

# Epigenetic Regulations, Motif and Pathway Identification of Gabaergic Neurotransmitter's Chip Sequence

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**Abstract. Background:** The next generation techniques has revolutionalized the biology era. In this work we are using Chromatin immunoprecipitation (ChiP) technique on the ChIP–seq of Gamma-Amino Butyric Acid (GABA). GABA is a brain-made chemical. It also found in some foods. The effect of GABA in brain is anti-seizure and anti-anxiety. GABA blocks the brain signals, the neurotransmissions and hence there is interest in using GABA as a dietary supplement. But it is taken by mouth, it is able to cross the blood-brain barrier. Hence the effects of GABA supplements on the brain is unknown.

**Methodology:** GABA is implicated in several neurological disorders like alzheimers, rett symdrome, schizophrenia, etc. In this work, the chip sequence of GABA is retrieved from SRA database, removed the PCR duplicates and finally identified the genome enriched region using the MAC2 call peak tool.

**Results:** Peak table was annotated to identify the genes and the corresponding pathways was identified from the KEGG pathway. This annotated peak table was used to identify the motifs present in the chip-seq of GABA.

**Keywords:** Chromatin immunoprecipitation (ChiP)  $\cdot$  Gamma-Amino Butyric Acid (GABA)  $\cdot$  Neurotransmission  $\cdot$  PCR duplicates  $\cdot$  MACS2 callpeak  $\cdot$  ChiP peak  $\cdot$  KEGG pathway  $\cdot$  motif

### 1 Introduction

The advent of Next-generation sequencing (NGS) techniques is utilized in divergent technique research to make an important assortment features of chromatin biology by categorizing genomic loci that are bound by transcription features, occupied by nucleosomes loci [1]. In Chip-seq analysis mapping of the DNA-protein interfaces occurs at very high decree and hence it gives proactive quality-control and appropriate dataset analytics which help in take important decisions resulting in meaningful analysis. This High resolution NGS technique resolves the way for genome-wide profiling and



Fig. 1. The GABA pathway [7]

tremendous progress of DNA-binding proteins, histone modifications, or nucleosomes [2–4].

We have taken neurotransmitter Gamma-Amino Butyric Acid (GABA)ergic ChIP sequence for our present study. The long-term stability and function of neuronal networks is dependent on a maintained balance between excitatory and inhibitory synaptic transmission that functions as the primary inhibitory neurotransmitter by inhibiting nerve transmission and reduces neuronal excitability in the central nervous system (CNS) (Fig. 1) [5, 6].

The GABA released or liberated from presynaptic terminals mediate the GABAergic signaling, through a specific actions by the neurotransmission of GABA. This happens by using a class of GABA transporters (GATs) which is followed by the actions of GABA which plays a very important and very a crucial role in controlling both neuronal inhibitory and excitatory states, as well as in wide range of developmental with behavioral patterns in the brain [8–10]. Any fluctuations in GABA and GABAergic signaling are implicated in various neurological disorders like autism spectrum disorder (ASD), Rett's syndrome, Prader-Willi Syndrome (PWS) and schizophrenia (SZ) [11, 12].

#### 2 Materials and Methods

The primary aim of the current research is to provide a detailed overview of epigenetic regulations of GABAergic neurotransmission and throw a light on the pathways involved. Galaxy tutorial by Lauren Mills, Analyzing ChIP-Seq Data in Galaxy is used to analyze *Mus musculus* GABAergic CB1-KO sequence having SRA accession number SRR13960849 and SRR13960850 from SRA database taken for this work. We mapped the reads using Bowtie2 [13, 14]. Using Collect Alignment Summary Metrics tool [15] we take the summary of our alignment. Next using RmDup [16] we remove PCR duplicates and Collect Alignment Summary Metrics tool was re-run. Finally, using MACS2 callpeak [17, 18] we identify peaks from alignment results. Using Peak calling we identify areas in our genome that have been enriched with our aligned reads, these areas are those where protein interacts with DNA [19].

