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Full Length Research Paper

In silico identification of miRNAs and their target prediction from *Japanese encephalitis*

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MicroRNA is a family of small non-coding RNAs that regulate gene expression in a sequence-specific manner. miRNAs are a class of post-transcriptional regulators. miRNAs are a family of 19 to 25 small nucleotide RNAs. Since miRNAs have been discovered and their role in gene regulation established, it has been theorized that viruses could generate miRNAs as well and that these viral encoded miRNAs could regulate cellular mechanisms and viral replication. There are several lines of evidence to support this theory. *Japanese encephalitis* is a viral disease (Flavivirus) but its geographic strains differ by RNA sequencing neurotropic virus that primarily affects the central nervous system. That is why this work mainly focuses on finding out its function. An oligonucleotide drug candidate can be designed against this virus. Computational prediction is analyzed and estimation of evolutionary relationship among types of organism is done in this project. 25 precursors and eight potential miRNAs were found, and these miRNAs target 123 target sites in 13 genes in human.

Key words: miRNA, pre-miRNA, pri-miRNA, *Japanese encephalitis,* precursor potential miRNA, +target prediction.

INTRODUCTION

Mature microRNAs (miRNAs) are a class of naturally occurring small non-coding RNA molecules; about 21 to 25 nucleotides in length. MicroRNAs are partially complementary to one or more messenger RNA (mRNA) molecules, and their main function is to down- regulate gene expression in a variety of manners, including translational repression, mRNA cleavage and deadenylation. They were first described by Lee Y et al., 2003, 2004) and the term microRNA was coined in 2001. The genes encoding miRNAs are much longer than the processed mature miRNA molecule (Carthew et al., 2009). Based on the biogenesis of miRNA, many miRNAs are known to reside in introns of their pre-mRNA host genes and share their regulatory elements, primary transcript and have a similar expression profile (Chen, 2005).

MicroRNAs are transcribed by RNA polymerase II as large RNA precursors called pri-miRNAs and comprise a 5' cap and poly-A tail (Erlanger et al., 2009). The pri-miRNAs are processed in the nucleus by the microprocessor complex, consisting of the RNase III enzyme Drosha and the double-stranded-RNA-binding protein, Pasha/DGCR8. The resulting pre-miRNAs are approximately 70-nucleotides in length and are folded into imperfect stem-loop structures (Hammond, 2000). The pre-miRNAs are then exported into the cytoplasm by the karyopherin exportin 5 (Exp5) and Ran-GTP complex Ran (ras-related nuclear protein), a small GTP binding protein belonging to the RNA superfamily that is essential for the translocation of RNA and proteins through the nuclear pore complex (Krek et al., 2005). The Ran GTPase binds Exp5 and forms a nuclear heterotrimer with pre-miRNAs; once in the cytoplasm, the pre-miRNAs undergo an additional processing step by the RNAse III enzyme dicer generating the miRNA, a double-stranded RNA approximately 22 nucleotides in length. Also, Japanese *encephalitis* virus (JEV), a neurotropic mosquito-borne flavivirus mainly prevalent in Asia,

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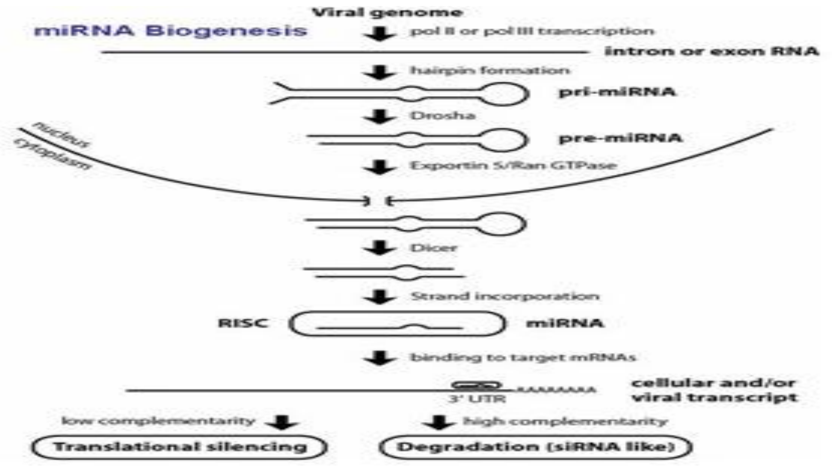

Figure 1. Biogenesis of miRNA.

is the most important causative agent of acute viral encephalitis in humans. JEV is also one of the main causes of infectious reproductive failure in swine,(Murkami et al.,2005), resulting in significant economic losses in the pig industry; this virus has a normal zootomic transmission cycle between swine or birds and mosquitoes. Swine are the main amplifier hosts, from which infected mosquitoes transmit the virus to human (Figure 1).

