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Research Article

Molecular Variation and its Evolutionary Relationship Studies of Pld3 in Alzheimer's Disease taking a NGS Approach

Abhay Mishra[1]*, Bashah J. Khan[2]

[1]Deparptment of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India
[2]Bioinformatics Technologies of India, Bareilly, Uttar Pradesh, India

ABSTRACT

Alzheimer disease is most common cause of dementia in humans leading to Neurodegenerative state in them. It is usually marked by slowly progressive episodic memory loss which evolves into global loss of cognitive ability and psychiatric features. Alzheimer Disease categorized in chronic disorder which slowly result in destruction of neurons and further causes serious cognitive disability. There were many risk variants with effects on LOAD risk and a rare variant in PLD3 (phospholipase D3; Val232Met) seen doubling the risk of Alzheimer's disease. PLD3 is present on chromosome 19q13.2 with various variants increase risk for Late On-Alzheimer's Disease. Hippocampus and cortex are vulnerable for AD and PLD3 is seen over there. PLD3 protein position(s) 1 to 490 with length 490. Rare coding variants in the phospholipase D3 (PLD3) gene, also known as HU-K4, have recently been identified to increase the risk for late-onset Alzheimer's disease (LOAD) and follow-up analyses of the candidate variants in several large independent LOAD case–control data series. PLD3 is highly expressed in the brain, especially mainly in neurons, but at a lower level in almost all tissues. The level of PLD3 was found to be down regulating in Alzheimer's disease (AD) brains, which was negatively correlated with amyloid precursor protein (APP) and amyloid-β (Aβ) levels. The sequence and other functions have been identified by doing work on the different software tool such as SNP Table View, Dot Matrix View, Graphical Sequence View, Phylogenetic Tree. These tools analyze in identifying the sequence of Pld3, graphical sequence views, various alignment views, phylogenetic tree views and tabular views of data. SNP table view which is the place where you can work with SNP's from a sequence in a tabular format. Dot Matrix view present line that represents the alignment. Graphical Sequence View helps to view graphical representation of our gene. Phylogenetic tree shows the genetic tree of Pld3.

KEY WORDS: Biotechnology, bioinformatics, pld3, Alzheimer Disease, SNP's.

Introduction

Bioinformatics is the bridge between science in which biology and information technology. (Benson et al., 2005). It deals with the application of computers to the collection, organization, analysis, manipulation, of Science and Technology encompassing a systematic development and application of IT solution to handle biological information (Jayaram et al., 2012). Aim of presentation sharing of biological data (Mcentyre et al., 2005). Bioinformatics is an interdisciplinary area bioinformatics is to organize biological data in easily read or make implementation (Murray 1994; Madzokere et al., 2013), Bioinformatics role is also in analyzing biological data (Madzokere et al., 2013). Alzheimer's disease (AD) is a chronic disorder that slowly destroys neurons and causes serious cognitive disability. It is human disease leading to Neurodegenerative state (Rogaev et al., 1995).

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***Address for Correspondence:** Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India; Tel.: +91-9045179160.
E-mail: bashahj.khan@gmail.com

Alzheimer disease (AD) is a type of dementia that causes problems with memory, thinking and behaviour among older people (Inoue et al., 2013). AD occurs worldwide and affects all ethnic groups. Alzheimer genetics is traditionally subdivided into early onset (EOAD) and late Alzheimer genetics is traditionally subdivided into Early Onset Alzheimer Disease (EOAD) and Late Onset Alzheimer Disease (LOAD). EOAD has an onset before age 60–65 years and accounts for 1–5% of all cases. Other are LOAD which onset after age 60–65 years and is the predominant form of AD (Bertam et al., 2005; Campion et al., 1999). The incidence of Alzheimer disease is increasing due, in part, to increased life expectancy and the aging baby boomer generation. The average lifetime risk of developing Alzheimer disease is 10–12%. This risk at least doubles with the presence of a first-degree relative with the disorder (Goldman et al., 2011).

PLD3 is highly expressed in the brain, especially mainly in neurons and at a lower level in almost all tissues. The level of PLD3 was found to be down regulated in Alzheimer's disease (AD) brains, which was negatively correlated with amyloid precursor protein (APP) and amyloid-β (Aβ) levels (Wang et al., 2014). PLD3 is highly expressed in brain regions that are vulnerable to Alzheimer's disease pathology, including hippocampus and cortex, and is expressed at significantly lower levels in neurons from Alzheimer's disease brains compared to control brains (Oliveira et al., 2010). Together, genetic and functional data indicate that carriers of PLD3 coding variants have a twofold increased risk for LOAD and that PLD3 influences APP processing (Cruchaga, et al., 2014). Over expression of PLD3 leads to a significant decrease in intracellular amyloid-β precursor protein (APP) and extracellular Aβ42 and Aβ40 (the 42- and 40-residue isoforms of the amyloid-β peptide), and knockdown of PLD3 leads to a significant increase in extracellular Aβ42 and Aβ40 (Oliveira TG et al., 2010).

Materials and Methods

Performed different modules: SNP Table View, Dot Matrix View, Graphical Sequence View, Phylogenetic Tree, EditSequence, GeneQuest, MapDraw, PrimerSelect to analyze pld3 gene.

Edit Sequence: Edit Sequence is the foundation module of the Lasergene suite and serves as operations centre for entering and manipulating DNA sequence data and it helps in identifying open reading frame (ORF).

Gene Quest: It helps in identifying: Base composition, codon, Patterns and Repeats. **Gene Finding:** Gene Quest offers 3 different ways to compare sequence to other; **DNA Finder:** After choosing comparison sequence, this identifies any regions that match the project sequence; **Protein Finder:** After choosing comparison sequence, this identifies any regions whose translations match the project sequence; **BLAST Search:** Submit a query of specified DNA segment to a BLAST server.

MapDraw: It reads and interprets Gene bank formatted features planes of sequence documents. Mapdraw translates nucleotides using the currently active genetic code.

PrimerSelect: It is an application of Lasergene that help in designing primers and probes for PCR, sequencing and hybridization experiments.

SNP Table View: SNP Table View can be utilized with NCBI Workbench. It helps in identification of DNA variation in single position of sequence among the individuals.

Dot Matrix View: Dot Matrix module is an application of NBCI Workbench which let a graphical representation of the given sequence with it's position.

Graphical Sequence View: In this module of NCBI Workbench number of exons and whole gene of interest can be visualized along marked length.

Phylogenetic Tree: Phylogenetic trees are diagrams showinng evolutionary interrelation of a group of an organisms that have arise from same species and above workbench let you with the tree and closely related to gene of interest.

