Phylogenetic analysis of genes and proteins of Alzheimer & Parkinson's

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In

Bioinformatics

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CERTIFICATE

This is to certify that the M.Tech. dissertation entitled "Phylogenetic analysis of genes and proteins of Alzheimer & Parkinson's" submitted by Km.Anjana **(DTU/12/M.Tech/393)** in partial fulfillment of the requirement for the award of the degree of Master of Engineering, Delhi Technological University (Formerly Delhi College of Engineering), is an authentic record of the candidate's own work carried out by her under my guidance.

The information and data enclosed in this dissertation is original and has not been submitted elsewhere for honoring of any other degree.

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LIST OF ABBREVIATIONS

1- aβ - β-amyloid

2-ACEIs- acetyl cholinesterase inhibitors

3-NSAIDs- Nonsteroidal anti-inflammatory drugs, or

4-UCH-L1- Ubiquitin carboxy-terminal hydrolase L1

5-PARK- Parkin

6-NCBI-National Center for Biotechnology Information

7-PSEN-presenilin

8-APP-Amyloid precursor protein

9-APOE-appolippoprotein E

10-EGPRED-Eukaryotic gene prediction server

12-Gad-gracile axonal dystrophy

Phylogenetic analysis of genes and proteins of Alzheimer& Parkinson

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ABSTRACT

In this work, we have done the phylogenetic analysis of Alzheimer disease (AD) & Parkinson's (PD) as well as genomic and proteomic study of genes and proteins of these diseases, and finally designed the siRNA target for each individual genes. We selected the four genes are APOE, APP, PSEN1 and PSEN2 of AD, and four genes of the PD these are SYNUCLIEN, PARK2, PARK, PARK7. We used various kinds of software tools and Databases. For genes and proteins sequence retrieval from NCBI database, phylogenetic relationship establishment by MUSCLE, T-COFFEE, CLUSTALW2 and BLAST distance tree. These tools helped us to select significant homo loges Pan Troglodytes,Musmusculus, Macacamulatta, Bos Taurus, RattusNorvegicus, and canis lupus, their gene and protein sequences for further analysis process .We have calculated the different types of physical and chemical properties of genes and proteins using Motif Search, Oligonulceotidecalculator, Sequence Manipulation Suite. We used some software tools for detailed analysis of genes like FTGpred server and EGpred , FTGPred server for analyzing nucleotide sequence to predict the gens using Fourier transform Technique. EGPred is a Web-based server that combines abinitio methods and similarity searches to predict genes, particularly exon regions, with high accuracy. For detailed analysis protein sequence we used TOP Pred and DASTrans membrane server, these software tools gave graphical view of the protein sequence, which helped to find the trans membrane region in protein and topology of protein. Finally we designed the siRNA target of these genes for therapeutic purposes, which is significant in treating genetically inherit neurological disorders, several knockdown studies,finding in new therapeutic agents for new drug discoveries and many medical applications.

Key words- AD-Alzheimer diseases, PD-Parkinson disease, PSEN1-presenilin1, PSEN2 presenilin2,APOE- apolipoprotein E, APP- Amyloidal precursor protein

Introduction

Phylogenetics is the study of evolutionary relationship among organisms or genes. Phylogenetic analysis is used to establish and inferring these relationship (Baxevanis*et al*.,2004). All organism on Earth have descended from a common ancestor, it means any set of species extant or extinct is related to each other. This relationship can be represented by *Phylogenetic trees*.The homogeneous attribute of biological functions and molecular mechanisms in living organisms vigorously suggests that species descended from a prevalent ancestor. Molecular phylogenetics utilizes the structure and function of molecules and how they transmute over time to infer these evolutionary relationships (Dermin*et al.,* 1998). This branch of study emerged in the early 20th century but didn't commence in earnest until the 1960swith the advent of protein sequencing, PCR, electrophoresis, and other molecular biology techniques (Hall *et al.,* 2006). Over the past 30 years, as computers have become more potent and more generally accessible, and computer algorithms more sophisticated, researchers have been able to tackle the immensely perplexed stochastic and probabilistic quandaries that define evolution at the molecular level more efficaciously.(Sanderson *et al*., 2002) Within past decade, this field has been further reenergized and redefined as whole genome sequencing for intricate organisms has become more expeditious and less expensive. (Leo *et al.,* 1998) As mounds of genomic data becomes publically available, molecular phylogenetics is perpetuating to grow and find incipient applications. (Patthy*et al.,* 2009)

Alzheimer's disease (AD) and Parkinson's disease (PD) are among the most common neurodegenerative disorders affecting older populations. (Beal *et al.*,2005) AD is characterized by impaired memory and cognitive decline while the primary symptoms of PD include reposing tremor, bradykinesia and rigidity (Sexton *et al*.,2011; Cheng *et al*., 2010). In PD, mild cognitive changes are frequently present, which could progress to dementia (PD dementia (PDD)). PDD and AD dementias are different in pathology although the difference in microstructural changes remains unknown. To further understand these diseases, it is essential to understand the distinct mechanism of their microstructural changes.

RNA interference (RNAi) is a natural cellular process that regulates gene expression and provides an innate defense mechanism against invading viruses and transposable elements (Kim *et al*., 2007). The finding that dsRNA initiates RNAi was among the most consequential recent contributions to cell biology (Fire *et al*., 1998), and since the revelation that RNAi can be mediated by 21nucleotide (Ntd) duplexes (Elbashir*et al*., 2001), researchers have worked to harness their potential for addressing biological questions and treating human disease.

2-Review of literature

2.1DEMENTIA

The meaning of dementia by and large acknowledged by clinical clinicians and specialists is that delineated in DSM-IV (APA, 1994). In synopsis, it states that for an analysis of dementia, there ought to be verifiable confirmation of hindrance in short-term what's more long haul memory. Hindrance in short-term memory (ie powerlessness to learn new data) may be showed by a powerlessness to recollect three articles following five minutes. Long haul memory weakness (ie Powerlessness to recollect data that was known in the past) may be shown by a powerlessness to recollect past particular data (eg what happened recently; origination; occupation) or actualities of basic learning (eg past Prime Ministers; well known dates). (Thompson, S. 2011)The notable purposes of the full-length definition (all of which don't essentially must be available for an analysis of dementia) are:

1. Impedance of fleeting and long haul memory;
2. Impedance of conceptual considering;
3. Impeded judgment;
4. Unsettling influences of higher cortical capacity (eg aphasia; apraxia; agnosia; constructional trouble);
5. Identity change;
6. Particular natural element;
7. Unlucky deficiency of a non-natural variable as a purpose behind the manifestations (eg significant misery).

2.2Alzhimer

Alzhiemer disease is acase of brain that causes problem with memory thinking and behavior but it is not a normal part of ageing. It gets worse as time progresses, some people may be toatally incapable of even more basic self-care and imposing a great burden on their families and communities.Alzhiemer is commonly called “THE AGING OF BRAIN” symptoms may vary widely but most of the people observed the first sign is forgetfulness severe enough to affect their ability to function at home as well as at work place or to enjoyelife long hobbies. The disease may cause problem that a person always remains confused,lost in familiar places, misplace things or having trouble with language.As the population ages, Alzhiemer becoming more of the medical, social and health concern. It is a dementing disorder can cause severe and irreversible lose of intellectual function (Nowotny*et al.*,2001; Kown*et al.*, 2001; Goate*et al.*, 2001) AD is a degenerative brain disease that causes the deterioration of cognitive abilities such as memory, language, communication, reasoning, and judgment. AD is a chronic brain disease that causes the impairment of cognitive abilities such as memory, language, communication, reasoning, and judgment, the symptoms increases with time and the disease advances bythe early, mid, and late stages. The demonstration of the symptoms can range for each individual at each stage. Currently, there is no cure for the disease;nevertheless,there are ways in which the

symptoms can be treated. Our memories are crucial because they help us to mold our experiences, our relationships, and our sense of self. The loss of them can be desolating and drive changes in personality and behavior. (Cohene*et al.,* 2007; Baecker*et al.,* 2007; Marzali*et al.,*2007; Mindey*et al*.,2007)

The criteria for the clinical diagnosis of Alzheimer's disease (AD) were accomplished by the National Institute of Neurological and Communicative Disorders and Stroke (NINCDS) and the Alzheimer's disease and Related Disorders Association (ADRDA) workgroup in 1984. These criteria were universally adopted successfully and have been extremely useful, survived intact without any alteration for more than a quarter of a century. Nevertheless, in the intervening 27 years, significant towards in our understanding of AD, in our ability to find the pathophysiological process of AD, and changes in conceptualization in respect of the clinical spectrum of the disease.(Jack jr Clifford R *et al.,* 2011).

Pharmacological treatments of the Alzheimer's disease are verylimited. According to recent observational studies,it may have observed that use of non-steroidal anti-inflammatorydrugs (NSAIDs) protect against the growthof the disease, possibly through their antiinflammatory properties. Consequences of research have varied and at least one study found no effect.Small sample sizes are also one of the limitation leads to negative result of the studies.In such considerations there should be asystematic review which quantify a pooled measure of effect from the existing studies.(Etminan*et al*., 2003; Gill *et al.,* 2003; Samii*et al.,*2003)

The various agencies worldwide responsible for marketing empowerments and forsuperwising medicinal productscurrently appropriate to use two different classes of treatmentfor Alzheimer's disease (AD): acetyl cholinesterase inhibitors and NMDA glutamate receptor antagonists.
In France, initially these drugs are prescribed by neurologist, geriatrician or psychiatrist are fully addressed by national health insurance. Theaim is to treat the AD not to achieve a cure but to assistin controlling the symptoms. The symptomatic efficacyof drug treatments has been demonstrated innumerous randomized trials. However, their effectiveness in relation to cognition is modest, andthere have been few assessments of their effect overtime.In addition, psychotropic drugs can also beuseful to control behavioral and psychological symptomsof the disease. However, pharmacologicaltreatment options, such as antipsychotics, antidepressantsand anticonvulsants, need careful considerationof the benefits and limitations of each drug class. Thisis most particularly important for antipsychotics withwidely reported side effects (Tifratene*et al*., 2012)

2.3<u>Types of Alzheimer</u>

2.3(a)Early-onset familial: Only 10 % of all somebody diagnosed with Alzheimer disease develop symptoms before the age of 65 years. They are said to have early-attack Alzheimer disease, and approximately 10% of these early-onset cases have a familial form of the condition, which is transmitted as an autosomal dominant allele trait.

2.3(b)Late-onset sporadic: Alzheimer disease is generally diagnosed after the age of 65 years, when it is referred to as late-onset Alzheimer disease. The condition affects 5% of the population aged over 65 years and more than twenty % of the population over 85 years.

2.4Clinical and Neuropathology symptoms of the Disease

Trouble in discovering the right words or comprehension what individuals are stating, trouble in performing long ago normal undertakings, and exercises, issues with dialect, identity and disposition.

AD is the most wide-spread progressive neurological disorder in men after 65 years of ageand it becomes very solemn all-society quandary in consequence of incrementing of average age. (Babusikova*et al*., 2011) Although the cause or causes of Alzheimer's disease are not yet kenned, most experts concurthat AD, like other mundane chronic conditions, probably develops as a result of multiplefactors rather than a single cause. Risk factors for AD are-

- Age
- Gender
- Gene polymorphism
- Hypercholesterolemia
- Diabetes mellitus
- Stroke
- Brain injuries
- Education
- Alcohol & Smoking

Alzheimer`s disease is a feature outgrowth with identifiable clinical state which are in a continuity with normal ageing process. It is a multifactorial disease and genetic as well as environmental factors are included in its pathogenesis (Babusikova*et al*., 2011).

2.5 Neuropathological phenotype of AD

- **Neurotic plaques**-These are one of the two diagnostic brain lesions observed in Alzheimer's original patient, are microscopic foci of extracellular amyloid deposition and associated axonal and dendritic injury, generally discovery in large numbers in the limbic and association cortices. (Dickson *et al*., 1997)Such plaques contain extracellular

deposits of amyloid β-protein (aβ) that occur principally in a filamentous form, i.e., as star-shaped masses of amyloid fibrils.

- **The pre-amyloidal Plaques-** many laboratories developed sensitive antibodies to endogenous or synthetic Ab. Immunohistochemical staining with such antibodies revealed a far more extensive number of Ab deposits apperceive many Ab deposits that lacked the compacted, fibrillar appearance of the classical neuritic plaques. Many of the plaques found in limbic and association cortices, and virtually all of those in brain regions not pellucid implicated in the typical symptomatically of AD. The apperception of these amorphous plaques in the tardy 1980s (Joachim *etal.,* Morris etal.,Selkoe*etal.,* 1989) and their detection in regions that additionally contained many neuritic plaques (i.e., limbic and association cortices) led to the concept that they might represent precursor lesions of neuritic plaques.(Tagliavini*etal.*,1988) (Hirai*etal*., 1988). These lesions were thus referred to as "diffuse" plaques or "pre amyloid deposits.

- **Neurofibrillary Tangles of Hyper phosphorylated Tau Proteins-** Numerous neurons in the cerebrum locales entorrhinal cortex, hippo campus, para hippocampal gyrus, amygdala, frontal, worldly, parietal and occipital affiliation cortices, and certain sub cortical cores anticipating, hold vast, non membrane bound groups of anomalous strands that involve a significant part of the perinuclear cytoplasm. Electron microscopy uncovers that the greater part of these strands comprise of sets of 10-nm fibers wound into helices (combined helical fibers or PH F), with a helical time of 160 nm. Some tangle bearing neurons likewise hold skeins of straight, 10- to15-nm fibers sprinkled with PHF. Starting in 1985, immune cytochemical and biochemical breaks down of neurofibrillary tangles proposed that they were made out of the micro tubule-related protein tau (Brionetal.,1985; Grundkeetal., 1986; Kosik*etal.*,1986; Nukiana*etal.,* 1986; Wood *etal.,* 1986).
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- **Dystrophic Cortical Neurites Within and Outside Neuritic Plaques-**Huge numbers of the widened and convoluted neurites found inside and promptly encompassing amyloid plaques hold PHF that are structurally, biochemically, and immunocytochemically unclear from those that include the neurofibrillary tangles. Furthermore, plaques regularlyhold various dystrophic neurites that are not immunoreactive or PHF tau. Tau-positive dystrophic neurites are additionally showed in a more across the board circulation in thecortical neuropil outside of the neuritic plaques. The pervasivenesswhat's more thickness of dystrophic cortical neurites that hold modified manifestations of tau changes significantly among Alzheimer cases. There is proof that cases that are especially rich in neurofibrillary tangles are additionally those that show boundless tau-immunoreactive dystrophic cortical neurites (Probst*et al.*,1989).

- **Microangiopathy of amyloid-**aβ was pristinely isolated from amyloid-laden meningeal arterioles and venules that are regularly discovered just outside of the brains of patients with AD or Down's disorder (Glenner*et al*.,1984a; 1984). Correspondingly, little arterioles, venules, furthermore vessels inside cerebral cortex likewise habitually bear amyloid stores. This microvascular angiopathy is described at the ultrastructural level by amyloid fibrils found in the albuminal storm cellar film of the vessels,once in a while with obvious enlargement or "overflow" ofthe fibrils into the encompassing perivascular neuropil (an injury alluded to as dyshorricangiopathty) (Verbeek*et al*.,2011). The Ab peptides that happen as fibers in the micro vessel storm cellarfilms show up, on the premise of immune reactivity, to be basically b40 species, in spite of the fact that confirmation has been displayed that the at first stored species in vessels, destined to create amyloid angiopathy may be aβ42 (Suzuki *et al.*,1994).

2.6 <u>Genetics of AD: Gene & mutation</u>

Alzheimer's disease is a progressive, neurodegenerative disease that represents a growth global health crisis. Two major forms of the disease exist: early onslaught (familial) and late onslaught (sporadic). Early onrush Alzheimer's is rare, accounting for less than 5% of disease burden. It is inherited in Mendelian dominant style and is caused by mutations in three genes (APP, PSEN1, and PSEN2).

Late onset Alzheimer's is park among individuals over 65 old of years. Heritability of this form of the disease is high (79%), but the etiology is campaign by a combination of genetic and environmental factors. A large number of genes have been implicated in the development of late onset Alzheimer's.

<u>2.6(a)APP mutation</u>-

The principle protein segment of the extracellular plaque is b-amyloid. Dissolvable b-amyloid is a typical constituent of human cerebrum produced by cleavage of the bigger APP by two proteins called β-secretase and gamma secretase b-Amyloid in the mind is heterogeneous, comprising of an arrangement of peptides fluctuating long from 39 to 43 amino acids. β-Amyloid of size 40 amino acids is alluded to asaβ40, and is ordinarily the most inexhaustible structure. (Genner*et al*., 1984) aβ42 and aβ43 allude to the 42 and 43 amino corrosive structures, and the extents of these two structures build in the amyloid plaques of Alzheimer ailment brains autosomal overwhelming Alzheimer ailment seem to change typical APP preparing by creating expanded generation of aβ42 and aβ43 An alternate kind of APP irregularity happens in patients with Down syndrome, a condition created by an additional duplicate of part or all of chromosome 21. Patients with Down syndrome are cannily hindered and have various formative variations from the norm noted right on time in life.

2.6 (b)Presenilin mutations-

The presenilin proteins are transmembrane proteins that are primarily localized in the endoplasmic reticulum and the Golgi apparatus. They are widely expressed but their function are unknown Mutation in this gene cause a phenotype, linked to defects in the Notch signaling pathway, which is consequential for cell-fate decisions during development. (Hutton et al., 1997) Mice destitute of PS1 additionally show rigorous defects that resemble a phenotype in which Notch is missing, fortifying the role of PS1 in this signaling pathway. So far, most of the mutations found to cause mutations found in PS2, which is 67% homologous to PS1 all the mutation increase the amount of aβ42 and aβ43 engendered, which expedites b-amyloid aggregation and amyloid plaque formation (Selkoe*et al*., 1998).

2.6(c) Apolipoprotein E e4 allele polymorphism-The genes whose mutations cause familial Alzheimerdisease are among the few kenned 'causes' of Alzheimer disease, but they are responsible for less than 1% of the total number of cases (Nowtony*et al*.,2001). Of more preponderant public health paramountacyhas been the finding that the e4 allele of the apolipoproteinE gene (ApoE-e4) occurs in sporadic cases of Alzheimerdisease with incremented frequency compared with controls.Apo E is a major serum lipoprotein involved in cholesterolmetabolism. There are three naturally occurring alleles oftheApoEgene, e2, e3 and e4, which differ from one anotherby a single codon. While the e4 allele is overrepresented among patients with Alzheimer disease compared with control populations, the e2 allele frequency is lower inpatients with Alzheimer disease than in controls, implicatively insinuatingthat this allele may be protective against developing thecondition. The ApoE-e4 allele shows a dose-dependentincrease in risk for Alzheimer disease, ostensibly mediatedthrough a decrementation in the age of onset, such that individualswith two replicas of the e4 allele have an earlier onset thanthose with one copy, who have an earlier onset thanindividuals with no e4 allele. The molecular mechanism bywhich the ApoE genotype is involved in Alzheimer disease pathogenesis is obscure, but patients with ApoE-e4 show a paramount, dose-dependent increase in the density of beta amyloid deposits (Lewy et al., 1997).

2.7 Treatment & Prevention

The most paramount class of drugs utilized in the concrete treatment of Alzheimer disease was developed for the ability to increment acetylcholine levels in the central nervous system. Not only are acetylcholine levels reduced in Alzheimer disease brains, but recollection and cognitive impairment can be induced in salubrious puerile persons and animals whose cholinergic transmission systems are pharmacologically blocked. There are now two classes of compounds that can increment brain acetylcholine levels:

(1) acetyl cholinesterase inhibitors (ACEIs), which increase synaptic concentrations of acetylcholine; and

(2) muscarinic agonists, which mimic acetylcholine by directly stimulating the muscarnic acetylcholine receptor.

ACEIs have been shown to be of modest clinical benefit in Alzheimer disease, and some ACEIs have been commercially available to treat the condition for several years. Since acetylcholinesterase breaks down acetylcholine, These receptors, when activated, have effects on learning, recollection and comportment; they may withal be involved in the processing of APP. The muscarinic agonists currently being developed are categorical for the muscarinic M1 receptor subtype. The M1receptors are localized in the cortex and hippocampus, whereas other muscarinic receptors are withal found in smooth muscle and glandular tissue. The latter may beresponsible for the uncomfortable side effects – namely, salivation, sweating, nausea and regurgitating – visually perceived when endeavoring to manipulate the cholinergic system pharmacologically. (Levy *et al*.,1998)

The research has been promising;Oestrogen has other salutaryeffects, which include incrementing levels of choline acetyltransferase.

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Other treatment roads that are continuously investigated include:

1. Lessening calcium poisonous quality with calcium channel blockers, which ensure neurons from calcium particle actuated damage by restricting calcium particle entrance;
2. Utilizing cholesterol-bringing medications down to bring down the cerebrum amassing of Apoe-e4;
3. Lessening the concoction changes in the tau protein;
4. Keeping the improvement of plaques, neuritic dystrophy and gliosis by ahead of schedule inoculation with Ab42; and
5. Keeping the development of b-amyloid by repressing the secretases that discharge it fromappor by forestalling b-amyloid from collecting into its dangerous structure. Albeit a percentage of the proposed medicines depicted above, for example, estrogen substitution and constant NSAID utilization, do appear to bring down the danger for creating Alzheimer infection in specific populaces, it is vague how powerful they may be at keeping the condition. (Marx *et al.*, 1996) Avoidance is an viable method in ailments where the danger components or etiology are comprehended and modifiable (e.g. suspension of smoking to avoid lung disease or immunizations to anticipate adolescence .sickness Unfortunately, the best known etiologies alternately hazard variables for Alzheimer ailment are hereditary transformations, on account of the familial condition, or the Apoe-e4 allele on account of sporadic Alzheimer malady. While these are not promptly modifiable, they may propose certain populaces at most astounding danger for Alzheimer illness in whom more forceful intercession may be advantageous.(Nowtony *et al*., 2001).

2.8 Parkinson

Parkinson's disease is a neurodegenerative movement disorder caused by a combining of environmental and genetic factors. Recent man genetic survey have identified five gene that are linked to Parkinson's disease (PD): a-synuclein, parkin, UCH-L1, DJ-1 and NR4A2. Among these genes, a salmagundi of mutations in the human parkin locus have been found in many PD compositor's case , both familial and sporadic. Parkin appears to be the most prevalent genetic factor in PD. It encodes for a protein -ubiquitin E3 ligase, whose loss-ofpurpose mutations cause specific retrogression of Intropin (DA) neurons in substantia jigaboo in human patients. The aggregation of parkin substrates is 1 sense of think to be the Key factor in the selective death of DA neurons. Rapid progress in the identification of these substrates and the generation of genetic animal poser has produced a plethora of knowledge about the biological function of parkin and its role in PD. These sketch also offer novel pharmacological targets for the ontogeny of more selective therapeutic strategies.(Feing*et al*., 2003)

2.9Genetics of the Parkinson disease

Long term epidemiological studies demonstrate that numerous variables help the frequency of PD (Tanner *et al*.,1990). These incorporate presentation to specific poisons, living in a provincial nature's domain, and so forth. Regardless of far reaching hunt down particular poisons in brains of PD patients, no convincing discoveries in human populace have been acquired so far on the commitment of ecological elements to PD. On the other hand, it has been noted for quite a while that more or less 5~10% of PD cases are familial (Olanow*et al.*, 1999). Late advancement in sub-atomic heredity investigations of families with PD has prompted the distinguishing proof of a few loci that are joined to certain inherited manifestations of PD. These incorporate a-synuclein (Park1) on chromosome 4q21-23 (Polymeropoulos*et al*.,1996; 1997), parkin (Park2) on chromosome 6p25.2-27 (Kitada*et al*., 1998), UCH-L1 (Park5) on chromosome 4p14 (Leroy *et al.*, 1998), DJ-1 (Park7) on chromosome 1p36 (Olanow*et al.*, 1999) , and Nr4a2 on chromosome 2q22-23 (Le *et al*.,2003). In expansion to these genes, a few other loci have been ensnared in PD, in spite of the fact that the mindful genes have not been recognized.

2.9(a)Synuclein-

The main confirmation that a few manifestations of PD could have a absolutely hereditary premise was accounted for by Golbe and colleagues in the introductory record of the Contursirelated (Bjarkm*et al.*, 2001). Through linkage investigation, (Polymeropoulos*et al*.1996) mapped a gene for PD to a locus at chromosome 4q21-23 (Park1 locus) (Polymeropoulos*et al*.1996). They later distinguished a point transformation (A53t) in the alpha synuclein gene, which was placed in this locus and was joined to an early onset, autosomal predominant manifestation of familial PD (Polymeropoulos*et al*.1997). An alternate missense transformation (A30p) in a- synuclein was distinguished in a free study (Kruger *et al.*, 1998) On the other hand, consequent examinations including an expansive number of patients with sporadic or familial PD have fizzled to distinguish any transformations in the a-synuclein gene (Chan *et al*.,1998, Scott *et*

al.,1997;Vaughan*et al*., 1998a; 1998b), showing that this gene is included in little populace of PD patients. Notwithstanding the rareness of transformations in a-synuclein, in vitro studies exhibit that contrasted with the wild-sort protein, both a-synuclein mutants show quickened establishment of Lewy body-like fibrils and circular congregations when they are available at high fixation in result (Conway *et al.,* 1998;Narhi*et al*., 1999).

2.9(b)Parkin-

Linkage mulls over in a couple of Japanese families with Autosomal Latent Juvenile Parkinsonism (AR-JP) prompted the distinguishing proof of a locus on chromosome 6q25.2-q27 (Park2 locus) (Matsumine*et al*.,1997). Utilizing positional cloning and exon trapping systems, (Kitada*et al*., 1998) cloned a vast gene (1.3 Mb) in this locale whose inward erasure was connected with PD manifestations. It was named parkin to reflect its association with the illness (Kitada*et al*., 1998). Various catch up studies have demonstrated that point changes, interior erasure and truncations happen in numerous AR-JP patients with differing ethnic foundations (Abbas *et al*.,1999;Hattori *et al*.,1998a; 1998b; Leroy *et al*.,1998; Lucking *et al*., 2000). As opposed to patients with sporadic PD, the larger part of AR-JP patients don't have Lewy bodies, considerations. Notwithstanding the causative part of parkin in AR-JP, its changes are a huge element in idiopathic manifestations of Parkinson's disease.

2.9(c) UCH-L1

A missense mutation (I93M) in the ubiquitin carboxylterminalhydrolase L1 (UCH-L1) gene was identified in a German family with autosomal ascendant PD (Leroy *et al*.,1998). UCH-L1is a brain-concrete deubiquitinating enzyme that is present inLewy bodies. The I93M point mutation causes a 50% reduction in catalytic activity of the enzyme, which ispostulated to affect protein degradation in the brain.However, the mutation identified in the diminutive German familyhas incomplete penantrance(Leroy *et al*.,1998). Additional studies on several PD kindred's fail to identify any mutation in this gene(Gasser *et al*., 2001). Furthermore, null mutation of UCH-L1 gene in the gracile axonal dystrophy (gad) mouse exhibits phenotypes that are markedly different from those of PD (Saigoh*et al.,* 1999). It is still obscure to which extent UCH-L1 is involved in the etiology of PD, albeit a recent study suggests that UCH-L1 may have two opposing enzymatic activities: an ubiquitin C terminal hydrolase and a dimerization-dependent ubiquityl ligase, which may be paramount in PD pathogenesis (Liu *et al.,* 2002).

2.9 (d)DJ-1

Very recently, two mutations in the DJ-1 gene were found in two families that have an autosomal recessive form of right on time onset Parkinsonism (Park7) (Bonafati*et al*.,2002). One of the mutations is a point mutation (L166p); the other is a 14 kb deletion that uproots the initial five exons. In both cases, just homozygous relatives are influenced, while heterozygous bearers are clearly free of any PD manifestations (Bonafati*et al.,*2002). It recommends that the changes cause misfortune of capacity of the gene item, which may be discriminatingly included in PD.

The known capacities of DJ-1 have all the earmarks of being very different and don't give any evident association with Parkinson's disease. It has arrangement homology to some prokaryotic proteins, for example, Thij, which is included in thiamine amalgamation; Pfpi, a protease; arac, a translation variable; and certain glutamine amidotransferases (Bonafati*et al*.,2002). Human DJ-1 is initially distinguished as an oncogene that interfaces with c-myc or h-ras to expand cellconversion C) An alternate gathering has observed that it is a administrative subunit of a RNA-tying protein.

2.10 Symptoms of the Parkinson disease

The primary symptoms of Parkinson's disease are all cognate to voluntary and involuntary motor function and conventionally start on one side of the body. Symptoms are mild at first and will progress over time. Some individuals are more affected than others. Studies have shown that by the time that primary symptoms appear, individuals with Parkinson's disease will have lost 60% to 80% or more of the dopamine-engendering cells in the brain. Characteristic motor symptoms include the following

2.10(a)Tremors: Trembling in fingers, hands, arms, feet, legs, jaw, or head tremors regularly happen while the individual is resting, however not while included in an assignment. Tremors may decline when an individual is energized, tired, or pushed.

2.10(b)Inflexibility: Stiffness of the appendages and trunk, which may expand amid development. Unbending nature may deliver muscle throbs and ache. Misfortune of fine hand developments can prompt cramped penmanship (micrographia) and may make consuming troublesome.

2.10(c)Bradykinesia: Slowness of intentional development. About whether, it may get to be hard to start development and to finish development. Bradykinesia together with solidness can likewise influence the facial muscles and bring about a vacuous, "veil like" appearance.

2.10(c)Postural instability: Impaired or lost reflexes can make it hard to alter carriage to keep up equalization. Postural flimsiness may prompt falls.

2.10(d)Parkinsonian gait: Individuals with more dynamic Parkinson's malady create a different rearranging stroll with a stooped position and a lessened or truant arm swing. It may get to be hard to begin strolling and to make turns. People may solidify in mid-stride and seem to fall forward while strolling.

2.10 (e) Secondary symptoms of Parkinson's disease

nervousness, instability, and anxiety, perplexity, memory misfortune, and dementia (more normal in elderly people) ,stoppage, misery trouble gulping and unreasonable salivation ,reduced

feeling of scent expanded sweating ,male erectile brokenness's, skin issues impede, quieter discourse, and monotone voice, urinary recurrence/direness

2.11Causes of Parkinson disease

A substance called dopamine goes about as an errand person between two cerebrum territories - the substantianigra and the corpus striatum - to deliver smooth, controlled developments. The greater part of the development related indications of Parkinson's ailment are created by an absence of dopamine because of the misfortune of dopamine-creating cells in the substantianigra. At the point when the measure of dopamine is excessively low, correspondence between the substantianigra and corpus striatum gets to be inadequate, and development gets to be hindered; the more noteworthy the misfortune of dopamine, the more terrible the development related side effects. Different cells in the cerebrum likewise decline to some degree and may help non-development related indications of Parkinson's disease.

In spite of the fact that it is well realized that absence of dopamine causes the engine indications of Parkinson's ailment, it is not clear why the dopamine-creating mind cells disintegrate. Hereditary and neurotic studies have uncovered that different useless cell methods, aggravation, and anxiety can all help cell harm. Furthermore, unusual bunches called Lewy bodies, which hold the protein alpha-synuclein, are found in numerous mind cells of people with Parkinson's ailment. The capacity of these bunches with respect to Parkinson's sickness is not caught on. All in all, researchers suspect that dopamine misfortune is because of a fusion of hereditary and natural variables.

Who is at risk for Parkinson's disease?

- Age is the biggest danger variable for the improvement and movement of Parkinson's infection. Most individuals who create Parkinson's ailment are more seasoned than 60 years old .
- Men are influenced about 1.5 to 2 times more regularly than ladies.
- A little number of people are at expanded danger due to a family history of the issue
- Head trauma, disease, or presentation to natural poisons, for example, pesticides and herbicides may be a danger element**.**

2.12 Treatments & Surgery

2.12(a)Drug treatment-

Medications for PD fall into three categories. The first category includes drugs that work directly or indirectly to increment the caliber of dopamine in the brain. People cannot simply take dopamine pills because dopamine does not facilely pass through blood vessels into the brain. The most mundane drugs for PD are dopamine precursors – substances such as levodopa that cross the blood-brain barrier and are then transmuted into dopamine. Other drugs mimic dopamine, avert or slow its breakdown, or increase the amount of it that is relinquished.