METHODOLOGY

The genome sequences of J. encephalitis were retrieved from NCBI (www.ncbi.nlm.nihgov).Precursor predicting tools, Mireval (http://tagc.univ mrs.fr/mireval) and MiRPARA (http://www.whiov.ac.cn/bioinformatics/mirpara) were used to find out the precursor sequences; and then the secondary structure with optimal minimum free energy was found out with the help of different types of web servers such as Mfold web server (http://mfold.rit.albany.edu/doc/mfold-manual/), RNA fold web server (http://rna.tbi.univie.ac.at) and DINA melt web server (http://www.microrna.gr/microT). For validating the energy values, miRBASE (mirbase@manchester.ac.uk) was checked, where we browsed all the mature virus sequences and stem loop structure sequences in FASTA format. These sequences were already experimentally identified. Then we find out the optimal minimum energy value of viruses in the miRBASE (only precursors), based on multiple sequence alignment, and then find out the conserved regions for miRNA. This miRNA predicted the target sites, and the target prediction was completed with the help of DIANA tar web server (http://www.microrna.gr); it predicted the different types of target sites.

RESULTS AND DISCUSSION

Precursor analysis

miRNA was the first to be discovered, a class of non-coding RNAs involved in gene regulation, transcribed as ~70 nucleotide precursors and subsequently processed by the dicer enzyme to give a 21 nucleotide product (Moore,1993). The extents of the hairpin precursors are not generally known and are estimated based on hairpin prediction (Ruykum, 1993; Siolas et al., 2005; Sui et al., 2002). This study is exclusively based on *in silico* firstly to find out the precursors from Japanese encephalitis having 1 to 25 precursors in a single whole genome (NC_001437.1); and then this sequence is submitted in miRevaluator and mirPARA software for precursor prediction (Table 1).

Potential miRNA analysis

Prediction of potential miRNA was based on (MSA) multiple sequence alignment (pair wise) and its alignment was completed with the help of EBI clastal-W and mirpara miRNA predicting software. The sequence was submitted in EBI which is retrieved from miRBases; and pair wise alignment was done with precursors of viruses and precursors of Japanese encephalitis (Kozomara et al., 2011). Mainly, MSA focused on conserved regions for miRNA (Table 2).

Target prediction analysis

Based on observation, potential miRNA predicted the different types of target sites in a human gene. Then it predicted the different type of genes and those involved in different type of target sites with conserved information (Tables 3, 4 and 5).

All the tables explain the different types of analyses related to *in silico* prediction only, and the whole information of targeted genes in humans and targeted miRNA of Japanese encephalitis. Table 5 gives important

Table 1. Predicted precursor miRNA from miREvaluator, mirPARA.