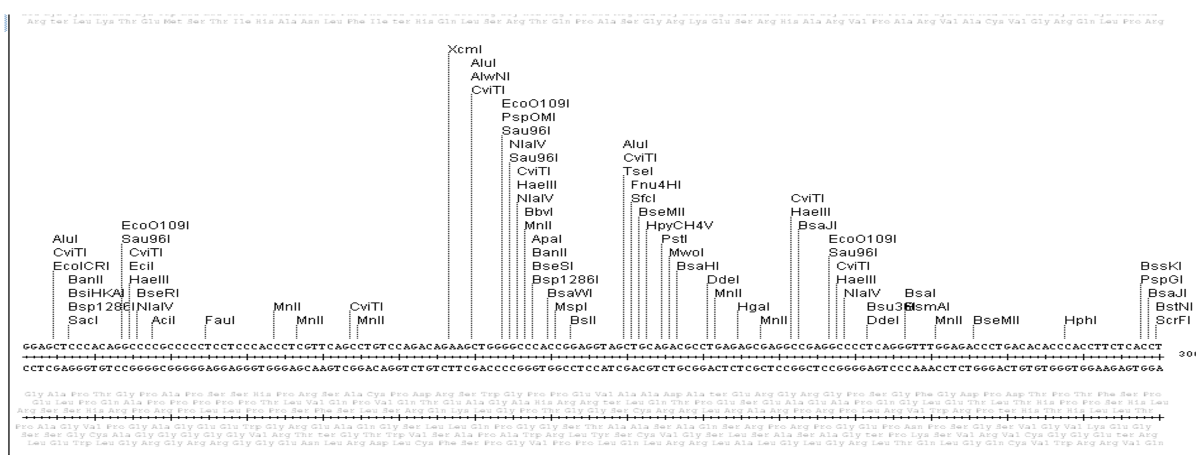
Selection: 1 > 2142
Length: 2142

WARNING: The presence of 23 nonsense codons suggests that this is NOT a coding region.

Base Composition								
			Codons					
Base	Overall		Position 1		Position 2		Position 3	
A	423	19.7%	177	24.8%	81	11.3%	165	23.1%
C	719	33.6%	195	27.3%	298	41.7%	226	31.7%
G	603	28.2%	166	23.2%	232	32.5%	205	28.7%
T	397	18.5%	176	24.6%	103	14.4%	118	16.5%
X	0	0.0%	0	0.0%	0	0.0%	0	0.0%

Codon Preference:											
GCA	Ala(A)	15	CAG	Gln(Q)	13	UUG	Leu(L)	8	UAA	Ter(.)	2
GCC	Ala(A)	25	(Q)		17	(L)		51	UAG	Ter(.)	2
GCG	Ala(A)	13	GAA	Glu(E)	4	AAA	Lys(K)	12	UGA	Ter(.)	19
GCU	Ala(A)	18	GAG	Glu(E)	8	AAG	Lys(K)	7	(.)		23
(A)		71	(E)		12	(K)		19	ACA	Thr(T)	23
AGA	Arg(R)	14	GGA	Gly(G)	11	AUG	Met(M)	15	ACC	Thr(T)	20
AGG	Arg(R)	26	GGC	Gly(G)	18	(M)		15	ACG	Thr(T)	7
CGA	Arg(R)	4	GGG	Gly(G)	18	UUC	Phe(F)	6	ACU	Thr(T)	16
CGC	Arg(R)	9	GGU	Gly(G)	8	UUU	Phe(F)	4	(T)		66
CGG	Arg(R)	9	(G)		55	(F)		10	UGG	Trp(W)	36
CGU	Arg(R)	2	CAC	His(H)	8	CCA	Pro(P)	29	(W)		36
(R)		64	CAU	His(H)	4	CCC	Pro(P)	44	UAC	Tyr(Y)	1
AAC	Asn(N)	4	(H)		12	CCG	Pro(P)	6	UAU	Tyr(Y)	2
AAU	Asn(N)	3	AUA	Ile(I)	3	CCU	Pro(P)	23	(Y)		3
(N)		7	AUC	Ile(I)	1	(P)		102	GUA	Val(V)	4
GAC	Asp(D)	6	AUU	Ile(I)	2	AGC	Ser(S)	20	GUC	Val(V)	2
GAU	Asp(D)	1	(I)		6	AGU	Ser(S)	4	GUG	Val(V)	13
(D)		7	CUA	Leu(L)	2	UCA	Ser(S)	16	GUU	Val(V)	2
UGC	Cys(C)	27	CUC	Leu(L)	15	UCC	Ser(S)	20	(V)		21
UGU	Cys(C)	7	CUG	Leu(L)	18	UCG	Ser(S)	6	NNN	???(X)	0
(C)		34	CUU	Leu(L)	5	UCU	Ser(S)	17	(X)		0
CAA	Gln(Q)	4	UUA	Leu(L)	3	(S)		83	Total		714

Figure 1 Indicating codon usage in the sequence: in the sequence the base composition of adenine is 19.7%, cytosine is 33.6%, guanine is 28.2% and tyrosine is 18.5%.



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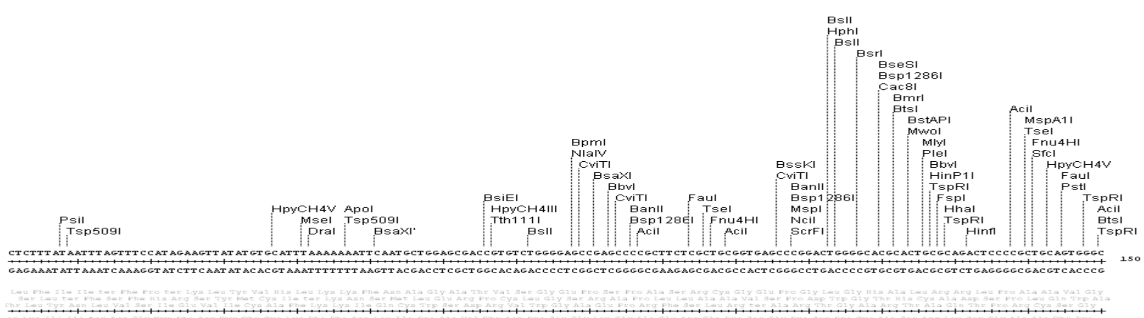

