Strategies of Disease Causing Gene

Mutation in *PVRL4* gene encoding nectin-4 underlies ectodermal-dysplasia-syndactyly syndrome (EDSS1)

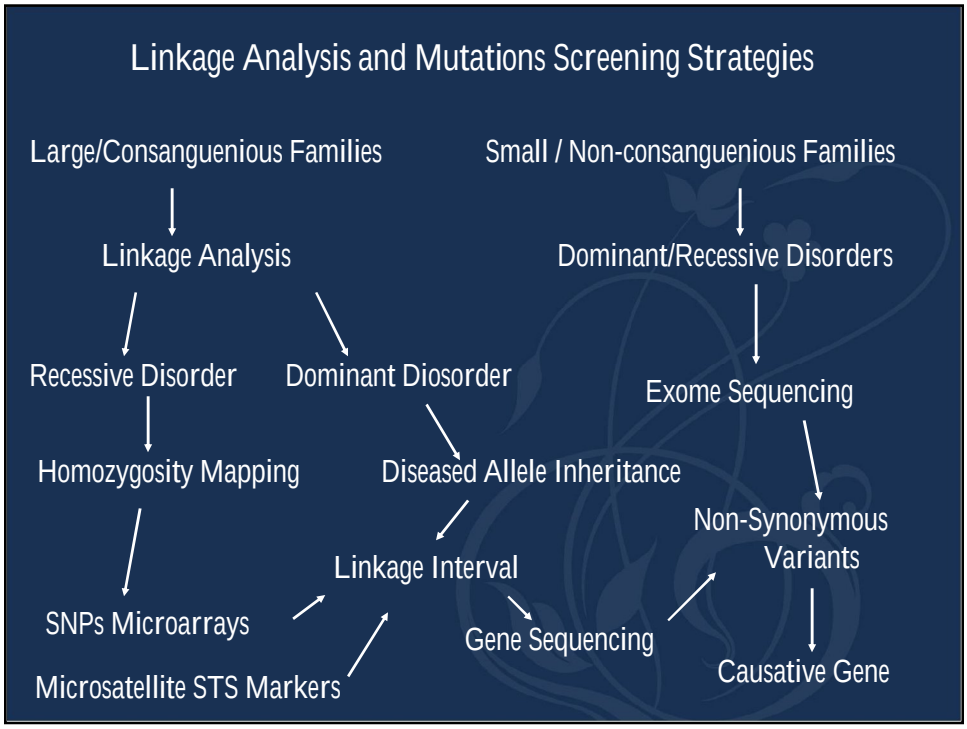
MUSHARRAF JELANI, PhD

Department of Biochemistry
Institute of Basic Medical Sciences
Khyber Medical University
Peshawar

Ectodermal Dysplasia (ED)

- Hair
- Teeth
- Nails
- Sweat Glands

- More than 200 Ectodermal Dysplasias
- 62 Genes
  - Cell-cell signaling
  - Cell-cell adhesion
  - Transcription regulation
  - Development
  - Others



EDSS1 Phenotype

- Ectodermal-dysplasia-syndactyly syndrome (EDSS1, MIM 613573) is a rare form of ED affecting skin and its appendages mainly hair, teeth and nails.
- All these individuals showed features of hypotrichosis, hypoplastic nails, tooth enamel hypoplasia, hyperhidrosis, palmoplantar keratoderma and bilateral partial cutaneous syndactyly.

EDSS1 Genotyping and Mutation Screening

- The present study described a large consanguineous Pakistani family with EDSS1 phenotypes, originated from a remote area of Punjab province Pakistan.
- By homozygosity mapping we assigned the disease locus on chromosome 1q23.1-q23.3. DNA sequence analysis detected a homozygous missense mutation in PVRL4 gene.