S.NO	Annotated precursor name	Precursors (sequences)	Length (nt)	Optimal secondary structure (MFE) value
1	>MIR _JE_P1	ATGCTGAAACGCGGCCTACCCCGCGTATTCCCACTAGTGGAGTGAAGAGGGTAGTAATGAGCTTGTTGGACGGCAGAGGGCAGTACGTTTCGTGCTGGCTCTTATCACGTTCTTCAAGTTTACAGCATTAGCCCCGAC	140	-65.46
2	>MIR_JE_P2	GGAGGAAATGAAGGCTCAATCATGTGGCTCGCAAGCTTGGCAGTTGTCATAGCTTACGCAGGAGCAATGAAGTTGTCAA	80	-22.75
3	>MIR_JE_P3	TAGCTGCTTGACAATCATGGCAAACGACAAACCAACATTGGACGTCCGCATGATTAACATCGAAGCTAGCCAACTTGCTG	80	-18.25
4	>MIR_JE_P4	GCTCTCCCCTGGACGTCCCCTTCGAGCACAGCGTGGAGAAACAGAGAACTCCTCATGGAATTTGAAGAGGCGCACGCCAC	80	-20.28
5	>MIR_JE_P5	GCATGCTGACACTGGATGTGCCATTGACATCACAAGAAAAGAGATGATGTGGAAGTGGCATCTTTGTGCACAACGACG	78	-21.14
6	>MIR_JE_P6	ACACACCCTTTGGGGAGATGGTGTTGAGGAAAGTGAACTCATCATTCCGCATACCATAGCCGGACCAAAAAGCAAGCACA	80	-21.93
7	>MIR_JE_P7	CTGCTGGTACGGAATGGAAATCAGACCTGTCAGGCATGAAACAACACTCGTTAGATCACAGGTTGATGCTTTCAATGGTGAAATGGTTGACCCTTTTCAGCTGGGCCTTCTGGTGATGTTTCTGGCCACCCAGGAGGTCCTTCGCAAGAGGTGGACG	160	-53.83
8	>MIR_JE_P8	TTGATGTTTGCCATCGTAGGTGGCTTGGCCGAGTTGGATATTGAATCCATGTCAATACCCTTCATGCTGGCAGGTCTCAT	80	-25.91
9	>MIR_JE_P9	CGAGGAACATCCGGCTCACCCATTCTGGATTCTAATGGAGACATCATAGGCCTATACGGCAATGGAGTTGAGCTTGGCGA	80	-25.13
10	>MIR_JE_P10	CTGGCGGTGTTTCTCATCTGCGTCTTGACCGTGGTTGGAGTGGTGGCAGCAAACGAGTACGGGATGCTAGAGAAAACCAA	80	-26.73
11	>MIR_JE_P11	GCAGCGTTCCTCGTCAACCCTAATGTCACCACTGTGAGAGAAGCAGGGGTATTGGTGACGGCGGCTACGCTCACTTTGT	79	-26.50
12	>MIR_JE_P12	TGAAAACAACATAGTGGGAGGACATCCGGTTTCGCGAGGCTCAGCAAAACTCCGTTGGCTCGTGGAGAAAGGATTTGTCTCGCCAATAGG	90	-31.52
13	>MIR_JE_p13	TCGGAGGTGGGCTAGTGCGTCTCCCCCTGTCCCGGAACTCCAATCACGAGATGTATTGGGTTAGTGGAGCCGCTGGCAATTGGTGCACG	90	-29.76
14	>MIR_JE_p14	GCTGGATGGAATGTGAAGGACACAGCTTGTCTGGCCAAAGCATATGCACAGATGTGGCTACTCCTATACTTCCATCGTAG	80	-28.10
15	>MIR_JE_p15	CTTCTGCTCTATCTCAACATCAGCTACTAGGCACAGAGCGCCGAAGTATGTAGCTGGTGGTGAGGAAGAACACAGGATCT	80	-31.85
16	>MIR_JE_p16	TAGTAATGAGCTTGTTGGACGGCAGAGGGCCAGTACGTTTCGTGCTGGCTCTTATCACGTTCTTCAAGTTTACAGC	76	-35.60
17	>MIR_JE_p17	AGGAGGAAATGAAGGCTCAATCATGTGGCTCGCAAGCTTGGCAGTTGTCATAGCTTACGCAGGAGCAATGAAGTTGTCGA	81	-21.75
18	>MIR_JE_p18	TCACAGGAAGGAGGCCTCCATCAGGCGTTGGCAGGAGCCACAAGCTCAGTGAAGTTAACA	60	-21.80
19	>MIR_JE_p19	TGGTTGACCCTTTTCAGCTGGGCCTTCTGGTGATGTTTCTGGCCACCCAGGAGGTCCTTCGCAAGAGGTGGACGGCCA	78	-32.90
20	>MIR_JE_p20	ACTATAGCTGCCGGACTAATGGTCTGCAACCCAAACAAGAAGAGAGGGTGGCCAGCCACTGAGTTTTTGTCGGCAGTT	78	-26.72
21	>MIR_JE_p21	GCGGTGTTTCTCATCTGCGTCTTGACCGTGGTTGGAGTGGGCAGCAAACGAGTACGGGATGCTAGAGAAAACCAAAGCA	80	-36.70
22	>MIR_JE_p22	AATGGTCGCCACTGATGTGCCTGAACTGGAAAGGACCACCCTCTGATGCAAAAGAAAGTCGGACAGGTGCTCCTCATAGGGGTAAGCGTAGCAGCGTTCC	100	- 24.10

Table 1. Contd.