Next, we annotate our peaks table to take top 100 most significant peaks and identify the genes overlapping with these peaks. The pathways of these genes were identified from KEGG pathways. Finally, we identify the motifs using SeqPos motif analysis tool [20]. Biological sequence motifs are short conserved sequence pattern associated with distinct functions that usually represents important structural or functional features [21].

#### **3** Results and Discussion

*Mus musculus* chip-sequences with SRA accession number SRR13960849 and SRR13960850 were mapped using *Mus musculus* ref seq using Bowtie2. The Bowtie2 mapping output is given in Fig. 2 for SRR13960849 and Fig. 3 for SRR13960850.

We use the tool Collect Alignment Summary Metrics tool take the summary of our mapping done above. Table 1 contains the alignment summary for SRR13960849 and Table 2 for SRR13960850.

Next, we removed the PCR duplicates using RmDup. Table 3 & 4 contains the Alignment summary for SRR13960849 and SRR13960850 and removing PCR duplicates.

As per the alignment summary (Table 3, 4), we see that the reads are less post RmDup which implies that the duplicate reads are removed. Next, we use MAC2 call peak tool to identify areas in the genome that are enriched with the aligned reads. Model-based Analysis of ChIP-seq (MACS) is a commonly used tool for identifying transcription factor binding sites. The algorithm confines the influence of genome complexity to evaluate the significance of enriched ChIP regions. This tool improves the spatial resolution of binding sites by combining the information of both sequencing tag position and orientation [22]. Here, MACS is used along with a control sample (SRR13960849) which increases specificity of the peak calls (Fig. 4). MACS2 models the distance between the paired forward and reverse strand peaks and uses 1000 enriched regions to model the distance between the forward and reverse strand peaks [23].

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Fig. 2. Mapping output for SRR13960849

The cross-correlation metric diagram given in Fig. 5 is worked out as the Pearson's linear correlation joining the Crick strand and the Watson strand and this metric standard usually produces two peaks when this cross-correlation is plotted against the shift value, one corresponding to the read length ("phantom" peak) and the other average fragment length of the library.

The absolute and relative height of the above said peaks are useful in determining of the success of any ChIP-seq experiment. A high-quality immunoprecipitation is characterized by a ChIP peak that is which should be higher than the "phantom" peak and very small or no ChiP peak is seen in failed experiments. Our results show high and only ChiP peak and no "phantom" peak which clearly says our results contain a high-quality immunoprecipitation [17].

Next, from the annotation results our peaks table by taking top 100 most significant peaks, we identify the genes overlapping with these peaks which are given in Table 5. The pathways of these genes were identified from KEGG pathways (Table 6).

Finally, we identify the motifs present in our GABA ChiP-seq. We used used SeqPos motif analysis tool. The file, top 100 most significant peaks in bed format was selected for motif identification (Table 7, 8).

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Table 1.	Alignment	summary	for	SRR	3960849
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CATEGOR Y	Т	OTAL_REAI S	)	PF_REA S	D	PF_READS_ D	ALIC	GNE	PCT_PF	_F	READS_ALIGN ED		PF_ALI	GNED_BAS S	E	PF_HQ_MEDIA E	AN S	MISMATCH
UNPAIRE D		43468872		434688 2	7	404420	)44		0	. 9	930368		2060	0634150		0.00	37	136
						1												
PF_MISMAT RATE	CH	PF_H RATE	)_F	ERROR_	P A	F_INDEL_R TE	ME NG	CAN_R STH	EAD_LE		READS_ALIGNE PAIRS	ED.	_IN_	PCT_REA _PAIRS	NDS.	_ALIGNED_IN		PCT_ADAP TER
0.000501		51			0		0				0			0				0.000008

CAT EGO RY	TOTAL_R EADS	PF_READ S	PF_READ S_ALIGN ED	PCT_PF_ READS_A LIGNED	PF_ALIGNE D_BASES	PF_HQ_M EDIAN_M ISMATCH ES
UNP AIR ED	4330991 7	4330991 7	4025183 0	0.92939 1	205084987 1	0