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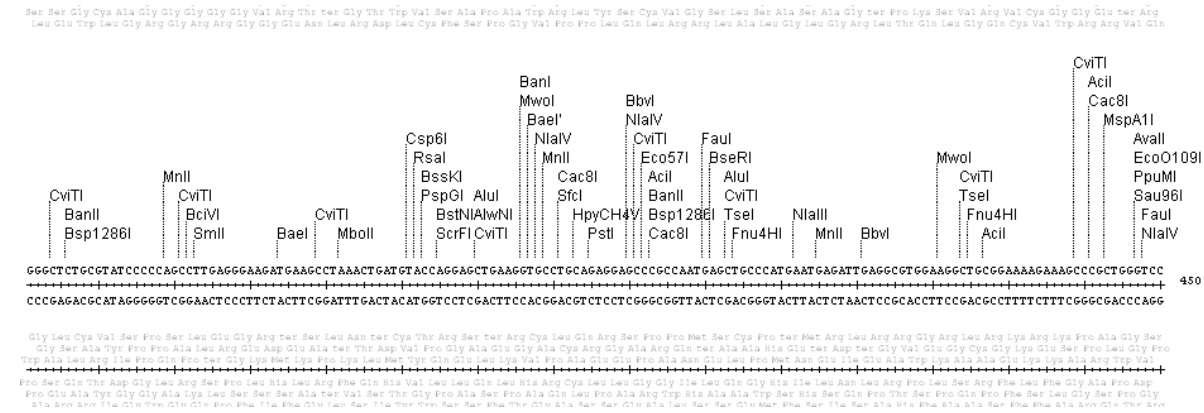
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Cac8I
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AvaII
EcoO109I
PpuMI
Sau96I
FauI
NlaIV
BanI
MwoI
BaeI'
NlaIV
MnlI
Cac8I
SfcI
HpyCH4V
PstI
BbvI
NlaIV
CviTI
Eco57I
AciI
BanII
Bsp1286I
Cac8I
FauI
BseRI
AluI
CviTI
TseI
Fnu4HI
NlaIII
MnlI
BbvI
MwoI
CviTI
TseI
Fnu4HI
AciI
Csp6I
RsaI
BsskI
PspGI
AluI
BstNIAlwNI
ScrFI
CviTI
CviTI
BanII
Bsp1286I
MnlI
CviTI
BciVI
SmlI
BaeI
CviTI
MboII
GGGCTCTGCGTATCCCCCAGCCTTGAGGGAAGATGAAGCCTAAACTGATGTACCAGGAGCTGAAGGTGCCTGCAGAGGAGCCCCGCCAATGAGCTGCCCATGAATGAGATTGAGGCGTGGAAGGCTGCGGAAAAGAAAGCCCGCTGGGTCC
CCCGAGACGCATAGGGGGTCGGAACTCCCTTCTACTTCGGATTTGACTACATGGTCCTCGACTTCCACGGACGTCTCCTCGGGCGGTTACTCGACGGGTACTTACTCTAACTCCGCACCTTCCGACGCCTTTTCTTTCGGGCGACCCAGG
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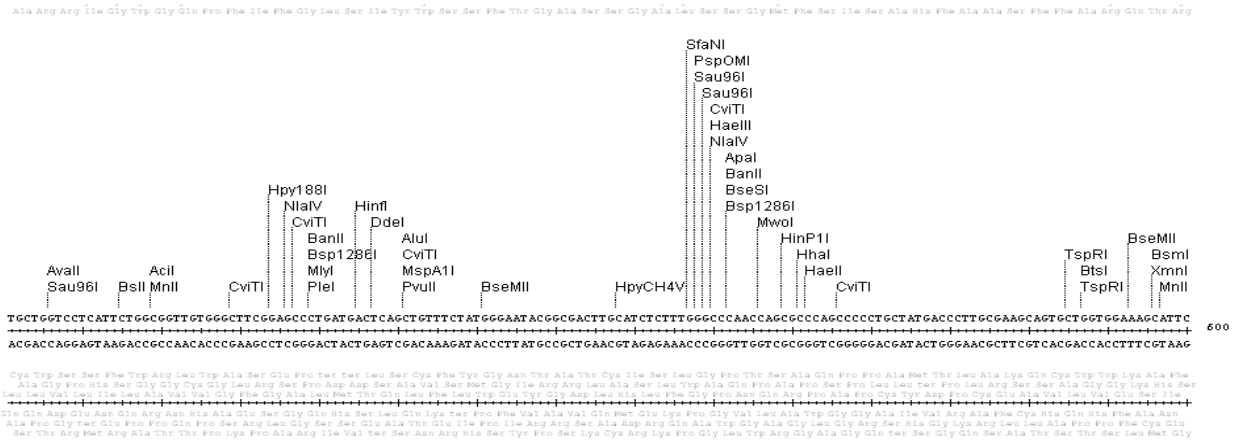
C.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Sau96I
Sau96I
CviTI
HaeIII
NlaIV
ApaI
BanII
BseSI
Bsp1286I
MwoI
HinP1I
HhaI
HaeII
CviTI
Hpy188I
NlaIV
CviTI
BanII
Bsp1286I
MlyI
PleI
HinfI
DdeI
AluI
CviTI
MspA1I
PvuII
BseMII
HpyCH4V
AvaII
Sau96I
BsII
AciI
MnlI
CviTI
TspRI
BtsI
TspRI
BseMII
BsmI
XmnI
MnlI
TGCTGGTCCTCATTCTGGCGGTTGTGGGCTTCGGAGCCCTGATGACTCAGCTGTTTCTATGGGAATACGGCGACTTGCATCTCTTGGGCCCAACCAGCGCCCAGCCCCCTGCTATGACCCTTGCGAAGCAGTGCTGGTGGAAAGCATTC
ACGACCAGGAGTAAGACCGCCAACACCCGAAGCCTCGGGACTACTGAGTCGACAAAGATACCCTTATGCCGCTGAACGTAGAGAAACCCGGGTTGGTCGCGGGTCGGGGGACGATACTGGGAACGCTTCGTCACGACCACCTTTCGTAAG
600

D.


CviTI
HaeIII
StuI
Cac8I
BglI
MwoI
SfiI
PspOMI
Sau96I
EcoO109I
Sau96I
CviTI
HaeIII
NlaIV
NlaIV
ApaI
BanII
BseSI
Bsp1286I
AciI
BsaJI
BtgI
AciI
BstUI
MspA1I
SacII
AvaII
Sau96I
NlaIV
BsaJI
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Hpy188I

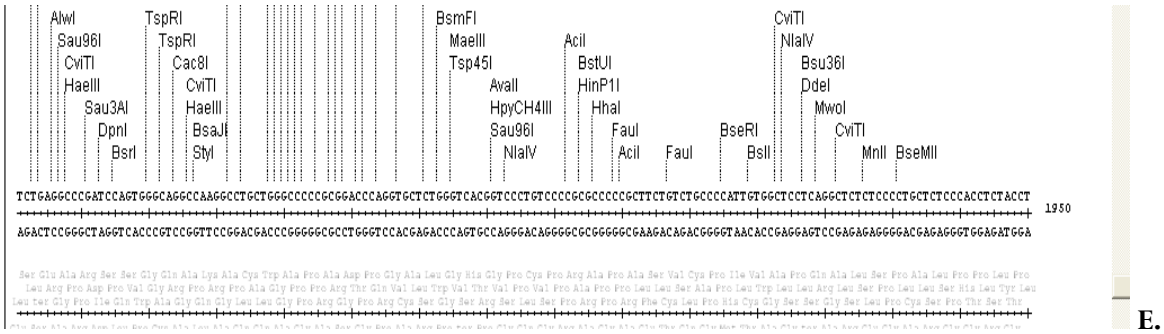

Figure 2 Restriction map (A-E) indicated restriction enzymes that slice the sequence at a specific position

