitatory neurons. NMDA and AMPA receptors, which are activated by excitatory neurotransmitter glutamate, are known to be essential for synaptic plasticity and memory [187, 188]. Additionally, the selective deletion of CBP or HDACs selectively in excitatory neurons using transgenes driven by the CaMKIIα promoter impairs or enhances long-term memory respectively [24, 26]. Therefore, our work initially focuses on the set of excitatory neurons marked by the CaMKIIα promoter. However, we anticipate that this tool will be widely applicable to the study of epigenetics in the context of neurological and psychiatric disorders, metabolic disorders, cardiac disease, developmental disorders and cancer.

We were able to produce mouse lines that express the HA-tagged H3.3. It was found that using the tTA system allowed for expression of tagged H3.3 to be restricted to adulthood by doxycycline and that expression only occurred in CaMKIIα positive neurons. Also, we found that ChIP for the HA tag of H3.3 isolates promoters from genes expressed in neurons selectively, proving that we can sort nucleosomes at the chromatin level.

Materials and Methods

Subjects

tetO-H3.3-HA mice were created using a mouse-codon optimized H3.3-HA sequence from Geneart (Life Technologies Grand Island, NY) cloned into the mm400 plasmid [61, 186]. The plasmid was cut using Not1, the proper band was gel excised and sequenced to ensure it was mutation-free. The gel-extracted DNA was submitted to the Transgenic and Chimeric Mouse Facility at UPENN, where it was injected into C57BL/6J mouse eggs for the production of transgenic mice. Mice were genotyped using the following primers: F: 5'-GCGTCCATCTGGTCAGAAA-3', R: 5'-TGGAATCTCAGGTCGGTCTT-3'. 4 pups expressing the tetO-H3.3-transgene were obtained and tested for expression. All tested lines expressed the transgene, but only 3 lines appeared to have a typical CaMKIIα pattern. Line 52 was chosen for future experiments based on high expression and good breeding. All mice were on a 12hr light/ 12hr dark schedule (lights on 7AM) with food and water available *ad libitum*. All procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Immunohistochemistry

All mice were bred in breeding cages with doxycycline food. At weaning, some mice were removed from doxycycline while others remained on doxycycline food. Mice were transcardially perfused with 4% paraformaldehyde in PBS, postfixed overnight, and cryoprotected in 30% sucrose for 2 days. Brains were flash frozen in 2-methylbutane on dry ice and 30 μM coronal sections were prepared using a cryostat. Free floating sections were permeabilized using 0.1% Triton X-100 (TX-100) in PBS for 5 min and autofluorescence was quenched by addition of 1% H2O2 in PBS 15 min at room temperature. Sections were blocked in 8% normal goat serum (NGS) with 0.3% TX-100 for 50 min at room temperature and washed 3x in PBS for 5 min each. Sections were incubated with the HA antibody (1:500, Roche Clone 3F10) in 2% NGS and 0.3% TX-

100 by rocking overnight at 4°C. After washing, sections were incubated in PBST containing biotinylated goat anti-rat (1:1000, Jackson ImmunoResearch # 112-065-003) for 2 hrs at room temperature. Sections were washed again, and incubated in ABC solution (Vector #PK-4000) for 1.5hrs at room temperature. Sections were washed again and incubated in 2 ml DAB solution (0.2 mg/ml). 100 ul of 0.1% H2O2 was added and sections were incubated for 20 min to complete staining. Sections were mounted, dried overnight, coverslipped and visualized using a light microscope.