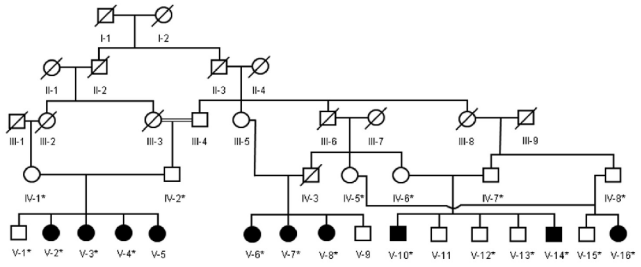
Meterials and Methods

- Study Approval
  - Institutional Review Board (IRB), Quaid-i-Azam University Islamabad, Pakistan
- Research Volenteers
  - Inform consent from all participants
  - Blood samples of 5-6 ml (EDTA tubes)
- Genomic DNA Extraction
  - Commercially available kits
  - Organic Method (Phenol-Chloroform)
  - Nanodrop-1000 spectrophotometer DNA quantification
- Polymerase Chain Reaction
  - Standard temperature conditions for STS markers and exonic DNA amplification
- Gel Electrophesis
  - Agarose 2%
  - Polyacrylamide 8%

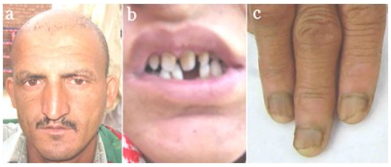
Meterials and Methods

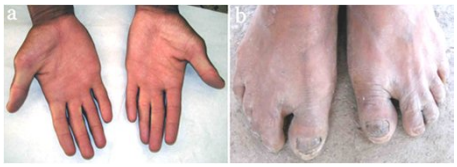
- Genotyping Data Analysis
  - PEDSTATS
  - Allegro 2
  - Superlink Online Software
- DNA Sequencing Analysis
  - Chain Termination Reaction or DNA Cycle Sequencing (ABI Prism 310 Genetic Analyzer)
- Standard Gene Sequences
  - NCBI
  - Ensembl
  - UCSC Genome Browser Santa Cruz
- Sequencing Alignment
  - Bioedit
  - ClustalW

EDSS 1 Pedigree Analysis



EDSS 1 Clinical Features





Exclusion of known genes

No	Phenotype	Gene	Chromosome	Markers	cM*	Mb+
1	Tricho-thio-dystrophy	*ERCC3*	2q21	D2S1328	136.80	125.90
				D2S2339	136.80	126.29
				D2S2271	139.06	127.81
				D2S2215	140.87	129.70
				D2S1260	143.00	131.30
2	Odonto-onycho-dermal dysplasia	*WNT10A*	2q35	D2S2248	218.87	217.64
				D2S1338	220.78	218.58
				D2S2244	222.96	219.94
				D2S163	225.03	220.50
				D2S126	226.94	221.72
3	Lacrimo-auriculo-dento-digital syndrome	*FGFR3*	4p16.3	D4S111	0.97	0.98
				D4S3038	0.97	01.08
				D4S115	0.97	01.22
				D4S1614	02.61	02.86
				D4S126	03.60	03.02
4	Lacrimo-auriculo-dento-digital syndrome	*FGF10*	5p13-p12	D5S430	65.30	41.40
				D5S665	65.87	42.35
				D5S1396	66.41	44.33
				D5S2106	66.75	45.23
				D5S1388	66.95	51.62

Exclusion of known genes

No	Phenotype	Gene	Chromosome	Markers	cM*	bp+
5	Tricho-thio-dystrophy	*GTF2H5*	6q25.3	D6S947	165.52	154.44
				D6S442	167.93	155.79
				D6S363	171.11	158.46
				D6S969	171.11	159.20
				D6S1581	174.00	160.19
6	Ectodermal dysplasia-cutaneous syndactyly	None	7p21.1-p14.3	D7S488	31.04	18.35
				D7S2562	35.75	21.45
				D7S2190	39.92	24.43
				D7S1808	42.92	28.00
				D7S2491	48.65	30.78
				D7S817	50.85	32.10
7	Tricho-rhino-phalangeal syndrome	*TRPS1*	8q24.12	D8S565	119.68	116.46
				D8S1694	121.77	118.41
				D8S384	121.77	118.52
				D8S199	123.12	120.34
8	Lacrimo-auriculo-dento-digital syndrome	*FGFR2*	10q26	D10S542	141.57	120.74
				D10S1722	143.88	122.13
				D10S1483	145.95	123.27
				D10S587	149.12	125.17
				D10S2322	151.09	126.14