AatII	Bpu10I	Bsu36I	HpyCH4V	PvuII	XmaI
Acc65I	BsaAI	BtgI	KasI	RsaI	XmnI
AccI	BsaBI	BtrI	KpnI	RsrII	
AciI	BsaHI	BtsI	MaeIII	SacI	
AclI	BsaI	Cac8I	MboII	SacII	
AfeI	BsaJI	ClaI	MfeI	SalI	
AflII	BsaWI	Csp6I	MluI	SanDI	
AflIII	BsaXI	CviTI	MlyI	SapI	
AgeI	BsaXI'	DdeI	MnlI	Sau3AI	
AhdI	BseMII	DpnI	MscI	Sau96I	
AluI	BseRI	DraI	MseI	SbfI	
AlwI	BseSI	DraIII	MslI	ScaI	
AlwNI	BsgI	DrdI	MspA1I	ScrFI	
ApaI	BsiEI	EaeI	MspI	SexAI	
ApaLI	BsiHKAI	EagI	MwoI	SfaNI	
ApoI	BsiWI	EarI	NaeI	SfcI	
AscI	BslI	EciI	NarI	SfiI	
AseI	BsmAI	Eco57I	NciI	SfoI	
AvaI	BsmBI	EcoICRI	NcoI	SgfI	
AvaII	BsmFI	EcoNI	NdeI	SgrAI	
AvrII	BsmI	EcoO109I	NgoMIV	SmaI	
BaeI	Bsp1286I	EcoRI	NheI	SmlI	
BaeI'	BspEI	EcoRV	NlaIII	SnaBI	
BamHI	BspHI	FauI	NlaIV	SpeI	
BanI	BspMI	Fnu4HI	NotI	SphI	
BanII	BsrBI	FokI	NruI	SrfI	
BbeI	BsrDI	FseI	NsiI	SspI	
BbsI	BsrFI	FspI	NspI	StuI	
BbvCI	BsrGI	HaeII	PacI	StyI	
BbvI	BsrI	HaeIII	PciI	SwaI	
BcgI	BssHII	HgaI	PflMI	TaqI	
BcgI'	BssKI	HhaI	PleI	TatI	
BciVI	BssSI	HincII	PmeI	TfiI	
BclI	BstAPI	HindIII	PmlI	TscI	
BfaI	BstBI	HinfI	Ppu10I	TseI	
BglI	BstEII	HinP1I	PpuMI	Tsp45I	
BglII	BstF5I	HpaI	PshAI	Tsp509I	
BlpI	BstNI	HphI	PsiI	TspRI	
BmrI	BstUI	Hpy188I	PspGI	Tth111I	
BplI	BstXI	Hpy99I	PspOMI	XbaI	
BplI'	BstYI	HpyCH4III	PstI	XcmI	
BpmI	BstZ17I	HpyCH4IV	PvuI	XhoI	

Figure 3 Enzyme's name with their position that cut the sequence at a particular position

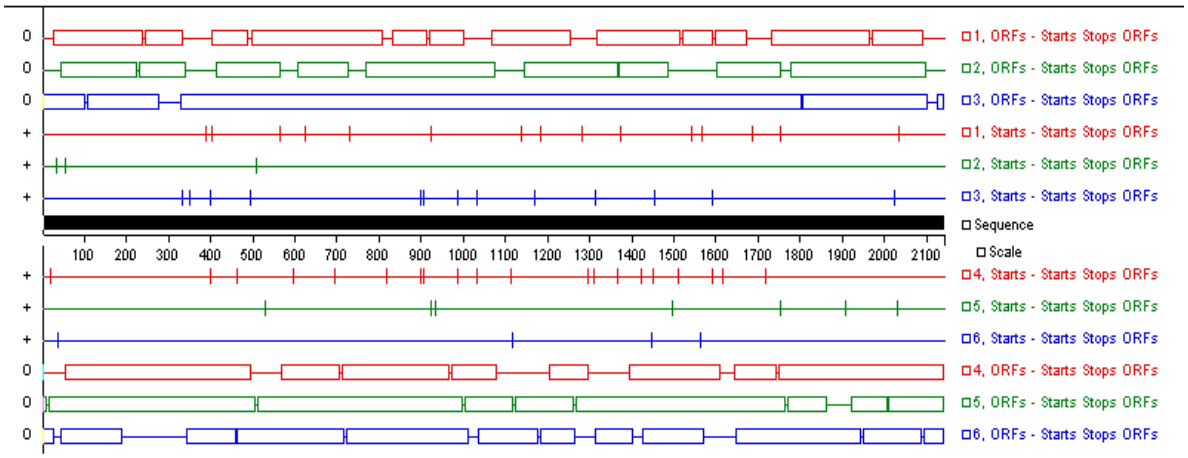

Figure 4 The product length of the sequence

Start	End	Length	Tm	dG	dProfile	Name
Upper Primers: 164 Located						
108	126	19-mer	61.9	-42.1	88.0	
170	190	21-mer	63.9	-47.1	132.6	
171	191	21-mer	62.5	-45.6	110.5	
171	192	22-mer	64.9	-47.5	124.4	
173	194	22-mer	62.7	-46.1	38.3	
267	285	19-mer	47.4	-33.9	15.3	
282	299	18-mer	47.4	-33.5	28.8	
282	300	19-mer	49.1	-35.1	27.3	
291	314	24-mer	60.1	-46.0	74.6	
292	315	24-mer	62.3	-47.5	90.5	
307	327	21-mer	60.7	-44.5	107.1	
327	344	18-mer	44.0	-33.8	12.9	
327	345	19-mer	45.4	-35.1	18.7	
327	346	20-mer	47.0	-36.7	18.3	
327	347	21-mer	50.0	-38.7	21.2	
327	348	22-mer	52.0	-40.2	21.6	
327	349	23-mer	52.6	-41.7	25.4	
327	350	24-mer	55.1	-43.7	24.9	
328	351	24-mer	52.7	-41.9	20.7	
Lower Primers: 163 Located						
1949	1967	19-mer	64.0	-44.4	195.3	
1964	1981	18-mer	61.9	-41.9	161.6	
1965	1982	18-mer	61.9	-41.9	186.6	
2006	2024	19-mer	51.4	-38.1	46.0	
2015	2032	18-mer	51.4	-36.7	71.5	

Figure 5 Size of the upper primer and lower primer

NCBI Genome Workbench : 7

C: NM_012268.3 (SNP Table View) [New Project (1)]

