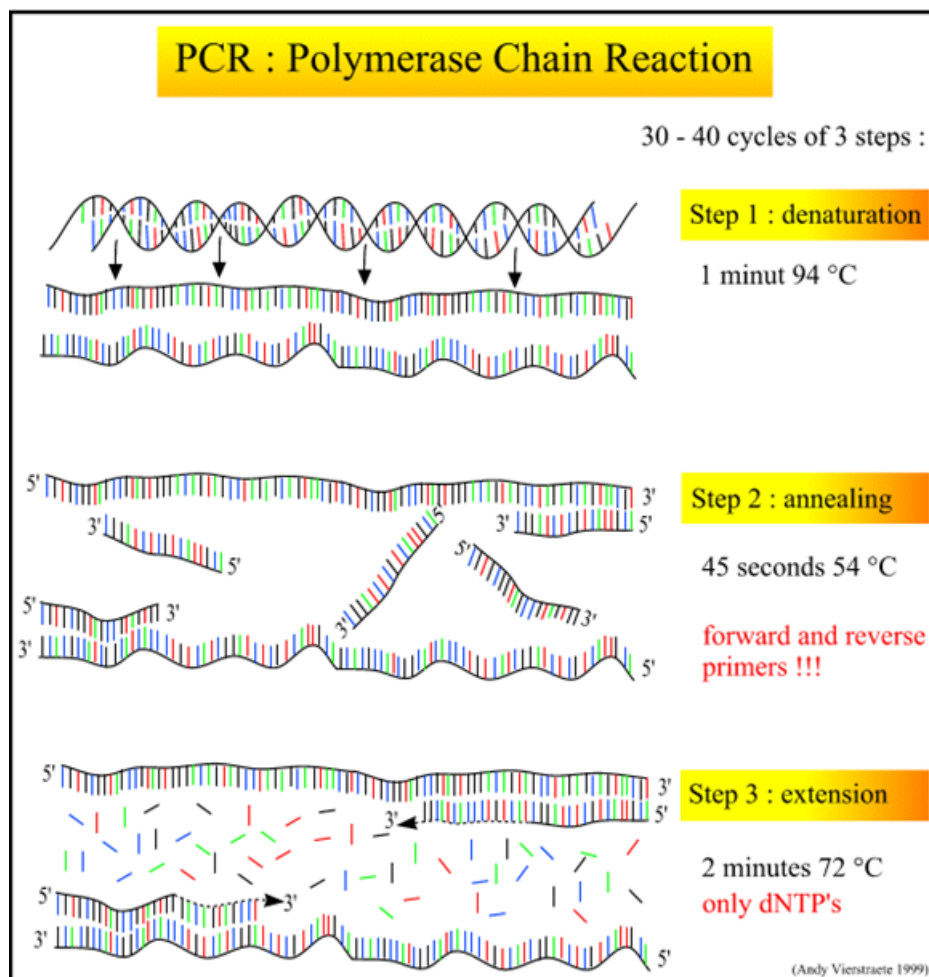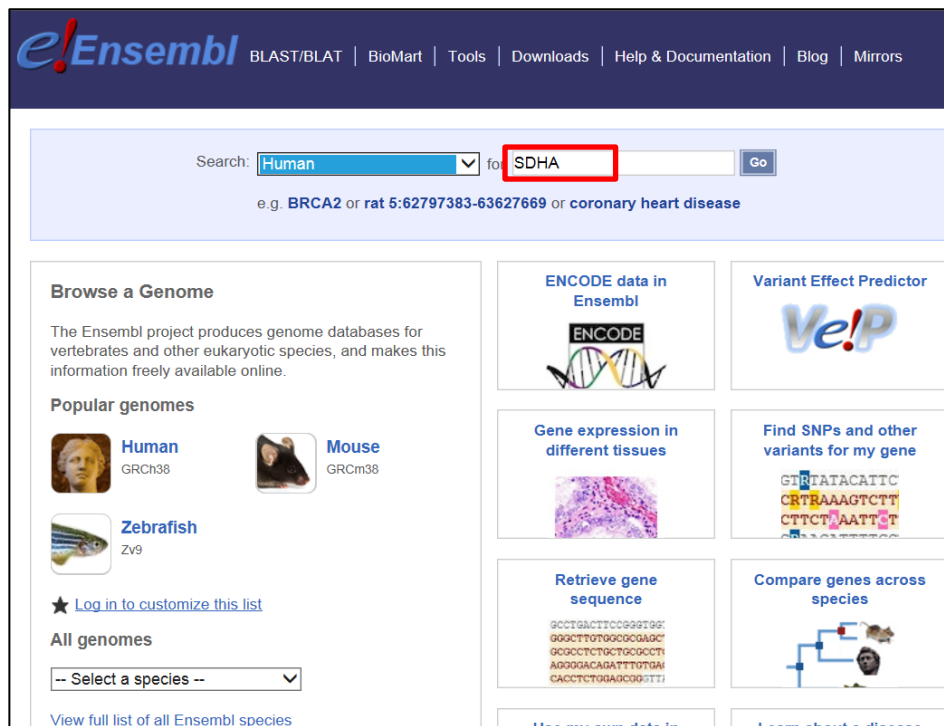# Designing PCR Primers