The second category of PD drugs affects other neurotransmitters in the body in order to facilitate some of the symptoms of the disease. For example, anticholinergic drugs decrease the activity of the neurotransmitter acetylcholine. These drugs avail to reduce tremors and muscle stiffness, which can result from having more acetylcholine than dopamine.
The third category of drugs prescribed for PD includes medications that avail control the non-motor symptoms of the disease. For example, people withPD-cognate despondence may be prescribed antidepressants**.**

2.12(b) Surgical treatment

At present, there are two commonly used surgical treatments for PDpallidotomy and deep brain stimulation. Because these procedures are invasive, they are conventionally reserved for rigorously afflicted Parkinson's patients who do not get adequate assuagement from medications.
Brain surgery was one of the first treatments for PD. Surgeons discovered that, by abstracting or ravaging components of the brain that were "misfiring," some of the symptoms of PD could be alleviated. The most mundane early brain operations for PD were pallidotomy, which eradicated part of the globuspallidus, and thalamotomy, which eradicated part of the thalamus. These procedures were irreversible and often led to complications. Clinicians have amended these techniques a great deal, but while they are much safer now, they are still irreversible In recent years, scientists have found that they can mimic the effects of pallidotomy and thalamotomy by deep brain stimulation (DBS). With DBS, an electrode is implanted in the brain in a way that calms the anomalous neuronal firing. This procedure is much safer than pallidotomy or thalamotomy because the electrodes can be turned off if the patient experiences quandaries. The stimulation additionally can be adjusted to match the patient's needs. Because of this, DBS is now the primary surgical intervention for PD. In 1997, the U.S. Food and Drug Administration (FDA) approved DBS for the treatment of essential tremor utilizing a single implanted electrode on one side of the brain. In January 2002, the FDA approved DBS for PD utilizing two implanted electrodes — one on each side of the brain. Recently, the FDA additionally approved a technologically advanced electrode apparatus that can becontrolled by the patient through utilization of a remote control contrivance.

2.12(c)Coplementry and supportive therapies

A wide variety of complementary and auxiliary therapies may be utilized for PD. Among these therapies are standard rehabilitation techniques, whichcan avail with quandaries such as gait and voice disorders, tremors and rigidity, and cognitive decline. Exercise may avail people ameliorate their mobility.Physical therapy or muscle-invigorating exercises may tone muscles and put underused and rigid muscles through a gamut of kineticism. Exercisecannot stop disease progression, but it may amend body vigor so that the person can more preponderant cope with his or her incapacitation. Researchers arestudying whether exercise withal may ameliorate the replication to levodopa and or increase levels of propitious compounds called neurotrophic factors in thebrain. Targeted exercises additionally may ameliorate balance, avail people

overcome gait quandaries, and reinforce certain muscles so that people can verbalizeand swallow more preponderant. Although structured exercise programs avail many patients, more general physical activity, such as ambulating, gardening, swimming,and utilizing exercise machines, is additionally propitious.Some early reports suggested that dietary supplements may be protective in PD. In integration, a phase II clinical tribulation of a supplement called coenzymeQ10 suggested that immensely colossal doses of this substance can slow disease progression in patients with early-stage PD. The NINDS and the National Centerfor Complementary and Alternative Medicine (NCCAM) are funding research to determine if folate, coffee, dietary antioxidants, fat, alcohol, and/or dairyproducts are salutary. While there is currently no evidence that any concrete dietary factor is benign in PD, a routine, salubrious diet can promoteoverall salubrity for PD patients just as it would for anyone else.Other complementary therapies that are utilized by some individuals with PD include massage therapy, yoga, tai chi, acupuncture, ginkgo biloba (forconcentration quandaries), and the Alexander technique, which optimizes posture and muscle activity. There have been inhibited studies suggesting mildbenefits with many of these therapies, but they do not slow PD and there is no cogent evidence that they are propitious.

3-METHODOLOGY

We have used various types of Bioinformatics software tool for Phylogenetic analysis of nucleotide and protein sequences of theAlzheimer and Parkinson's. First we used BLAST to find out homolog's of the genes and protein sequence, we picked the five homolog'sof each gene and protein of the both Neurological Disorder for example we took **Bos*Taurus*, canis*lupus*, Pan *Troglodytes*, Mus*musculus*, Rattus*norvegicus*&*Macacamulatta***. These are homolog's having the genes and proteins which being mutated in the Alzheimer as well as in Parkinson's.

Alzheimer- Apoe, App, PSEN1,PSEN2

Parkinson's-Synuclein, PARK2, PARK5, PARK7

These are those genes and protein which are responsible for neurological disorder. We analyzed these genes and their corresponding protein; basically we did proteomic and genomics. We have followed some steps which are as follows.

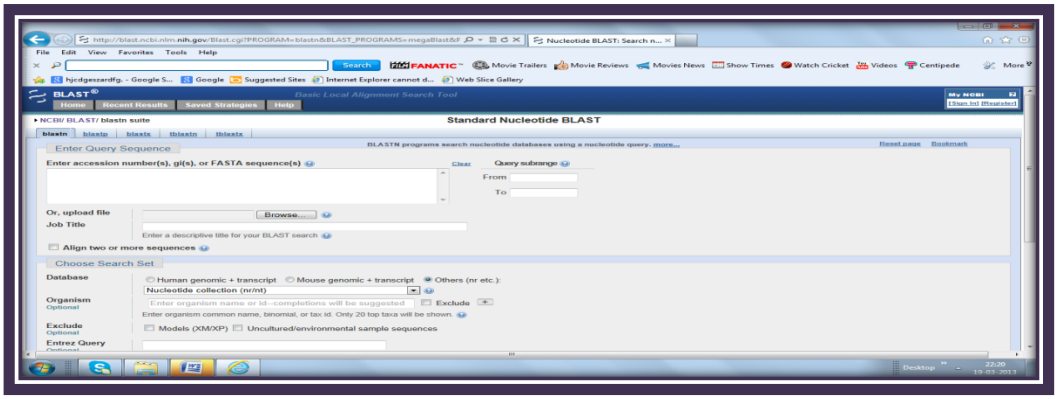
3.1Sequence Retrieval-

We retrieved all the genes and proteins from NCBI Database of Alzheimer&Parkinson's, then we did BLAST all of them for finding their homolog for comparative and phylogenetic analysis. We picked five homolges genes and proteins for genomic and proteomic studies

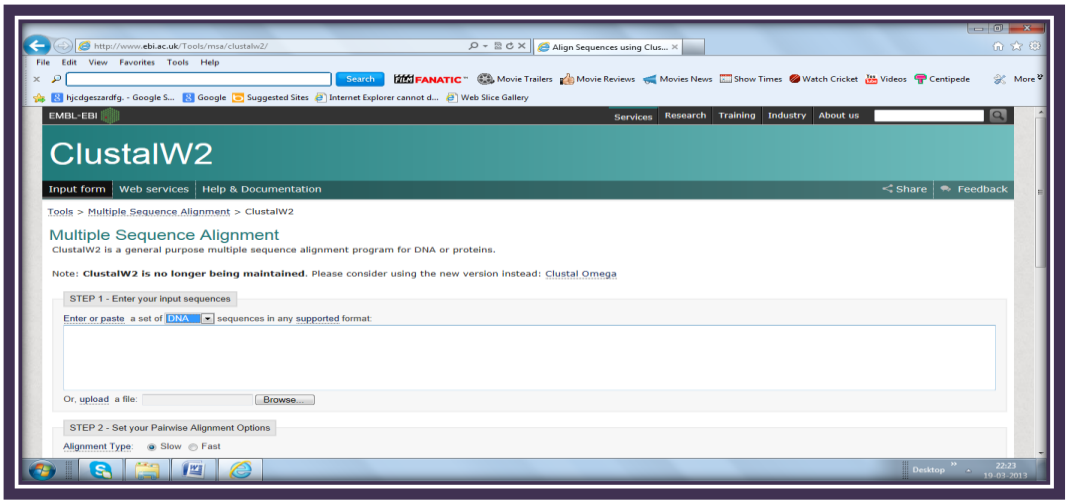
The gene sequences and their variants responsible for Alzheimer&Parkinson's have been identified and downloaded from the NCBI site in the Fasta format. These sequences have further been analyzed using the BLAST for their similarity with other related organisms. The sequences and their variants are further analyzed by performing multiple sequence alignment using software like **CLUSTALW**, **MUSCLE** and **T-COFFEE** for the similarity between these sequences. Finally the phylogenetic relationship between these sequences has been established using phylogenetic tool like **BLAST Distance tree**. Studying the phylogenetic analysis of the sequences mentioned helped us for prediction how these sequences have evolved with time in different species and as per the current sage how closely the sequences are related and how they differ from each other.

3.2Tools used-

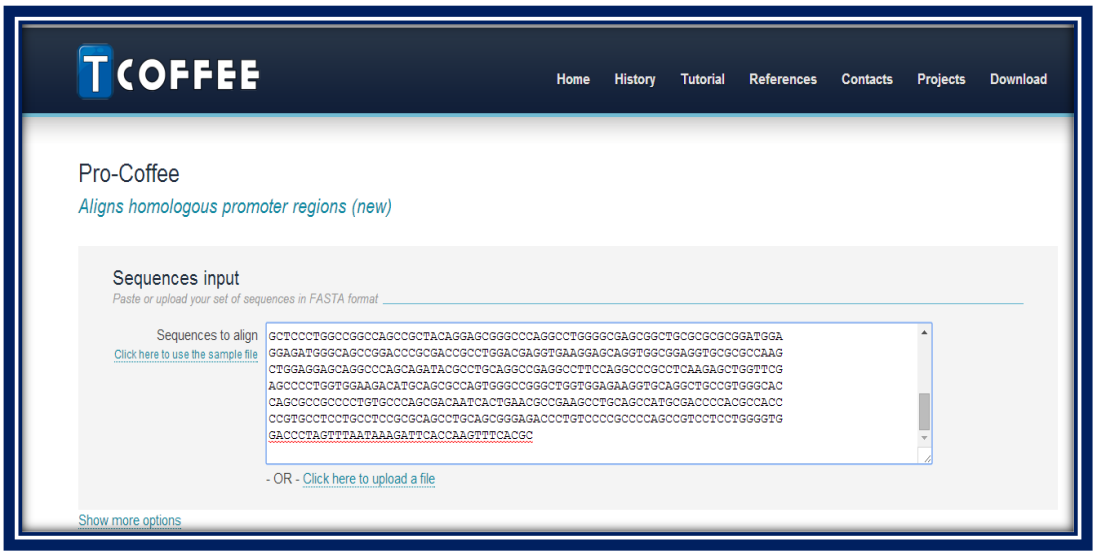
BLASTn



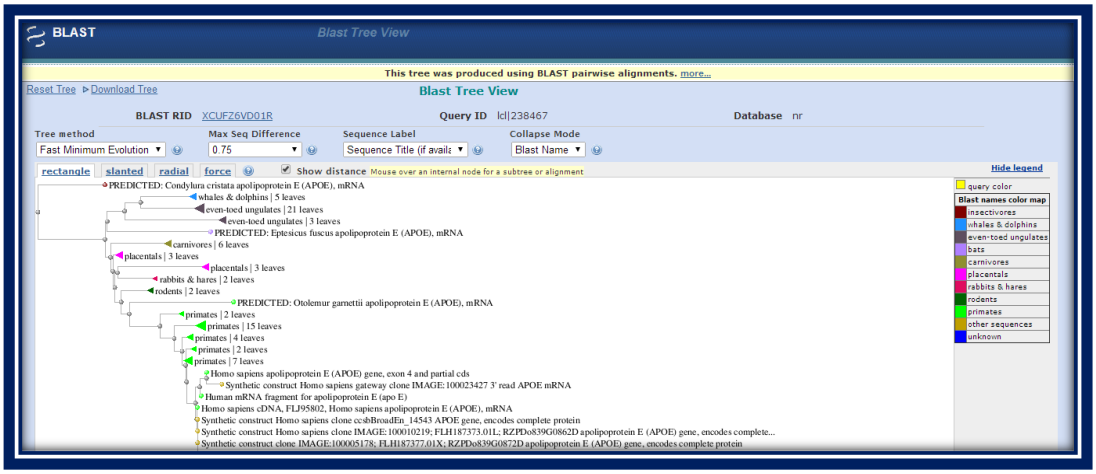
CLUSTALW2



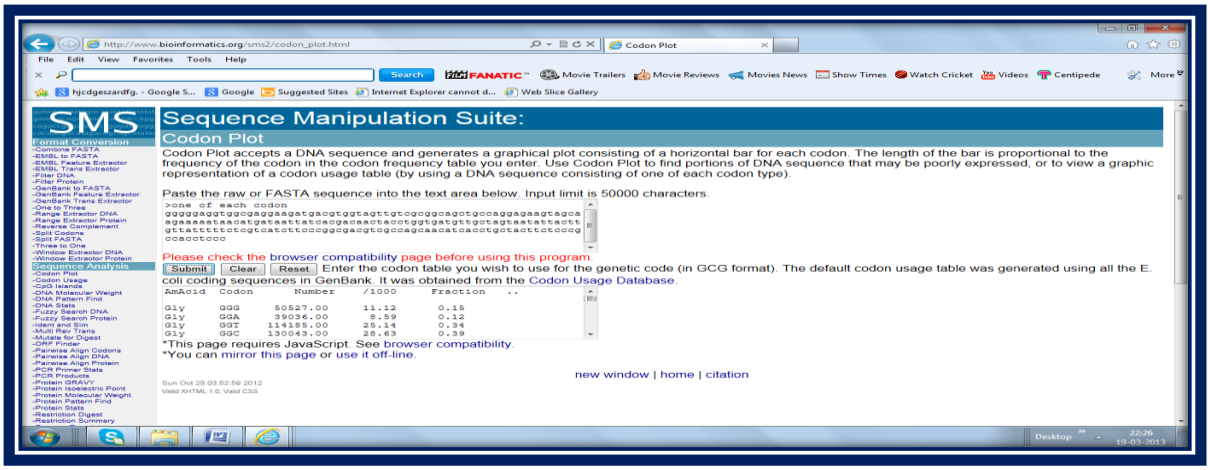
T-COFFEE



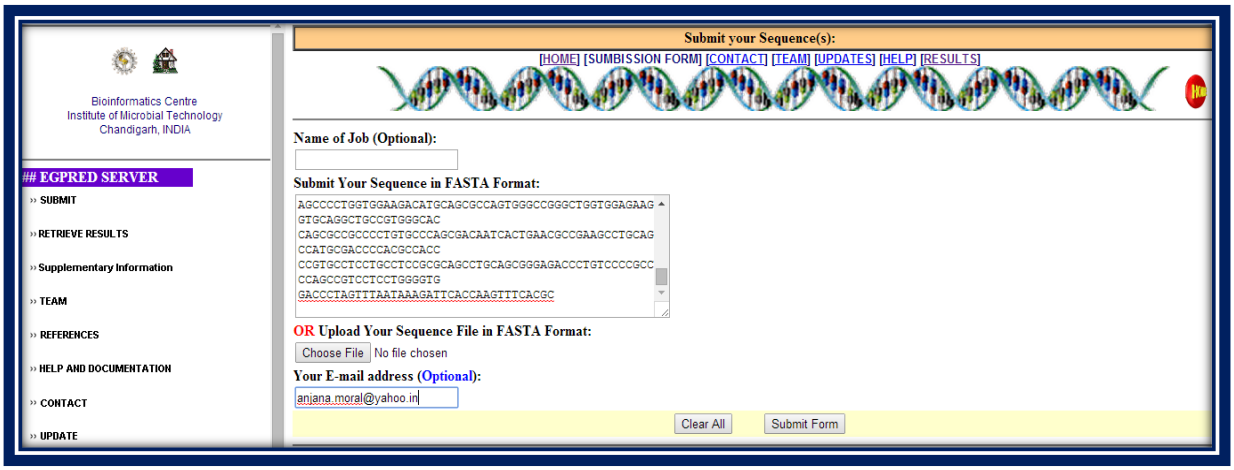
BLAST distance tree for establishing Phylogenetic analysis.



Sequence Manipulation Suite-

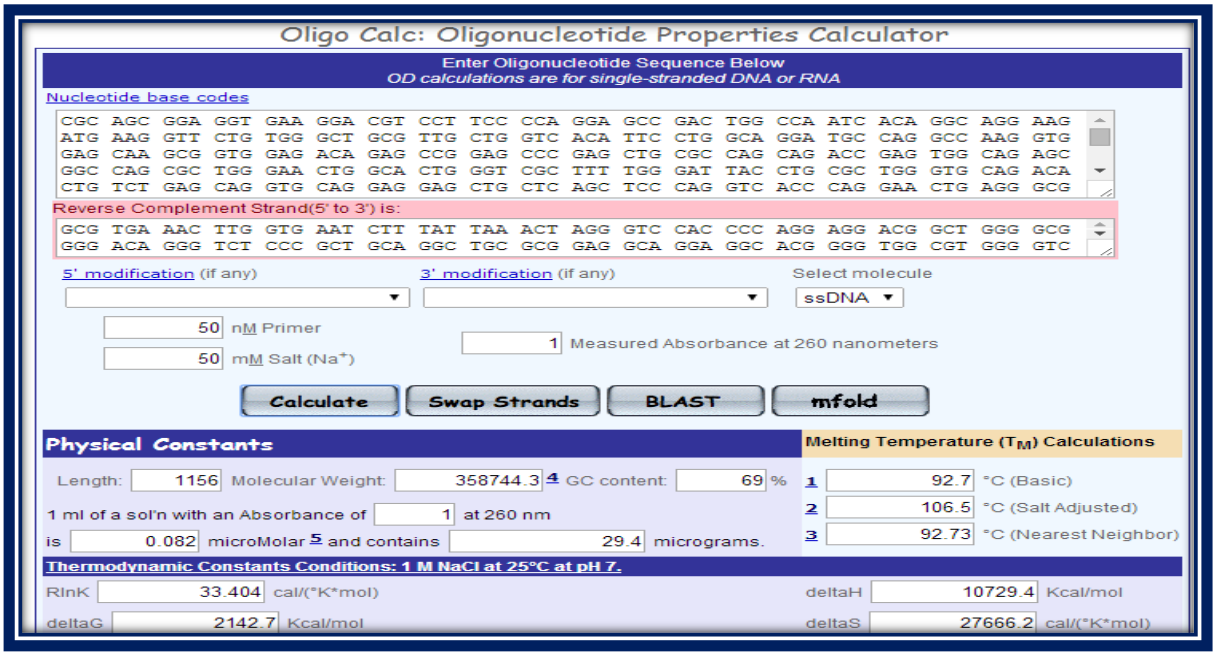


FTGPred server for analyzing nucleotide sequence to predict the gens using Fourier transform Technique.

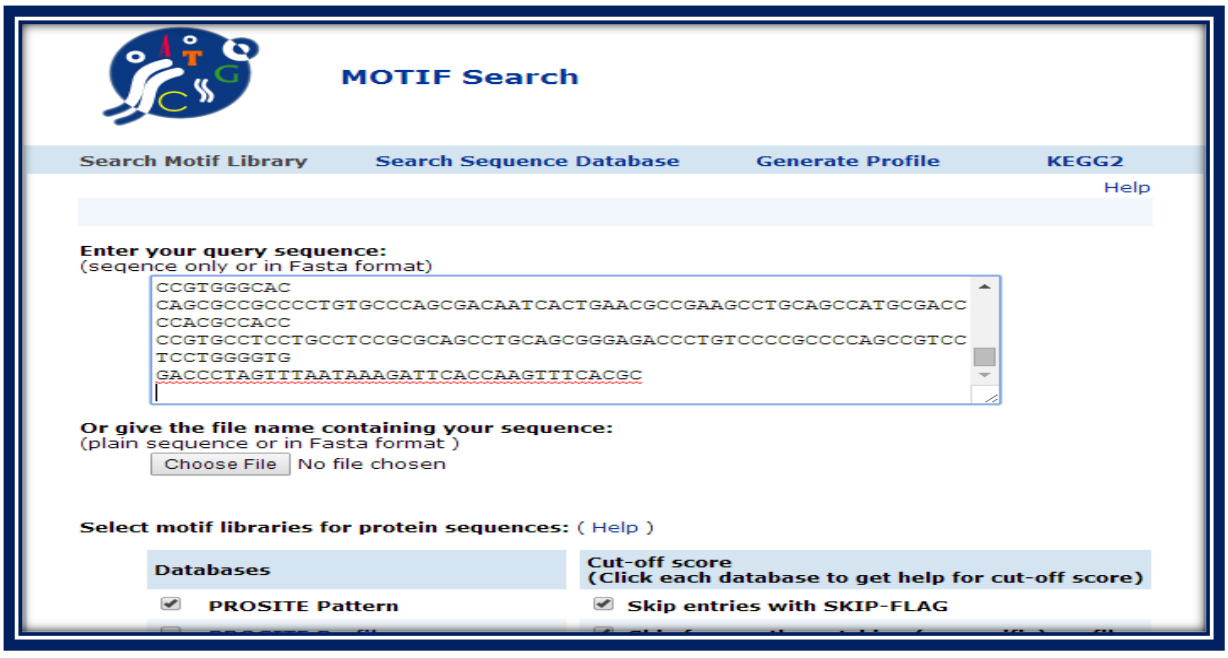


EGPred is a Web-based server that combines ab initio methods and similarity searches to predict genes, particularly exon regions, with high accuracy

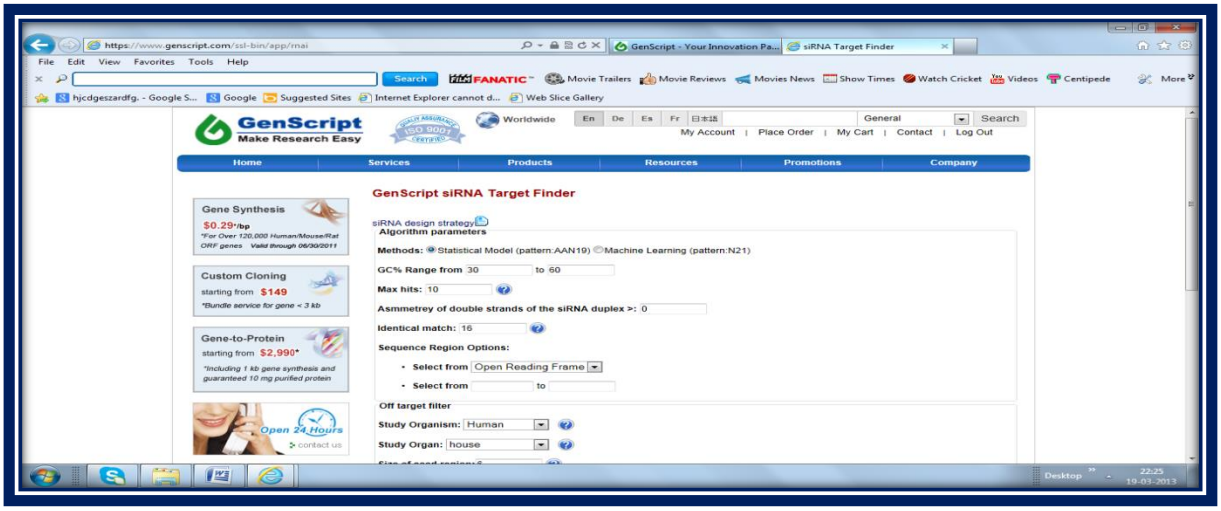
Oligonucleotide property calculator calculate the physical constants and thermodynamic constants of nucleotide sequence of genes.



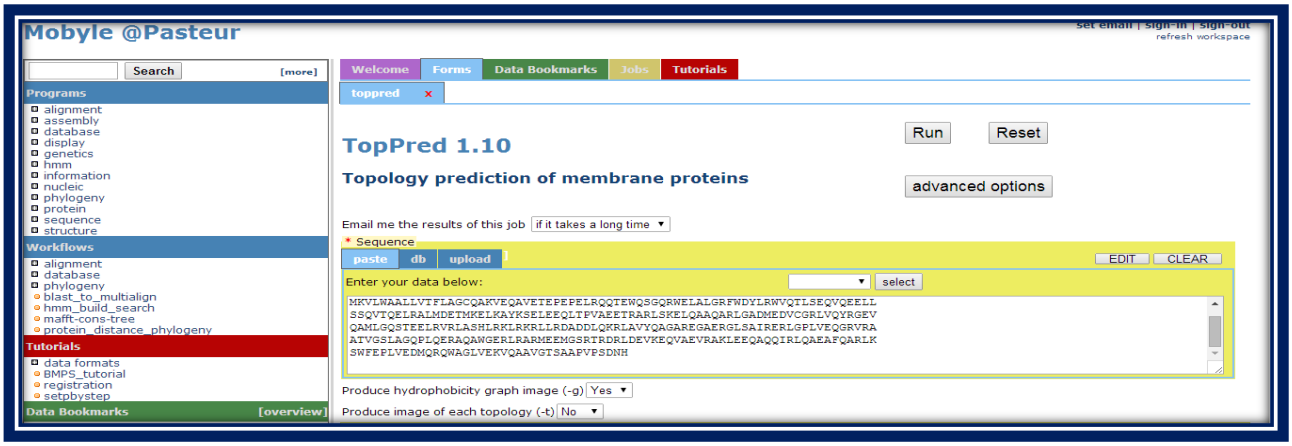
Motif Search-help to find Motifs and domain in DNA sequence



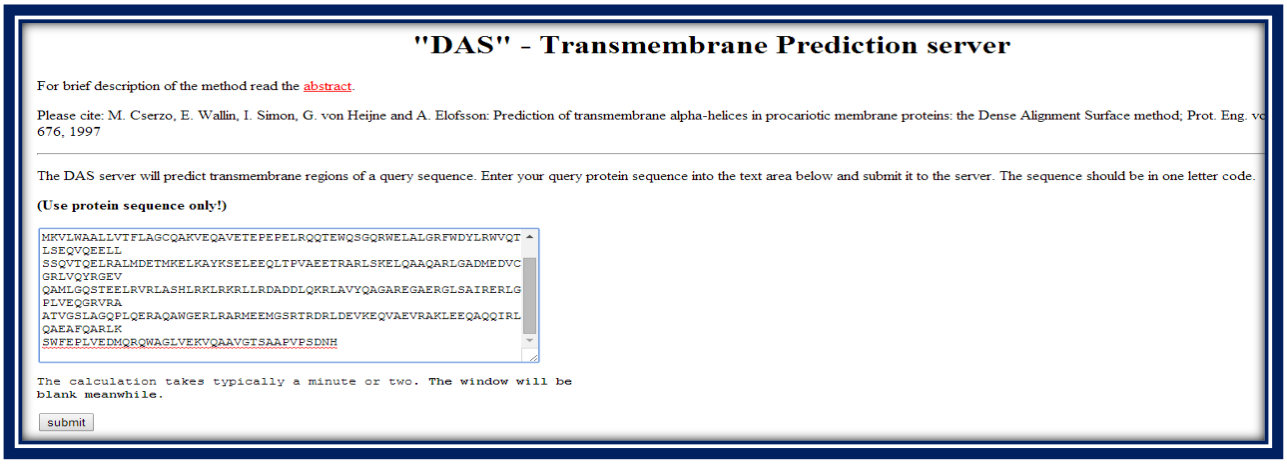
GenScript-help to design si-RNA for particular nucleotide sequence



TopPred-It is used to predict topology of a membrane protein from its amino acid sequence

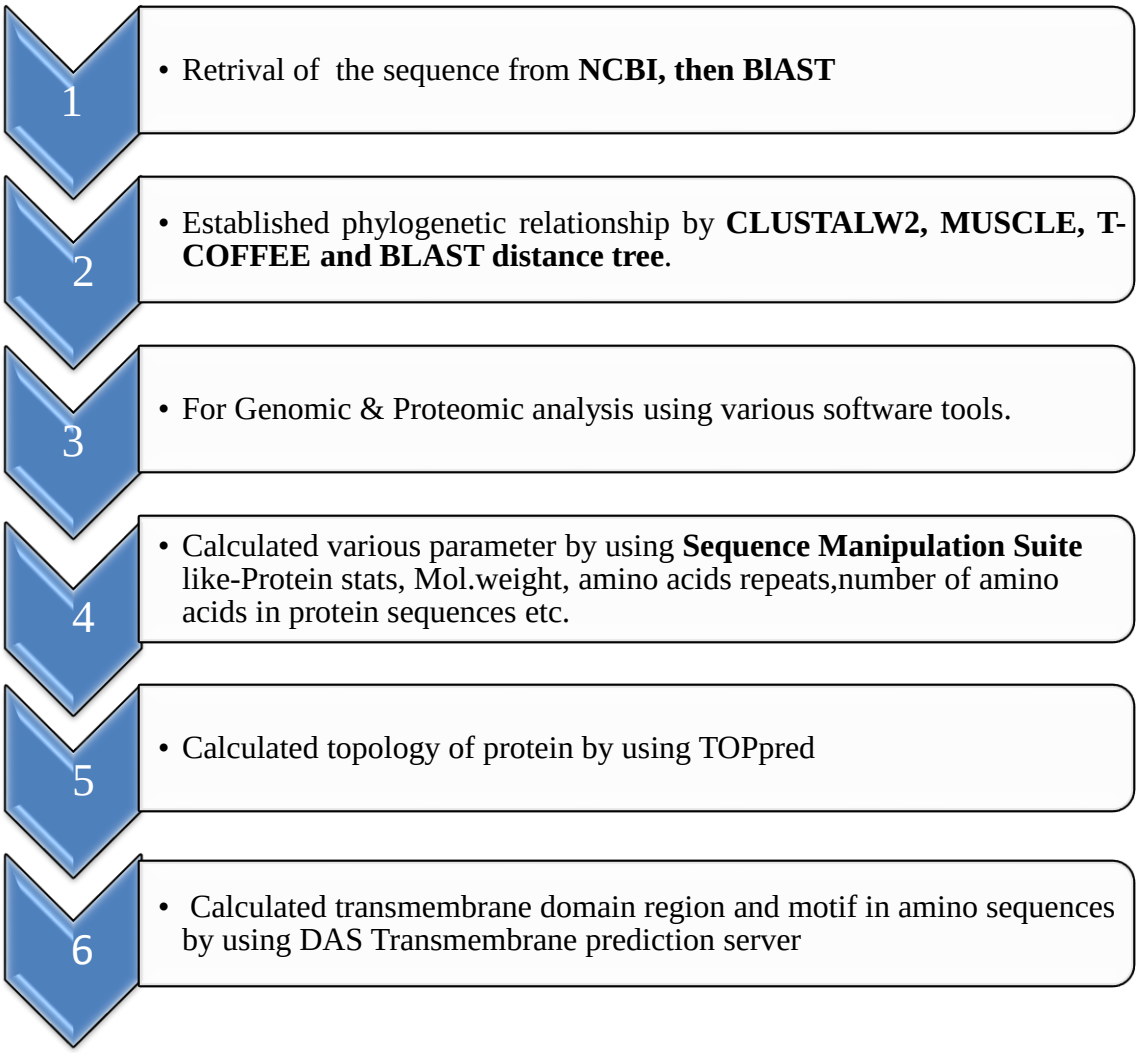


DAS-Transmembrane prediction server help to find out transmembrane domein in amino acid sequence of a protien which define the nature of a protein.