Immunofluorescence

Immunofluorescence was performed as described for immunohistochemistry to the primary antibody stage. The following primary antibodies were used: HA, CaMKIIα (1:1000 Santa Cruz #sc-32288), GFAP (1:1000 Millipore#MAB3402X [alexafluor 488 conjugated]), Pvalb (1:1000 Abcam #ab11427) and rocked in the dark overnight at 4°C. After washing, the following secondary antibodies were used: Goat anti-rat Alexa Fluor 555 for HA (1:1000 Invitrogen #A-21434), goat anti-mouse Alexa Fluor 488 for CaMKIIα (1:1000 Invitrogen#A-11001), and goat anti-rabbit Alexa Fluor 488 for Pvalb (1:1000 Invitrogen#A-11034). 10 drops of DAPI (Life Technologies #R37606) were added at this time as well and sections were incubated in the dark at room temperature for 2hrs. After washing, cells were slide mounted, dried overnight in the dark, coverslipped using permafluor, and dried at 4°C for two days. Images were collected using a Leica Widefield Microscope at the CDB Microscopy Core at UPENN.

Chromatin immunoprecipitation (ChIP)

Mice underwent cervical dislocation and hippocampal dissection. Hippocampi were finely chopped using a razorblade and incubated in 2% PFA for 10 min at room temperature.

100µl 1M glycine was added to quench the reaction, and crosslinked tissue was washed 3X in PBS with protease inhibitors. Crosslinked tissue was then frozen at -80°C. After thawing on ice, tissue was dounce homogenized in 1 ml ChIP cell lysis buffer (10 mM Tris-HCl pH 8.1, 10 mM NaCl, 3 mM $MgCl_2$, 0.5% NP-40), centrifuged at 5500g and the supernatant was removed. The pellet was redissovled in 200 µl ChIP nuclear lysis buffer (50 mM Tris-HCl pH 8.1, 5 mM EDTA, 1% SDS) by pipetting. The samples were transferred to TPX tubes (Diagenode, Denville, NJ) and left on ice for 10 min. Samples were sonicated using a Bioruptor sonicator (Diagenode) with two 15 minute cycles of 1min on, 1.5min off. Samples were centrifuged at max speed for 5 min at 4°C and the supernatant was collected. 50 µl of chromatin was used per IP and 5 µl of chromatin was set aside for input. 2 µg HA antibody (Roche Clone 3F10) and 430 µl ChIP dilution buffer (16.7 mM Tris-HCl pH 8.1, 1.1% TX-100, 0.01% SDS, 167 mM NaCl, 1.2 mM EDTA) was added to each IP and rocked overnight at 4C. 100 µl of a 50% slurry of protein G beads was added and rocked for 2 hr. Beads were then washed for 5 min at 4°C in the following order: Low salt buffer (0.1% SDS, 1% TX-100, 2mM EDTA, 20 mM Tris-HCl pH8, 150 mM NaCl), High salt buffer (0.1% SDS, 1% TX-100, 2 mM EDTA, 20 mM Tris-HCl pH8, 500 mM NaCl), LiCl buffer (1% NP-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl pH8, 250 mM LiCl) and twice in 1X TE buffer. DNA was eluted using 200 µl ChIP elution buffer (1% SDS/0.1M NaHCO3) at room temperature, 8 µl 5M NaCl was added to each sample, and reverse crosslinking was performed overnight at 65°C. The next day, 4 µl 0.5M EDTA, 8 µl 1M Tris-HCl pH 7.5 and 1 µl proteinase K were added to each sample and incubated for 1 hr at 55°C. The Qiagen Qiaquick PCR Purification Kit was then used to clean up DNA and elution was performed using 200 µl water.