23	>MIR_JE_p23	AGGACATCCGGTTTCGCGAGGCTCAGCAAAACTCCGTTGGCTCGTGGAGAAAGGATTTGTCT	62	-25.90
24.	>MIR_JE_p24	GGAGTGGTGAAGCTCATGAGCAAACCTTGGGACGCCATTGCCAACGTCACCACCATGGCCATGACTGACACCAC	78	-29.30
25.	>MIR_JE_25	CTGCTCTATCTCAACATCAGCTACTAGGCACAGAGCGCCGAGTATGTAGCTGGTGGTGAGGAAGAACACAGGATCAC	78	-28.30

Table 2. Precursor and sequence of pre-miRNA and sequence of potential miRNA.

S.NO	Annotated precursors name	Precursor (seq) pre-miRNA (precursor-microRNA)	Potential (miRNA) seq (microRNA)
1	>MIR _JE_P1	AUGCUGAAACGCGGCCUACCCCGCGUAUUCCCACUAGUGGGAGUGAAGAGGGUAGUAAUGAGCUUGUUGGACGGCAGAGGGCCAGUACGUUUCGUGCUGGCUCUUAUCACGUUCUUCAAGUUUACAGCAUUAGCCCCGAC	AUGCUGAAACGCGGCCUACCCC
2	>MIR_JE_P2	GGAGGAAAUGAAGGCUCAAUCAUGUGGCUCGCAAGCUUGGCAGUUGUCAUAGCUUACGCAGGAGCAAUGAAGUUGUCGAA	GGAGGAAAUGAAGGCUCAAUCAUG
3	>MIR_JE_P6	ACACACCCUUUGGGGAGAUGGUGUUGAGGAAAGUGAACUCAUCAUUCCGCAUACCAUAGCCGGACCAAAAAGCAAGCACA	ACACACCCUUUGGGGAGAUGGUGUU
4	>MIR_JE_P8	UUGAUGUUUGCCAUCGUAGGUGGCUUGGCCGAGUUGGAUUUGAAUCCAUGUCAAUACCCUUCAUGCUGGCAGGUCUCAU	UUGAUGUUUGCCAUCGUAGGUGGC
5	>MIR_JE_p16	UAGUAAUGAGCUUGUUGGACGGCAGAGGGCAGUACGUUCGUGCUGGCUCUUAUCACGUUCUUCAAGUUUACAGC	CUUGUUGGACGGCAGAGGGCCA
6	>MIR_JE_p18	UCACAGGAAGGAGGCCUCCAUCAGGCGUUGGAGGAGCCAUCGUGGUGGAGUACUCAAGCUCAGUGAAGUUAACA	GUGGUGGAGUACUCAAGCUCAGU
7	>MIR_JE_p19	UGGUUGACCCUUUUCAGCUGGGCCUUCUGGUGAUGUUUCUGGCCACCCAGGAGGUCCUUCGCAAGAGGUGGACGGCCA	UUUCAGCUGGGCCUUCUGGU
8	>MIR_JE_p20	ACUAUAGCUGCCGGACUAAUGGUCUGCAACCAACAAGAAGAGAGGGUGGCCAGCCACUGAGUUUUUGUCGGCAGUU	GCCAGCCACUGAGUUUUUGUCG

information of genes for easy understanding of targeted genes by miRNA of Japanese encephalitis.

Conclusion

Finally in this study, eight potential miRNAs were predicted *in silico* and 123 target sites were found in 13 genes. Out of these thirteen genes, VPS13B, NAV1, IQSEC2 and NTRK3 played an important role in the regulation of the central nervous system and its activity in human brain and body; it is shown that if it is damaged it could lead to brain fever and ultimately, death. So, if we can control the malfunction protein at the transcription level, we would be able to save patients suffering from

Table 3. Annotated name of miRNA and sequence of potential miRNA and their genes and number of targets.