Search: Exact Match Stop Filter All

	RS ID	Alleles	Sequence	Location	FXN Class	Weight	Variation Class	Resource Link	quency Valida	Quality Check	Phenotype	1000 Genome
1	760010676	A/G	NM_012268.3	1	In 5' Gene, Intr	1	SNP					Not present in
2	768088100	C/G	NM_012268.3	24	In 5' Gene, In 5	1	SNP					Not present in
3	776184936	C/T	NM_012268.3	35	In 5' Gene, In 5	1	SNP					Not present in
4	761194118	A/G	NM_012268.3	40	In 5' Gene, In 5	1	SNP		Validated (has			Not present in
5	764866409	A/G	NM_012268.3	44	In 5' Gene, In 5	1	SNP					Not present in
6	578237018	C/T	NM_012268.3	60	In 5' Gene, In 5	1	SNP					1000G Phase 3,
7	750027114	A/C	NM_012268.3	78	In 5' Gene, In 5	1	SNP		Validated (has			Not present in
8	867377223	A/G	NM_012268.3	80	In 5' Gene, In 5	1	SNP					Not present in
9	758014713	A/G	NM_012268.3	89	In 5' Gene, In 5	1	SNP		Validated (has			Not present in
10	11667768	C/T	NM_012268.3	101	In 5' Gene, In 5	1	SNP		>5% minor all			1000G Phase 3,
11	764771899	A/G	NM_012268.3	110	In 5' Gene, In 5	1	SNP					Not present in
12	559950337	C/T	NM_012268.3	111	In 5' Gene, In 5	1	SNP		Validated (has			1000G Phase 3,
13	11667774	C/G	NM_012268.3	123	In 5' Gene, In 5	1	SNP		>5% minor all			1000G Phase 3,
14	781086015	A/C	NM_012268.3	127	In 5' Gene, In 5	1	SNP					Not present in
15	547005546	C/T	NM_012268.3	138	In 5' Gene, In 5	1	SNP		Validated (has			1000G Phase 3,
16	560563887	C/G	NM_012268.3	159	In 5' Gene, In 5	1	SNP		Validated (has			1000G Phase 3,
17	755500415	-/C	NM_012268.3	162	In 5' Gene, In 5	1	INDEL					Not present in
18	531484557	C/T	NM_012268.3	162	In 5' Gene, In 5	1	SNP					1000G Phase 3,
19	74890358	A/G	NM_012268.3	206	In 5' Gene, In 5	1	SNP		Validated (has			1000G Phase 3,
20	571103065	G/T	NM_012268.3	229	In 5' Gene, In 5	1	SNP					1000G Phase 3,
21	532170004	C/T	NM_012268.3	264	In 5' Gene, In 5	1	SNP					1000G Phase 3,
22	751444939	G/T	NM_012268.3	272	In 5' Gene, In 5	1	SNP					Not present in
23	547574424	A/G/T	NM_012268.3	292	In 5' Gene, In 5	1	SNP		Validated (has			1000G Phase 3,
24	183725030	C/G	NM_012268.3	296	In 5' Gene, In 5	1	SNP					1000G Phase 3,
25	140944893	A/G	NM_012268.3	301	In 5' Gene, Dor	1	SNP		Validated (has			1000G Phase 3,
26	776542246	C/G	NM_012268.3	319	In 5' Gene, Intr	1	SNP					Not present in
27	567691992	A/G	NM_012268.3	343	In 5' Gene, Intr	1	SNP					1000G Phase 3,
28	541028592	G/T	NM_012268.3	347	Intron, In 5' UT	1	SNP		Validated (has			1000G Phase 3,
29	553361383	A/C	NM_012268.3	356	Intron, In 5' UT	1	SNP		Validated (has			1000G Phase 3,

A.

30	575092435	A/G	NM_012268.3	363	Intron, In 5' UT	1	SNP					1000G Phase 3,
31	757630473	A/G	NM_012268.3	365	Intron, In 5' UT	1	SNP					Not present in
32	542040557	C/T	NM_012268.3	380	Intron, In 5' UT	1	SNP		Validated (has			1000G Phase 3,
33	563932699	A/G	NM_012268.3	385	Intron, In 5' UT	1	SNP					1000G Phase 3,
34	781425223	C/T	NM_012268.3	389	Intron, In 5' UT	1	SNP					Not present in
35	530616280	C/T	NM_012268.3	415	Intron, In 5' UT	1	SNP		Validated (has			1000G Phase 3,
36	867975055	G/T	NM_012268.3	431	Intron, In 5' UT	1	SNP					Not present in
37	756646683	C/T	NM_012268.3	458	Intron, In 5' UT	1	SNP					Not present in
38	779475121	C/G	NM_012268.3	462	Intron, In 5' UT	1	SNP					Not present in
39	779494691	C/T	NM_012268.3	474	Intron, In 5' UT	1	SNP					Not present in
40	190321533	C/T	NM_012268.3	475	Intron, In 5' UT	1	SNP		Validated (has			1000G Phase 3,
41	199573132	C/T	NM_012268.3	477	Intron, In 5' UT	1	SNP		Validated (has			1000G Phase 3,
42	111819971	C/T	NM_012268.3	484	Intron, In 5' UT	1	SNP					Not present in
43	7253975	C/G	NM_012268.3	510	Intron, In 5' UT	1	SNP		>5% minor all			1000G Phase 3,
44	10417488	A/T	NM_012268.3	521	Intron, In 5' UT	1	SNP		>5% minor all			1000G Phase 3,
45	183478868	C/T	NM_012268.3	529	Intron, In 5' UT	1	SNP		Validated (has			1000G Phase 3,
46	529222162	C/T	NM_012268.3	548	Intron, In 5' UT	1	SNP					1000G Phase 3,
47	748157595	C/T	NM_012268.3	576	In 5' UTR	1	SNP					Not present in
48	756145262	A/G	NM_012268.3	579	In 5' UTR	1	SNP					Not present in
49	777968797	C/G	NM_012268.3	584	In 5' UTR	1	SNP		Validated (has			Not present in
50	749331824	C/G	NM_012268.3	589	In 5' UTR	1	SNP					Not present in
51	771238072	A/C/G/T	NM_012268.3	594	In 5' UTR	1	SNP		Validated (has	Non-overlappi		Not present in
52	772601678	C/T	NM_012268.3	599	In 5' UTR	1	SNP		Validated (has			Not present in
53	775854759	A/G	NM_012268.3	600	In 5' UTR	1	SNP					Not present in
54	752708568	-/C	NM_012268.3	604	In 5' UTR	1	INDEL					Not present in
55	761299929	C/G	NM_012268.3	605	In 5' UTR	1	SNP		Validated (has			Not present in
56	769060160	A/C	NM_012268.3	606	In 5' UTR	1	SNP					Not present in
57	200811665	A/G	NM_012268.3	610	In 5' UTR	1	SNP		Validated (has			1000G Phase 3,

B.