Exclusion of known genes

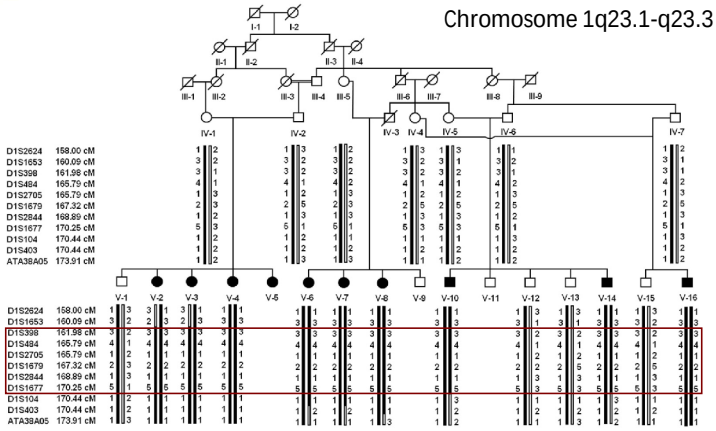
9	Ectodermal dysplasia cleft/lip palate syndrome	*PVRL1*	11q23-q24	D11S4104	127.01	118.14
				D11S924	128.16	118.94
				D11S994	128.79	119.45
				D11S925	130.70	120.33
10	Pachyonychia congenita 1 and 2, Ectodermal dysplasia with pure hair-nail type	*KRT81, KRT83, KRT85, KRT86*	12q13	D12S270	69.16	50.99
				D12S1604	70.68	52.01
				D12S103	71.09	52.72
				D12S1724	72.13	53.15
				D12S1632	74.18	54.70
11	T-cell immunodeficiency, congenital alopecia and nail dystrophy	*WHN*	17q11-q12	D17S841	55.69	24.56
				D17S1294	56.07	25.40
				D17S1800	56.74	26.96
				D17S798	58.69	28.31
				D171850	61.03	29.16
12	Trichodentoosseous syndrome	*DLX3*	17q21.3-q22	D17S943	76.92	45.19
				D17S747	79.29	46.02
				D17S1865	81.29	47.99
				D17S752	82.62	50.05
13	Tricho-thio-dystrophy	*ERCC2*	19q13.2-q13.3	D19S190	64.03	44.64
				D19S554	65.80	45.30
				D19S223	66.09	46.08
				D19S537	69.10	48.72

Genome-Wide Linkage Analysis

- A total of 534 short tandem repeat polymorphic microsatellite markers, with an
- average heterozygosity of 0.75 from Linkage Mapping Set (Invitrogen, San Diego, CA, USA), were genotyped in the present genome scans
- These markers are spaced approximately 6-7 cM apart on 22 autosomes, and X and Y chromosomes.

Linkage of Family K

Exclusion mapping followed by Human Genome Scan
Chromosome 1q23.1-q23.3



Statistics of Linkage Analysis

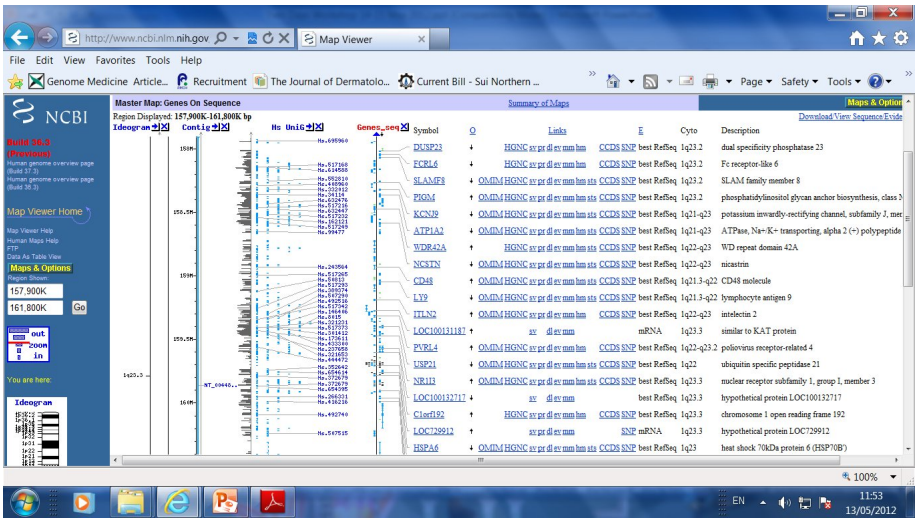
- PEDSTATS was used to evaluate the genotyped data for Mendelian incompatibilities and to assess the data for occurrence of unlikely genotypes.
- SIMWALK2 was used to construct the haplotypes.
- Parametric linkage analysis was carried out using the online version of superlink software (http://bioinfo.cs.technion.ac.il/superlink-online/) and
- MLINK program of FASTLINK computer package.