S.NO	Annotated miRNA name	miRNA nucleotide sequence	Target site	Name and number of genes
1.	>miR_JEV_1	AUGCUGAAACGCGGCCUACCCC	7	1gene (VPS 13B)
2.	>miR_JEV_2	GGAGGAAAUGAAGGCUCAAUCAUG	10	1gene(LRRc27)
3.	>miR_JEV_3	ACACACCCUUUGGGGAGAUGGUGUU	11	1gene (UPS36)
4.	>miR_JEV_4	UUGAUGUUUGCCAUCGUAGGUGGC	11	1gene (CDH23)
5.	>miR_JEV_5	CUUGUUGGACGGCAGAGGGCCA	7	1 gene (NAV1)
6.	>miR_JEV_6	GUGGUGGAGUACUCAAGCUCAGU	34	3genes(SIRPA),(NKD2),(IQSEC2)
7.	>miR_JEV_7	UUUCAGCUGGGCCUUCUGGU	19	2 genes (NTRK3),(PCNT)
8.	>miR_JEV_8	GCCAGCCACUGAGUUUUUGUCG	24	3 genes(TOM1),(KLF13),(SLIT1)

Table 4. Name of gene and position on chromosomes and target involved in protein codes.

S.NO	Name of genes	Position on Chromosome	3'UTR	Target sites involved in (conservation information)
1.	VPS 13B(7 target sites)vacuolar protein sorting-associated protein 13B	100899004-100899032	3998 - 4026	rn4, bosTau2, canFam2, dasNov1, loxAfr1, echTel1, galGal2
		100860285-100860313	3472 - 3500	bosTau2, canFam2, loxAfr1, echTel1
		100592670-100592698	243 - 271	oryCun1, bosTau2, canFam2
		100935514-100935542	6550 - 6578	rn4, mm8, bosTau2, canFam2, echTel1, galGal2, xenTro1, tetNig1
		100723348-100723376	1205 - 1233	anFam2, dasNov1, monDom4
		100890901-100890929	3905 - 3933	oryCun1, dasNov1, galGal2
		100949767-100949795	7116 - 7144	mm8, bosTau2, dasNov1
2.	LRR LRRc27(10target sites)LRRC27leucine rich repeat containing 27	134039555-134039583	1350 - 1378	Not conserved
		134042269-134042297	4064 - 4092	rn4, mm8, oryCun1, bosTau2, canFam2, dasNov1, loxAfr1, echTel1, monDom4, xenTro1
		134042479-134042507	4274 - 4302	Not conserved
		134042545-134042573	4340 - 4368	Not conserved
		134042611-134042639	4406 - 4434	Not conserved
		134042677-134042705	4472 - 4500	Not conserved
		134042743-134042771	4538 - 4566	Not conserved
		134042809-134042837	4604 - 4632	Not conserved
		134042875-134042903	4670 - 4698	Not conserved
		134042941-134042969	4736 - 4764	Not conserved
3.	USP.36 Ubiquitin carboxyl-terminal hydrolase 36	74306034-74306062	35 - 63	Not conserved
		74306007-74306035	62 - 90	Not conserved
		74305997-74306025	72 - 100	Not conserved

Table 4. Continued.