Quantitative PCR

All reactions were performed on 384 well plates and run using a Viia7 Real Time PCR System (Life Technologies). Each 5 µl reaction contained 2.5 µl Fast SYBR Green Master Mix (Life Technologies #4385614), 0.25 µl 5 µM primer mix and 2.25 µl ChIP or input DNA. The following primers were used: Nr4a1 F: 5'-GGAGCCTAGTGGGTCTGGAAGC-3' R: 5'- GGAGCGCGGATTGTTTGATCT-3', Nr4a2 F: 5'-GGGCTTGGGGGCGATGGTTC-3' R: 5'-AGGATCCGGCAACAGGTGCG-3', Nr4a3 F: 5'- GAGGGAGGAGGAGGGTGACGTA-3' R: 5'-CATAGAGTGCCTGGAATGCGAGA-3' SNAP25 F: 5'- AGCCCCGGGCAAACAACTCG-3' R: 5'- TTGGGTTTGCAGGGCTAGGGC-3', GFAP F: 5'-GCTGTTCCCTCGGCCCTCTCT-3' R: 5'-CACCAGCCTGGCTTCGCCAT-3', Olig2 F: 5'-AGGGAGTGGGGGCCTTCTGC-3' R: 5'-CCTCCTGTTTCCCGCTGCCG-3', Apcs F: 5'-AGACCCAGCTGCAGAATGGAGA-3' R: 5'- TGCTGGGAAGGGAAGAGCTGC-3', Fgb F: 5'- ACGAGACCTCCGAGACAGGGC-3' R: 5'- TGTGGACACAGGGGGTTCCTCG-3', Line1 F: 5'-AAACGAGGAGTTGGTTCTTTGAG-3' R: 5'-TTTGTCCCTGTGCCCTTTAGTGA-3', SNAP25 5' F: 5'-CAGCAGCCTCCATGCCCCAC-3' R: 5'- CTGAGCTCCCGCCATCGCAC-3', SNAP25 3' F: 5'- ACGCATGCTCAGTATTGGGACACT-3' R: 5'-ACACAGCTGCAGGTTTTGCTGGT-3', SNAP25 TES F: 5'-TCACACCAGAAAACACAGTCTGCAT-3' R: 5'- ACCAAGCCAAAGTGTCCATTGTCAT-3', Nr4a1 Exon1 F: 5'- TCTGGACGCACCCGTGACCT-3' R: 5'-CCCTCGCTGCCACCTGAAGC-3', Nr4a1 TES F: 5'- GGACAGCGGCTAACCCAGGGA-3' R: 5'- ACCTGAGACCCAAGGCCAGGTC-3'. Data were normalized as percent input and compared to get fold input of H3.3-HA expressing mice to non-expressing mice (%Input Double Transgenic/%Input Single Transgenic).

Results

Strategy for isolation of active chromatin from excitatory neurons

We created mouse lines expressing HA-tagged histone H3.3, which serves as a "barcode" for actively transcribed genomic regions [184, 185], under the control of the tetracycline transactivator (tTA) system to drive expression in specific cell types. Standard chromatin immunoprecipitation (ChIP) to isolate the tagged histone H3.3 will enable us to enrich chromatin at actively transcribed genes specifically from the cells of interest (**Figure A.1**).

Expression of the tagged H3.3 is limited to excitatory neurons in adulthood

Because tTA can be suppressed by doxycycline, we raised pups on a doxycycline diet until weaning and removed them to standard chow at that time. This should allow for expression of H3.3-HA to be restricted to adulthood. We tested this by immunohistochemistry for the HA tag of a mouse removed from doxycycline at weaning (postnatal day 28) and perfused at 2 months of age compared to a mouse left on doxycycline until 2 months of age (**Figure A.2**). Mice weaned onto standard chow showed highest transgene expression in the dentate gyrus, but also expression in CA1 and CA3. Mice that remained on doxycycline diet show no transgene expression. We then wanted determine if this expression was specific to excitatory neurons expressing CaMKIIα. We used immunofluorescence to compare the overlap between the HA tag on H3.3 to CaMKIIα, GFAP (a marker of astrocytes), and Pvalb (a marker for inhibitory neurons) (**Figure A.3**). As expected, we found that H3.3-HA was restricted to CaMKIIα

cells and was not expressed in glia or inhibitory neurons. This suggests that ChIP for the HA tag should selectively isolate only chromatin from excitatory neurons.