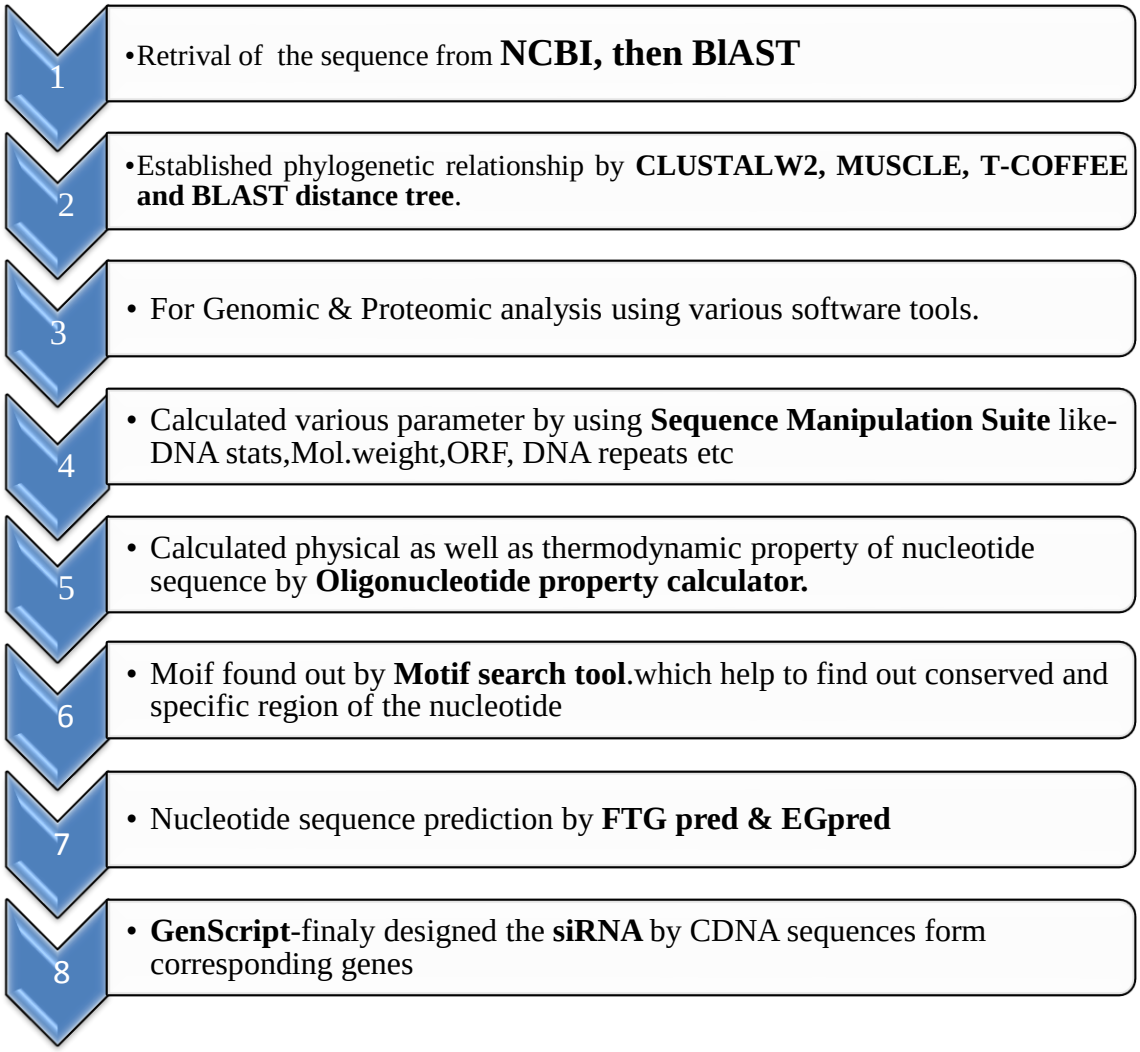


Genomics and Proteomics studies of the DNA and Protein sequences

Protein Sequences Analysis



Nucleotide (DNA) sequence analysis

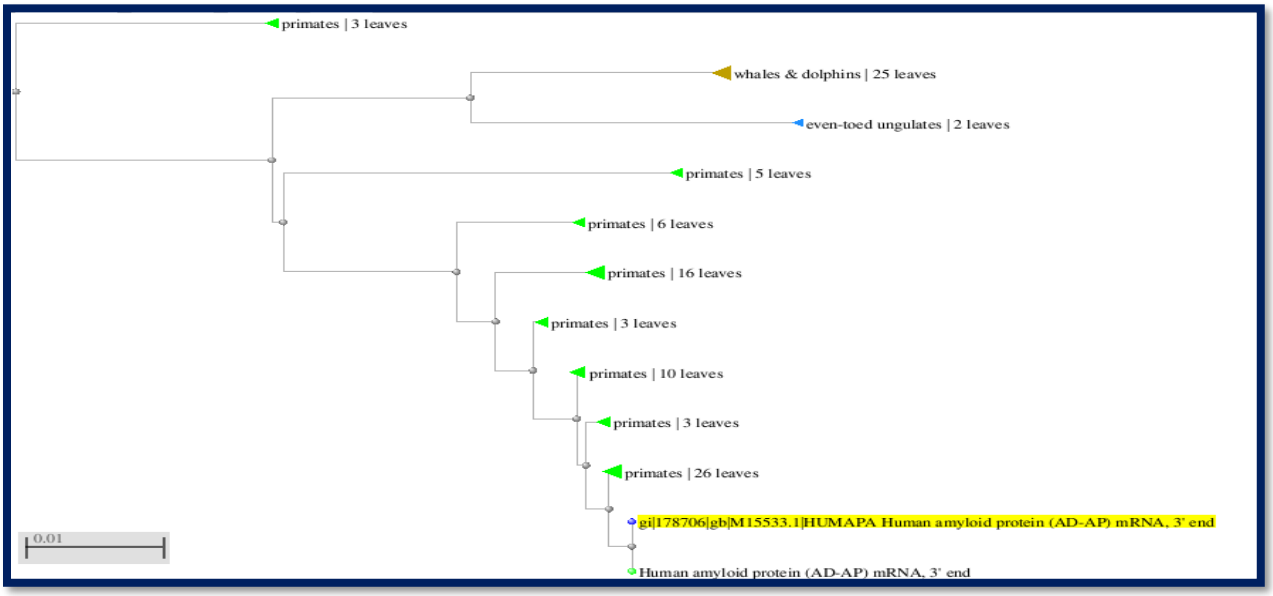


4-RESULT

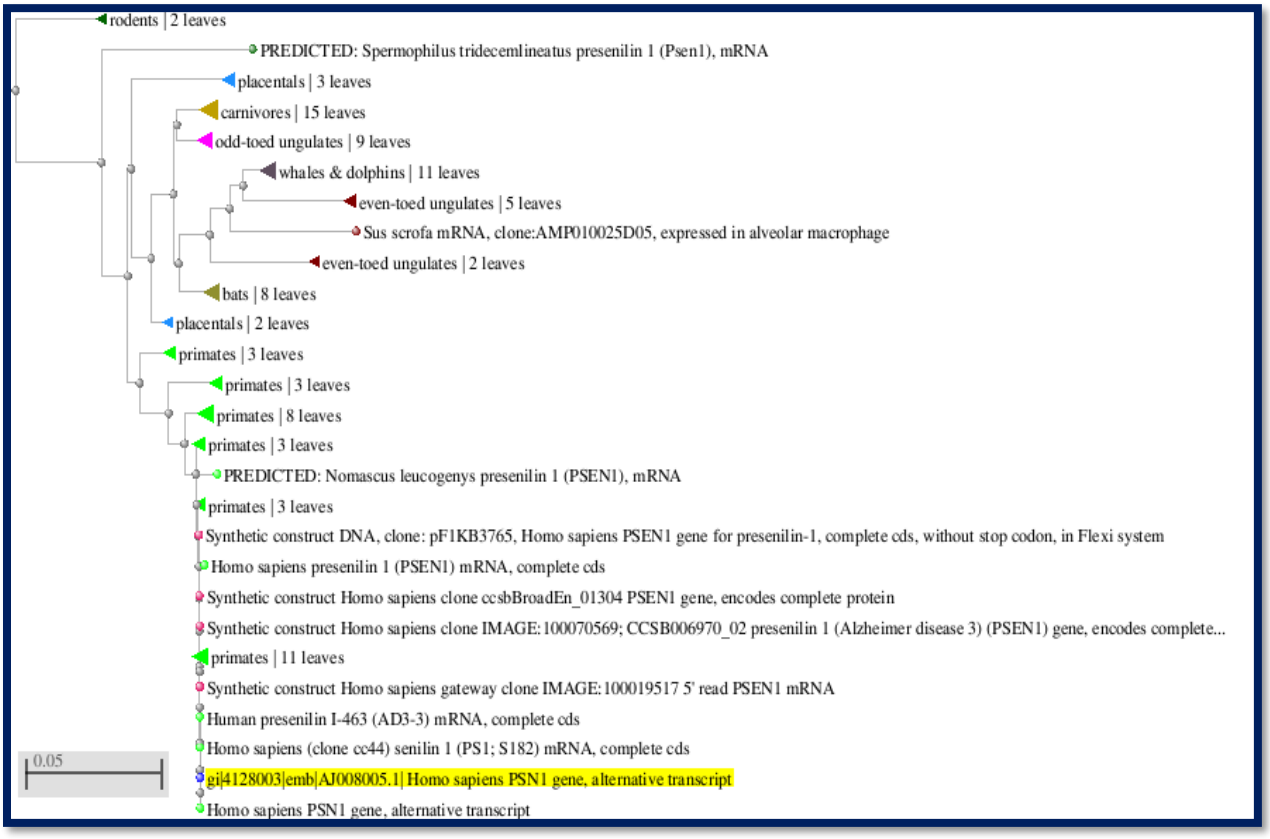
4.1Phylogenetic analysis of genes of the Alzheimer

Apoe

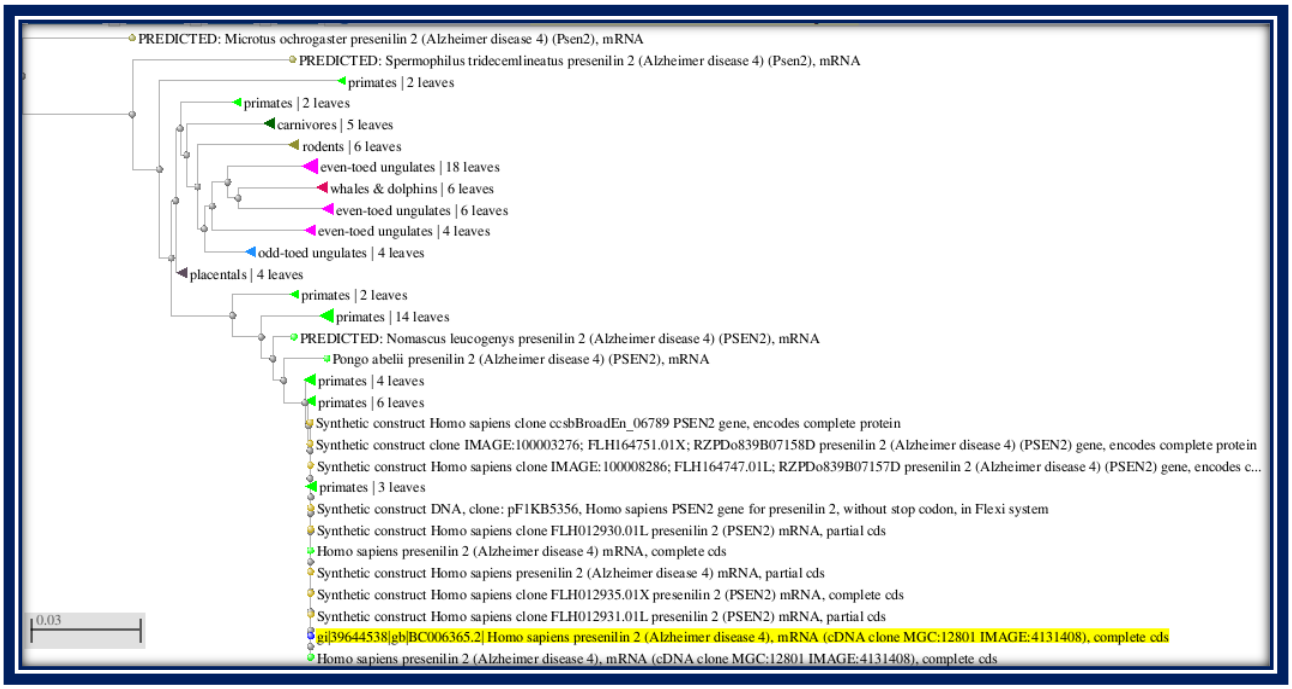
App



PSEN1

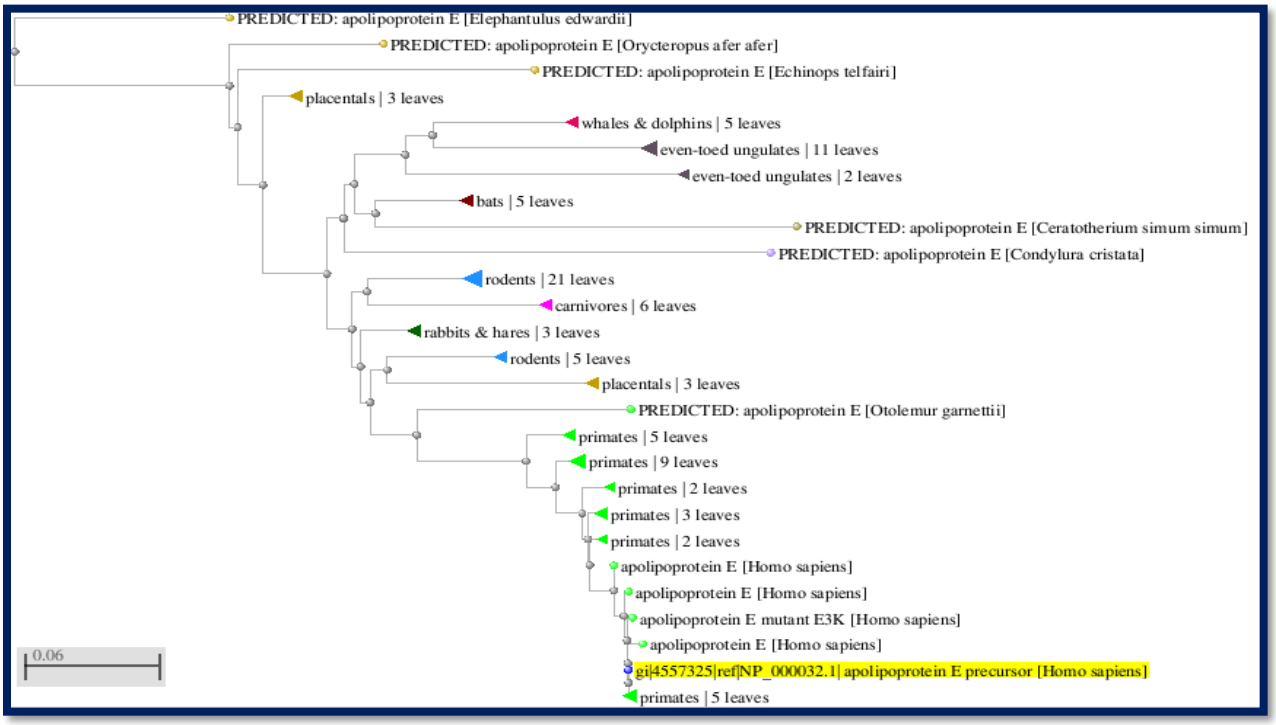

rodents | 2 leaves
PREDICTED: Spermophilus tridecemlineatus presenilin 1 (Psen1), mRNA
placentals | 3 leaves
carnivores | 15 leaves
odd-toed ungulates | 9 leaves
whales & dolphins | 11 leaves
even-toed ungulates | 5 leaves
Sus scrofa mRNA, clone:AMP010025D05, expressed in alveolar macrophage
even-toed ungulates | 2 leaves
bats | 8 leaves
placentals | 2 leaves
primates | 3 leaves
primates | 3 leaves
primates | 8 leaves
primates | 3 leaves
PREDICTED: Nomascus leucogenys presenilin 1 (PSEN1), mRNA
primates | 3 leaves
Synthetic construct DNA, clone: pF1KB3765, Homo sapiens PSEN1 gene for presenilin-1, complete cds, without stop codon, in Flexi system
Homo sapiens presenilin 1 (PSEN1) mRNA, complete cds
Synthetic construct Homo sapiens clone ccsbBroadEn_01304 PSEN1 gene, encodes complete protein
Synthetic construct Homo sapiens clone IMAGE:100070569; CCSB006970_02 presenilin 1 (Alzheimer disease 3) (PSEN1) gene, encodes complete...
primates | 11 leaves
Synthetic construct Homo sapiens gateway clone IMAGE:100019517 5' read PSEN1 mRNA
Human presenilin I-463 (AD3-3) mRNA, complete cds
Homo sapiens (clone cc44) senilin 1 (PS1; S182) mRNA, complete cds
gi|4128003|emb|AJ008005.1| Homo sapiens PSN1 gene, alternative transcript
Homo sapiens PSN1 gene, alternative transcript
0.05

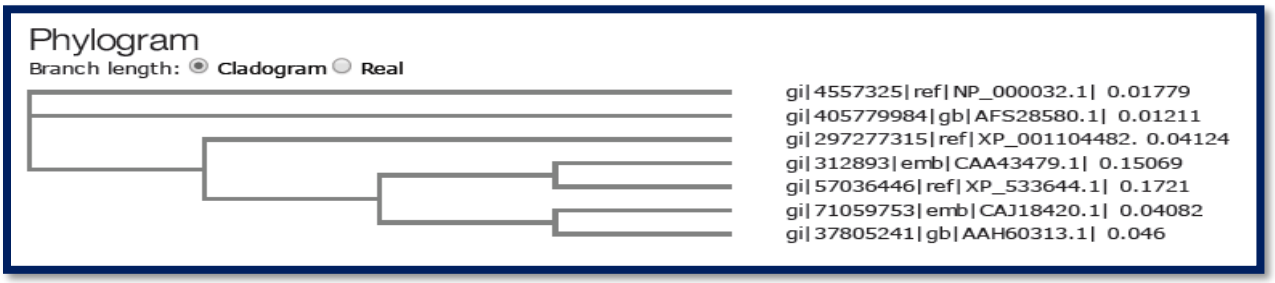
PSEN2


PREDICTED: Microtus ochrogaster presenilin 2 (Alzheimer disease 4) (Psen2), mRNA
PREDICTED: Spermophilus tridecemlineatus presenilin 2 (Alzheimer disease 4) (Psen2), mRNA
primates | 2 leaves
primates | 2 leaves
carnivores | 5 leaves
rodents | 6 leaves
even-toed ungulates | 18 leaves
whales & dolphins | 6 leaves
even-toed ungulates | 6 leaves
even-toed ungulates | 4 leaves
odd-toed ungulates | 4 leaves
placentals | 4 leaves
primates | 2 leaves
primates | 14 leaves
PREDICTED: Nomascus leucogenys presenilin 2 (Alzheimer disease 4) (PSEN2), mRNA
Pongo abelii presenilin 2 (Alzheimer disease 4) (PSEN2), mRNA
primates | 4 leaves
primates | 6 leaves
Synthetic construct Homo sapiens clone ccsbBroadEn_06789 PSEN2 gene, encodes complete protein
Synthetic construct clone IMAGE:100003276; FLH164751.01X; RZPDo839B07158D presenilin 2 (Alzheimer disease 4) (PSEN2) gene, encodes complete protein
Synthetic construct Homo sapiens clone IMAGE:100008286; FLH164747.01L; RZPDo839B07157D presenilin 2 (Alzheimer disease 4) (PSEN2) gene, encodes c...
primates | 3 leaves
Synthetic construct DNA, clone: pF1KB5356, Homo sapiens PSEN2 gene for presenilin 2, without stop codon, in Flexi system
Synthetic construct Homo sapiens clone FLH012930.01L presenilin 2 (PSEN2) mRNA, partial cds
Homo sapiens presenilin 2 (Alzheimer disease 4) mRNA, complete cds
Synthetic construct Homo sapiens presenilin 2 (Alzheimer disease 4) mRNA, partial cds
Synthetic construct Homo sapiens clone FLH012935.01X presenilin 2 (PSEN2) mRNA, complete cds
Synthetic construct Homo sapiens clone FLH012931.01L presenilin 2 (PSEN2) mRNA, partial cds
gi|39644538|gb|BC006365.2| Homo sapiens presenilin 2 (Alzheimer disease 4), mRNA (cDNA clone MGC:12801 IMAGE:4131408), complete cds
Homo sapiens presenilin 2 (Alzheimer disease 4), mRNA (cDNA clone MGC:12801 IMAGE:4131408), complete cds
0.03

4.2Phylogenetic analysis of the proteins of Alzheimer

Apoe protein



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gi|297277315|ref|XP_001104482.:0.04124,
(
(
gi|312893|emb|CAA43479.1|:0.15069,
gi|57036446|ref|XP_533644.1|:0.17210)
:0.01984,
(
gi|71059753|emb|CAJ18420.1|:0.04082,
gi|37805241|gb|AAH60313.1|:0.04600)
:0.11976)
:0.07550)
:0.02003);
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APP

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(
(
(
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gi|297277315|ref|XP_001104482.2|:0.06158)
:0.06492,
(
gi|71059753|emb|CAJ18420.1|:0.04396,
gi|37805241|gb|AAH60313.1|:0.05572)
:0.12566)
:0.01707,
gi|312893|emb|CAA43479.1|:0.15803,
gi|57036446|ref|XP_533644.1|:0.17109);
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Phylogram

Branch length: Cladogram Real

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gi|297277315|ref|XP_001104482.2| 0.06158
gi|71059753|emb|CAJ18420.1| 0.04396
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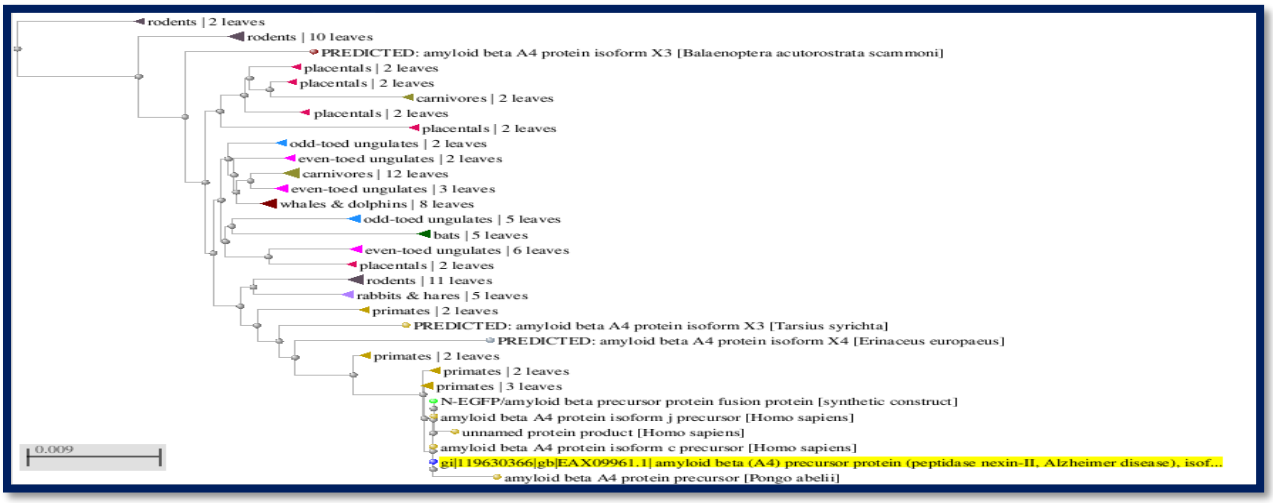
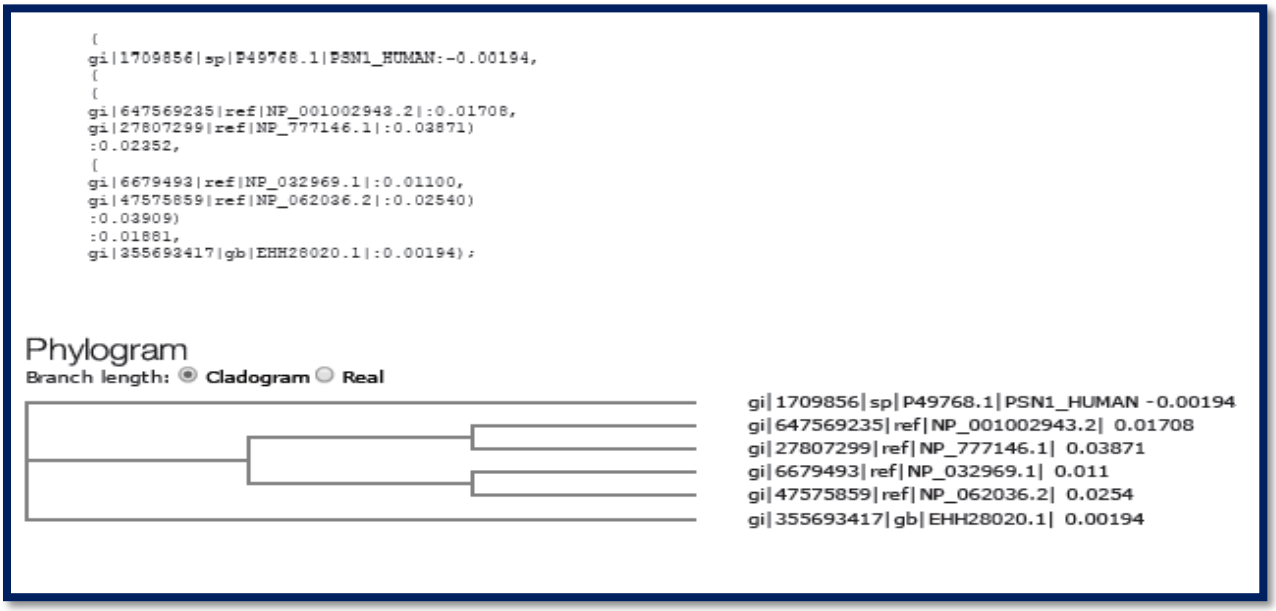


PSEN1`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:0.02352,
(
gi|6679493|ref|NP_032969.1|:0.01100,
gi|47575859|ref|NP_062036.2|:0.02540)
:0.03909)
:0.01881,
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Phylogram
Branch length:
Cladogram
Real
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gi|647569235|ref|NP_001002943.2| 0.01708
gi|27807299|ref|NP_777146.1| 0.03871
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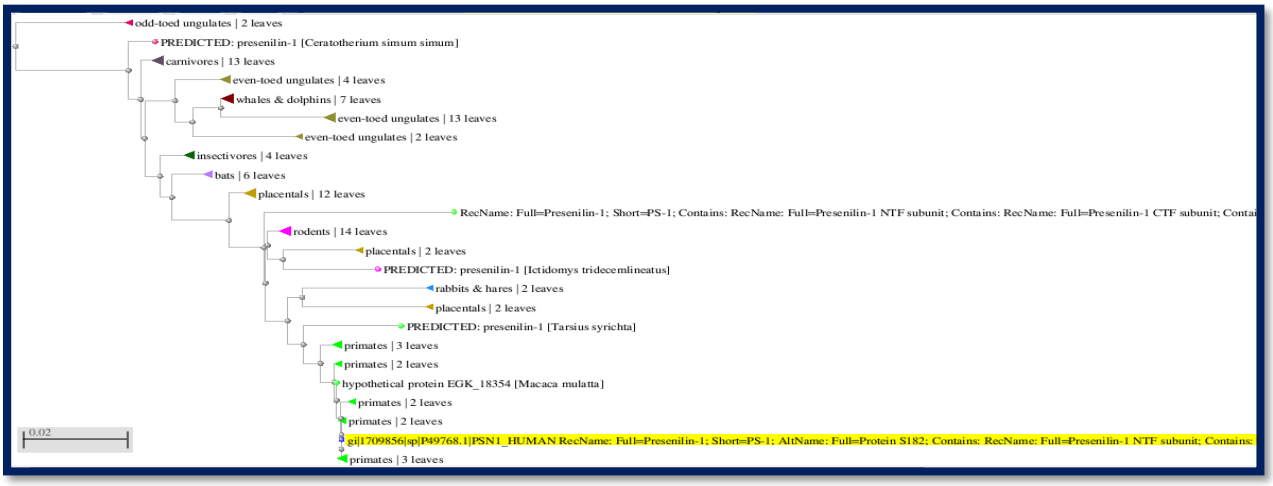
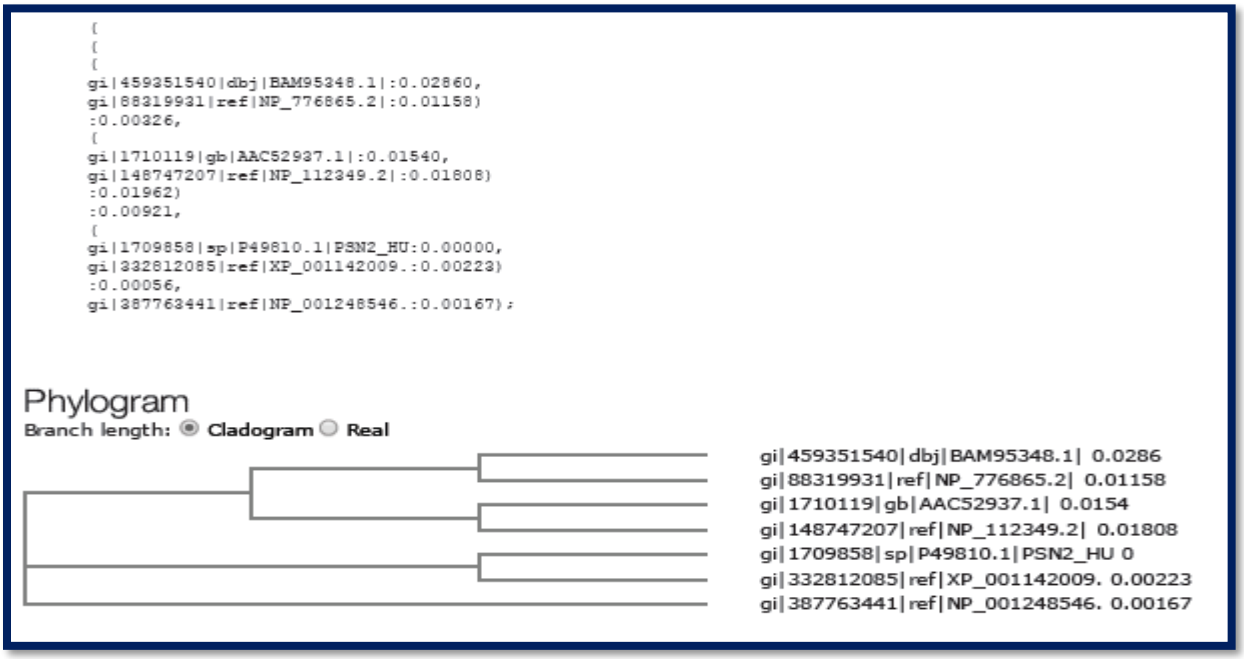
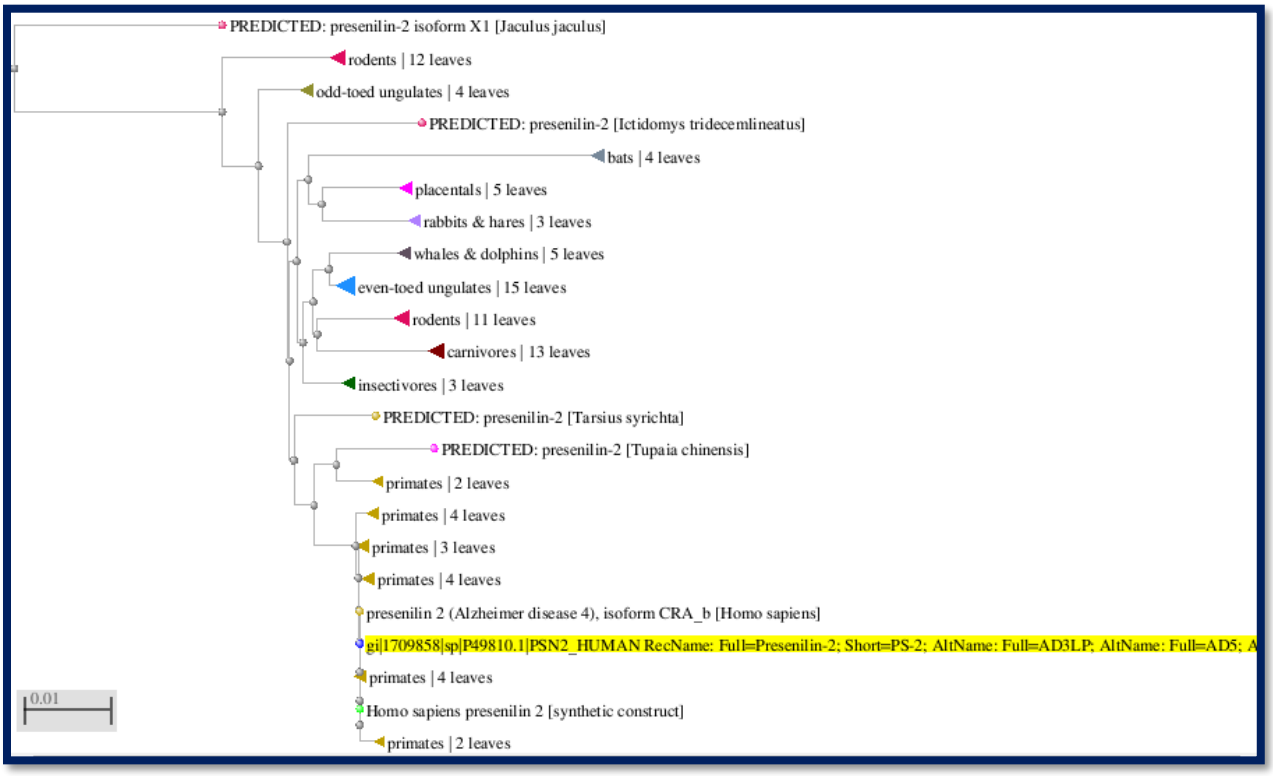

odd-toed ungulates | 2 leaves
PREDICTED: presenilin-1 [Ceratotherium simum simum]
carnivores | 13 leaves
even-toed ungulates | 4 leaves
whales & dolphins | 7 leaves
even-toed ungulates | 13 leaves
even-toed ungulates | 2 leaves
insectivores | 4 leaves
bats | 6 leaves
placentals | 12 leaves
RecName: Full=Presenilin-1; Short=PS-1; Contains: RecName: Full=Presenilin-1 NTF subunit; Contains: RecName: Full=Presenilin-1 CTF subunit; Contai
rodents | 14 leaves
placentals | 2 leaves
PREDICTED: presenilin-1 [Ictidomys tridecemlineatus]
rabbits & hares | 2 leaves
placentals | 2 leaves
PREDICTED: presenilin-1 [Tarsius syrichta]
primates | 3 leaves
primates | 2 leaves
hypothetical protein EGK_18354 [Macaca mulatta]
primates | 2 leaves
primates | 2 leaves
gi|1709856|sp|P49768.1|PSN1_HUMAN RecName: Full=Presenilin-1; Short=PS-1; AltName: Full=Protein S182; Contains: RecName: Full=Presenilin-1 NTF subunit; Contains:
primates | 3 leaves
0.02