58	762406245	G/T	NM_012268.3	639	Missense	1	SNP					Not present in 1000G
59	760407817	A/G	NM_012268.3	667	Synonymous	1	SNP		Validated (has a			Not present in 1000G
60	764069680	G/T	NM_012268.3	670	Missense	1	SNP					Not present in 1000G
61	200094590	C/T	NM_012268.3	673	Synonymous	1	SNP		Validated (has a			1000G Phase 3, 1000G F
62	757404195	A/G/T	NM_012268.3	674	Missense	1	SNP		Validated (has a			Not present in 1000G
63	11552733	C/G/T	NM_012268.3	688	Synonymous	1	SNP					Not present in 1000G
64	750540651	A/G	NM_012268.3	689	Missense	1	SNP					Not present in 1000G
65	747195891	-/A	NM_012268.3	691	Frameshift	1	INDEL					Not present in 1000G
66	758630487	C/T	NM_012268.3	699	Missense	1	SNP					Not present in 1000G
67	780172579	C/T	NM_012268.3	705	Missense	1	SNP					Not present in 1000G
68	747354721	A/G	NM_012268.3	709	Stop-gain	1	SNP					Not present in 1000G
69	768953958	A/G	NM_012268.3	718	Synonymous	1	SNP		Validated (has a			Not present in 1000G
70	745358823	C/T	NM_012268.3	731	Missense	1	SNP					Not present in 1000G
71	551176741	A/G/T	NM_012268.3	732	Missense	1	SNP					1000G Phase 3, Has 100
72	781581165	-/CGTGGGTGC	NM_012268.3	745	Frameshift	1	INDEL					Not present in 1000G
73	371085398	C/T	NM_012268.3	751	Synonymous	1	SNP					1000G Phase 3, Has 100
74	763121730	C/T	NM_012268.3	762	Missense	1	SNP					Not present in 1000G
75	535145267	A/G	NM_012268.3	766	Synonymous	1	SNP					1000G Phase 3, Has 100
76	199944221	C/T	NM_012268.3	770	Missense	1	SNP					1000G Phase 3, 1000G F
77	765220946	C/T	NM_012268.3	772	Synonymous	1	SNP					Not present in 1000G
78	773334902	G/T	NM_012268.3	790	Missense	1	SNP					Not present in 1000G
79	138062457	A/G	NM_012268.3	799	Synonymous	1	SNP					1000G Phase 3, Has 100
80	766435886	A/G	NM_012268.3	803	Missense	1	SNP					Not present in 1000G
81	535493639	C/T	NM_012268.3	808	Synonymous	1	SNP					1000G Phase 3, Has 100
82	142070038	A/G	NM_012268.3	809	Missense	1	SNP					1000G Phase 3, Has 100
83	375652785	C/T	NM_012268.3	811	Synonymous	1	SNP					Not present in 1000G
84	767787539	A/G	NM_012268.3	812	Missense	1	SNP		Validated (has a			Not present in 1000G
85	753009473	C/T	NM_012268.3	814	Synonymous	1	SNP					Not present in 1000G
86	367655989	C/G	NM_012268.3	817	Missense	1	SNP					Not present in 1000G

C.

87	756517905	C/T	NM_012268.3	820	Synonymous	1	SNP					Not present in 1000G
88	778221354	C/T	NM_012268.3	821	Missense	1	SNP					Not present in 1000G
89	749859040	A/T	NM_012268.3	822	Missense	1	SNP					Not present in 1000G
90	757908510	C/T	NM_012268.3	823	Synonymous	1	SNP					Not present in 1000G
91	779369271	C/T	NM_012268.3	826	Synonymous	1	SNP					Not present in 1000G
92	746530607	C/G/T	NM_012268.3	839	Missense	1	SNP		Validated (has a			Not present in 1000G
93	11552729	G/T	NM_012268.3	840	Missense	1	SNP					Not present in 1000G
94	745895688	-/C	NM_012268.3	841	Frameshift	1	INDEL					Not present in 1000G
95	145826567	C/T	NM_012268.3	842	Missense	1	SNP					Not present in 1000G
96	11552732	A/G	NM_012268.3	844	Synonymous	1	SNP					Not present in 1000G
97	138674695	C/G	NM_012268.3	848	Missense	1	SNP		Validated (has a			1000G Phase 3, 1000G F
98	769755129	C/T	NM_012268.3	853	Synonymous	1	SNP					Not present in 1000G
99	773281759	G/T	NM_012268.3	864	Missense	1	SNP					Not present in 1000G
100	145465613	C/T	NM_012268.3	865	Synonymous	1	SNP					1000G Phase 3, 1000G F
101	770914756	A/G	NM_012268.3	866	Missense	1	SNP					Not present in 1000G
102	763496042	C/G	NM_012268.3	869	Missense	1	SNP					Not present in 1000G
103	766998348	A/G	NM_012268.3	871	Synonymous	1	SNP					Not present in 1000G
104	752215238	A/G	NM_012268.3	880	Synonymous	1	SNP					Not present in 1000G
105	755709397	G/T	NM_012268.3	893	Stop-gain	1	SNP		Validated (has a			Not present in 1000G
106	763620683	A/G	NM_012268.3	897	Missense	1	SNP					Not present in 1000G
107	753592556	C/T	NM_012268.3	907	Synonymous	1	SNP					Not present in 1000G
108	376975617	A/G	NM_012268.3	912	Missense	1	SNP		Validated (has a			Not present in 1000G
109	778711055	G/T	NM_012268.3	914	Missense	1	SNP					Not present in 1000G
110	149236824	C/T	NM_012268.3	921	Missense	1	SNP					Not present in 1000G
111	11552734	A/G/T	NM_012268.3	922	Synonymous	1	SNP					Not present in 1000G
112	267605483	A/G	NM_012268.3	923	Missense	1	SNP			Somatic, not g	From LSDB	Not present in 1000G
113	200276989	C/T	NM_012268.3	929	Missense	1	SNP					1000G Phase 3, 1000G F
114	747066329	C/T	NM_012268.3	932	Missense	1	SNP					Not present in 1000G
115	199719440	C/T	NM_012268.3	937	Synonymous	1	SNP					1000G Phase 3, 1000G F

D.