		74305939- 74305967	130 - 158	rn4
		74305913- 74305941	156 - 184	rn4
		74305886- 74305914	183 - 211	Not conserved
		74305857- 74305885	212 - 240	Not conserved
		74305828- 74305856	241 - 269	Not conserved
		74305808- 74305836	261 - 289	Not conserved
		74305801- 74305829	268 - 296	Not conserved
		74305741- 74305769	328 - 356	Not conserved
4	CDH.23 Cadherin-23 precursor (Otocadherin).	73239812;73240235 - 73239839;73240236	5629 - 5657	rn4, mm8, oryCun1, bosTau2, canFam2, dasNov1, loxAfr1, echTel1, galGal2, tetNig1
		73209039- 73209067	1894 - 1922	rn4, mm8, bosTau2, canFam2, echTel1, galGal2, xenTro1
		73214671- 73214699	2215 - 2243	rn4, mm8, oryCun1, loxAfr1, monDom4, galGal2, tetNig1
		73237491- 73237519	5200 - 5228	rn4, mm8, oryCun1, bosTau2, echTel1, monDom4, tetNig1
		73214809- 73214837	2353 - 2381	rn4, mm8, oryCun1, bosTau2, canFam2, tetNig1
		73223185- 73223213	3184 - 3212	rn4, mm8, bosTau2, dasNov1, echTel1, monDom4
		73229000- 73229028	3868 - 3896	bosTau2, dasNov1, loxAfr1, echTel1, monDom4, galGal2
		73214713 - 73214741	2257 - 2285	rn4, canFam2, loxAfr1, galGal2
		73170638- 73170666	1243 - 1271	bosTau2, loxAfr1
		73220154;73220895 - 73220176;73220901	2719 - 2747	canFam2, loxAfr1
5	NAV1 Neuron navigator 1	200048219 - 200048247	1393 - 1421	oryCun1, bosTau2, canFam2, dasNov1, loxAfr1, echTel1, monDom4, galGal2
		200048300 – 200048328	1474 - 1502	oryCun1, canFam2, dasNov1, loxAfr1, echTel1, monDom4, galGal2
		200052901 - 200052929	1766 - 1794	mm8, bosTau2, canFam2
		200056148 - 200056176	2461 - 2489	rn4, mm8
		200056360 - 200056388	2673 - 2701	Not Conserved
		200059007 - 200059035	5320 - 5348	monDom4
		200060973 - 200061001	7286 - 7314	Not Conserved
	SIRPA (Signal- regulatory protein alpha-1) Brain Ig-like molecule with ty	1866596 - 1866624	381 - 409	Not Conserved
		1866605 - 1866633	390 - 418	Not Conserved
		1866608 - 1866636	393 - 421	Not Conserved
		1866611 - 1866639	396 - 424	Not Conserved
		1866614 - 1866642	399 - 427	Not Conserved
		1866617 - 186664	402 - 430	Not Conserved
		1866620 - 1866648	405 - 433	Not Conserved
		1866623 - 1866651	408 - 436	Not Conserved
		1866626 - 1866654	411 - 439	Not Conserved
		1866629 - 1866657	414 - 442	loxAfr1
		1866638 - 1866666	423 - 451	Not Conserved
		1866641 - 1866669	426 - 454	Not Conserved
		1867457 - 1867485	1242 - 1270	Not Conserved

Table 4. Continued.

6. NKD2 : (naked cuticle homolog 2)	1091218 - 1091246	327 - 355	rn4, mm8, canFam2
	1091410 - 1091438	519 - 547	canFam2
	1091413 - 1091441	522 - 550	bosTau2, canFam2, dasNov1, monDom4
	1091416 - 1091444	525 - 553	bosTau2, canFam2, dasNov1, monDom4
	1091428 - 1091456	537 – 565	rn4, mm8, bosTau2, canFam2, monDom4, tetNig1
	1091431 - 1091459	540 - 568	canFam2, monDom4
	1091434 - 1091462	543 - 571	canFam2, monDom4
	1091437 - 1091465	546 - 574	canFam2, monDom4
	1091440 - 1091468	549 - 577	rn4, mm8, dasNov1
	1091443 - 1091471	552 - 580	rn4, mm8
	1091446 - 1091474	555 - 583	rn4, mm8, canFam2, tetNig1
IQSEC2 (IQ motif and Sec7 domain-containing protein 2)	53280926 - 53280954	123 - 151	rn4, mm8
	53280870 - 53280898	179 - 207	mm8, bosTau2
	53280867 - 53280895	182 - 210	bosTau2, canFam2
	53280864 - 53280892	185 - 213	bosTau2, canFam2
	53280861 - 53280889	188 - 216	bosTau2, canFam2
	53280858 - 53280886	191 – 219	mm8, bosTau2, canFam2
	53280668 - 53280696	381 - 409	Not Conserved
	53280414 - 53280442	635 - 663	bosTau2, canFam2
	53280333 - 53280361	716 - 744	bosTau2
	53278970 - 53278998	2079 - 2107	oryCun1, dasNov1
7. NTRK3 (Neurotrophic tyrosine kinase receptor type 3)	86212921 - 86212949	8220 - 8248	loxAfr1, echTel1
	86220308 - 86220336	833 - 861	Not Conserved
	86219395 - 86219423	1746 - 1774	Not Conserved
	86219348 - 86219376	1793 - 1821	Not Conserved
	86219358 - 86219386	1783 - 1811	Not Conserved
	86215541 - 86215569	5600 - 5628	Not Conserved
	86212564 - 86212592	8577 - 8605	Not Conserved
	86211976 - 86212004	9165 - 9193	Not Conserved
	86207707 - 86207735	13434 - 13462	dasNov1
PCNT Pericentrin (Pericentrin B) (Kendrin).	46655555 - 46655583	2875 - 2903	rn4, mm8
	46608026 - 46608054	107 – 135	oryCun1
	46626163;46630175 - 46626183;46630183	1039 - 1067	bosTau2, loxAfr1
	46608115 - 46608143	196 - 224	mm8, bosTau2, loxAfr1, echTel1
	46633611 - 46633639	1421 - 1449	canFam2
	46674569;46674849 - 46674574;46674872	5635 - 5663	Not Conserved
	46674877 - 46674905	5668 - 5696	rn4, mm8
	46676187 - 46676215	6106 - 6134	rn4, mm8, oryCun1
	46681390 - 46681418	6790 - 6818	rn4, mm8
	46682554 – 46682582	6871 - 6899	Not Conserved
TOM1 Target of Myb protein 1	34073331 - 34073359	128 - 156	Not Conserved
	34073461 - 34073489	258 - 286	echTel1
	34073465 - 34073493	262 - 290	Not Conserved
	34073469 - 34073497	266 - 294	Not Conserved