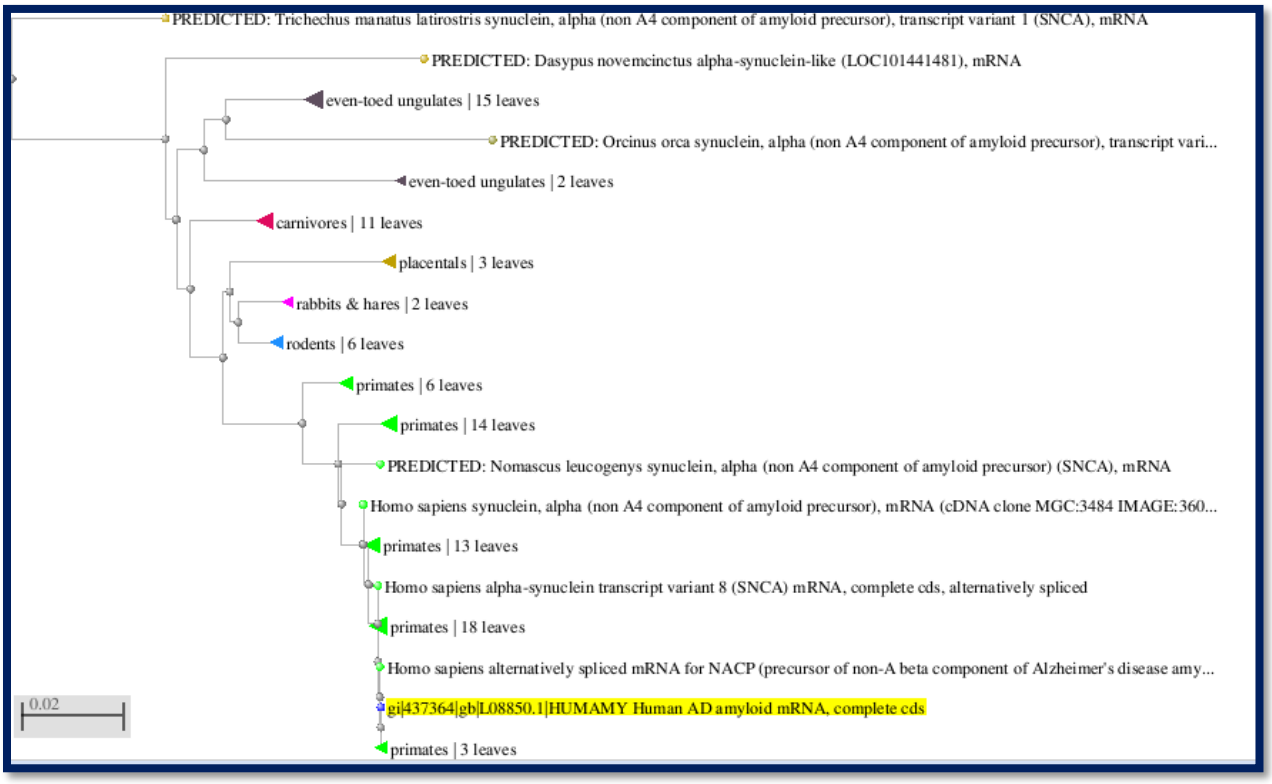
PSEN2


(
(
(
gi|459351540|dbj|BAM95348.1|:0.02860,
gi|88319931|ref|NP_776865.2|:0.01158)
:0.00326,
(
gi|1710119|gb|AAC52937.1|:0.01540,
gi|148747207|ref|NP_112349.2|:0.01808)
:0.01962)
:0.00921,
(
gi|1709858|sp|P49810.1|PSN2_HU:0.00000,
gi|332812085|ref|XP_001142009.:0.00223)
:0.00056,
gi|387763441|ref|NP_001248546.:0.00167);
Phylogram
Branch length: Cladogram Real
gi|459351540|dbj|BAM95348.1| 0.0286
gi|88319931|ref|NP_776865.2| 0.01158
gi|1710119|gb|AAC52937.1| 0.0154
gi|148747207|ref|NP_112349.2| 0.01808
gi|1709858|sp|P49810.1|PSN2_HU 0
gi|332812085|ref|XP_001142009. 0.00223
gi|387763441|ref|NP_001248546. 0.00167

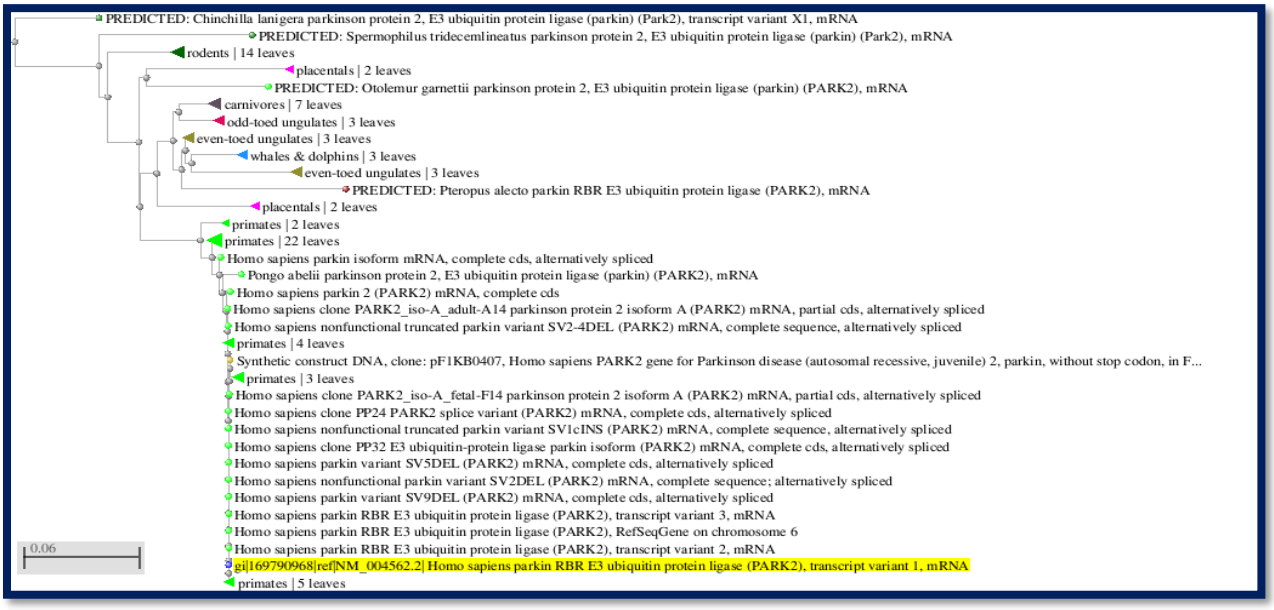

PREDICTED: presenilin-2 isoform X1 [Jaculus jaculus]
rodents | 12 leaves
odd-toed ungulates | 4 leaves
PREDICTED: presenilin-2 [Ictidomys tridecemlineatus]
bats | 4 leaves
placentals | 5 leaves
rabbits & hares | 3 leaves
whales & dolphins | 5 leaves
even-toed ungulates | 15 leaves
rodents | 11 leaves
carnivores | 13 leaves
insectivores | 3 leaves
PREDICTED: presenilin-2 [Tarsius syrichta]
PREDICTED: presenilin-2 [Tupaia chinensis]
primates | 2 leaves
primates | 4 leaves
primates | 3 leaves
primates | 4 leaves
presenilin 2 (Alzheimer disease 4), isoform CRA_b [Homo sapiens]
gi|1709858|sp|P49810.1|PSN2_HUMAN RecName: Full=Presenilin-2; Short=PS-2; AltName: Full=AD3LP; AltName: Full=AD5;
primates | 4 leaves
Homo sapiens presenilin 2 [synthetic construct]
primates | 2 leaves
0.01

4.3Phylogenetic analysis of the genes of PARKINSON

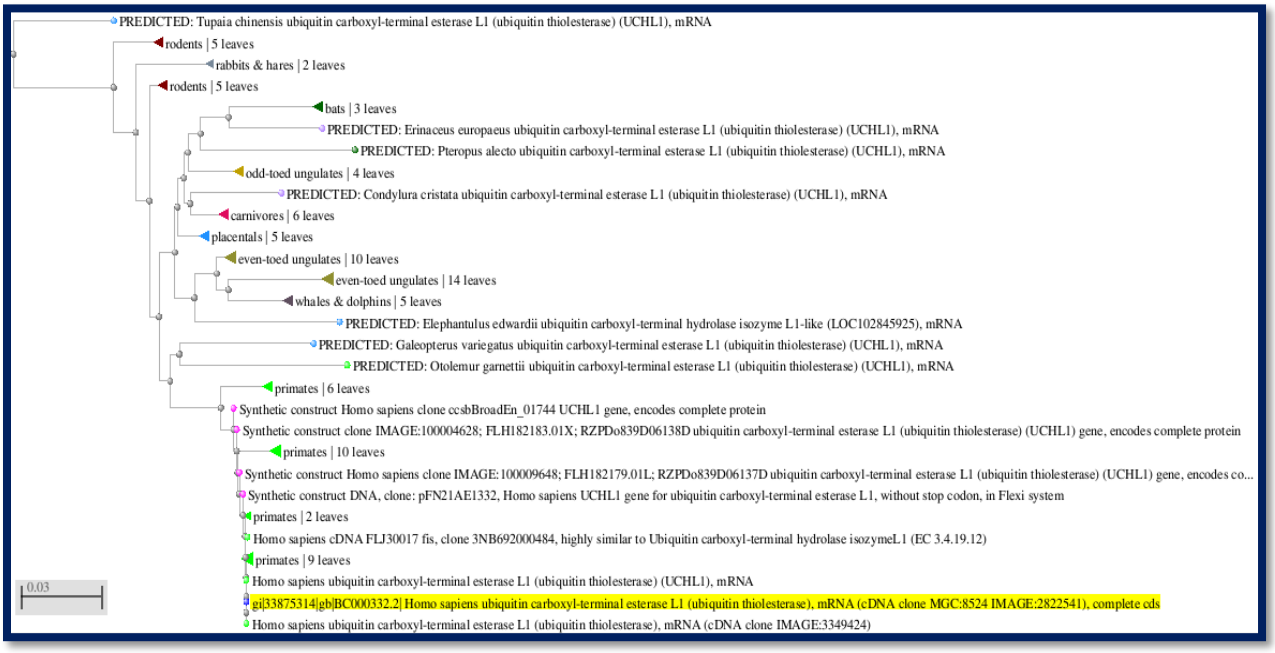
Synuclein



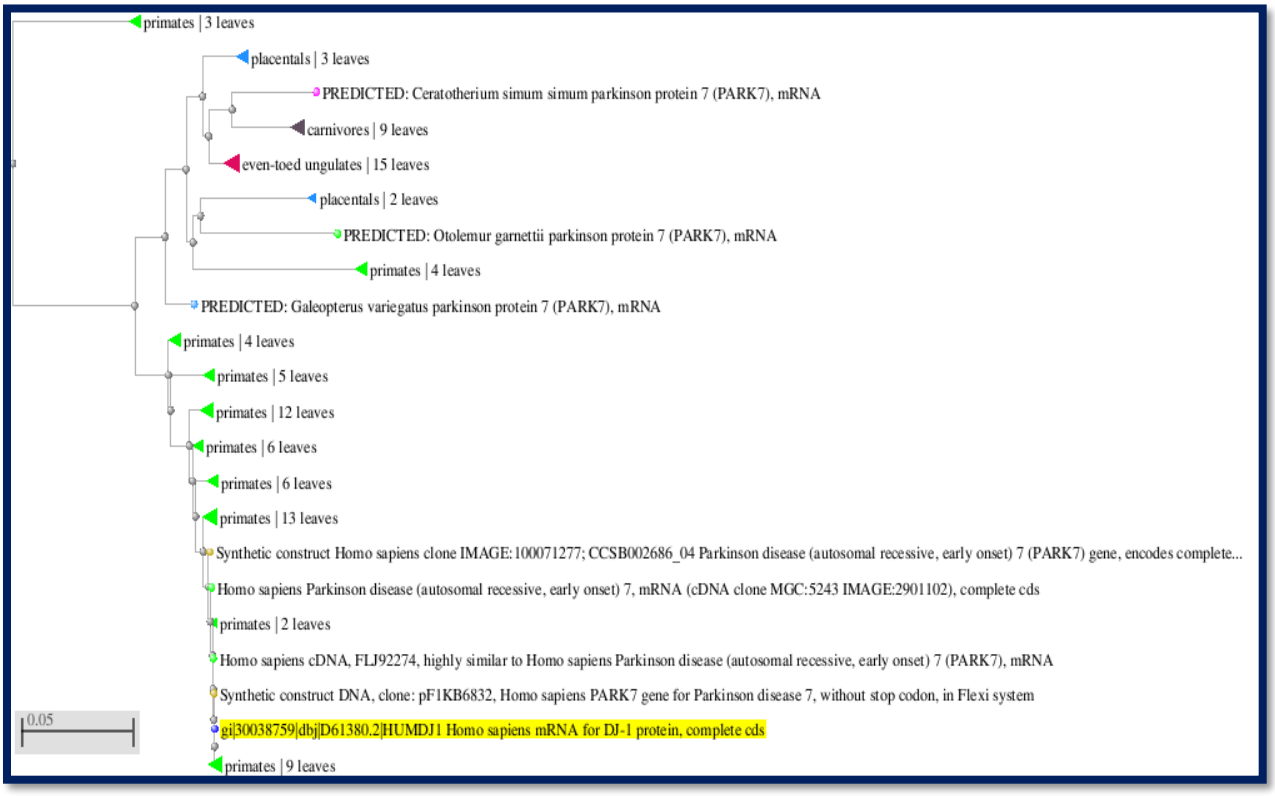
PARK2



PARK5

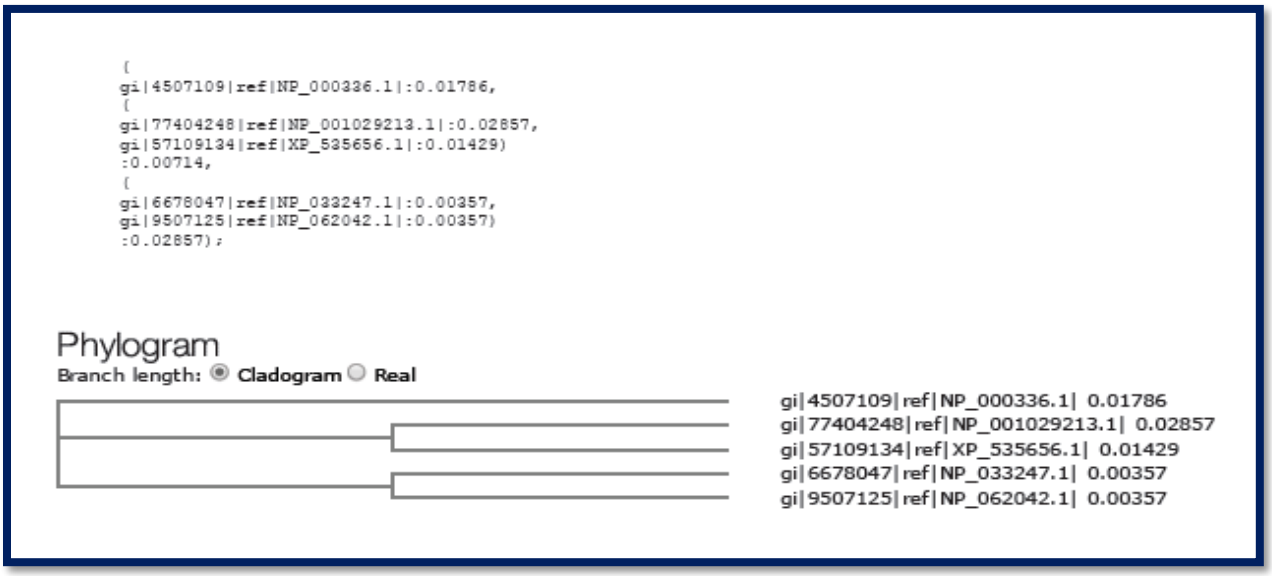

PREDICTED: Tupaia chinensis ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1), mRNA
rodents | 5 leaves
rabbits & hares | 2 leaves
rodents | 5 leaves
bats | 3 leaves
PREDICTED: Erinaceus europaeus ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1), mRNA
PREDICTED: Pteropus alecto ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1), mRNA
odd-toed ungulates | 4 leaves
PREDICTED: Condylura cristata ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1), mRNA
carnivores | 6 leaves
placentals | 5 leaves
even-toed ungulates | 10 leaves
even-toed ungulates | 14 leaves
whales & dolphins | 5 leaves
PREDICTED: Elephantulus edwardii ubiquitin carboxyl-terminal hydrolase isozyme L1-like (LOC102845925), mRNA
PREDICTED: Galeopterus variegatus ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1), mRNA
PREDICTED: Otolemur garnettii ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1), mRNA
primates | 6 leaves
Synthetic construct Homo sapiens clone ccsbBroadEn_01744 UCHL1 gene, encodes complete protein
Synthetic construct clone IMAGE:100004628; FLH182183.01X; RZPDo839D06138D ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1) gene, encodes complete protein
primates | 10 leaves
Synthetic construct Homo sapiens clone IMAGE:100009648; FLH182179.01L; RZPDo839D06137D ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1) gene, encodes co...
Synthetic construct DNA, clone: pFN21AE1332, Homo sapiens UCHL1 gene for ubiquitin carboxyl-terminal esterase L1, without stop codon, in Flexi system
primates | 2 leaves
Homo sapiens cDNA FLJ30017 fis, clone 3NB692000484, highly similar to Ubiquitin carboxyl-terminal hydrolase isozymeL1 (EC 3.4.19.12)
primates | 9 leaves
Homo sapiens ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase) (UCHL1), mRNA
gi|33875314|gb|BC000332.2| Homo sapiens ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase), mRNA (cDNA clone MGC:8524 IMAGE:2822541), complete cds
Homo sapiens ubiquitin carboxyl-terminal esterase L1 (ubiquitin thiolesterase), mRNA (cDNA clone IMAGE:3349424)
0.03

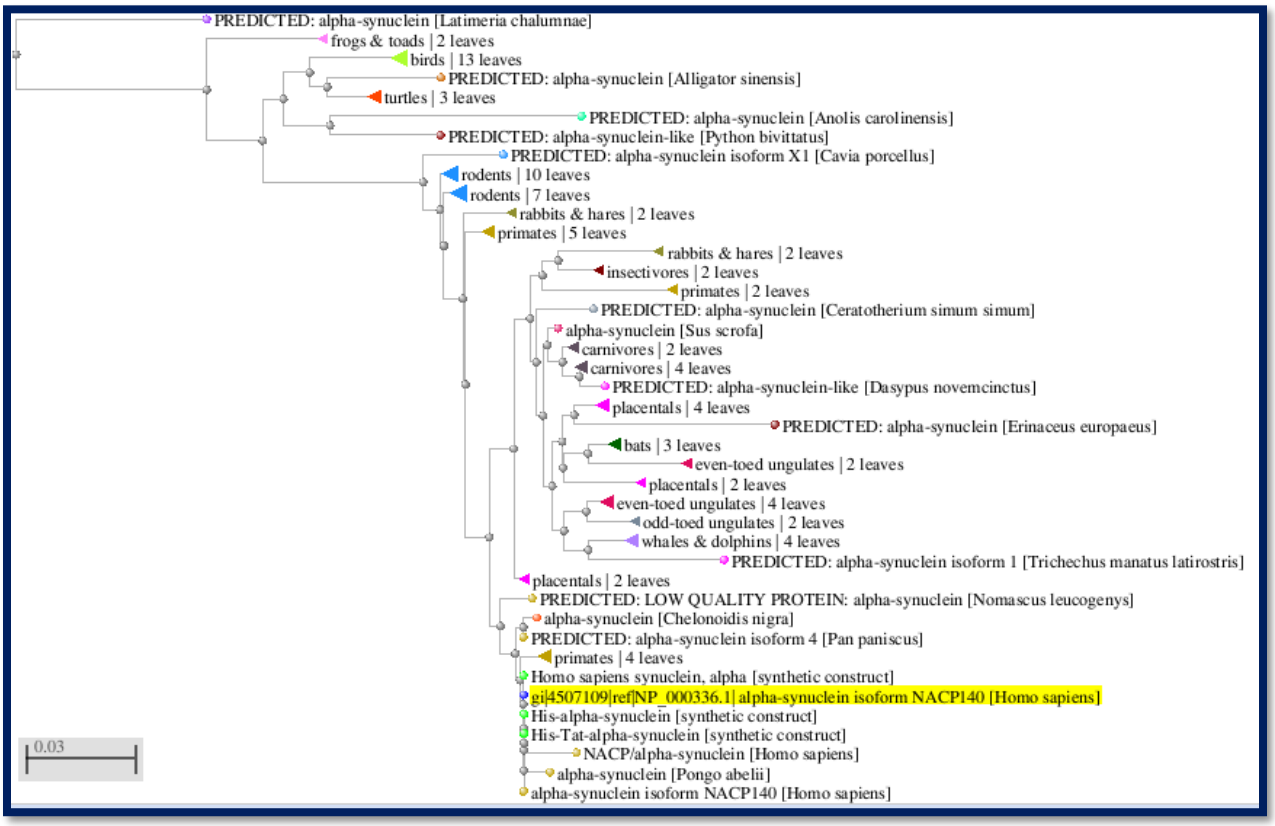
PARK7


primates | 3 leaves
placentals | 3 leaves
PREDICTED: Ceratotherium simum simum parkinson protein 7 (PARK7), mRNA
carnivores | 9 leaves
even-toed ungulates | 15 leaves
placentals | 2 leaves
PREDICTED: Otolemur garnettii parkinson protein 7 (PARK7), mRNA
primates | 4 leaves
PREDICTED: Galeopterus variegatus parkinson protein 7 (PARK7), mRNA
primates | 4 leaves
primates | 5 leaves
primates | 12 leaves
primates | 6 leaves
primates | 6 leaves
primates | 13 leaves
Synthetic construct Homo sapiens clone IMAGE:100071277; CCSB002686_04 Parkinson disease (autosomal recessive, early onset) 7 (PARK7) gene, encodes complete...
Homo sapiens Parkinson disease (autosomal recessive, early onset) 7, mRNA (cDNA clone MGC:5243 IMAGE:2901102), complete cds
primates | 2 leaves
Homo sapiens cDNA, FLJ92274, highly similar to Homo sapiens Parkinson disease (autosomal recessive, early onset) 7 (PARK7), mRNA
Synthetic construct DNA, clone: pF1KB6832, Homo sapiens PARK7 gene for Parkinson disease 7, without stop codon, in Flexi system
gi|30038759|dbj|D61380.2|HUMDJ1 Homo sapiens mRNA for DJ-1 protein, complete cds
primates | 9 leaves
0.05

4.4Phylogenetic analysis of protein of PARKINSON

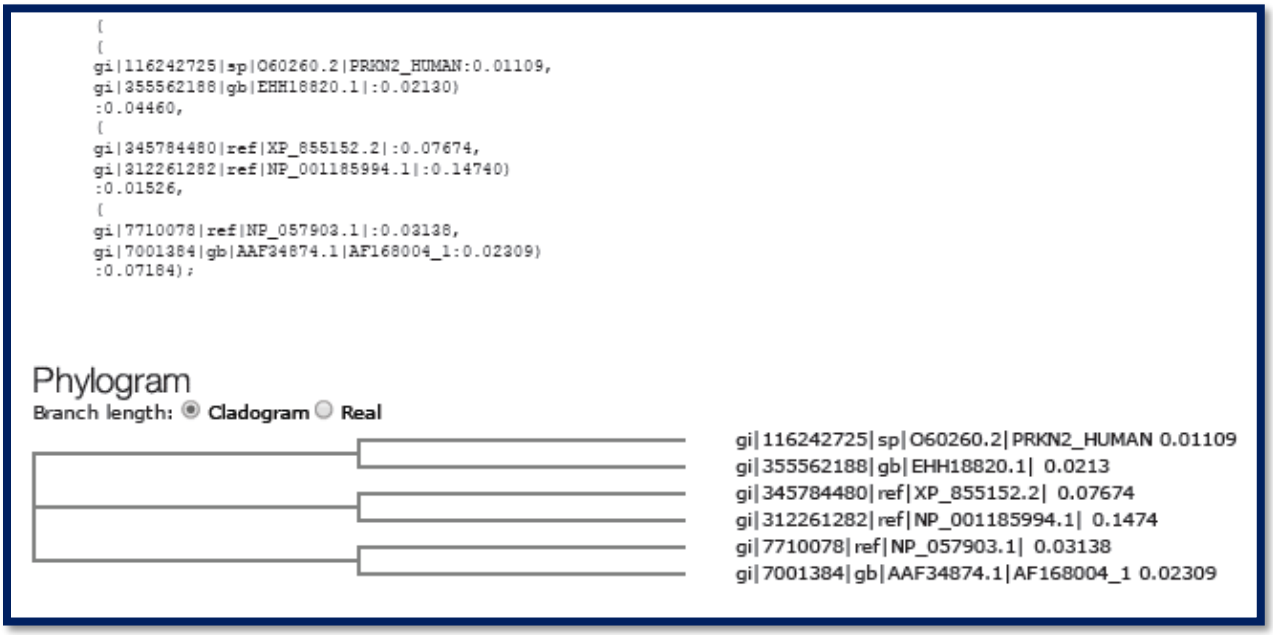
Synuclein protein

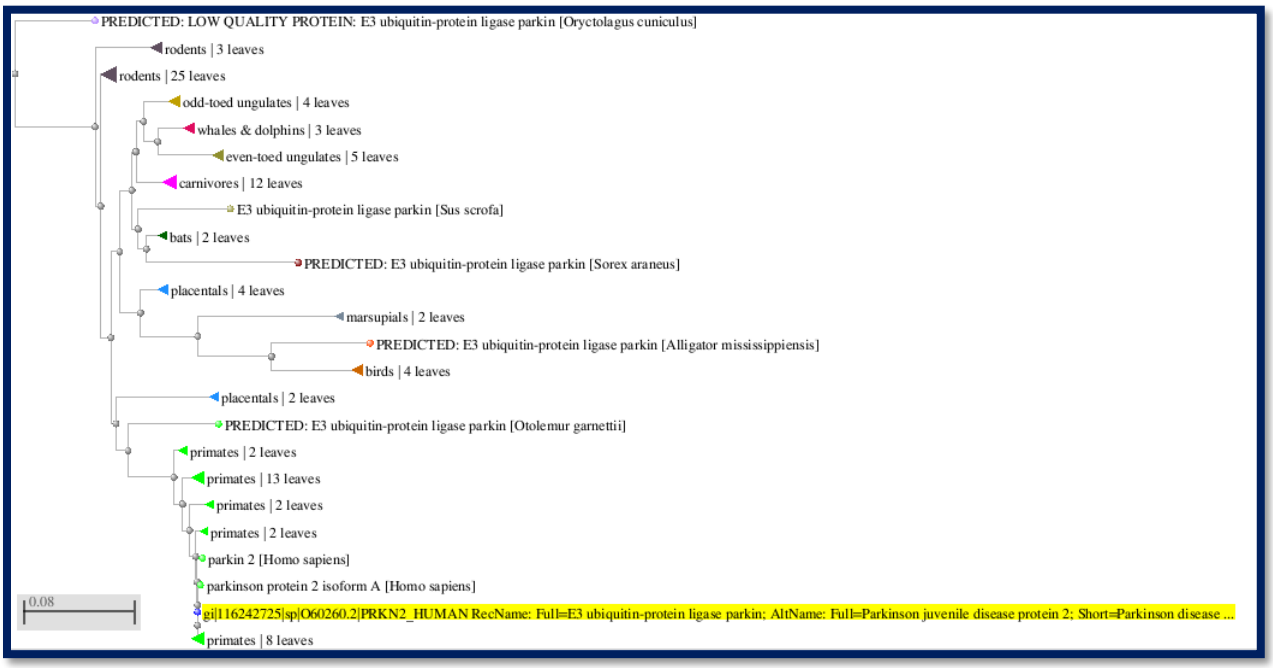




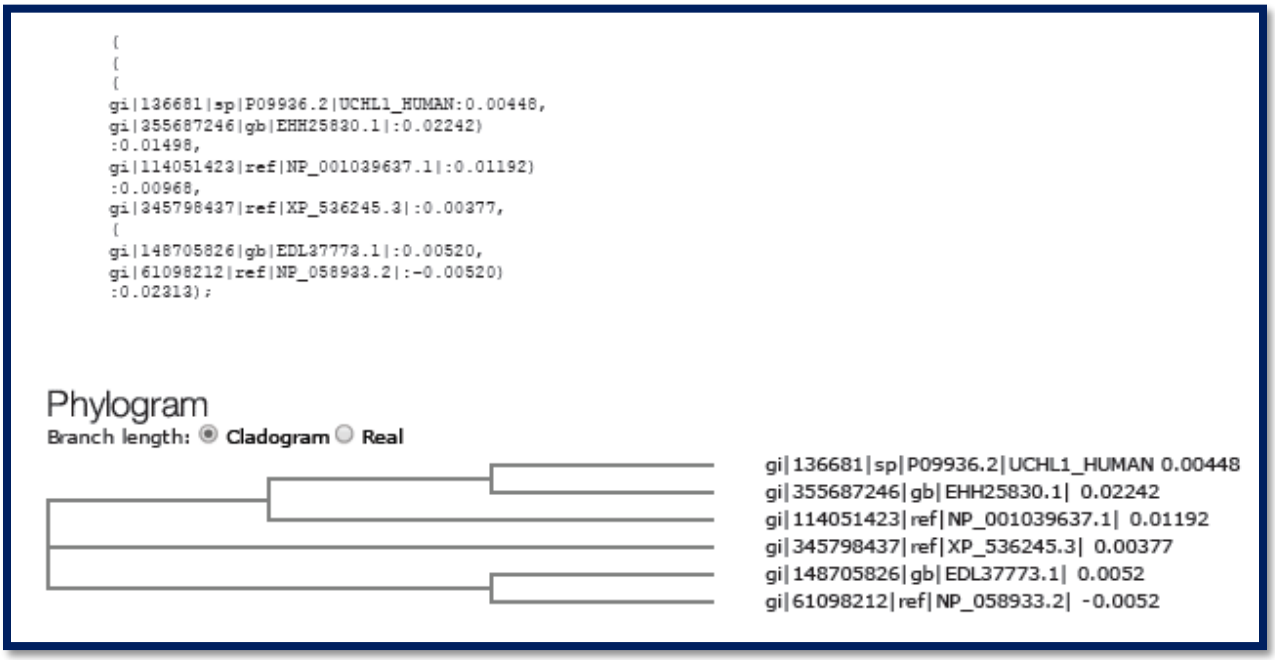
PARK2

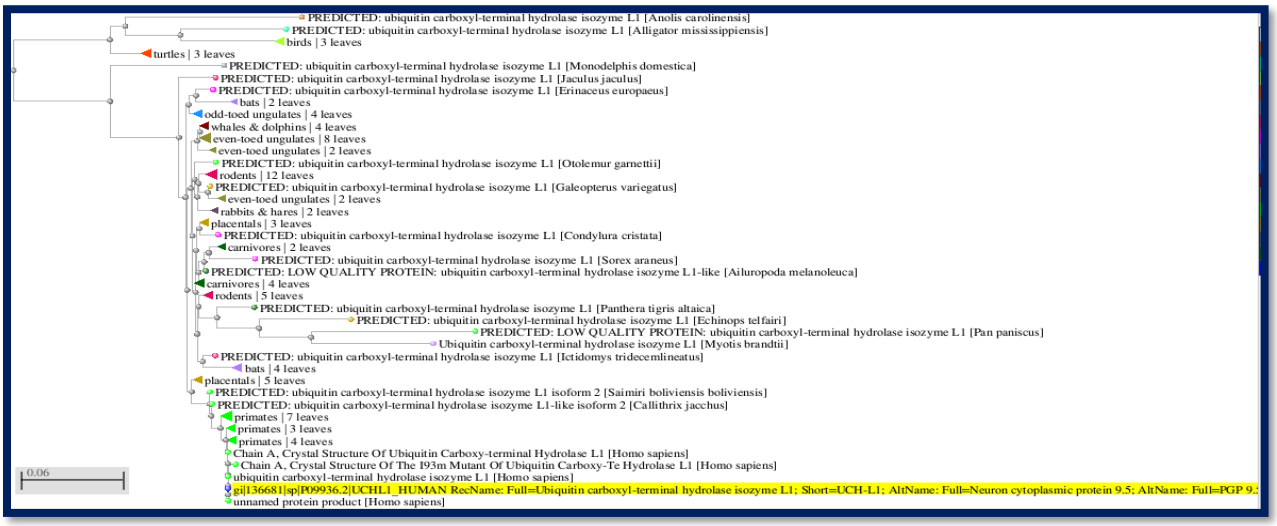
```
(
(
gi|116242725|sp|O60260.2|PRKN2_HUMAN:0.01109,
gi|355562188|gb|EHH18820.1|:0.02130)
:0.04460,
(
gi|345784480|ref|XP_855152.2|:0.07674,
gi|312261282|ref|NP_001185994.1|:0.14740)
:0.01526,
(
gi|7710078|ref|NP_057903.1|:0.03138,
gi|7001384|gb|AAF34874.1|AF168004_1:0.02309)
:0.07184);
```