116	776716590	A/G	NM_012268.3	944	Missense	1	SNP					Not present in 1000G
117	748464331	A/G	NM_012268.3	958	Synonymous	1	SNP					Not present in 1000G
118	192746231	C/T	NM_012268.3	963	Missense	1	SNP					1000G Phase 3, 1000G F
119	759001865	C/T	NM_012268.3	964	Synonymous	1	SNP					Not present in 1000G
120	769925913	A/G	NM_012268.3	965	Missense	1	SNP		Validated (has a			Not present in 1000G
121	773560279	A/G	NM_012268.3	968	Missense	1	SNP		Validated (has a			Not present in 1000G
122	534367783	C/T	NM_012268.3	969	Missense	1	SNP					1000G Phase 3, Has 100
123	758432318	-/GCC	NM_012268.3	975		1	INDEL					Not present in 1000G
124	144639479	C/T	NM_012268.3	979	Synonymous	1	SNP					Not present in 1000G
125	775049564	C/T	NM_012268.3	988	Synonymous	1	SNP		Validated (has a			Not present in 1000G
126	760256094	A/G	NM_012268.3	989	Missense	1	SNP		Validated (has a			Not present in 1000G
127	369138723	C/G	NM_012268.3	991	Synonymous	1	SNP		Validated (has a			Not present in 1000G
128	11552731	A/C	NM_012268.3	993	Missense	1	SNP					Not present in 1000G
129	753300888	C/G	NM_012268.3	1006	Synonymous	1	SNP					Not present in 1000G
130	868004647	C/T	NM_012268.3	1007	Missense	1	SNP					Not present in 1000G
131	756867186	A/G	NM_012268.3	1016	Missense	1	SNP					Not present in 1000G
132	764824983	A/C	NM_012268.3	1026	In 5' Gene, Missense	1	SNP					Not present in 1000G
133	367802500	A/G	NM_012268.3	1030	In 5' Gene, Synonymous	1	SNP					Not present in 1000G
134	761353429	G/T	NM_012268.3	1053	In 3' Gene, Missense	1	SNP		Validated (has a			Not present in 1000G
135	769363500	G/T	NM_012268.3	1054	In 3' Gene, Synonymous	1	SNP					Not present in 1000G
136	772839881	A/G	NM_012268.3	1056	In 3' Gene, Missense	1	SNP					Not present in 1000G
137	762536505	A/G	NM_012268.3	1068	In 3' Gene, Missense	1	SNP		Validated (has a			Not present in 1000G
138	867730804	A/C	NM_012268.3	1070	In 3' Gene, Missense	1	SNP					Not present in 1000G
139	867574189	A/C	NM_012268.3	1086	In 3' Gene, Missense	1	SNP					Not present in 1000G
140	766025627	C/G	NM_012268.3	1088	In 3' Gene, Missense	1	SNP					Not present in 1000G
141	144087366	A/G	NM_012268.3	1093	In 3' Gene, Synonymous	1	SNP		Validated (has a			1000G Phase 3, 1000G F
142	374184677	A/G	NM_012268.3	1097	In 3' Gene, Missense	1	SNP		Validated (has a			Not present in 1000G
143	767470890	C/T	NM_012268.3	1102	In 3' Gene, Synonymous	1	SNP					Not present in 1000G
144	752620117	A/G	NM_012268.3	1103	In 3' Gene, Missense	1	SNP		Validated (has a			Not present in 1000G

E.

371	567288047	C/T	NM_012268.3	2174	In 3' UTR	1	SNP					Not present in 1000G
372	775001616	-/G	NM_012268.3	2175	In 3' UTR	1	INDEL					Not present in 1000G
373	4635	A/C/G	NM_012268.3	2175	In 3' UTR	1	SNP		>5% minor allele			1000G Phase 3, 1000G P
374	781656714	A/C/G	NM_012268.3	2176	In 3' UTR	1	SNP		Validated (has a			Not present in 1000G
375	770404113	C/G	NM_012268.3	2178	In 3' UTR	1	SNP					Not present in 1000G
376	773898715	C/T	NM_012268.3	2180	In 3' UTR	1	SNP		Validated (has a			Not present in 1000G
377	546424854	A/G	NM_012268.3	2181	In 3' UTR	1	SNP					1000G Phase 3, Has 100
378	771543623	C/T	NM_012268.3	2188	In 3' UTR	1	SNP					Not present in 1000G
379	775083306	C/G	NM_012268.3	2193	In 3' UTR	1	SNP					Not present in 1000G
380	760201260	C/G	NM_012268.3	2195	In 3' UTR	1	SNP					Not present in 1000G
381	565813622	C/T	NM_012268.3	2197	In 3' UTR	1	SNP					1000G Phase 3, Has 100
382	762386016	-/G	NM_012268.3	2201	In 3' UTR	1	INDEL		Validated (has a			Not present in 1000G
383	776382708	C/T	NM_012268.3	2206	In 3' UTR	1	SNP					Not present in 1000G
384	761647265	A/C	NM_012268.3	2218	In 3' UTR	1	SNP					Not present in 1000G
385	764045401	-/T	NM_012268.3	2227	In 3' UTR	1	INDEL		Validated (has a			Not present in 1000G
386	765147994	A/C/G	NM_012268.3	2228	In 3' UTR	1	SNP		Validated (has a			Not present in 1000G
387	758362177	C/T	NM_012268.3	2238	In 3' UTR	1	SNP		Validated (has a			Not present in 1000G
388	766312803	A/C	NM_012268.3	2242	In 3' UTR	1	SNP					Not present in 1000G
389	751484821	-/C	NM_012268.3	2243	In 3' UTR	1	INDEL		Validated (has a			Not present in 1000G
390	546638979	C/T	NM_012268.3	2248	In 3' UTR	1	SNP		Validated (has a			Not present in 1000G
391	755210504	C/T	NM_012268.3	2251	In 3' UTR	1	SNP					Not present in 1000G
392	530183297	A/G	NM_012268.3	2253	In 3' UTR	1	SNP		Validated (has a			1000G Phase 3, Has 100
393	781603697	C/T	NM_012268.3	2259	In 3' UTR	1	SNP		Validated (has a			Not present in 1000G
394	748498735	A/G	NM_012268.3	2260	In 3' UTR	1	SNP					Not present in 1000G
395	548170265	C/T	NM_012268.3	2264	In 3' UTR	1	SNP					1000G Phase 3, Has 100
396	756602038	C/T	NM_012268.3	2275	In 3' UTR	1	SNP					Not present in 1000G
397	778336622	A/G	NM_012268.3	2288	In 3' UTR	1	SNP					Not present in 1000G
398	749686824	C/G	NM_012268.3	2294	In 3' UTR	1	SNP					Not present in 1000G
399	569676865	A/G	NM_012268.3	2299	In 3' UTR	1	SNP					1000G Phase 3, Has 100

F.