Chromatin immunoprecipitation of H3.3-HA isolates active regions of the excitatory neuron genome

Because H3.3 is known to incorporate into regions of active transcription, we anticipated that ChIP for the HA tag would enrich for regions around genes that are actively transcribed in excitatory neurons. Indeed, we found that ChIP for H3.3-HA from whole hippocampus enriches for promoters of genes active in excitatory neurons over those active specifically in glia or other tissues (**Figure A.4**). We also wanted to test whether this enrichment was particular to promoter regions of the genes or whether H3.3-HA incorporated throughout the gene body. We found that gene promoters had the highest proportion of H3.3-HA incorporation (**Figure A.5**). These data suggest that this method of chromatin sorting will isolate promoters of expressed genes selectively from excitatory neurons for further study.

Discussion

Although previous attempts have been made to combine sorting with epigenetic analysis in the brain [175], these attempts relied on FACS sorting that has the potential to distort results [183]. FACS sorting relies on harsh cell separating techniques that could alter chromatin state. In addition, crosslinking is necessary to preserve signal for FACS, so antibodies which work better with native ChIP [189] cannot be used. Our goal was to provide a method of chromatin sorting that relies only on standard chromatin

immunoprecipitation. This would dramatically improve the ability of researchers to discover epigenetic modifications that occur in specific subpopulations of cells in the brain. To this end, we produced a mouse model expressing an HA-tagged histone H3.3 specifically in excitatory neurons. Histone H3.3 has two major advantages that make it a great tool for studies in the brain: First, H3.3 can incorporate into chromatin outside of cell replication, which is crucial in post-mitotic neurons. Second, H3.3 preferentially incorporates into active regions of chromatin [184, 185] and therefore will isolate the chromatin most likely to be altered during learning. Because transcription is required for learning [108], we would expect actively transcribed regions of the genome to show the largest changes in histone alterations.

This tool is particularly exciting because of the use of histone H3.3, which preferentially occupies actively transcribed regions of the genome in cell [184, 185] and neuronal culture [190]. However, there have been few studies investigating where histone variants are located in the neuronal genome [160]. Thus, our experiments promise to uncover new mechanisms of gene regulation in neurons and will enable us to probe for specific histone modifications within active regions of the genome. Immunoprecipitation of the HA tag would be expected pull down nucleosomes specifically from active regions of the excitatory neuron genome, which can then be studied for histone modification changes. This would be particularly useful for transient modifications such as histone acetylation, which can be easily altered and may not survive FACS sorting.

We found that this mouse model does indeed express only in excitatory neurons and that ChIP for H3.3-HA enriches for promoters of genes active in excitatory neurons. Therefore, we believe this will be an important tool for studying histone modifications that

occur in the hippocampus in response to learning. Our goal is to combine this approach with mass spectrometry for combinatorial histone modifications [171-173] to unveil the histone code [30] that regulates learning in active regions of excitatory neurons.

As expected from previous research in our lab using the tTA system [191, 192], we were able to restrict expression to adulthood using doxycycline prior to weaning. H3.3-HA expression at 2 months of age is higher in the dentate gyrus than CA1 neurons, which are commonly studied in memory [51, 52, 108, 141]. This may be due to the dentate gyrus being a region of active neurogenesis or simply a result of the region of transgene insertion being more open in the dentate. Although CA1 is more commonly studied, the dentate gyrus shows histone acetylation differences of a similar magnitude [53] and therefore is likely to use the same histone code to regulate transcription. We found no overlap with glia or inhibitory neurons, indicating this tool should be able to isolate changes in excitatory neurons selectively.

Most importantly, we found that ChIP for H3.3-HA isolates promoters of genes active in excitatory neurons. This isolated chromatin could be studied in a number of ways. ChIP-seq could be used to determine whether H3.3 localization changes in response to stimuli. ChIP for the HA tag followed by mass spectrometry could be used to study all histone modifications at once and determine the code that changes in response to learning to regulate expression of these active genes. Finally, sequential ChIP could be used to study localization of a particular modification only at active regions of the excitatory neuron genome. We anticipate that this tool will greatly advance epigenomic analysis in the brain in response to learning. Also, because of the modularity of the tTA system, any tTA line can be crossed with tetO-H3.3-HA to drive expression in different cell types. GFAP-tTA could be used to drive expression in glia [193], Fos-tTA in recently

activated cells [194], or Myh6-tTA in cardiac myocytes [195]. Therefore, this tool has broad implications in epigenomic analysis of any tissue that displays cellular heterogeneity.