PF_MISMATCH_	PF_HQ_ERROR_	PF_INDEL_R	MEAN_READ_LE	READS_ALIGNED_IN_	PCT_READS_ALIGNED_IN	PCT_ADAP
RATE	RATE	ATE	NGTH	PAIRS	_PAIRS	TER
0.005489	0.003488	0.000524	51	0	0	0.000009

CAT EGO RY	TOTAL_R EADS	PF_READ S	PF_READ S_ALIGN ED	PCT_PF_ READS_A LIGNED	PF_ALIGNE D_BASES	PF_HQ_M EDIAN_M ISMATCH ES
UNP AIR ED	1713059 4	1713059 4	1410376 6	0.82330	718686483	0

#### Table 3. Alignment summary for SRR13960849 post RmDup

Γ	PF_MISMATCH_RA	PF_HQ_ERROR_RA	PCT_PF_READS_IMPROPER_PA	BAD_CYCLE	STRAND_BALAN	PCT_CHIMER	PCT_ADAPT
	TE	TE	IRS	S	CE	AS	ER
	0.005145	51	0.003344	0.000468	0.495284	0	0.00002

 Table 4.
 Alignment summary for SRR13960850 post RmDup

CAT EGO RY	TOTAL_R EADS	PF_READ S	PF_READ S_ALIGN ED	PCT_PF_ READS_A LIGNED	PF_ALIGNE D_BASES	PF_HQ_M EDIAN_M ISMATCH ES
UNP AIR ED	1772462 3	1772462 3	1466653 6	0.82746 7	747351950	0

PF_MISMATCH_RAT	PF_HQ_ERROR_RAT	PF_INDEL_RAT	MEAN_READ_LENGT	STRAND_BALANC	PCT_CHIMERA	PCT_ADAPTE
E	E	E	H	E	S	R
0.005025	0.003231	0.000477	51	0.49489	0	0.000022



#### Peak Model

Fig. 4. Peak Model in graphical format



Fig. 5. Cross-Correlation Metric

1.	NM_001161362
2.	NM_011245
3.	NM_001357743
4.	NM_133733
5.	NM_001284328
6.	NM_019796
7.	NM_001311113
8.	NM_001113180
9.	NM_019691
10.	NM_033607
11.	NM_008927

Table 5. Genes identified



Table 6. Pathways for the genes identified

(continued)

 Table 6. (continued)



 Table 7. Motifs identified [24]

clusters	collapsed_id	factor	DNA binding	domain	hits	cutoff	zscore	-10*log(pval)	similarity to to	p mean_position
1	denovo18		None		41	5.096	-3.905	99.63		-0.192
2	denovo3		None		44	5.553	-3.152	71.169		-0.159

**Table 8.** Motifs detected [24]

collapsed_ id		hits	cutoff	z-score	-10*log(pval)	mean_position
DENOVO 18	id: demovel 8 factors in the second s	41	5.096	- 3.095	99.63	-0.192
DENOVO : 3	id. denova3 factors DBD Nene denova3 statistics DBD Nene denova statistics DBD Nene denova Statistics DDD Nene denova Statistics DDD Nene denova Statistics DDD Nene denova Statistics DDD Nene denova Statistics DDD Nene denova Statistics DDD Nene denova Statistics DDD Nene denova Statistic	44	5.553	-3.1552	71.169	-0.159

### 4 Conclusion

A high-quality immunoprecipitation ChIP peak is detected in our results which imply high concentration of DNA binding proteins. Further, top DNA binding protein genes were identified and corresponding pathways was identified from KEGG pathway. The mRNA surveillance pathway, MAPK signaling pathway, cAMP signaling pathway and EGFR tyrosine kinase inhibitor resistance were detected. Further, motifs were detected from the ChIP peak.

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