Statistical Analysis of Family K

Table 5.1: Two-point LOD score between EDCS syndrome and microsatellite markers on chromosome 1q23.1-q23.3

Marker	Distance		Two-Point LOD Score at Recombination θ =						
Name	Physical (bp) [a]	Genetic (cM) [b]	0.00	0.01	0.05	0.10	0.20	0.30	0.40
D1S2624	154,897,915	158.00	-∞	0.00	1.09	1.26	0.92	0.39	0.01
D1S1653	156,199,398	160.09	-∞	1.92	2.42	2.29	1.56	0.74	0.15
D1S398	157,905,344	161.98	4.91	4.76	4.18	3.44	2.03	0.84	0.10
D1S484	159,033,934	165.79	5.05	4.91	4.34	3.62	2.22	0.98	0.16
D1S2705	159,124,442	165.79	4.92	4.77	4.19	3.45	2.03	0.79	0.04
D1S1679	160,628,539	167.32	4.86	4.72	4.14	3.42	2.02	0.79	0.04
D1S2844	161,215,397	168.89	4.86	4.72	4.15	3.43	2.06	0.87	0.13
D1S1677	161,826,325	170.25	-∞	2.81	2.94	2.54	1.48	0.53	-0.01
D1S104	161,903,530	170.44	1.28	1.38	1.48	1.40	1.03	0.57	0.16
D1S403	161,993,805	170.44	-∞	-0.28	0.63	0.96	0.88	0.51	0.15
ATA38A05	164,115,368	173.91	-∞	-1.03	0.20	0.54	0.47	0.17	-0.04