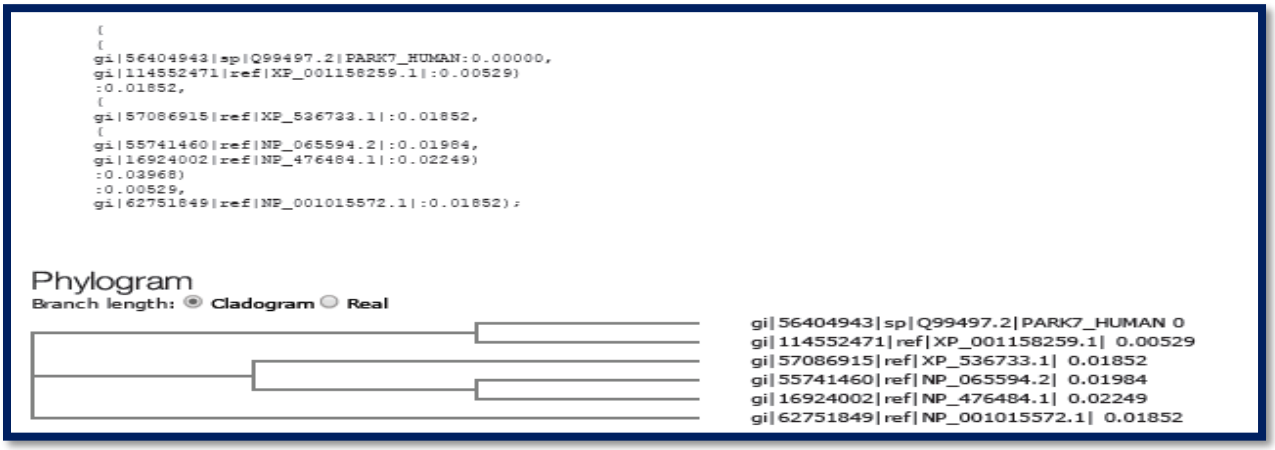


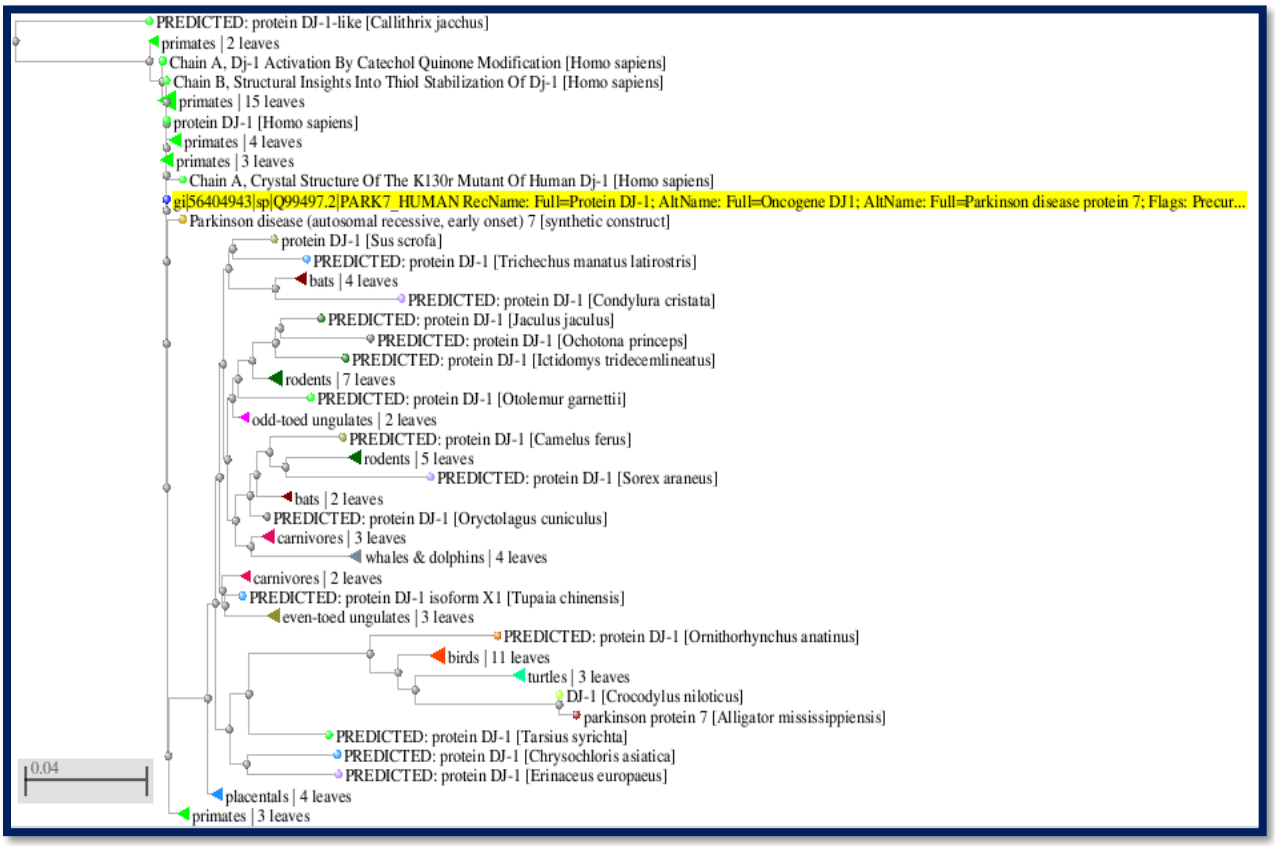
PARK5


(
(
(
gi|136681|sp|P09936.2|UCHL1_HUMAN:0.00448,
gi|355687246|gb|EHH25830.1|:0.02242)
:0.01498,
gi|114051423|ref|NP_001039637.1|:0.01192)
:0.00968,
gi|345798437|ref|XP_536245.3|:0.00377,
(
gi|148705826|gb|EDL37773.1|:0.00520,
gi|61098212|ref|NP_058933.2|:-0.00520)
:0.02313);
Phylogram
Branch length: Cladogram Real
gi|136681|sp|P09936.2|UCHL1_HUMAN 0.00448
gi|355687246|gb|EHH25830.1| 0.02242
gi|114051423|ref|NP_001039637.1| 0.01192
gi|345798437|ref|XP_536245.3| 0.00377
gi|148705826|gb|EDL37773.1| 0.0052
gi|61098212|ref|NP_058933.2| -0.0052


PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Anolis carolinensis]
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Alligator mississippiensis]
birds | 3 leaves
turtles | 3 leaves
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Monodelphis domestica]
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Jaculus jaculus]
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Erinaceus europaeus]
bats | 2 leaves
odd-toed ungulates | 4 leaves
whales & dolphins | 4 leaves
even-toed ungulates | 8 leaves
even-toed ungulates | 2 leaves
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Otolemur garnettii]
rodents | 12 leaves
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Galeopterus variegatus]
even-toed ungulates | 2 leaves
rabbits & hares | 2 leaves
placentals | 3 leaves
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Condylura cristata]
carnivores | 2 leaves
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Sorex araneus]
PREDICTED: LOW QUALITY PROTEIN: ubiquitin carboxyl-terminal hydrolase isozyme L1-like [Ailuropoda melanoleuca]
carnivores | 4 leaves
rodents | 5 leaves
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Panthera tigris altaica]
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Echinops telfairi]
PREDICTED: LOW QUALITY PROTEIN: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Pan paniscus]
Ubiquitin carboxyl-terminal hydrolase isozyme L1 [Myotis brandtii]
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 [Ictidomys tridecemlineatus]
bats | 4 leaves
placentals | 5 leaves
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1 isoform 2 [Saimiri boliviensis boliviensis]
PREDICTED: ubiquitin carboxyl-terminal hydrolase isozyme L1-like isoform 2 [Callithrix jacchus]
primates | 7 leaves
primates | 3 leaves
primates | 4 leaves
Chain A, Crystal Structure Of Ubiquitin Carboxy-terminal Hydrolase L1 [Homo sapiens]
Chain A, Crystal Structure Of The I93m Mutant Of Ubiquitin Carboxy-Te Hydrolase L1 [Homo sapiens]
ubiquitin carboxyl-terminal hydrolase isozyme L1 [Homo sapiens]
gi|136681|sp|P09936.2|UCHL1_HUMAN RecName: Full=Ubiquitin carboxyl-terminal hydrolase isozyme L1; Short=UCH-L1; AltName: Full=Neuron cytoplasmic protein 9.5; AltName: Full=PGP 9.
unnamed protein product [Homo sapiens]
0.06

PARK7


(
(
gi|56404943|sp|Q99497.2|PARK7_HUMAN:0.00000,
gi|114552471|ref|XP_001158259.1|:0.00529)
:0.01852,
(
gi|57086915|ref|XP_536733.1|:0.01852,
(
gi|55741460|ref|NP_065594.2|:0.01984,
gi|16924002|ref|NP_476484.1|:0.02249)
:0.03968)
:0.00529,
gi|62751849|ref|NP_001015572.1|:0.01852);
Phylogram
Branch length: Cladogram Real
gi|56404943|sp|Q99497.2|PARK7_HUMAN 0
gi|114552471|ref|XP_001158259.1| 0.00529
gi|57086915|ref|XP_536733.1| 0.01852
gi|55741460|ref|NP_065594.2| 0.01984
gi|16924002|ref|NP_476484.1| 0.02249
gi|62751849|ref|NP_001015572.1| 0.01852


PREDICTED: protein DJ-1-like [Callithrix jacchus]
primates | 2 leaves
Chain A, Dj-1 Activation By Catechol Quinone Modification [Homo sapiens]
Chain B, Structural Insights Into Thiol Stabilization Of Dj-1 [Homo sapiens]
primates | 15 leaves
protein DJ-1 [Homo sapiens]
primates | 4 leaves
primates | 3 leaves
Chain A, Crystal Structure Of The K130r Mutant Of Human Dj-1 [Homo sapiens]
gi|56404943|sp|Q99497.2|PARK7_HUMAN RecName: Full=Protein DJ-1; AltName: Full=Oncogene DJ1; AltName: Full=Parkinson disease protein 7; Flags: Precur...
Parkinson disease (autosomal recessive, early onset) 7 [synthetic construct]
protein DJ-1 [Sus scrofa]
PREDICTED: protein DJ-1 [Trichechus manatus latirostris]
bats | 4 leaves
PREDICTED: protein DJ-1 [Condylura cristata]
PREDICTED: protein DJ-1 [Jaculus jaculus]
PREDICTED: protein DJ-1 [Ochotona princeps]
PREDICTED: protein DJ-1 [Ictidomys tridecemlineatus]
rodents | 7 leaves
PREDICTED: protein DJ-1 [Otolemur garnettii]
odd-toed ungulates | 2 leaves
PREDICTED: protein DJ-1 [Camelus ferus]
rodents | 5 leaves
PREDICTED: protein DJ-1 [Sorex araneus]
bats | 2 leaves
PREDICTED: protein DJ-1 [Oryctolagus cuniculus]
carnivores | 3 leaves
whales & dolphins | 4 leaves
carnivores | 2 leaves
PREDICTED: protein DJ-1 isoform X1 [Tupaia chinensis]
even-toed ungulates | 3 leaves
PREDICTED: protein DJ-1 [Ornithorhynchus anatinus]
birds | 11 leaves
turtles | 3 leaves
DJ-1 [Crocodylus niloticus]
parkinson protein 7 [Alligator mississippiensis]
PREDICTED: protein DJ-1 [Tarsius syrichta]
PREDICTED: protein DJ-1 [Chrysochloris asiatica]
PREDICTED: protein DJ-1 [Erinaceus europaeus]
0.04
placentals | 4 leaves
primates | 3 leaves

4.5Statistical analysis of the various parameters of the proteins of Alzheimer

Table-1 number of amino acid content and percentage of amino acid repeats in indivisual protein sequence.

Protein	**Apoe**		**App**		**Psen1**		**Psen2**	
Amino acid	**No of repeats**	**% of repeats**	**No of repeats**	**% of repeats**	**No of repeats**	**% of repeats**	**No of repeats**	**% of repeats**
A	39	12	57	8	36	8	27	6
C	2	1	12	2	5	1	9	2
D	11	3	47	7	19	4	17	4
E	40	13	85	12	32	7	36	8
F	4	1	17	2	23	5	21	5
G	18	6	31	4	23	5	30	7
H	2	1	25	4	10	2	4	1
I	2	1	23	3	31	7	26	6
K	13	4	38	5	16	3	15	3
L	41	13	52	7	56	12	58	13
M	8	3	21	3	13	3	18	4
N	1	0	28	4	17	4	10	2
P	8	3	31	4	19	4	28	6
Q	32	10	33	5	19	4	12	3
R	34	11	33	5	20	4	13	3
S	14	4	30	4	34	7	29	6
T	12	4	45	6	26	6	27	6
V	24	8	62	9	41	9	38	8
W	8	3	8	1	8	2	8	2
Y	4	1	17	2	19	4	22	5
Aliphatic I,L,	67	21	137	20	128	27	122	27
Aromatics F,W,Y6	16	5	42	6	50	11	51	11
Positive K,R,H	49	15	96	14	46	10	32	7
Negative D,E	51	16	132	19	51	11	53	12
Tiny G,A,S	71	22	118	17	93	20	86	13
tRNAsynthetase class I Z,E,Q,R,C,M,V,I ,L,Y,W	195	61.51	379	49.74	281	52.33	262	52.30
tRNAsynthetase class II B,G,A,P,S,T,H,D ,N,K,F	122	38.49	383	50.26	256	47.67	239	47.70

Table-2 Physical and chemical property of protein sequences

Protein	APOE	APP	PSEN1	PSEN2
Number of Amino acids	317	759	536	499
Mol.wieght	55.58 kDa	85.96 kDa	60.48 kDa	55.58 kDa
Isoelectric point	5.42	4.46	4.81	4.29

4.6Transmembrane region or hydrophobic region and Topology prediction of protein sequences of individual protein.

APOE

Table-3

Start	Stop	Length	Cut-off
44	58	15	1.7
46	56	11	2.2

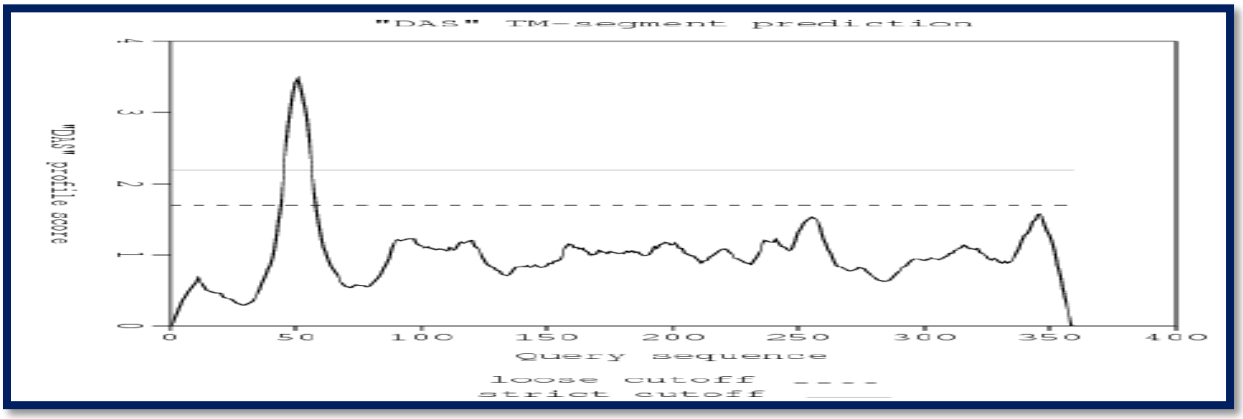


Figure-1 graph representaion of the transmembrane region of Apoe

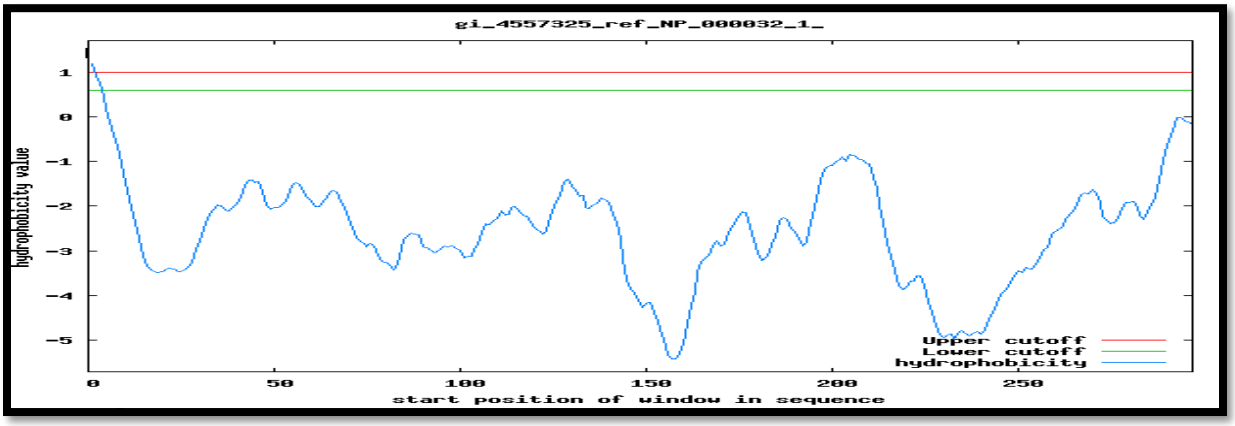


Figure-2 graphical analysis of the topology of APOE

Table-4

Start	Stop	Length	Cut-off
90	104	15	1.7
91	103	13	2.2
204	216	13	1.7
560	565	6	1.7
715	737	23	1.7
716	736	21	2.2

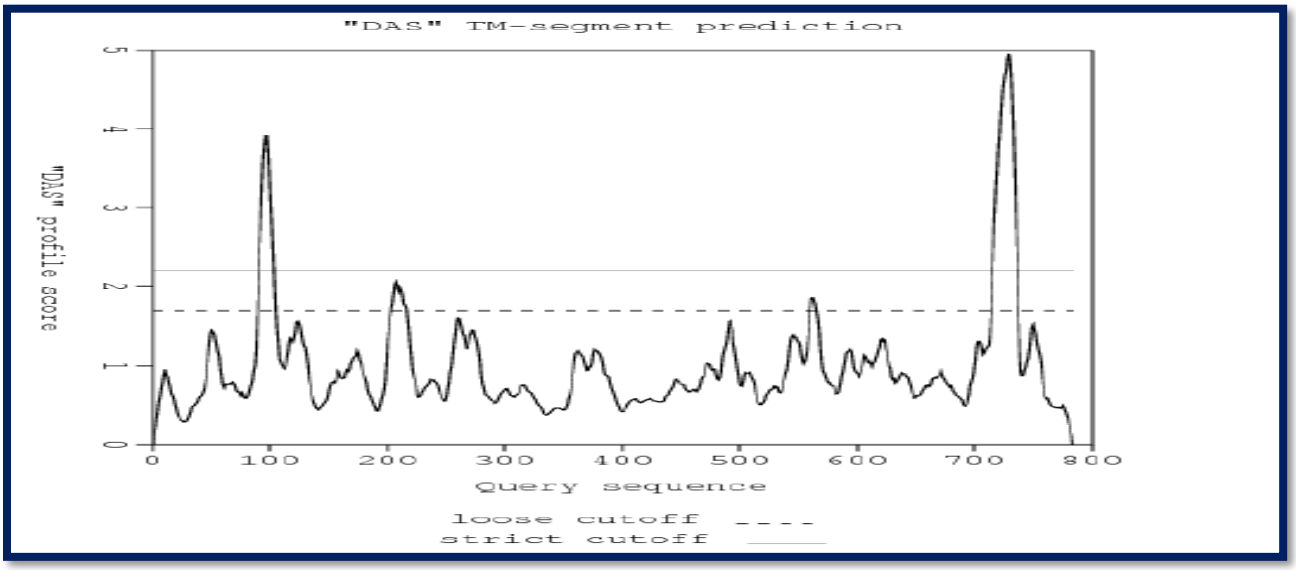


Figure-3APP protein transmembrane prediction by DAS Transmembrane pridiction graph.

.

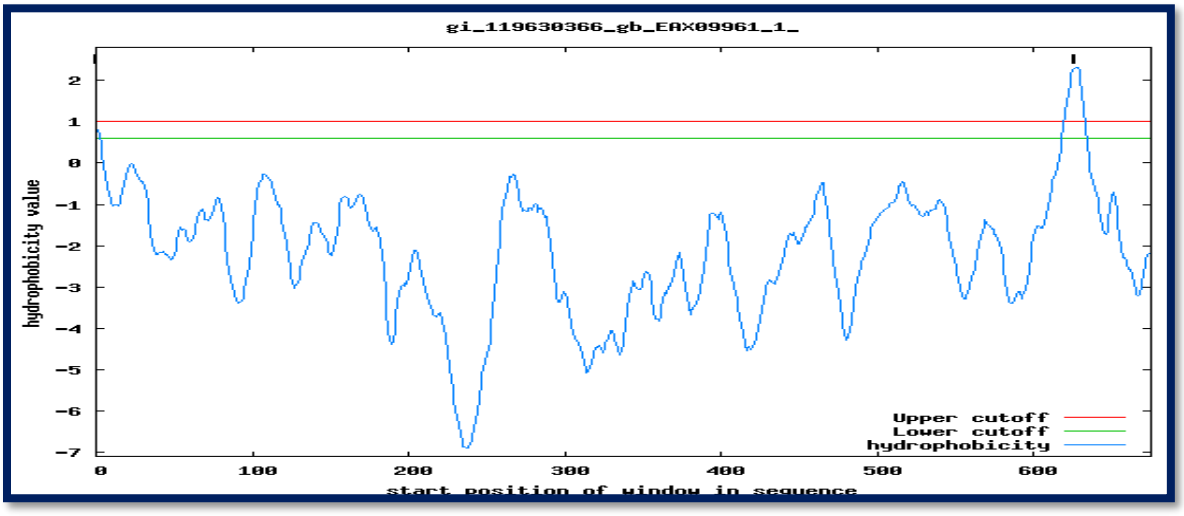


Figure- 4 APP topology prediction by TOP pred

PSEN1

Table-5

Start	Stop	Length	Cut-off
261	282	22	1.7
262	280	19	2.7
312	335	24	1.7
314	334	21	2.2
341	363	23	1.7
343	362	20	2.2
372	392	21	1.7
374	390	17	2.2
404	421	18	1.7
406	418	13	2.2
424	443	20	1.7
426	441	16	2.2
465	475	11	1.7
466	473	8	2.2
564	574	11	1.7
565	573	9	2.2
587	629	43	1.7
588	609	21	2.2
615	628	14	2.2

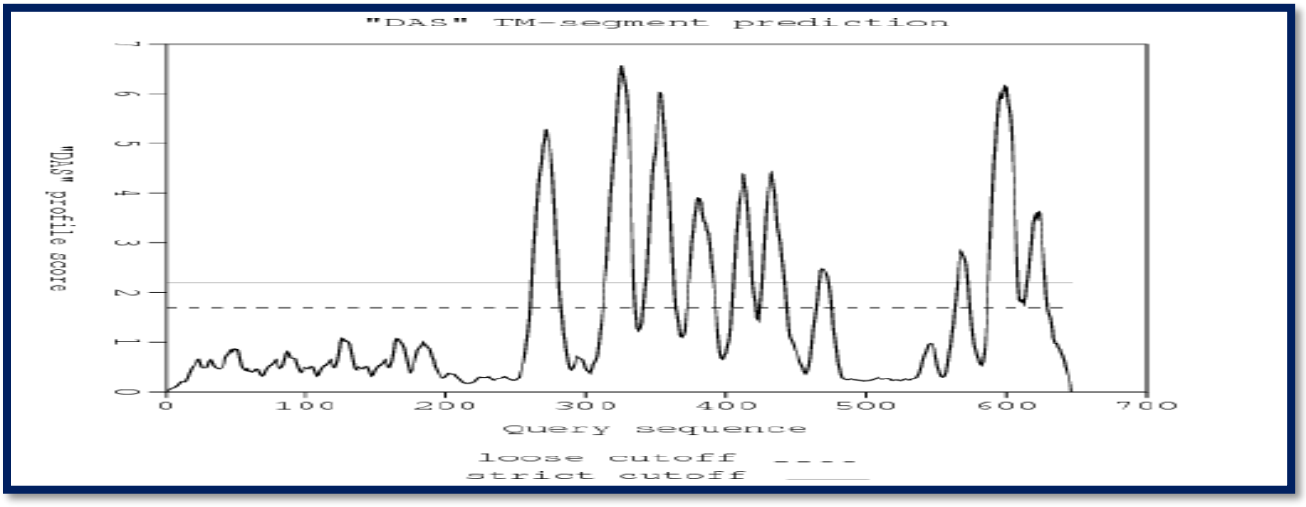


Figure-5 graph representaion of the transmembrane region of Apoe

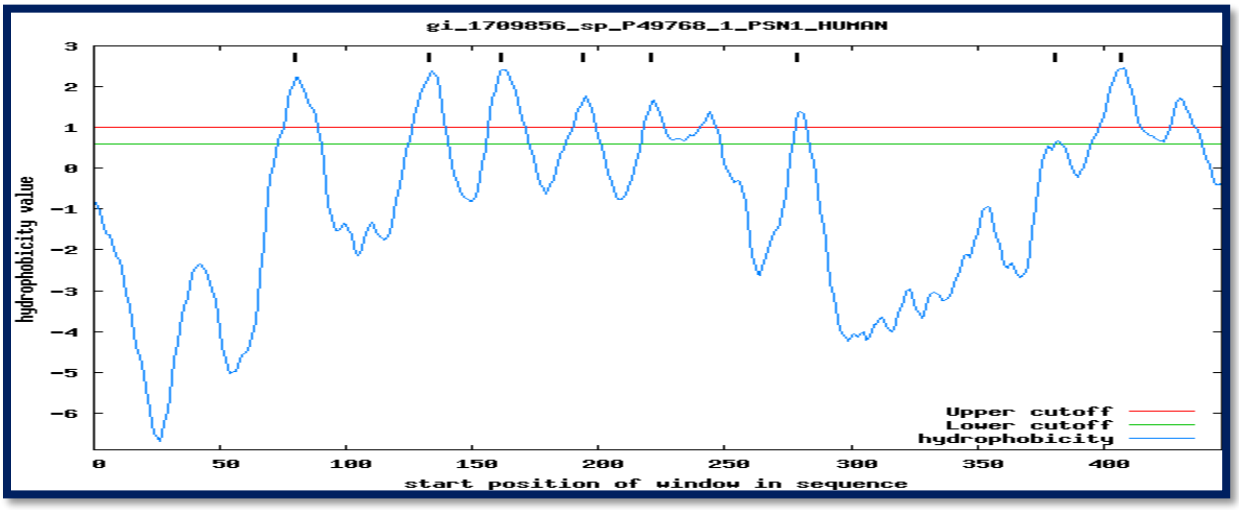


Figure- 6 PSEN1 topology prediction by TOP pred

Table-6 PSEN2

Start	Stop	Length	Cut-off
260	280	21	1.7
261	279	19	2.2
311	334	24	1.7
312	333	22	2.2
343	362	20	1.7
344	361	18	2.2
376	392	17	1.7
377	387	11	2.2
403	419	17	1.7
404	417	14	2.2
425	441	17	1.7
426	440	15	2.2
464	474	11	1.7
467	467	1	2.2
469	469	1	2.2
538	548	11	1.7
540	546	7	2.2
561	583	23	1.7
562	582	21	2.2
586	586	1	1.7
588	601	14	1.7
590	600	11	2.2

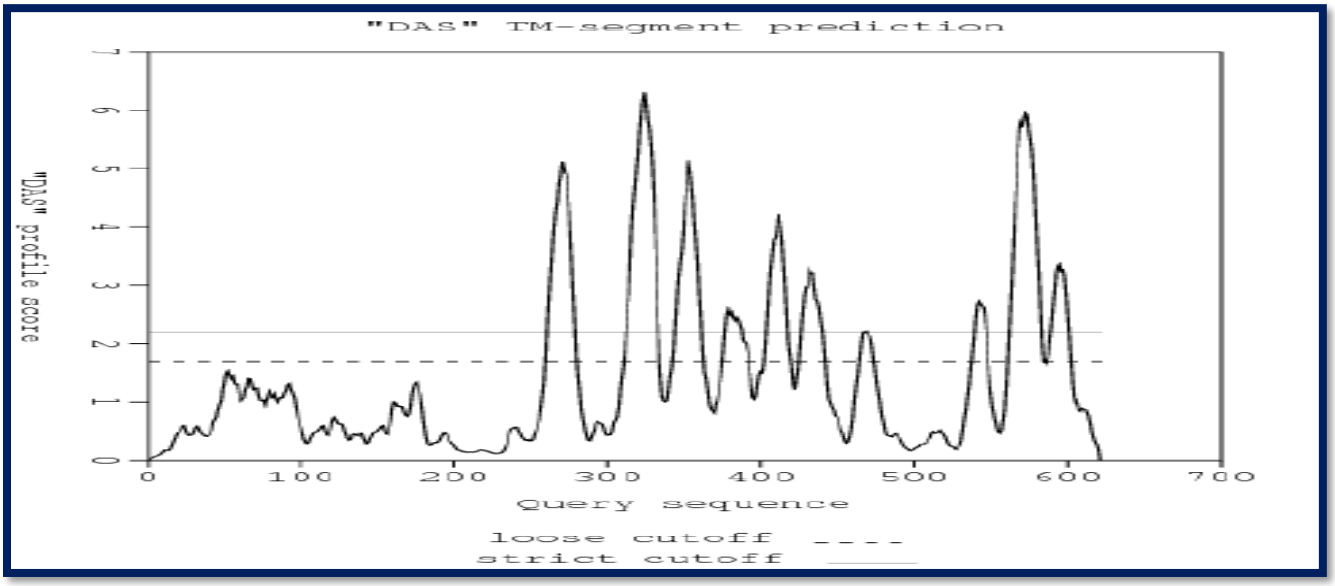


Figure-7 graph representaion of the transmembrane region of PSEN2

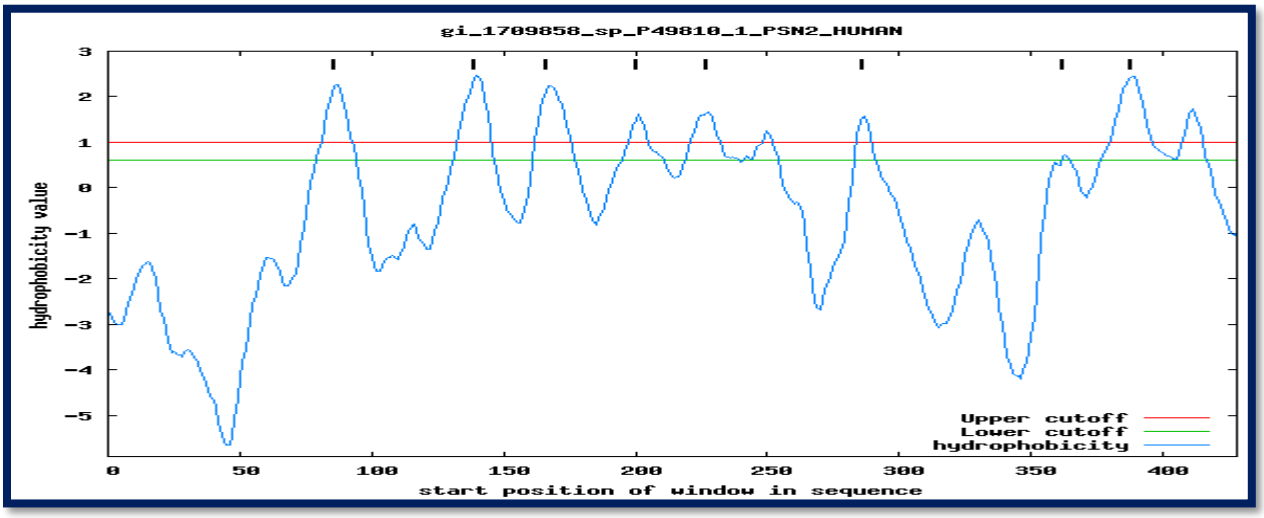


Figure- 8 PSEN2 topology prediction by TOP pred

4.7Statistical analysis of the various parameters of the GENES of Alzheimer

Table-7Percentage of ATCG content in the individual genes and in its variants

Genes Responsible	Percentage of ATCG content			
	A	T	C	G
APOE	18	13	32	37
APOE2	19.52	19.59	29.27	31.62
APOE3	30.93	25.54	22.95	20.57
APOE4	17.99	12.80	31.83	37.37
APP	23	33	20	21
PSEN1	30.87	25.46	22.22	23.46
PSEN2	22	23	26	28

Table-8 Number of ATCG repeats in indivisual gene.