Figure 6 Figures (A-F) shows total snp seen in PLD3

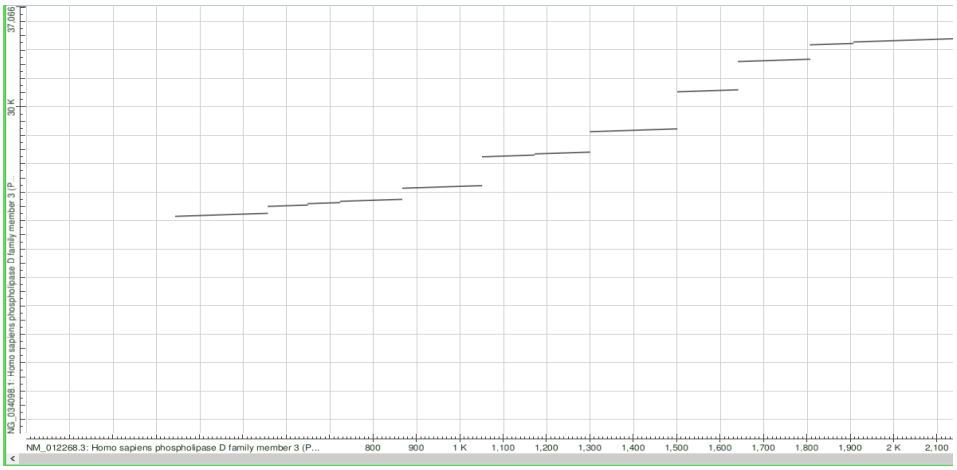

Figure 7 Graphical representation of MapDraw showing sequence

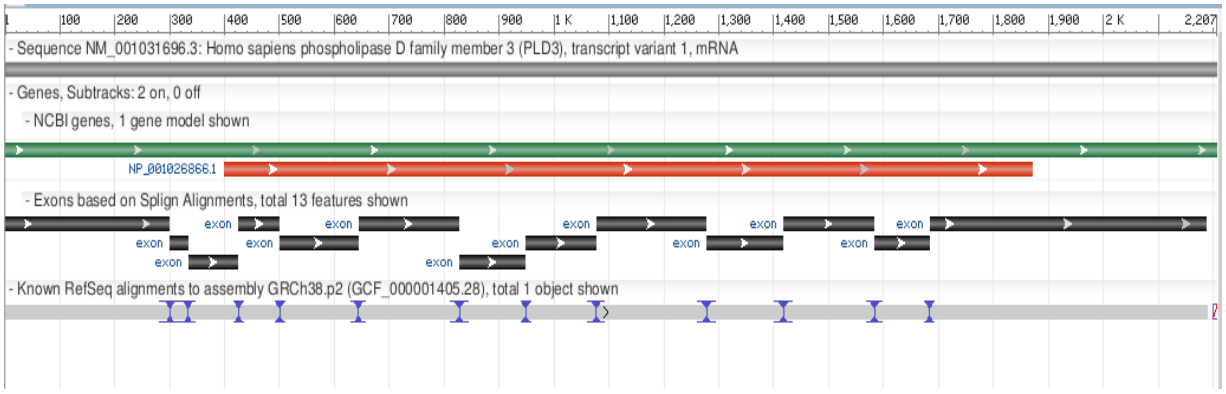

Figure 8 Graphical representation of sequences with exons notified

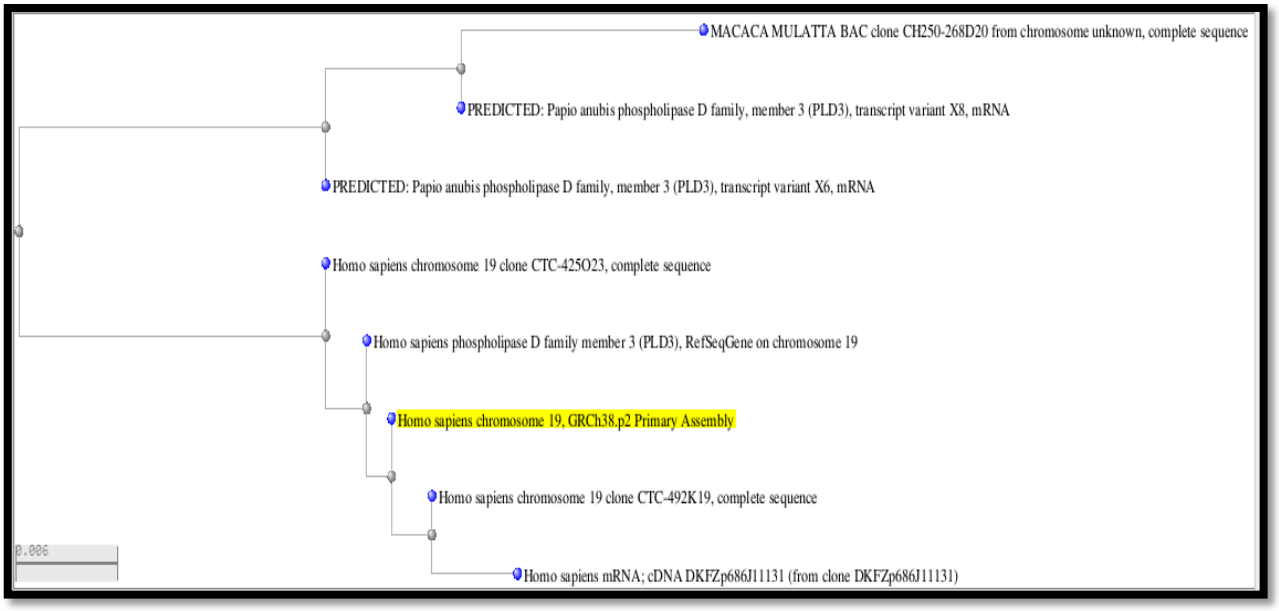

Figure 9 Closely related other genes of same organism and same species

Results and Discussion

On studying EditSeq 5 Orf's obtained in the sequence of length 2142 base pair, in gene quest 714 codons that are used in sequence with different percentage of base composition, in MapDraw obtained 212 enzymes with specific restriction sites, in PrimerSelect primers forward, reverse, melting temperature and rna codons (164, 163, average 47.5 and 95 respectively), SNP Table View shows Single Nucleotide Polymorphism which are 405 alleles with further descript as missense, synonyms, 3' and 5', frame shift, stopgain (157, 78, 102, 62, 2, 5 respectively), with Dot Matrix View the sequence arranged opposite to length, in Graphical Sequence View gives 13 feature exon in the 2142 long sequence and Phylogenetic Tree show closely related with PLD3 of same organism and same species.

Conclusions

Performed different modules to analyze sequence of PLD3 that are: Edit Sequence in which obtained 5 ORF in the sequence, In GeneQuest obtained 714 codon that were used in the sequence and nucleotide frequencies, In MapDraw obtained 5 types of restriction maps indicating different restriction enzymes for a particular restriction site, the total enzymes were 212, In PrimerSelect obtained upper primer (forward) which is 164 lower primer (reverse) i.e. 163, average melting temperature of the gene PLD3 is 47.26, In SNP Table View 405 SNP, In Dot Matrix View the length ranges from 2.5k to 35k with 9 segments, with Graphical Sequence View 13 exons were present in PLD3 and Phylogenetic Tree closely related to Homo sapiens chromosome 19 clone CTC-492K19 and Homo sapiens mRNA:cDNA DKFZp686J11131. The future aspects of these are helpful for specific SNP working, different function correlated with amyloid precursor protein (APP) and amyloid-β (Aβ) levels which are main cause of Alzheimer's Disease.

Conflict of Interests

The authors declare that they have no conflict of interest.

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