Figure Legends

Figure A.1. Using a tagged histone H3.3 as a novel epigenetic tool to sort chromatin. A) HA-tagged H3.3 is controlled by the tetO promoter, which is only active in the presence of tTA. Using CaMKIIα-tTA gives expression only in forebrain excitatory neurons. B) The tagged histone approach allows standard chromatin immunoprecipitation to select chromatin only from the cells expressing the construct and does not rely on harsh sorting techniques. C) Depiction of the "H3 barcode hypothesis", which states that H3 isoforms serve as long-term marks to regulate activity at genomic regions. H3.3 incorporates at active sites of the genome.

Figure A.2. Doxycyline suppresses expression of H3.3-HA. Both mice were kept on doxycycline until weaning and perfused at 2 months of age, but one mouse was removed from doxycycline at weaning while one remained on doxycycline. Expression (brown staining) only occurs in the mouse removed from doxycycline, indicating that expression can be restricted to adulthood (post-weaning). Expression is higher in dentate gyrus than CA1. Cresyl violet (purple) was used as a counterstain to show cell layers.

Figure A.3. Expression of H3.3-HA is limited to excitatory neurons. Each panel is shown as green channel only (top), red channel only (middle) and overlap (bottom) A) There is nearly complete overlap (yellow) of H3.3-HA (red) and CaMKIIα (green) as expected. B) There is no overlap (yellow) between H3.3-HA (red) and astrocyte marker GFAP (green). C) There is no overlap (yellow) between H3.3-HA (red) and inhibitory

neuron marker Pvalb (green). Pvalb positive cells appear to have processes surrounding excitatory neurons, but are not within those cell bodies.

Figure A.4. ChIP for H3.3-HA enriches for promoters of genes active in excitatory neurons. ChIP was performed for the HA tag of H3.3-HA from whole hippocampus and primers against promoters from various genes were tested. On average, neuronally expressed genes (red) were 6.8x higher than genes expressed in glia (blue), 13.4x higher than liver-specific genes (yellow) and 18x higher than genes not expressed in all cell types (green). Data are presented as fold ChIP enrichment over a single transgenic mouse that does not express H3.3-HA. N=3 mice were used and error bars represent s.e.m.

Figure A.5. ChIP for H3.3-HA enriches for promoters over gene bodies. To determine whether this enrichment was particular to promoters, we tested primers throughout two neuronally expressed genes. Enrichment is biased toward the promoter of both genes tested. TES=transcripition end site, 5' = 5' UTR, 3'= 3' UTR.

Contributions

This chapter was written by Shane G. Poplawski with suggestions by Ted Abel. Experiments were performed by Shane G. Poplawski, Anna McNally and Brittany Mayweather.