Genes Responsible	Number of Repeats			
	A	T	C	G
APOE	208	148	368	432
APOE2	581	583	871	941
APOE3	4368	3607	3241	2905
APOE4	208	148	368	432
APP	278	345	211	223
PSEN1	371	306	243	282
PSEN2	582	562	659	705

Table-9 Physical and Thermo dynamical properties of the Genes (DNA seq.)

Gene responsible	Apoe	Apoe2	Apoe3	Apoe4	App	Psen1	Psen2
Molecular weight	314844.061Da	921003.01Da	4358955.12Da	358823.25Da	326469.20Da	372411.08Da	775748.10Da
% of GC content	69	61	44	69	41	44	54
Melting Temperatue	92.7	89.98	85.38	92.73	83.8	84.82	88.44
δH Kcal/mol	10729.4	26641.9	117345.6	10729.4	8724.5	10019.18	21705.7
δScal/mol	27666.2	69251.7	308856.8	27666.2	23020.1	26372.6	56638.9

δG kcal/mol	2142.7	5158.8	21541.7	2142.7	1580.2	1835.2	4132.8

4.7(a)Different kind of Motifs present in individual gene.

Table-10 Motif found in APOE

Motif found	Position	Description	Prosite	Related sequence
EGF_1	157..168	EGF-like domain signature	PS00022	725
CTCK_1	1066..1102	C-terminal cystine knot signature.	PS01185	48
ANAPHYLATOXIN_1	623..655	Anaphylatoxin domain signature	PS01177	30
VWFC_1	520..564	VWFC domain signature	PS01208	120
4FE4S_FER_1	751..762	4Fe-4S ferredoxin-type iron-sulfur binding region signature	PS00198	1376
2FE2S_FER_1	175..183	2Fe-2S ferredoxin-type iron-sulfur binding region signature	PS00197	267
DEFENSIN	865..892	Mammalian defensins signature	PS00269	59

Table-11 Motif found in APP

Motif found	Position	Description	Prosite	Related sequence
EGF_1	94..105	EGF-like domainsignature 1.	PS00022	725
ANAPHYLATOXIN_1	587..616	Anaphylatoxin domainsignature	PS01177	30
2FE2S_FER_1	114..122	2Fe-2S ferredoxin-typeiron-sulfur binding regionsignature	PS00197	267

Table-12 Motif found in PSEN1

Motif found	Position	Description	prosite	Related sequence
J_ACTX	268..267	Janus-faced atracotoxin (JACTX) familysignature	PS60020	4
ANAPHYLATOXIN_1	538..568	Anaphylatoxin domainsignature	PS01177	30
THIOLASE_3	306..319	Thiolases active site	PS00099	253
TUBULIN	697..703	Tubulin subunits alpha,beta, and gamma signature.	PS00227	422
VWFC_1	231..282	VWFC domain signature	PS01208	120
4FE4S_FER_1	332..343	4Fe-4S ferredoxin-typeiron-sulfur binding regionsignature.	PS00198	1376

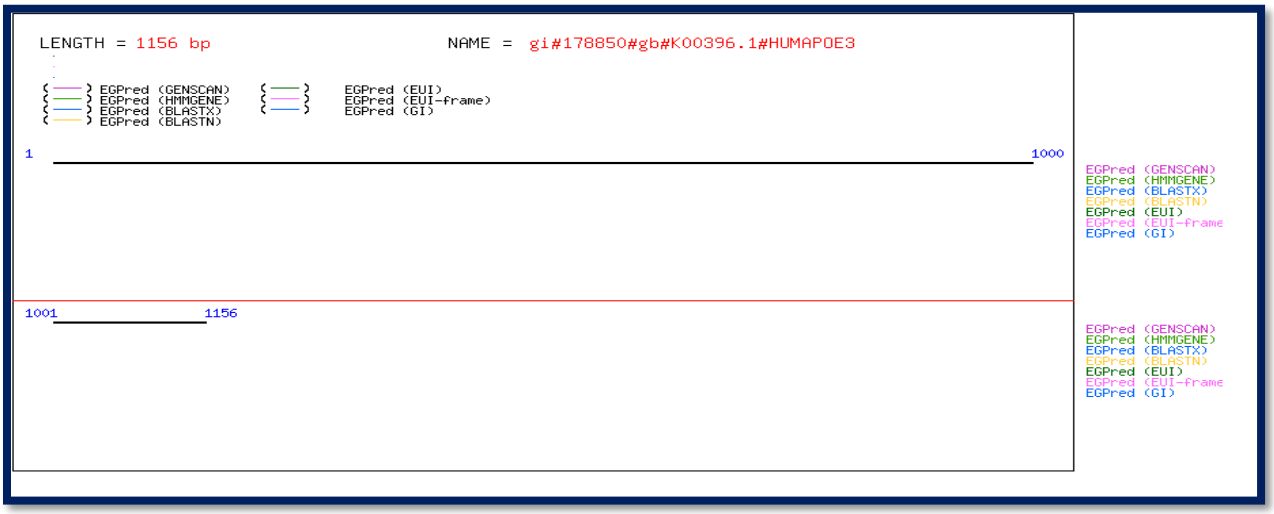
2FE2S_FER_1	13..21	2Fe-2S ferredoxin-typeiron-sulfur binding regionsignature	PS00197	267

Table-13 Motif found in PSEN2

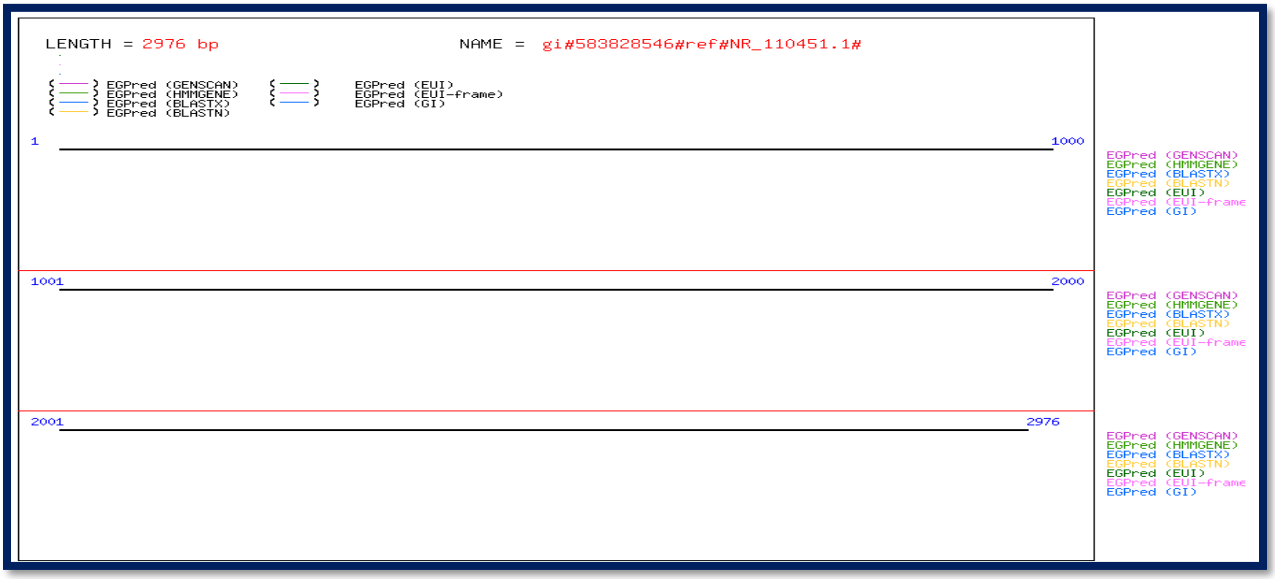
Motif found	Position	Description	Prosite	Related sequence
EGF_1	268..267	Janus-faced atracotoxin (JACTX) family signature	PS60020	4
CTCK_1	538..568	C-terminal cystine knot signature	PS01177	30
ANAPHYLATOXIN_1	306..319	Anaphylatoxin domain signature	PS00099	253
THIOLASE_3	697..703	Thiolases active site	PS00227	422
VWFC_1	231..282	VWFC domain signature	PS01208	120
2FE2S_FER_1	332..343	2Fe-2S ferredoxin-type iron-sulfur binding region signature	PS00198	1376
DEFENSIN	13..21	Mammalian defensins signature	PS00197	267

4.8EGPred prediction of the genes (nucleotide sequences) of Alzheimer

Apoe gene and its variant Apoe2, Apoe3 & Apoe4

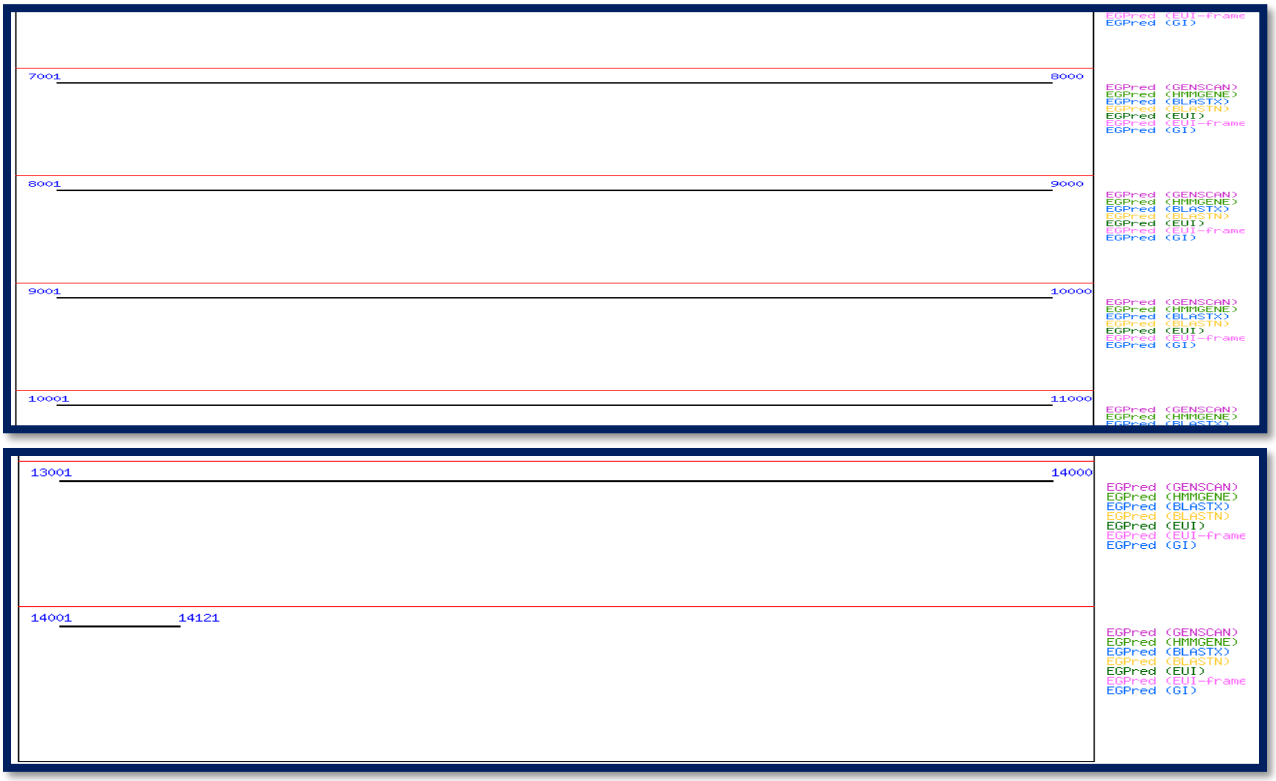


Apoe2

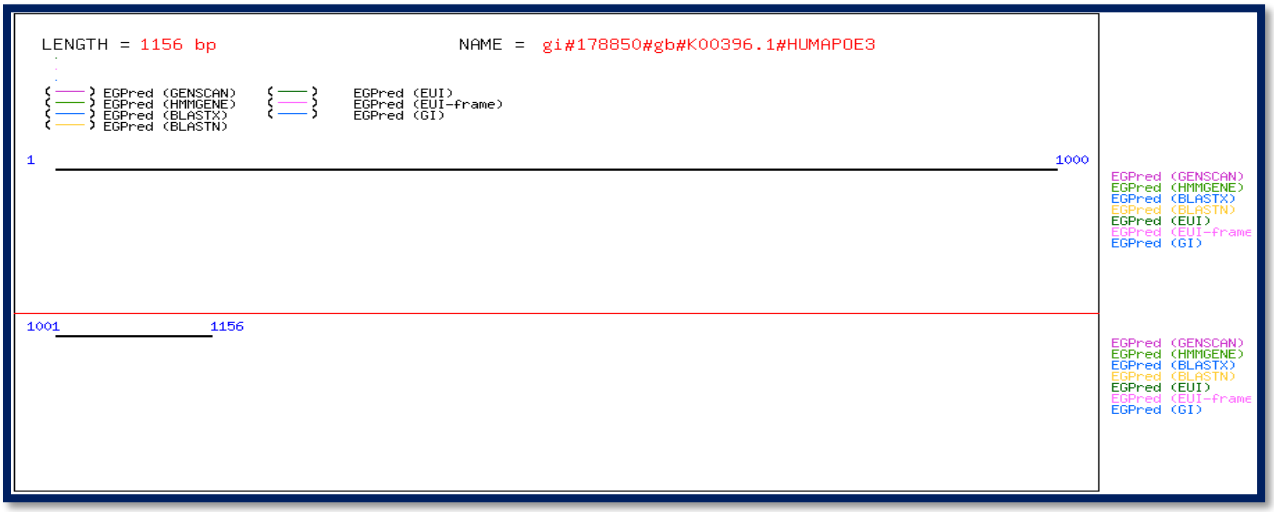


Apoe3

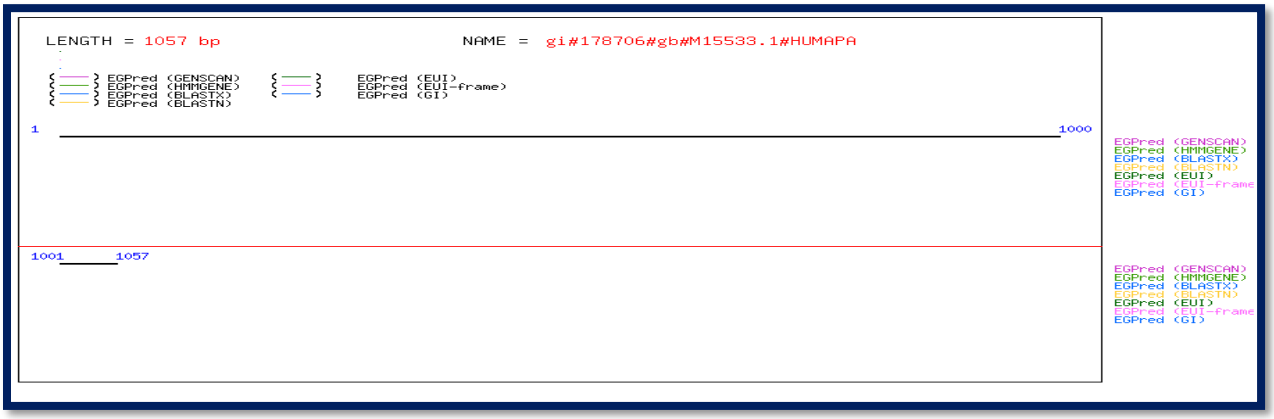

LENGTH = 14121 bp
NAME = gi#105990531#ref#NM_000384.2#
EGPred (GENSCAN)
EGPred (HMMGENE)
EGPred (BLASTX)
EGPred (BLASTN)
EGPred (EUI)
EGPred (EUI-frame)
EGPred (GI)
1
1000
1001
2000
2001
3000
3001
4000
4001
5000
5001
6000
6001
7000


7001
8000
8001
9000
9001
10000
10001
11000
13001
14000
14001
14121
EGPred (GENSCAN)
EGPred (HMMGENE)
EGPred (BLASTX)
EGPred (BLASTN)
EGPred (EUI)
EGPred (EUI-frame)
EGPred (GI)

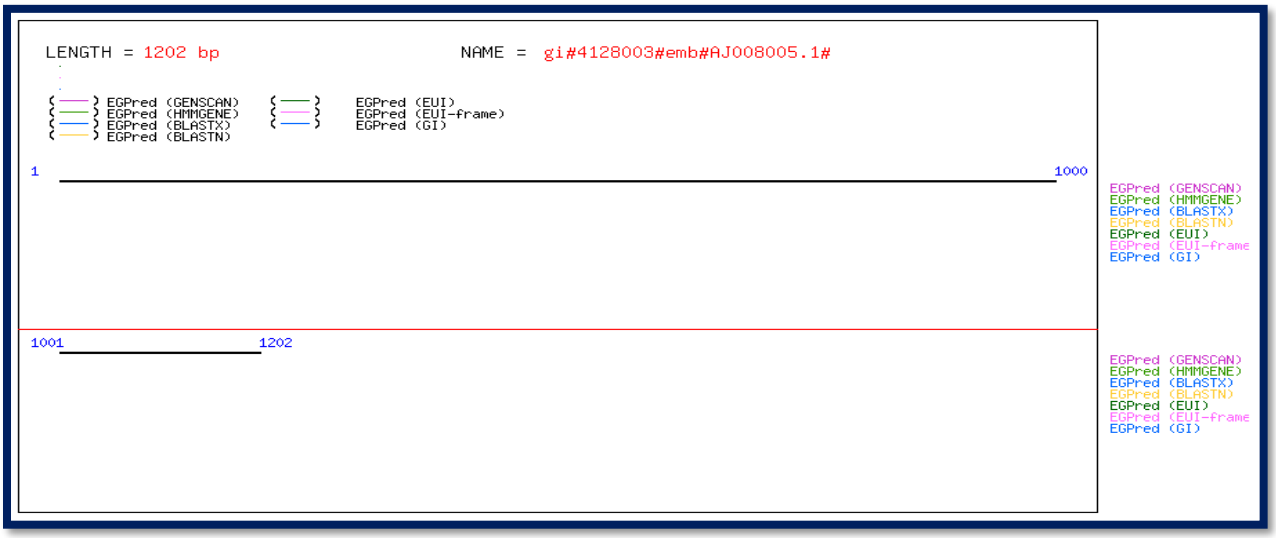
Apoe4


LENGTH = 1156 bp
NAME = gi#178850#gb#K00396.1#HUMAPOE3
EGPred (GENSCAN)
EGPred (HMMGENE)
EGPred (BLASTX)
EGPred (BLASTN)
EGPred (EUI)
EGPred (EUI-frame)
EGPred (GI)
1
1000
1001
1156

App


LENGTH = 1057 bp
NAME = gi#178706#gb#M15533.1#HUMAPA
EGPred (GENSCAN)
EGPred (HMMGENE)
EGPred (BLASTX)
EGPred (BLASTN)
EGPred (EUI)
EGPred (EUI-frame)
EGPred (GI)
1
1000
1001
1057

PSEN1


LENGTH = 1202 bp
NAME = gi#4128003#emb#AJ008005.1#
EGPred (GENSCAN)
EGPred (HMMGENE)
EGPred (BLASTX)
EGPred (BLASTN)
EGPred (EUI)
EGPred (EUI-frame)
EGPred (GI)
1
1000
1001
1202

Psen2

4.9FTG prediction of the genes (nucleotide sequences) of the Alzheimer

Apoe gene and its variants Apoe2,Apoe3 & Apoe4

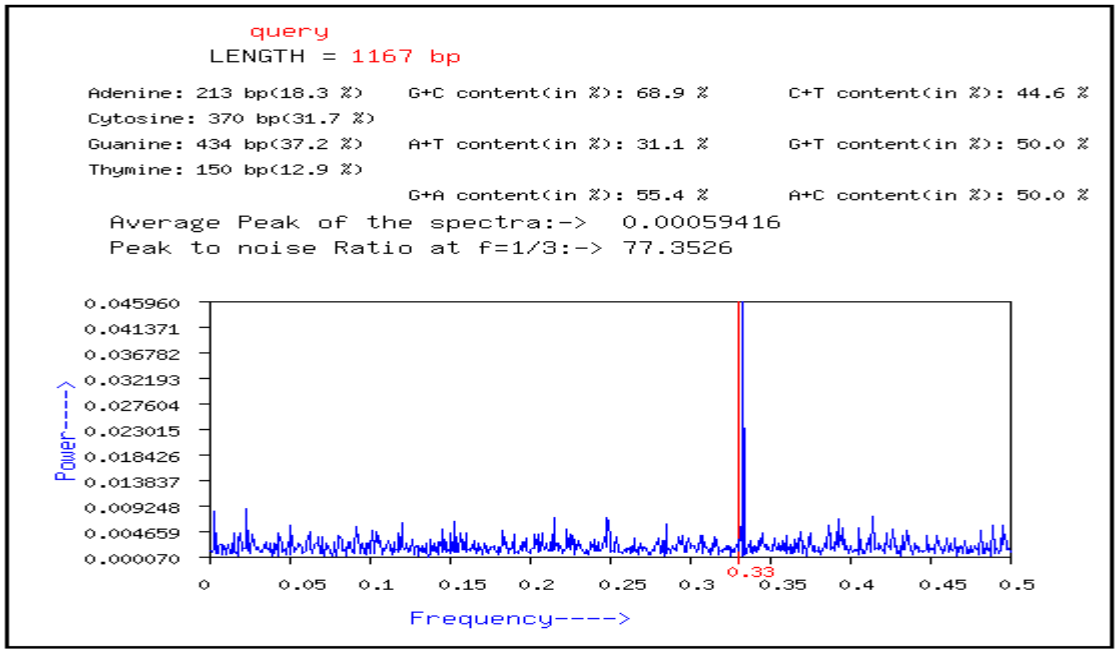


Figure-9 Apoe gene graph representation of ATCG frequency

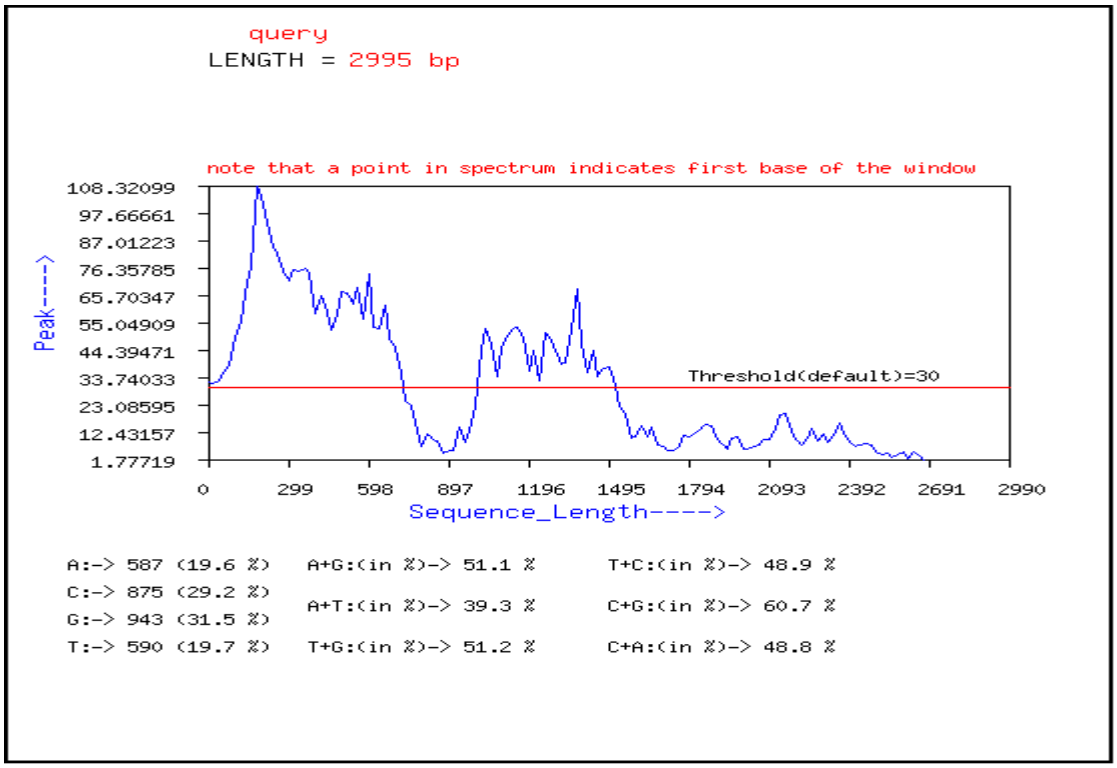


Figure-10Apoe2 gene graph representation of ATCG frequency

Apoe3

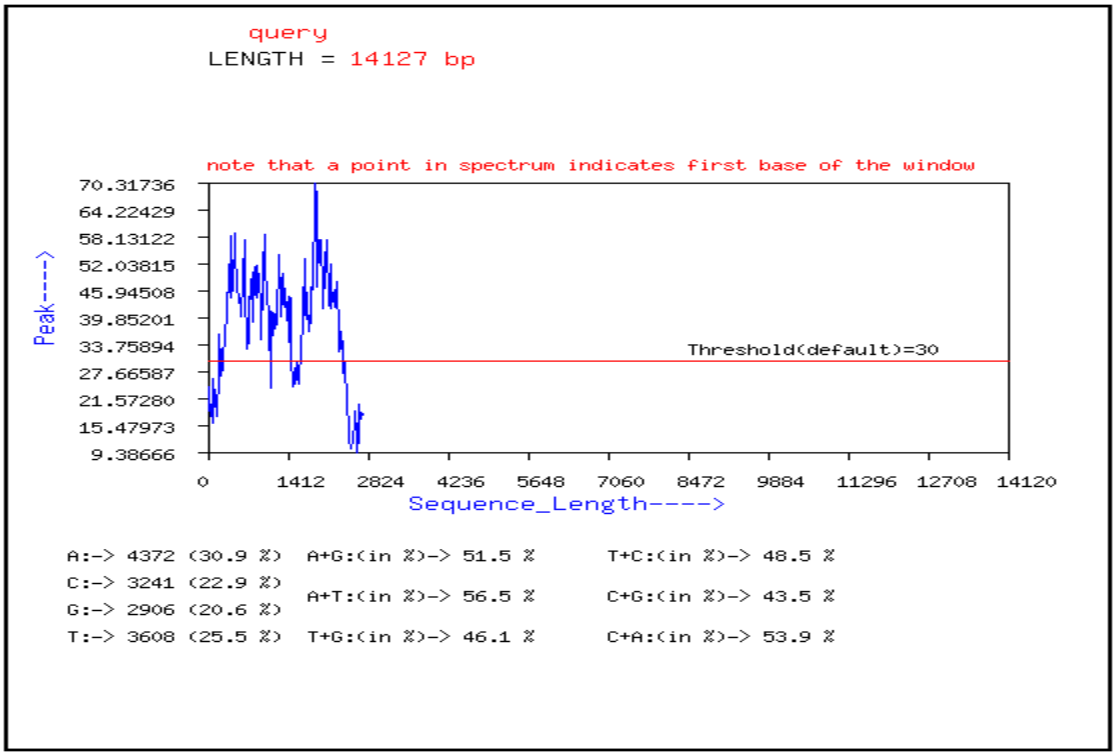
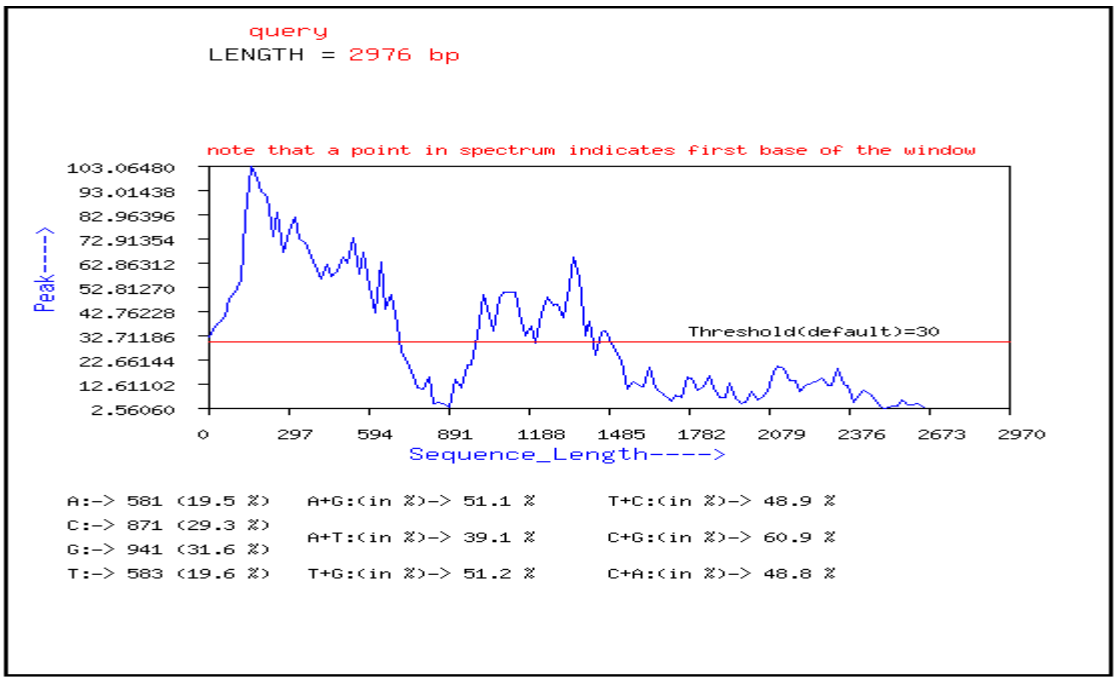