Table 4. Continued.

8		34073473 - 34073501	270 – 298	Not Conserved
		34073577 - 34073605	374 – 402	Not Conserved
		34073584 - 34073612	381 – 409	Not Conserved
		34073657 - 34073685	454 – 482	Not Conserved
	KLF13 Krueppel-like factor 13	29452170 – 29452198	375 – 403	Not Conserved
		29452174 - 29452202	379 – 407	Not Conserved
		29452312 - 29452340	517 - 545	canFam2
		29452379 - 29452407	584 - 612	Not Conserved
		29453220 - 29453248	1425 - 1453	Not Conserved
		29453605 - 29453633	1810 - 1838	Not Conserved
		29454155 - 29454183	2360 - 2388	Not Conserved
		29455533 - 29455561	3738 - 3766	Not Conserved
	SLIT1 Slit homolog 1 protein precursor (Slit-1) (Multiple epidermal growth factor-like domains 4).	98798838;98806068 - 98798865;98806069	111 - 139	mm8, oryCun1, bosTau2, canFam2, dasNov1, loxAfr1, echTel1, monDom4, xenTro1, tetNig1
		98792773 - 98792801	815 - 843	Not Conserved
		98752506 - 98752534	2860 - 2888	rn4, mm8
		98752502 - 98752530	2864 - 2892	rn4, mm8, galGal2
		98750456 - 98750484	3767 – 3795	mm8, oryCun1
		98749228 - 98749256	4995 - 5023	Not Conserved
		98748667 - 98748695	5556 - 5584	Not Conserved
		98748567 - 98748595	5656 - 5684	Not Conserved

Table 5. Information of genes.

S.No	Gene name	Precision	Ensemble ID	m iTG score	SNR
1.	VPS 13B	0.71	ENSGOOOOO135249	22.31	1.06
2.	LRR c27	0.88	ENSGOOOOO148814	25.00	2.85
3.	USP 36	1	ENSGOOOOO55483	29.00	2.32
4.	CDH 23	0.98	ENSGOOOOO107730	29.50	1.45
5.	NAV1	0.78	ENSOOOOO134369	19.80	0.94
6.	SIRPA	0.96	ENSGOOOOO198053	30.0	2.5
	NKD2	0.84	ENSGOOOOO145506	22.0	2.5
	IQSEC2	0.84	ENSGOOOOO124313	20.0	2.5
7.	NTRK3	0.96	ENSGOOOOO140538	24.66	2.01
	PCNT	0.96	ENSGOOOOO160299	23.26	2.01
8.	TOM1	0.41	ENSGOOOOO100284	21.00	1.18
	KLF13	0.41	ENSGOOOOO169926	19.00	1.18
	SLIT1	0.41	ENSGOOOOO187122	19.00	1.18

attack of *J. encephalitis.* Since this is a prediction based method, it has to be proved experimentally.

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