Figures

Figure A.1

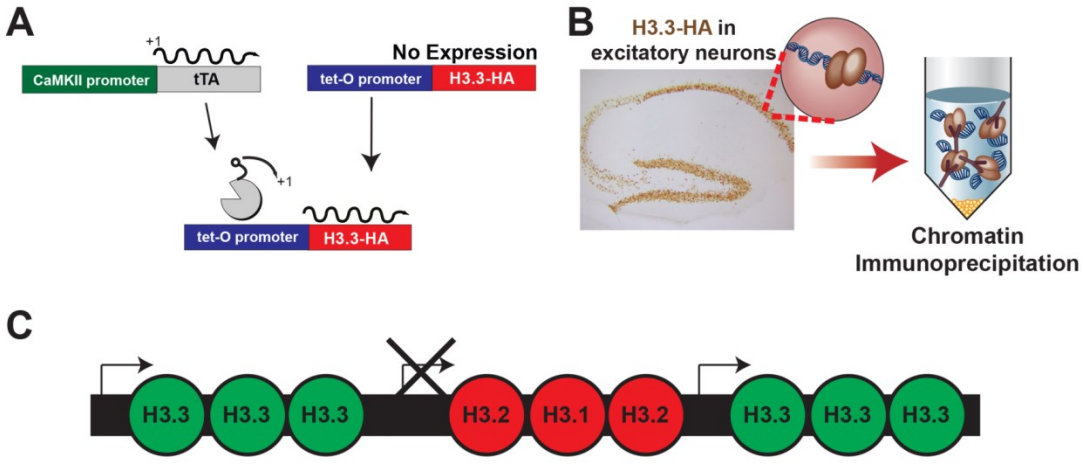


Figure A.2

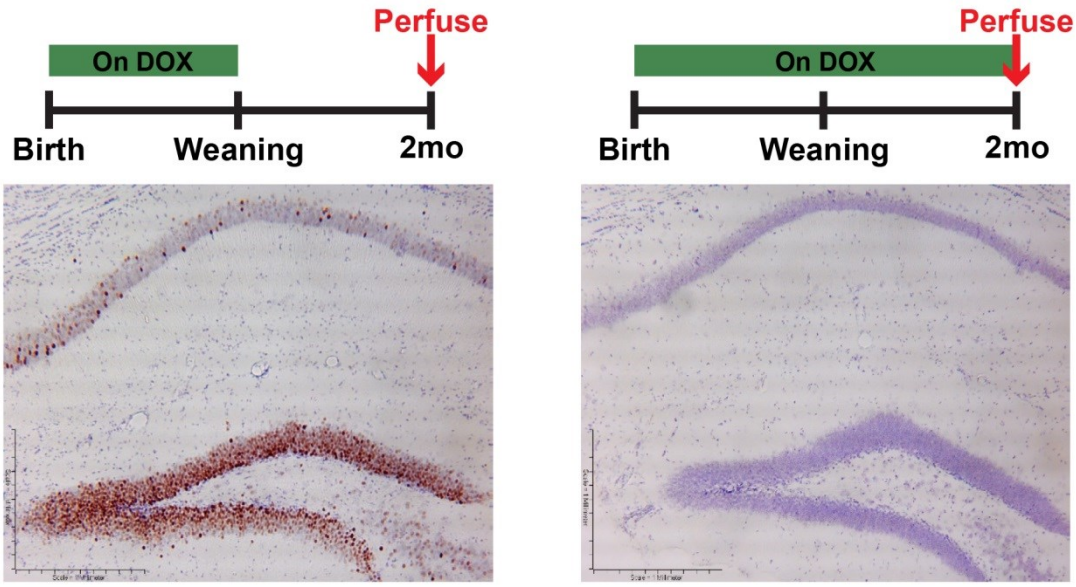

Perfuse
On DOX
Birth
Weaning
2mo
Perfuse
On DOX
Birth
Weaning
2mo

Figure A.3

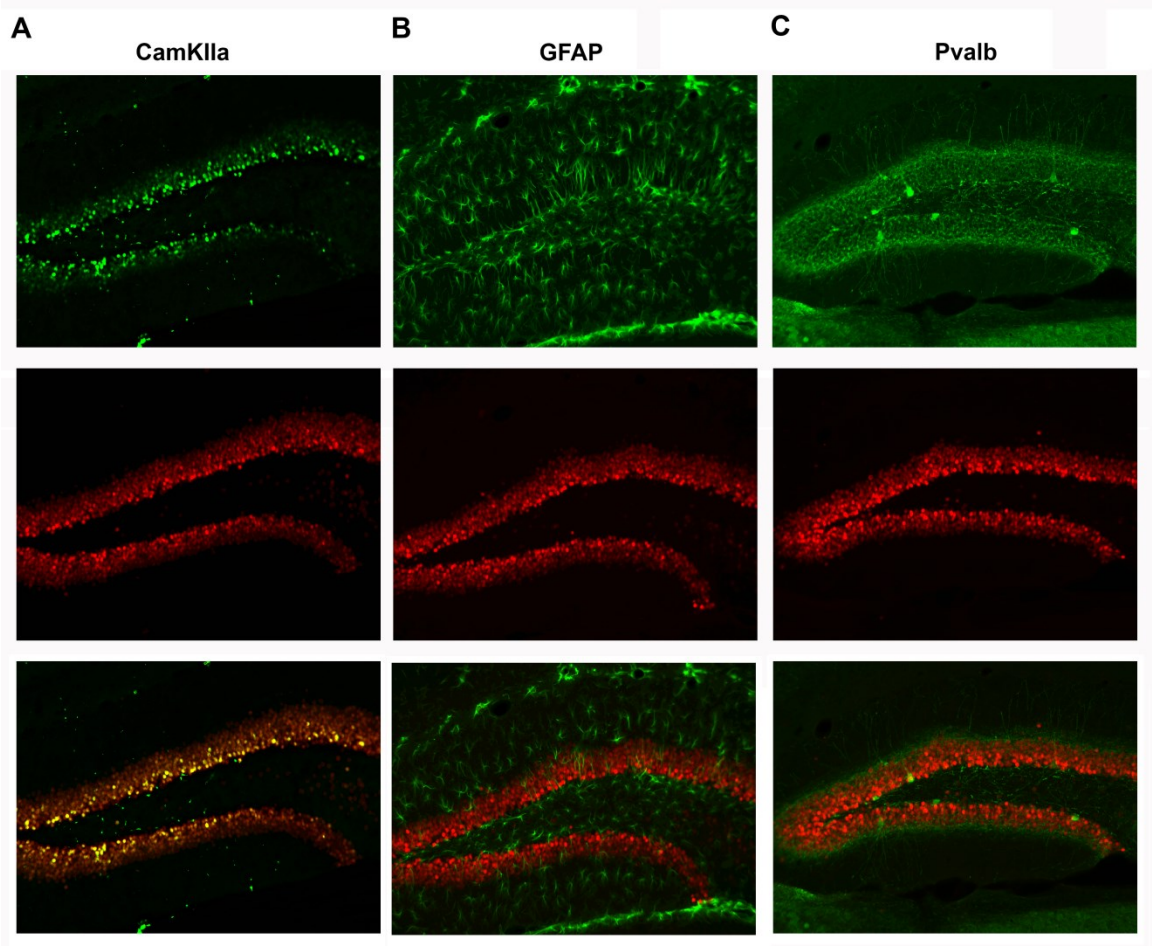