[a]Physical and [b]Genetic distances are according to the second-generation combined linkage physical map of the human genome (Matise *et al.*, 2007).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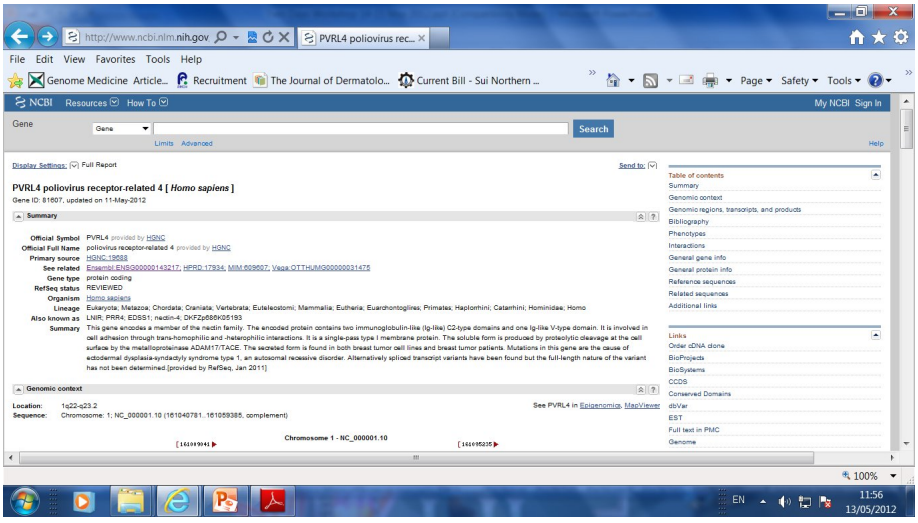
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PVRL4 poliovirus receptor-related 4 [Homo sapiens]
Gene ID: 81607, updated on 11-May-2012
Summary
Official Symbol PVRL4
Official Full Name poliovirus receptor-related 4
Primary source HGNC:19688
Gene type protein coding
RefSeq status REVIEWED
Organism Homo sapiens
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as LNIR; PRR4; EDS81; nectin-4; DKFZp686K05193
Summary This gene encodes a member of the nectin family. The encoded protein contains two immunoglobulin-like (Ig-like) C2-type domains and one Ig-like V-type domain. It is involved in cell adhesion through trans-homophilic and -heterophilic interactions. It is a single-pass type I membrane protein. The soluble form is produced by proteolytic cleavage at the cell surface by the metalloproteinase ADAM17/TACE. The secreted form is found in both breast tumor cell lines and breast tumor patients. Mutations in this gene are the cause of ectodermal dysplasia-syndactyly syndrome type 1, an autosomal recessive disorder. Alternatively spliced transcript variants have been found but the full-length nature of the variant has not been determined.