Figure-11Apoe3 gene graph representation of ATCG frequency



Figur-12Apoe4 gene graph representation of ATCG frequency

PSEN1

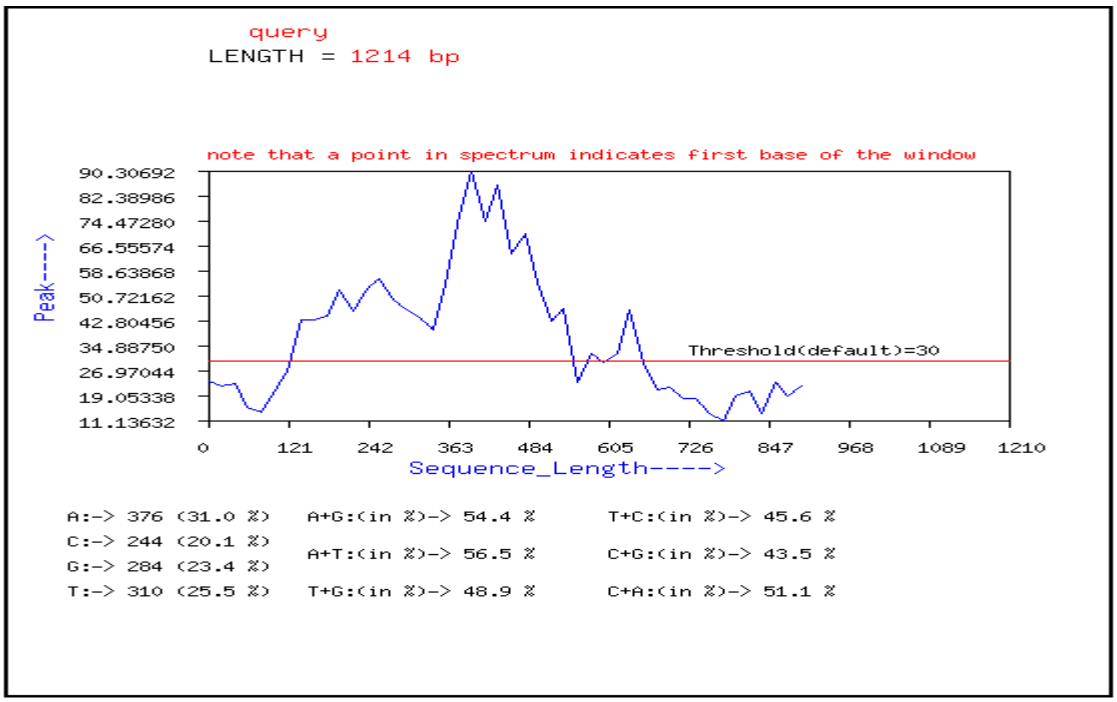


Figure-13PSEN1 gene graph representation of ATCG frequency

PSEN2

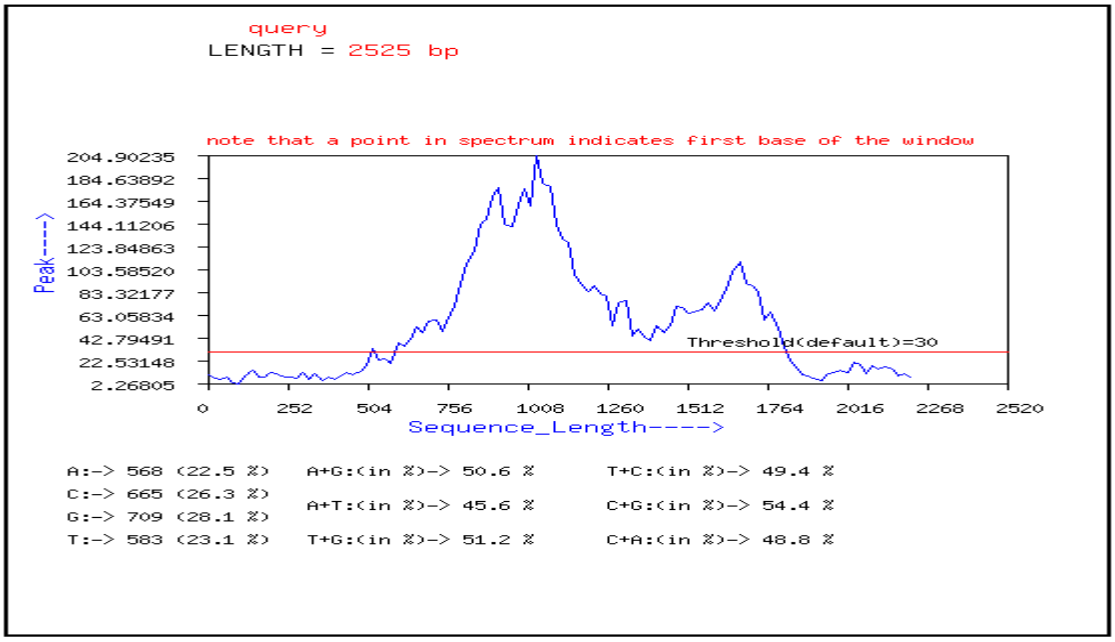


Figure-14PSEN2 gene graph representation of ATCG frequency

4.10Designing of siRNA of indivisual gene by using GenScripts database software tool siRNA target finder

Apoe and its variants Apoe2,Apoe3 &Apoe4 siRNA targets

Found variants:NM_000041;XM_001722911;XM_001724655;XM_001724653;XM_001722946

Query summary:

- Sequence Length: 722
- Specified Region: 1 -- 722
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AAGGCCTACAAATCGGAACTG	327	47.62	23.62	4.99/-35.50	NA	268/360	0	☑

siRNA insert 1: 70 bp.

BamH I Hind III
GGATCCCGGCCTACAAATCGGAACTGTTCAAGAGACAGTTCCGATTTGTAGGCCTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

Apoe2

Found variants:NM_174936

Query summary:

- Sequence Length: 2976
- Specified Region: 1 -- 2976
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AACAAACAAACGTTGTCCTAA	2936	33.33	10.00	1.27/-29.20	NA	709/360	1	☑
2.	AATGGAGGCTTAGCTTTCTGG	2207	47.62	9.66	1.75/-36.30	NA	546/360	0	☑
3.	AACAAACGTTGTCCTAACAAA	2940	33.33	7.33	1.64/-29.20	NA	165/360	1	☑
4.	AATGCAAAGTCAAGGAGCATG	1429	42.86	4.42	2.65/-33.80	NA	478/360	0	☑
5.	AAACAAACGTTGTCCTAACAA	2939	33.33	-0.50	0.33/-29.20	NA	204/360	1	☑

siRNA insert 1: 71 bp.

BamH I Hind III
GGATCCCGCAAACAAACGTTGTCCTAATTCAAGAGATTAGGACAACGTTTGTTTGTTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 2: 71 bp.

BamH I Hind III
GGATCCCGTGGAGGCTTAGCTTTCTGGTTCAAGAGACCAGAAAGCTAAGCCTCCATTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 3: 71 bp.

BamH I Hind III
GGATCCCGCAAACGTTGTCCTAACAAATTCAAGAGATTTGTTAGGACAACGTTTGTTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 4: 71 bp.

BamH I Hind III
GGATCCCGTGCAAAGTCAAGGAGCATGTTCAAGAGACATGCTCCTTGACTTTGCATTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 5: 70 bp.

BamH I Hind III
GGATCCCACAAACGTTGTCCTAACAATTCAAGAGATTGTTAGGACAACGTTTGTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

Apoe3

Found variants:NM_000384

Query summary:

- Sequence Length: 14121
- Specified Region: 1 -- 14121
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AAGATCATCAGAACCATTGAC	8141	38.10	29.69	0.01/-32.40	NA	383/360	0	☑
2.	AAGGTGCGAAGCAGACTGAGG	3249	57.14	27.40	2.74/-40.10	NA	70/360	0	☑
3.	AAGGCATGGCACTGTTTGGAG	9144	52.38	25.23	1.60/-38.20	NA	394/360	0	☑
4.	AAGCACCTCCGGAAGTACACA	269	52.38	24.78	3.97/-39.10	NA	533/360	1	☑
5.	AAGTCCATGAGTTAATCGAGA	7149	38.10	24.77	2.58/-32.90	NA	433/360	0	☑
6.	AAGCTAAGCAATGTCCTACAA	7256	38.10	24.08	1.88/-33.00	NA	380/360	0	☑
7.	AAGTCATCATCTCGTGTCTAG	5980	42.86	22.74	1.71/-34.20	NA	374/360	0	☑
8.	AAGCCAGGCCATTGCGACGAA	13564	57.14	22.50	4.63/-40.30	NA	539/360	0	☑
9.	AAGTGCTTATCAGGCCATGAT	5263	42.86	22.28	4.19/-35.60	NA	311/360	0	☑
10.	AAGCTATAAAGCAGACACTGT	5677	38.10	22.17	1.40/-33.10	NA	689/360	0	☑

siRNA insert 1: 70 bp.

BamH I — Hind III

GGATCCCGATCATCAGAACCATTGACTTCAAGAGAGTCAATGGTTCTGATGATCTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 2: 70 bp.

BamH I — Hind III

GGATCCCGGTGCGAAGCAGACTGAGGTTCAAGAGACCTCAGTCTGCTTCGCACCTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 3: 70 bp.

BamH I — Hind III

GGATCCCGGCATGGCACTGTTTGGAGTTCAAGAGACTCCAAACAGTGCCATGCCTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 4: 70 bp.

BamH I — Hind III

GGATCCCGCACCTCCGGAAGTACACATTCAAGAGATGTGTACTTCCGGAGGTGCTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 5: 70 bp.

BamH I — Hind III

GGATCCCGTCCATGAGTTAATCGAGATTCAAGAGATCTCGATTAACTCATGGACTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 6: 70 bp.

BamH I — Hind III

GGATCCCGCTAAGCAATGTCCTACAATTCAAGAGATTGTAGGACATTGCTTAGCTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 7: 70 bp.

BamH I — Hind III

GGATCCCGTCATCATCTCGTGTCTAGTTCAAGAGACTAGACACGAGATGATGACTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 8: 70 bp.

BamH I — Hind III

GGATCCCGCCAGGCCATTGCGACGAATTCAAGAGATTCGTCGCAATGGCCTGGCTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 9: 70 bp.

BamH I — Hind III

GGATCCCGTGCTTATCAGGCCATGATTTCAAGAGAATCATGGCCTGATAAGCACTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

siRNA insert 10: 70 bp.

BamH I — Hind III

GGATCCCGCTATAAAGCAGACACTGTTTCAAGAGAACAGTGTCTGCTTTATAGCTTTTTTCCAAAAGCTT

| Sense | Loop | Antisense | Termination Signal

Apoe4

Found variants:NM_000041;XM_001722946;XM_001722911;XM_001724655;XM_001724653

Query summary:

- Sequence Length: 1156
- Specified Region: 1 -- 1156
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AAGGCCTACAAATCGGAACTG	330	47.62	23.62	4.99/-35.50	NA	268/360	0	☑

siRNA insert 1: 70 bp.

BamH I Hind III
GGATCCCGGCCTACAAATCGGAACTGTTCAAGAGACAGTTCCGATTTGTAGGCCTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

APP

Found variants:NM_000484;NM_201413;NM_201414;NM_001136130;NM_001136129;XM_002343748;XM_002348066;XM_002345446

Query summary:

- Sequence Length: 912
- Specified Region: 1 -- 912
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AAGAGTACCAACTTGCATGAC	629	42.86	24.67	0.10/-34.20	NA	280/360	0	☑
2.	AACCAACCAGTGACCATCCAG	413	52.38	9.97	0.49/-38.50	NA	440/360	0	☑
3.	AACCTGCATTGATACCAAGGA	328	42.86	4.00	2.58/-35.40	NA	610/360	0	☑
4.	AAACCGTGATGACCAGACATA	864	42.86	2.83	4.96/-34.90	NA	126/360	0	☑
5.	AACCGTGATGACCAGACATAA	865	42.86	-0.94	6.01/-34.90	NA	448/360	0	☑

siRNA insert 1: 70 bp.

BamH I Hind III
GGATCCCGAGTACCAACTTGCATGACTTCAAGAGAGTCATGCAAGTTGGTACTCTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

siRNA insert 2: 71 bp.

BamH I Hind III
GGATCCCGCCAACCAGTGACCATCCAGTTCAAGAGACTGGATGGTCACTGGTTGGTTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 3: 71 bp.

BamH I Hind III
GGATCCCGCCTGCATTGATACCAAGGATTCAAGAGATCCTTGGTATCAATGCAGGTTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 4: 70 bp.

BamH I Hind III
GGATCCCACCGTGATGACCAGACATATTCAAGAGATATGTCTGGTCATCACGGTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

siRNA insert 5: 71 bp.

BamH I Hind III
GGATCCCGCCGTGATGACCAGACATAATTCAAGAGATTATGTCTGGTCATCACGGTTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

Psen1

Found variants:NM_000021;NM_007318

Query summary:

- Sequence Length: 547
- Specified Region: 1 -- 547
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AACGGCAGGAGCACAACGACA	289	57.14	14.77	3.85/-40.30	NA	545/360	0	☑
2.	AATATGGCGCCAAGCATGTGA	415	47.62	9.65	0.50/-36.90	NA	126/360	0	☑
3.	AATACTGTACGTAGCCAGAAT	258	38.10	6.91	3.28/-32.80	NA	428/360	0	☑

siRNA insert 1: 71 bp.

BamH I Hind III
GGATCCCGCGGCAGGAGCACAACGACATTCAAGAGATGTCGTTGTGCTCCTGCCGTTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 2: 71 bp.

BamH I Hind III
GGATCCCGTATGGCGCCAAGCATGTGATTCAAGAGATCACATGCTTGGCGCCATATTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 3: 71 bp.

BamH I Hind III
GGATCCCGTACTGTACGTAGCCAGAATTTCAAGAGAATTCTGGCTACGTACAGTATTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

Psen2

Found variants:NM_000447;NM_012486

Query summary:

- Sequence Length: 563
- Specified Region: 1 -- 563
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select

4.11Statistical analysis of the various parameters of the proteins of Parkinson's

Table-14 number of amino acid content and percentage of amino acid repeats in indivisual protein sequence

Amino acid	Synuclein		PARK2		PARK5		PARK7	
	No of repeats	% of repeats	No of repeats	% of repeats	No of repeats	% of repeats	No of repeats	% of repeats
A	19	14	27	6	20	9	24	13
C	0	0	35	8	6	3	3	2
D	6	4	23	5	11	5	9	5
E	18	13	28	6	22	10	15	8
F	2	1	17	4	13	6	3	2
G	18	13	38	8	15	7	18	10
H	1	1	15	3	6	3	3	2
I	2	1	15	3	7	3	10	5
K	15	11	18	4	16	7	16	8
L	4	3	30	6	23	10	18	10
M	4	3	7	2	6	3	5	3
N	3	2	17	4	11	5	6	3
P	5	4	29	6	9	4	9	5
Q	6	4	27	6	12	5	4	2
R	0	0	31	7	9	4	7	4
S	4	3	30	6	11	5	9	5
T	10	7	24	5	6	3	8	4
V	19	14	36	8	17	8	19	10
W	0	0	8	2	1	0	0	0
Y	4	3	10	2	2	1	3	2
Aliphatic I,L,	25	18	81	17	47	21	47	25
Aromatics F,W,Y6	6	4	35	8	16	7	6	3
Positive K,R,H	16	11	64	14	31	14	26	14
Negative D,E	24	17	51	11	33	15	24	13
Tiny G,A,S	41	29	95	20	46	21	51	27
tRNAsynthetase class I Z,E,Q,R,C,M, V,I,L,Y,W	83	42.35	273	49.19	161	49.69	123	46.42
tRNAsynthetase class II B,G,A,P,S,T,H, D,N,K,F	113	57.65	281	50.63	161	49.69	141	53.21

Table-15 Physical and chemical property of protein sequences

Protein	Synuclein	Park2	Park5	Park7
Number of Amino acids	194	552	319	263
Mol.wieght	20.49 kDa	61.37 kDa	35.71 kDa	28.30 kDa
Isoelectric point	4.34	6.29	4.71	4.87

4.12Transmembrane region or hydrophobic region and Topology prediction of protein sequences of individual protein

Table-16

Start	Stop	Length	Cut-off
110	120	11	1.7
112	118	7	2.2
130	134	5	1.7

.

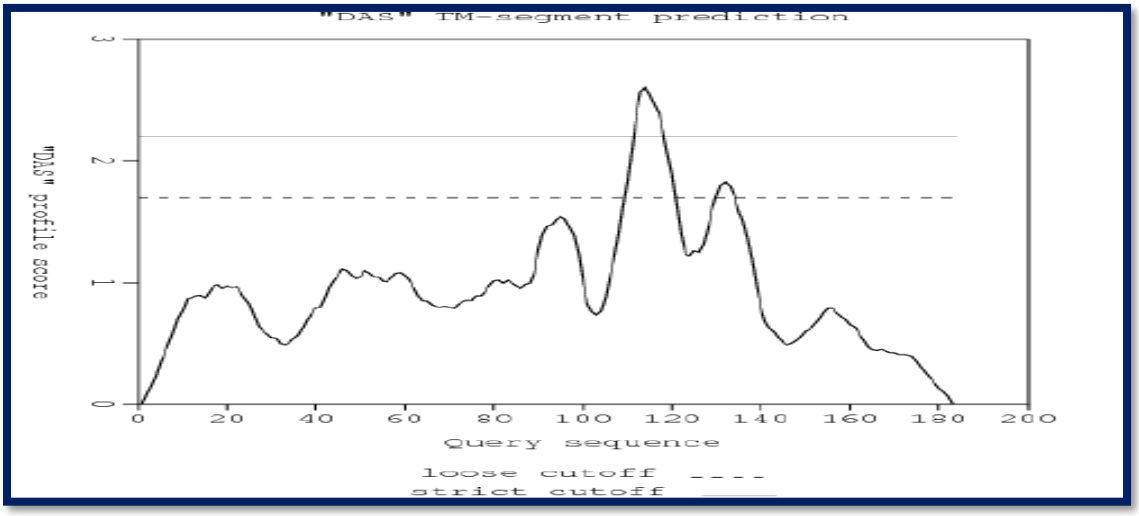


Figure-15 DAS Trans membrane prediction server for finding the transmembrane region in **Synuclein**protein sequence.

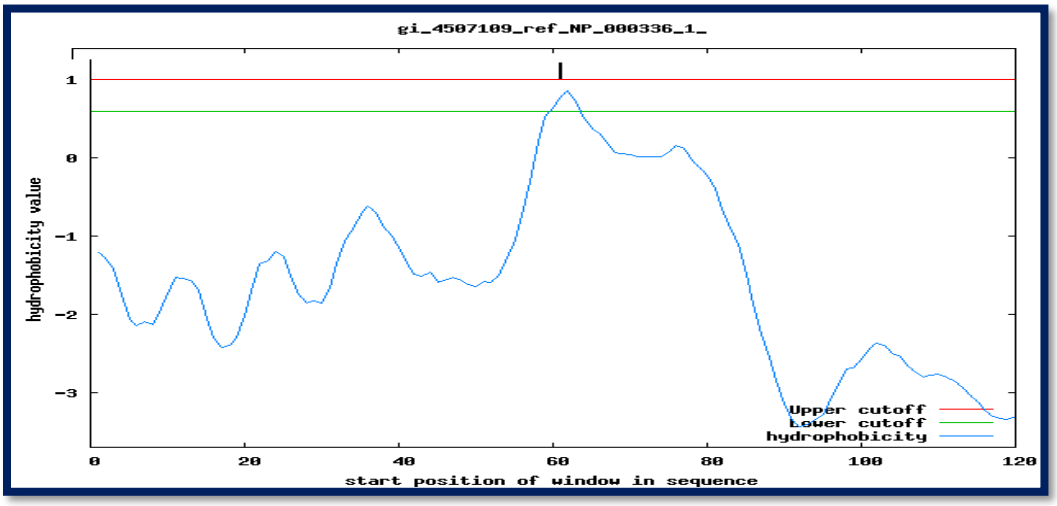


Figure-16Graphical representation of topologyof synuclein protein by TOPPred.

DAS Transmembrane prediction server for finding the transmembrane region in Park2 protein sequence.

Table-17

Start	Stop	Length	Cut-off
126	129	4	1.7
237	247	11	1.7
241	244	4	2.2
351	354	4	1.7
383	391	9	1.7

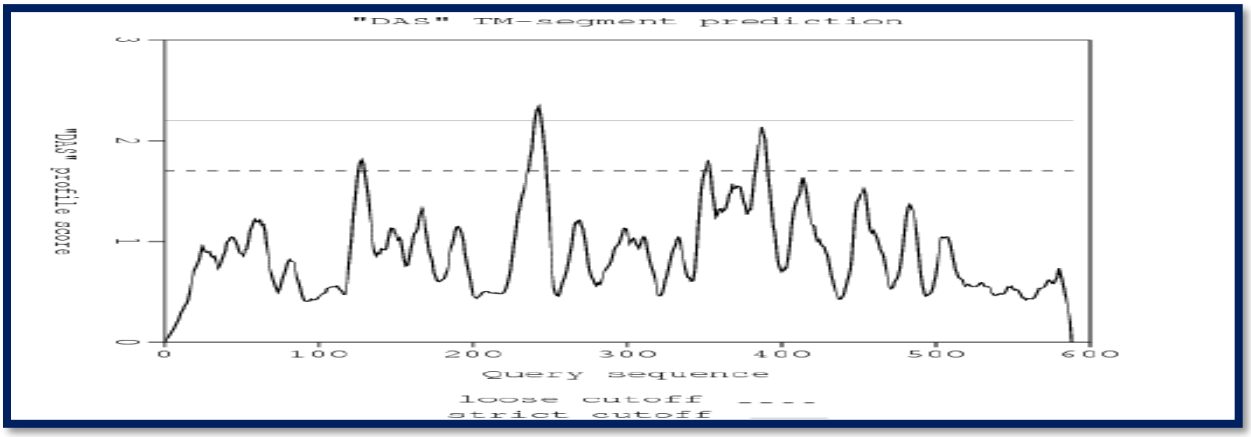


Figure-17 graph representaion of the transmembrane region of PARK2

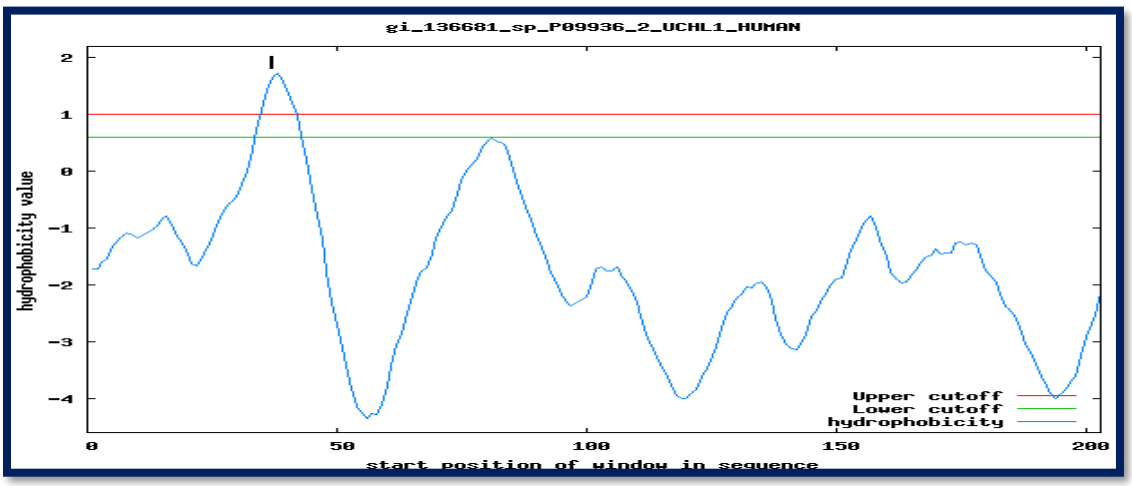


Figure-18 Graphical representation of topology of PARK2 protein by TOPpred

Table-18

Start	Stop	Length	Cut-off
224	236	13	1.7
225	235	11	2.2
271	278	8	1.7

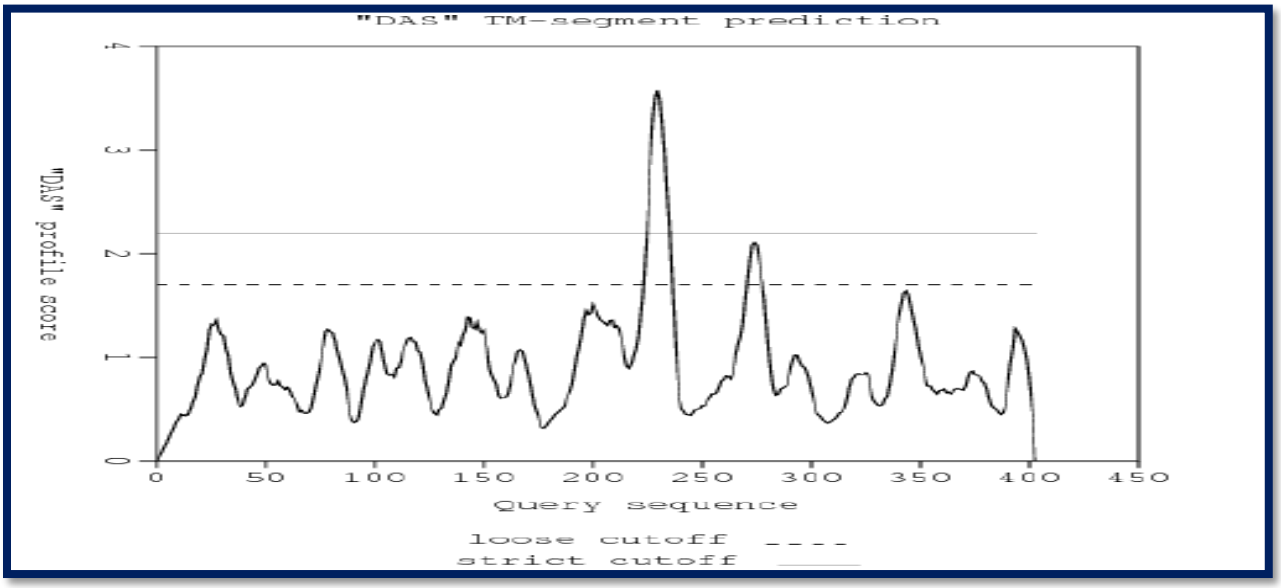


Figure-19 DAS Transmembrane prediction server for finding the trans membrane region in Park5 protein sequence.

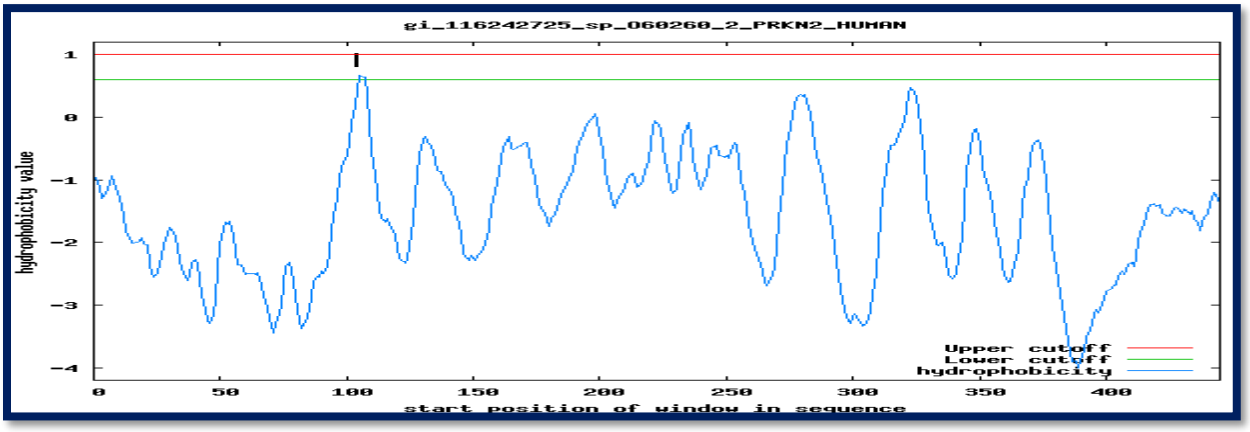


Figure 20 Graphical representation of topology of PARK 5 protein by TOPPred.

Table-18

Start	Stop	Length	Cut-off
206	216	11	1.7
208	210	3	2.2
267	272	6	1.7

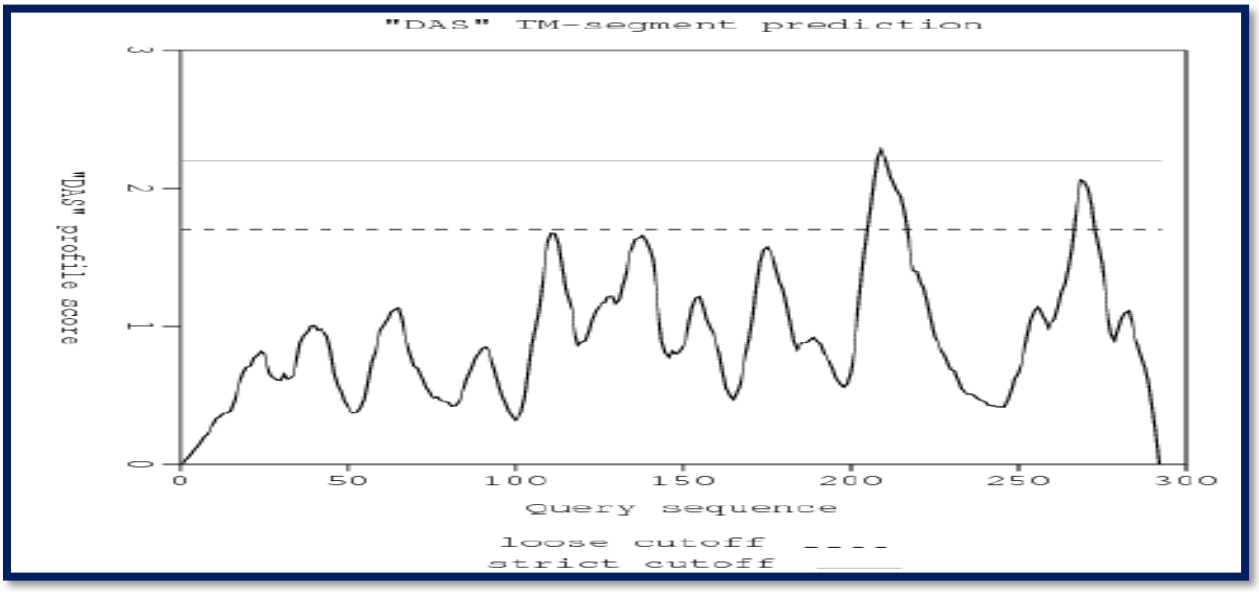


Figure-21 DAS Transmembrane prediction server for finding the trans membrane region in Park7 protein sequence

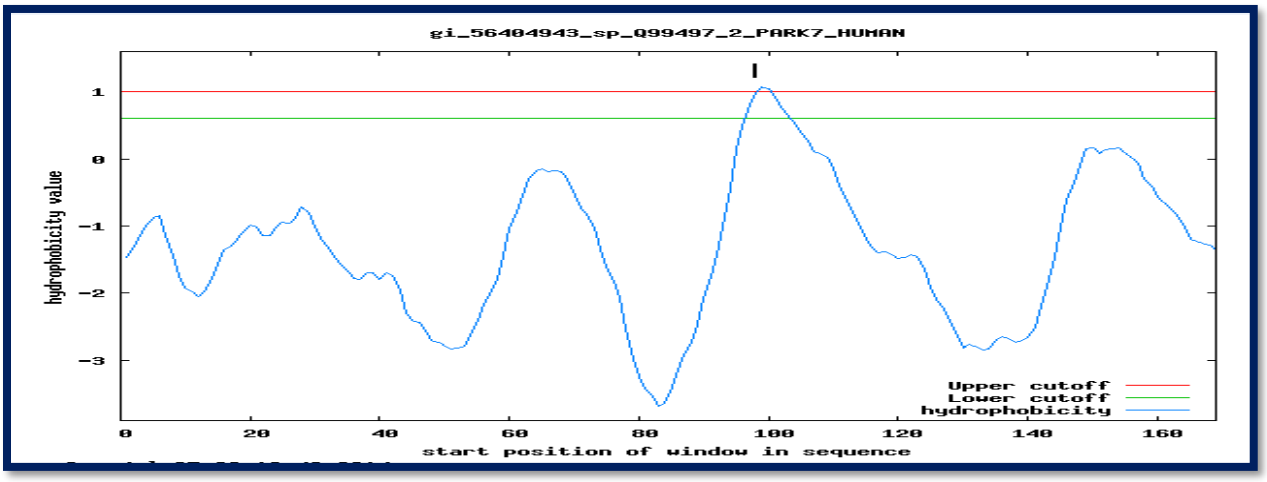


Figure-22 Graphical representation of topology of synuclein protein by TOPPred.