Figure A.4

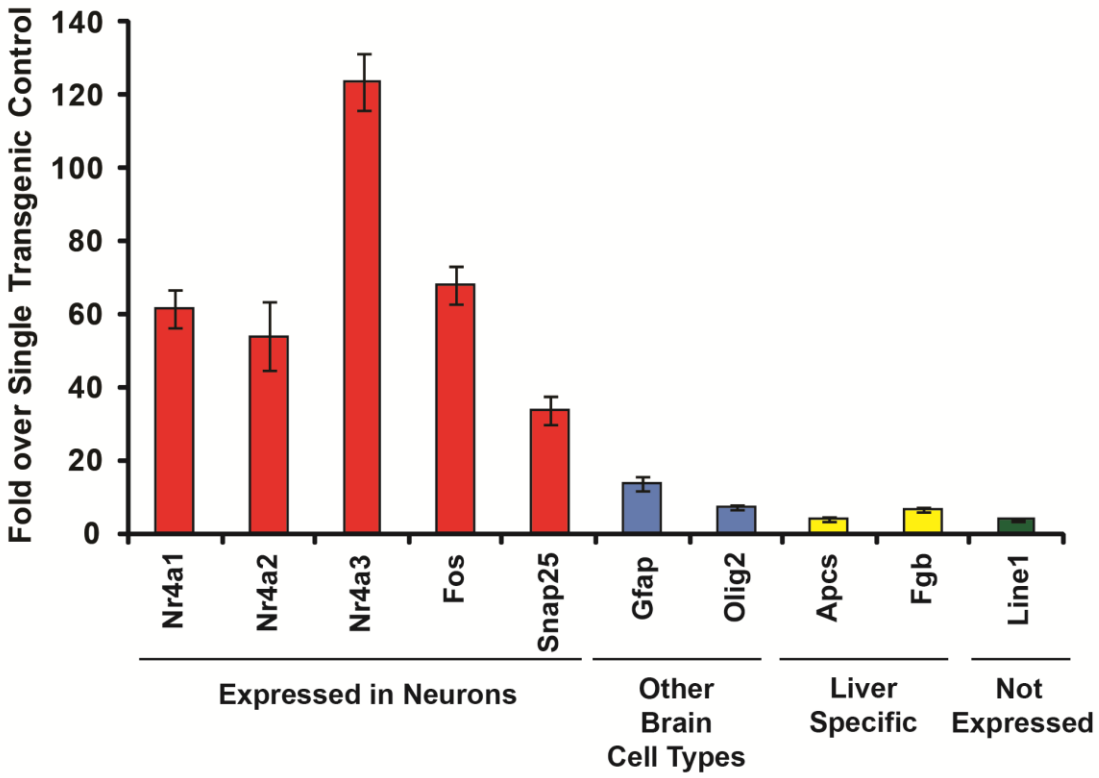
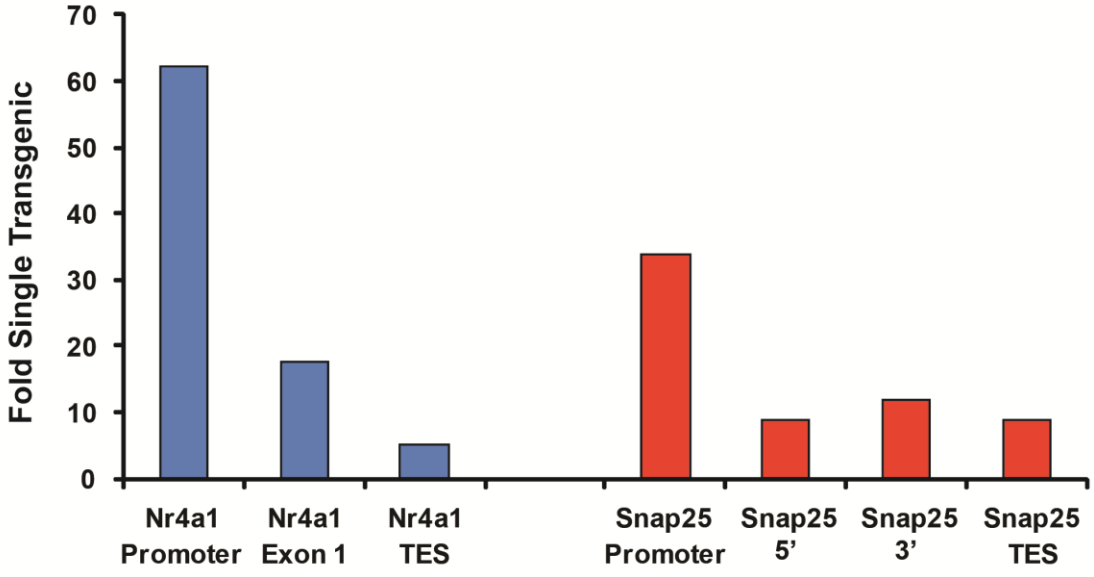

Fold over Single Transgenic Control
140
120
100
80
60
40
20
0
Nr4a1
Nr4a2
Nr4a3
Fos
Snap25
Gfap
Olig2
Apcs
Fgb
Line1
Expressed in Neurons
Other Brain Cell Types
Liver Specific
Not Expressed

Figure A.5


Fold Single Transgenic
70
60
50
40
30
20
10
0
Nr4a1 Promoter
Nr4a1 Exon 1
Nr4a1 TES
Snap25 Promoter
Snap25 5'
Snap25 3'
Snap25 TES

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