Genomic context
Location: 1q22-q23.2
Sequence: Chromosome 1: NC_000001.10 (161040781..161059385, complement)
Chromosome 1 - NC_000001.10
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Genomic regions, transcripts, and products
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General protein info
Reference sequences
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CCDS
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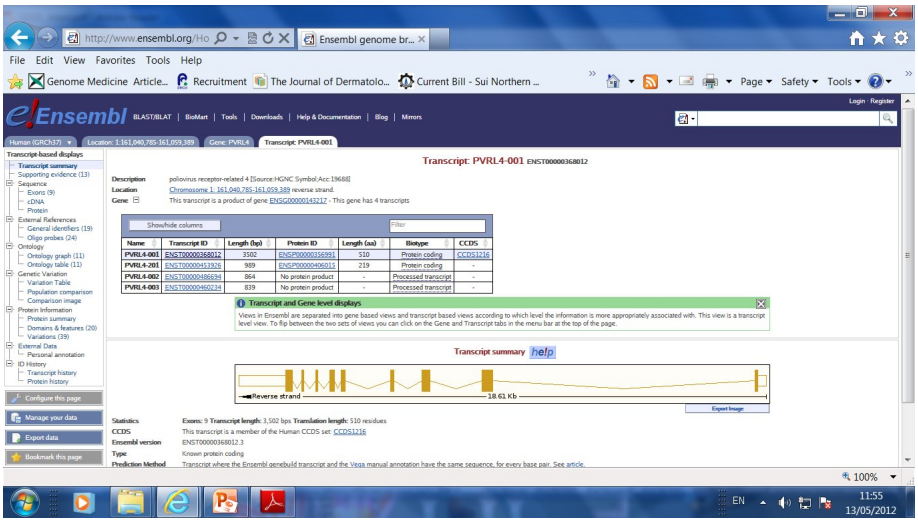
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Transcript and Gene level displays
Transcript summary help
Reverse strand
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Prediction Method Transcript where the Ensembl genebuild transcript and the Vega manual annotation have the same sequence, for every base pair. See article.
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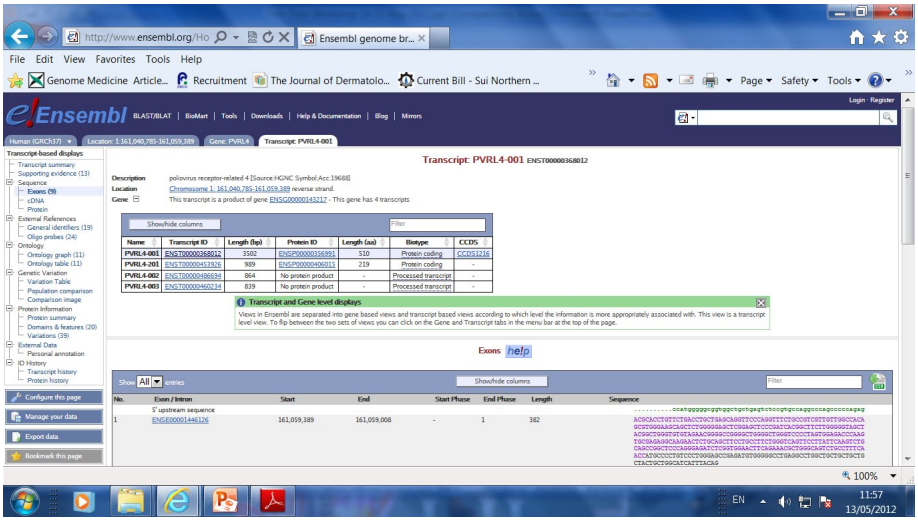
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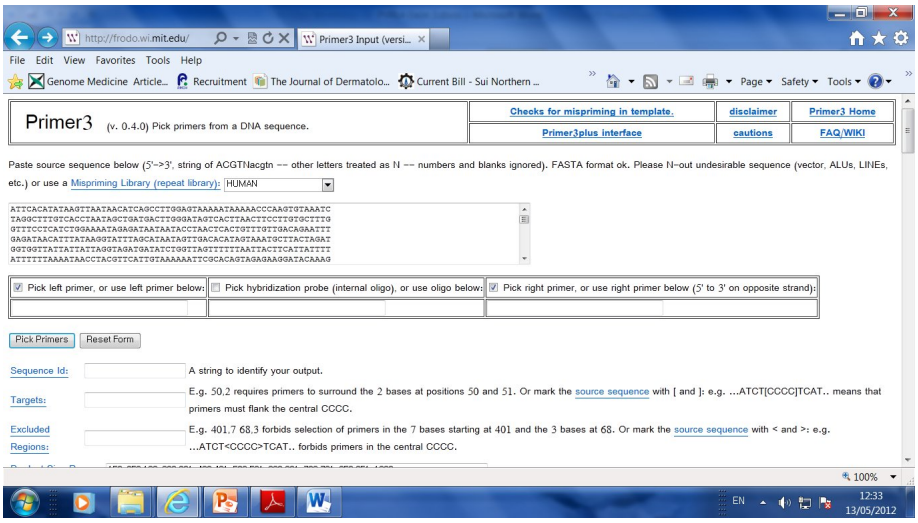
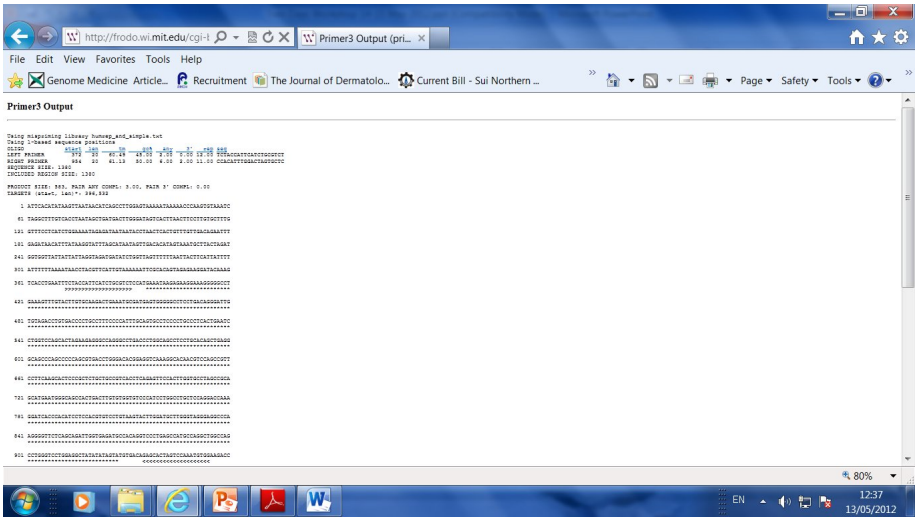
Exon 3 PVRL4 Sequence





Primers of Exon 3 PVRL4

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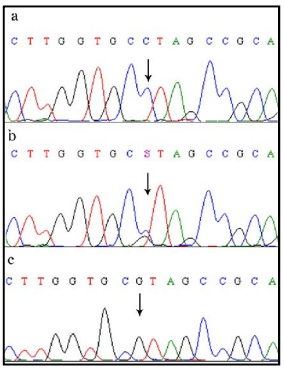
Forward Primer 5'TCTACCATTCATCTGCGTCT 3'

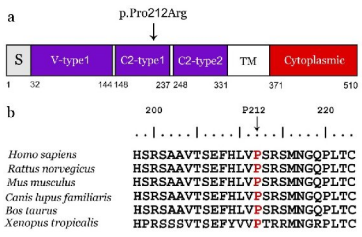
Reverse Primer 5'CCACATTTGGACTAGTGCTC3'

Product Size 583 bp

PVRL4 Mutation in Family K



c.635C>G, p.Pro212Arg



THANK YOU