4.13Statistical analysis of the various parameters of the GENES of Parkinson's

Table-19 Percentage of ATCG content in individual gene

Genes responsible	Percentage of ATCG content			
	A	T	C	G
Synuclein	30	32	16	22
PARK5	26	33	20	21
PARK2	31	25	20	23
PARK7	22	23	26	28

Table-20 Total number of repeats in individual gene

Genes Responsible	Number of Repeats			
	A	T	C	G
Synuclein	471	488	248	342
PARK5	278	345	211	223
PARK2	371	306	242	282
PARK7	562	582	659	705

Table-21Physical and Thermodynamical properties of genes

Gene responsible	Synuclein	PARK2	PARK5	PARK7
Molecular weight	480216	1258048	343213.3	775669.1
% of GC content	38	49	50	54
Melting Temperature	82.93	86.77	87.05	88.44
δH Kcal/mol	12644.5	34802.7	9514.4	21705.7
δScal/mol	33458.9	91238.8	24893.6	56638.9
δG kcal/mol	2262.8	6499.3	1787.6	4132.8

Table22-Motif found in synuclein

Motif found	Position	Description	prosite	Related sequence
EGF_1	4..15	EGF-like domain signature	PS00022	725
2FE2S_FER_1	112..125	2Fe-2S ferredoxin-type iron-sulfur binding region signature	PS00197	253
DEFENSIN	452..460	Mammalian defensins signature	PS00269	267

Table-23 Motif found in Park2

Motif found	Position	Description	prosite	Related sequence
EGF_1	87..98	EGF-like domain signature	PS00022	725
CTCK_1	3093..3131	C-terminal cystine knot signature.	PS01185	48
ANAPHYLATOXIN_1	395..432	Anaphylatoxin domain signature	PS01177	30
VWFC_1	632..676	VWFC domain signature	PS01208	120
4FE4S_FER_1	1808..1819	4Fe-4S ferredoxin-type iron-sulfur binding region signature	PS00198	1376
2FE2S_FER_1	226..234	2Fe-2S ferredoxin-type iron-sulfur binding region signature	PS00197	267
DEFENSIN	969..997	Mammalian defensins signature	PS00269	59
INTEGRIN_BETA	1543..1557	cysteine-rich domain signature	PS00243	48
THIOLASE_3	1740..1753	Thiolases active site	PS00099	253

Table-24 Motif found in park5

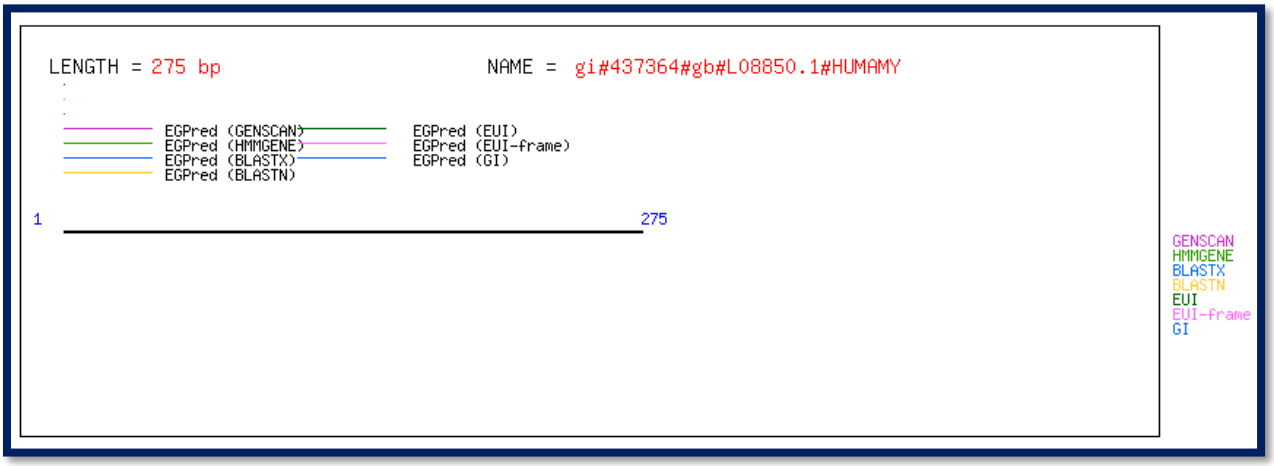
Motif found	Position	Description	prosite	Related sequence
EGF_1	144..155	EGF-like domain signature	PS00022	725
ANAPHYLATOXIN_1	1..35	Anaphylatoxin domain signature	PS01177	30
VWFC_1	69..123	VWFC domain signature	PS01208	120
2FE2S_FER_1	2..10	2Fe-2S ferredoxin-type iron-sulfur binding region signature	PS00197	267
INTEGRIN_BETA	46..61	cysteine-rich domain signature	PS00243	48
THIOLASE_3	444..457	Thiolases active site	PS00099	253

Table-25 Motif found in park7

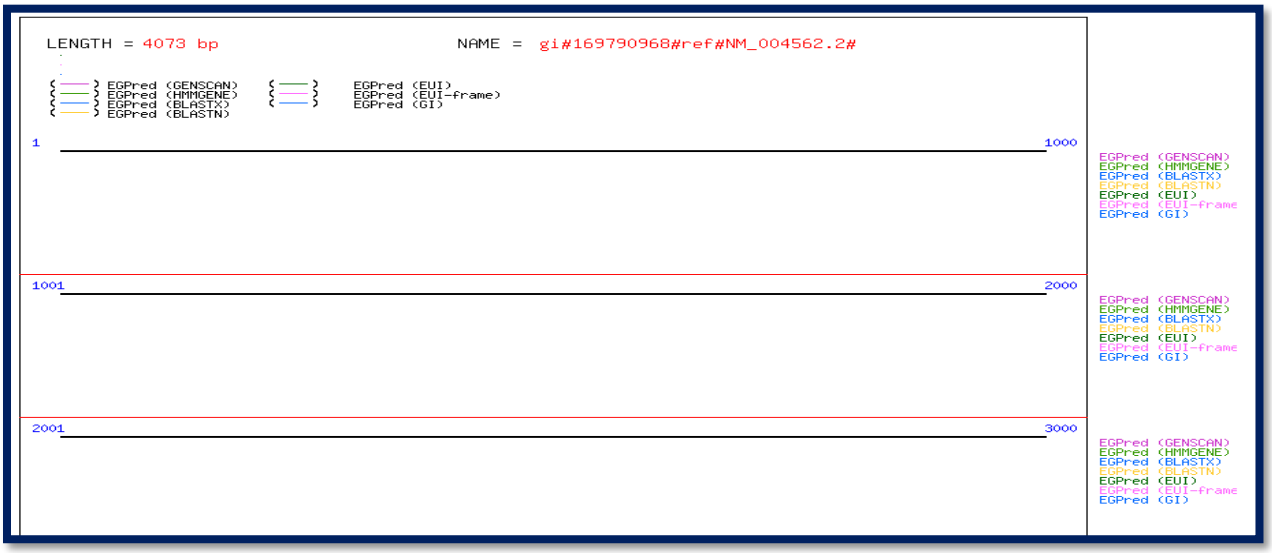
Motif found	Position	Description	prosite	Related sequence
EGF_1	315..326	EGF-like domain signature	PS00022	725
2FE2S_FER_1	292..305	2Fe-2S ferredoxin-type iron-sulfur binding region signature	PS00197	253
DEFENSIN	25..33	Mammalian defensins signature	PS00269	267

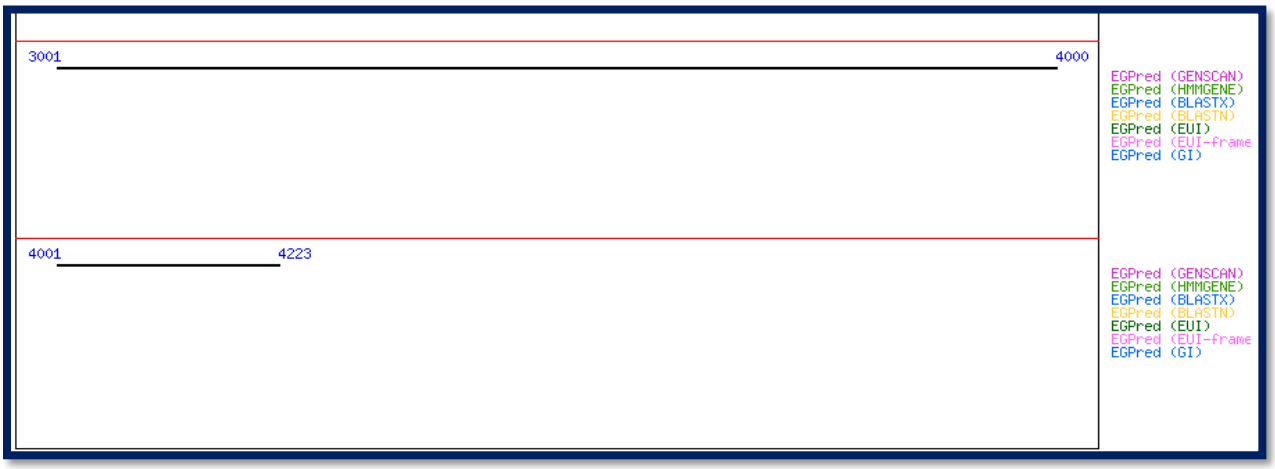
4.14EGPred prediction of the genes (nucleotide sequences) of Parkinson's

Synuclein



PARK2

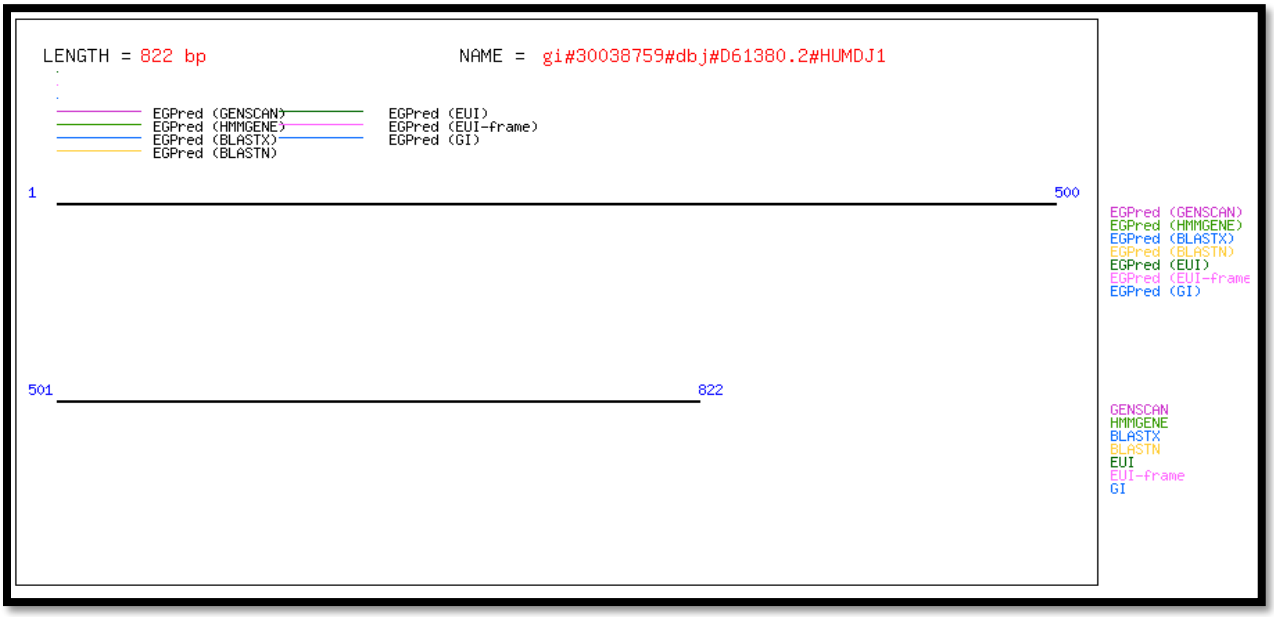




PARK5


LENGTH = 1110 bp
NAME = gi#33875314#gb#BC000332.2#
EGPred (GENSCAN)
EGPred (HMMGENE)
EGPred (BLASTX)
EGPred (BLASTN)
EGPred (EUI)
EGPred (EUI-frame)
EGPred (GI)
1
1000
1001
1110
EGPred (GENSCAN)
EGPred (HMMGENE)
EGPred (BLASTX)
EGPred (BLASTN)
EGPred (EUI)
EGPred (EUI-frame
EGPred (GI)

PARK7


LENGTH = 822 bp
NAME = gi#30038759#dbj#D61380.2#HUMDJ1
EGPred (GENSCAN)
EGPred (HMMGENE)
EGPred (BLASTX)
EGPred (BLASTN)
EGPred (EUI)
EGPred (EUI-frame)
EGPred (GI)
1
500
501
822
GENSCAN
HMMGENE
BLASTX
BLASTN
EUI
EUI-frame
GI

4.15FTG prediction of the genes (nucleotide sequences) of the Parkinson

Synuclein

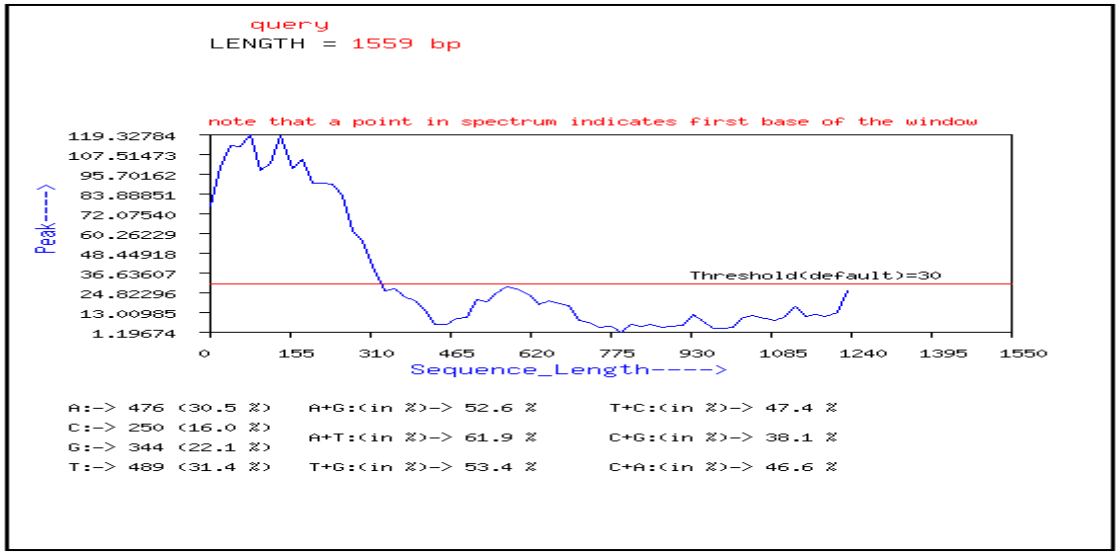


Figure-23 Graphical representation of the ATCG content in Synuclein gene

Park2

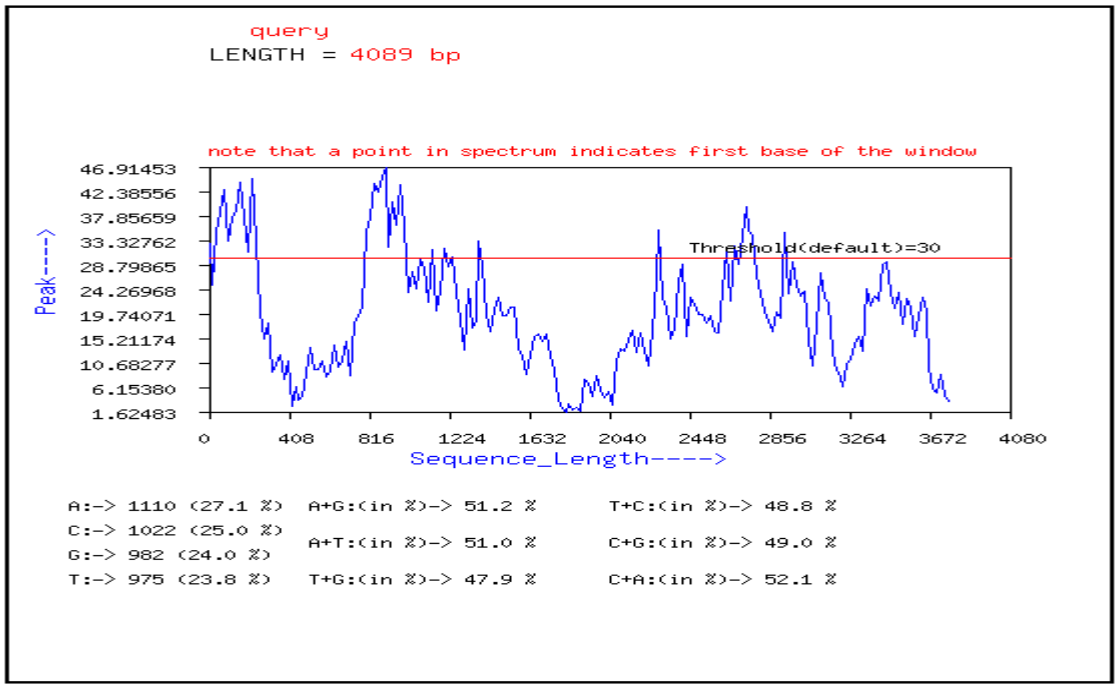
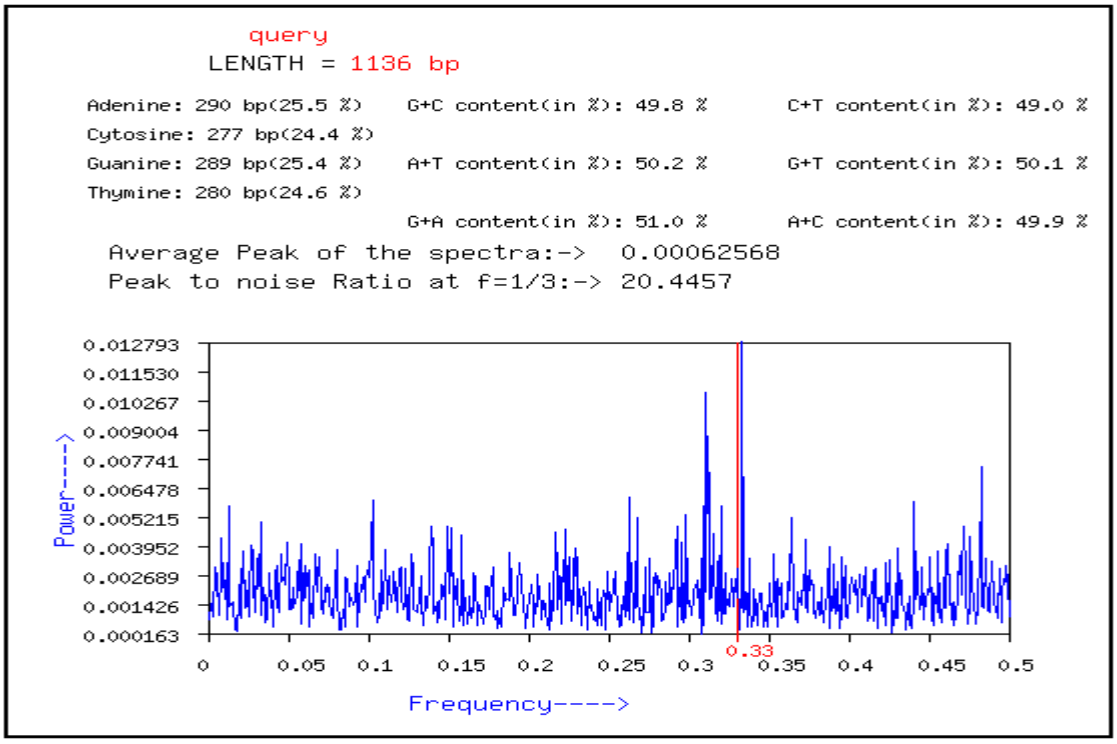


Figure-24 Graphical representation of the ATCG content in PARK2 gene

PARK5



Park7

Figure-26 Graphical representation of the ATCG content in PARK5 gene

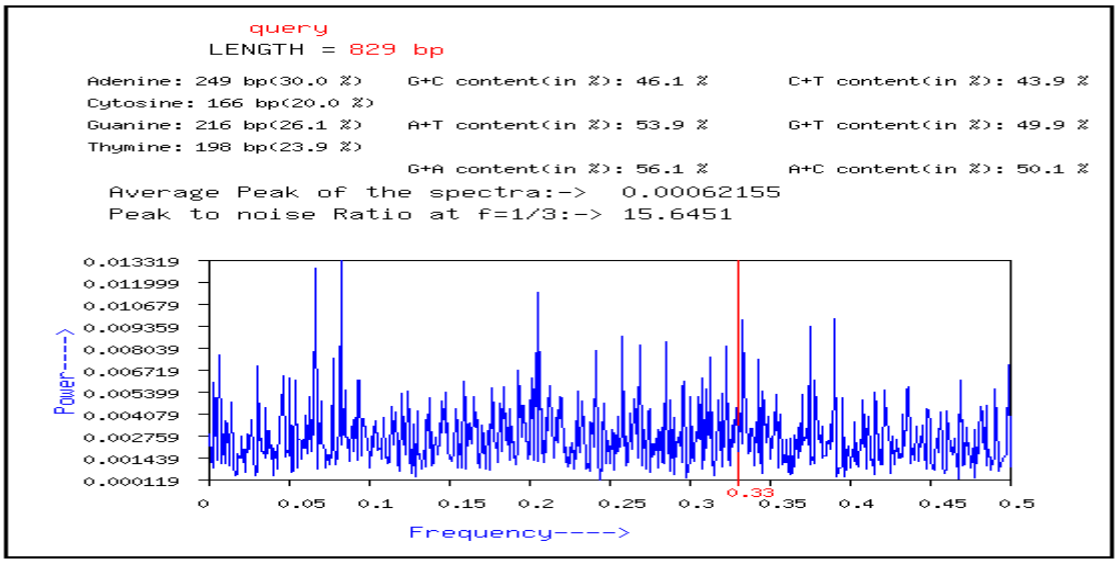


Figure-27Graphical representation of the ATCG content in PARK7 gene

4.16**Designing of siRNA of indivisual gene by using GenScripts database software tool siRNA target finder**

Synuclein

Found variants:NM_005460

Query summary:

- Sequence Length: 726
- Specified Region: 1 -- 726
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AAGTATTTGACCGTACTCAAA	66	33.33	24.91	1.03/-30.00	NA	617/360	0	☑
2.	AAGCAACGGCAGTCGAGGTAA	25	52.38	22.49	3.60/-38.20	NA	53/360	0	☑
3.	AACTGTGCCGAAGATGTGATA	192	42.86	14.96	3.16/-34.90	NA	146/360	0	☑
4.	AAAGCAACGGCAGTCGAGGTA	24	52.38	2.55	1.11/-38.20	NA	65/360	0	☑

siRNA insert 1: 70 bp.

BamH I Hind III
GGATCCCGTATTTGACCGTACTCAAATTCAAGAGATTTGAGTACGGTCAAATACTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

siRNA insert 2: 70 bp.

BamH I Hind III
GGATCCCGCAACGGCAGTCGAGGTAATTCAAGAGATTACCTCGACTGCCGTTGCTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

siRNA insert 3: 71 bp.

BamH I Hind III
GGATCCCGCTGTGCCGAAGATGTGATATTCAAGAGATATCACATCTTCGGCACAGTTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 4: 70 bp.

BamH I Hind III
GGATCCCAGCAACGGCAGTCGAGGTATTCAAGAGATACCTCGACTGCCGTTGCTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

PARK2

Found variants:NM_001080379;NM_001080378;NM_152410

Query summary:

- Sequence Length: 630
- Specified Region: 1 -- 630
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AATCGCCTGGAAGGTAGAAAT	519	42.86	0.60	8.33/-34.70	NA	683/360	0	☑
2.	AAATCGCCTGGAAGGTAGAAA	518	42.86	-0.12	4.42/-34.50	NA	286/360	0	☑

siRNA insert 1: 71 bp.

BamH I Hind III
GGATCCCGTCGCCTGGAAGGTAGAAATTTCAAGAGAATTTCTACCTTCCAGGCGATTTTTTCCAAAAGCTT
^| Sense | Loop | Antisense | Termination Signal

siRNA insert 2: 70 bp.

BamH I Hind III
GGATCCCATCGCCTGGAAGGTAGAAATTCAAGAGATTTCTACCTTCCAGGCGATTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

PARK5

Found variants:NM_006247

Query summary:

- Sequence Length: 522
- Specified Region: 1 -- 522
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AAGACTCAGGCCAATGACTAC	102	47.62	17.23	2.17/-36.80	NA	541/360	0	☑

siRNA insert 1: 70 bp.

BamH I Hind III
GGATCCCGACTCAGGCCAATGACTACTTCAAGAGAGTAGTCATTGGCCTGAGTCTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

PARK7

Found variants:NM_007262;NM_001123377

Query summary:

- Sequence Length: 608
- Specified Region: 1 -- 608
- GC% Range: 30% -- 60%
- Organism: human

siRNA candidate targets after Homology filtering:

Build Insert for Selected siRNA | Select All | Clear All

No.	Sequence	Start	GC%	Scores	ΔE/Thermodynamic	SNPs	Off-target	Pos-Motifs	Select
1.	AAGACGGCCTGATTCTTACAA	530	42.86	21.91	4.33/-34.60	NA	186/360	0	☑
2.	AAAGACGGCCTGATTCTTACA	529	42.86	10.35	2.75/-34.60	NA	115/360	0	☑

siRNA insert 1: 70 bp.

BamH I Hind III
GGATCCCGACGGCCTGATTCTTACAATTCAAGAGATTGTAAGAATCAGGCCGTCTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

siRNA insert 2: 70 bp.

BamH I Hind III
GGATCCCAGACGGCCTGATTCTTACATTCAAGAGATGTAAGAATCAGGCCGTCTTTTTTCCAAAAGCTT
| Sense | Loop | Antisense | Termination Signal

5-Conclusion

Phylogenetic analysis of the Alzheimer and Parkinson's genes and proteins helpful to find evolutionary relationship of the diseases as the genes involved in Alzheimer&Parkinson's normal work in human homolges like **in Pan *troglodytes*, Macaca*mulatta*,Bos*Taurus*, Canis*lupus*, Mus*musculus*&Rattus*norvegicus*.** These all are mammals having all the **APOE,APP,PSEN1 & PSEN2** genes working normal, but found defective in both Alzheimer neurological disorder, same as in Parkinson's the genes **SYNUCLEIN,PARK2, PARK5,PARK7** found defective and in homolog's these working normally. So what are the reasons of this phenomenon that the genes which evolved during evolution in human and their homolog's works in different manner. All these reasons can be found only by phylogenetic analysis of these genes and proteins. We designed siRNA target or each individual gene which would be helpful to find out various therapeutic agent,new drug discoveries, to treat inherit genetic neurological disorders etc.,RNA interference (RNAi) has been revolutionary to biology and biology predicted therapeutics.

6-DISCUSSION & FUTURE PERSPECTIVE

RNA interference (RNAi) is a natural cellular process that regulates gene expression and provides an innate defense mechanism against invading viruses and transposable elements (Kim *et al*., 2007). The finding that dsRNA initiates RNAi was among the most consequential recent contributions to cell biology (Fire *et al*., 1998), and since the revelation that RNAi can be mediated by 21 nucleotide (Ntd) duplexes (Elbashir*et al*., 2001), researchers have worked to harness their potential for addressing biological questions and treating human disease. Some reagents, such as minute interfering RNAs (siRNAs), are applied directly to cells, tissues and organisms; others are engineered to be expressed in cells, such as hairpin structures that provide siRNAs when processed. The rudimental premise underlying the broad utility of RNAi is that, in theory, we can design siRNAs (or vectors encoding them) to target virtually any gene of interest. RNAi technologies utilize a cell's natural machinery to move exogenously applied siRNAs to the felicitous cellular compartment, where they encounter the correct mRNA target and induce its degradation.

RNAi for neurological disorders-The blood–brain barrier limits access to the central nervous system (CNS) and thus the most practical manner to silence targets in neural cells is through direct injection of the RNAi trigger. As siRNAs have a short half-life, redosing utilizing indwelling catheters would be required for chronic diseases. The short half-life of siRNAs may be desirable. Vectors expressing therapeutic RNAi improved disease phenotypes for many months in preclinical studies in rodent models of poly glutamine reiterate diseases, amyotrophic lateral sclerosis (Ralph *et al.*, 2005) Parkinson's disease and Alzheimer's disease.

We have designed uniquesiRNA target using GenScript which is useful to target those genes mRNA which is responsible for Alzheimer& Parkinson. It will also help full in discovery of new therapeutic agents, drug designing, Vaccination and treatment of various inherit genetic disorders

However, RNA is inherently unstable, potentially immunogenic, and typically requires a distribution conveyance for efficient convey to the targeted cells. These issues have obstructed the clinicalprogress of some RNA-predicated drugs and have contributed to commixed results in clinical testing. Nevertheless, promising results from recent clinical tribulations suggest that these barriers may be overcome with ameliorated synthetic distribution carriers and chemical modifications of the RNA therapeutics. This work fixates on the clinical results of siRNA, RNA aptamer, and ribozyme therapeutics and the prospects for future successes (Bunnet*et al*., 2011).

Discovery of the RNA interference (RNAi) pathway has been revolutionaryto biology and biologically predicated therapeutics.. The whole process commences with the design and validation ofshort-interference RNA (siRNA) utilizing bioinformatics and cell culturesystems. siRNA that has high specificity and potency (knockdownefficiency) then could be applied to

animal models of human diseases formore evaluation, with the ultimate hope of advancing to clinical trials.Numerous chemical modifications of siRNA are currently being investigatedin an effort to enhance their stability, to elongate their stay in the body, and,perhaps, to eschew immune stimulation. Different distribution methods aredeveloped depending on the diseases, tissues, or organs being targeted. Inshort, this relatively incipient biotechnology is advancing expeditiously as we learnfrom its failures and successes.

Application and future prospective of this work-

- Phylogenetic analysis of the Alzheimer and Parkinson's genes and proteins helpful to find evolutionary relationship of the diseases as the genes involved in Alzheimer & Parkinson's normal work in human homolges like **in Pan Troglodytes,Macacamulatta,Bos Taurus, Canis lupus,MusMusculus&Rattusnorvegicus.**These all are mammals having all the**APOE,APP, PSEN1 & PSEN2** genes working normal, but found defective in both Alzheimer neurological disorder, same as in Parkinson's the genes **SYNUCLEIN,PARK2, PARK5,PARK7**found defective and in homolog's these working normally. So what are the reasons of this phenomenon that the genes which evolved during evolution in human and their homolog's works in different manner. All these reasons can be found only by phylogenetic analysis of these genes and proteins.

- We designed siRNA target or each individual gene which would be helpful to find out various therapeutic agent,new drug discoveries, to treat inherit genetic neurological disorders *etc*., RNA interference(RNAi) has been revolutionary to biology and biology predicted therapeutics.

- The herald RNA (mRNA)targets once believed to be ''undruggable'' by conventional methods arenow being ebulliently pursued with RNAi-predicated therapeutics.Fundamental research into the RNAi pathway and biotechnologicalresearch on the application of RNAi may one day make an incipient class of drugsan authenticity by targeting the mRNA of disease-causing or disease-promotinggenes directly.

- Recent advances of biological drugs have broadened the scope of therapeutic targets for a variety of human diseases. This holds true for dozens of RNA-predicated therapeutics currently under clinical investigation for diseases ranging from genetic disorders to HIV infection to sundry cancers. These emerging drugs, which include therapeutic ribozymes, aptamers, and diminutive interfering RNAs (siRNAs), demonstrate the unprecedented multifariousness of RNA.

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