A primer is a short synthetic oligonucleotide which is designed to have a reverse sequence that is complement to a region of interest in a target DNA

Some thoughts on designing primers:

1. Primers should be 17.28 bases in length
2. Base composition should be 50.60% (G+C)
3. Primers should end (3') in a G or C, or CG or GC: this prevents "breathing" of ends and increases efficiency of priming
4. Tms between 55.80^{o}C are preferred
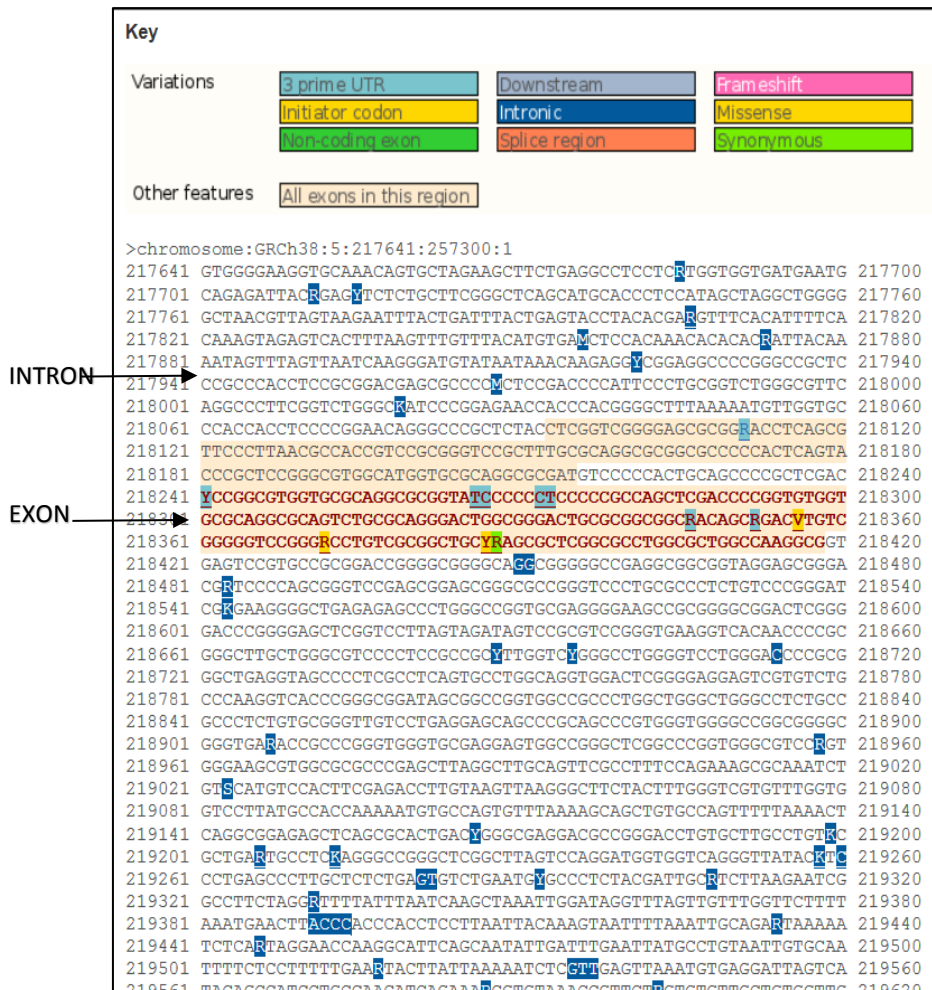
Primer design steps:

Figure 1. Go to ensemble data base and select (human) and type the gene name *SDHA* in this example.

Figure 2. Selects (sequence) from the list on the left then select (configure this page) to show variations.

Figure 3.The entire sequence (Exons and introns) of the whole gene will appear, copy the gene sequence and paste in new word document to make the designing process much easier.

```
>chromosome:GRCh38:5:217641:257300:1
217641 GTGGGGAAGGTGCAAACAGTGCTAGAAGCTTCTGAGGCCTCCTCRTGGTGGTGATGAATG 217700
217701 CAGAGATTACRGAGYTCTCTGCTTCGGGCTCAGCATGCACCCTCCATAGCTAGGCTGGGG 217760
217761 GCTAACGTTAGTAAGAATTTACTGATTTACTGAGTACCTACACGARGTTTCACATTTTCA 217820
217821 CAAAGTAGAGTCACTTTAAGTTTGTTTACATGTGAMCTCCACAAACACACACRATTACAA 217880
217881 AATAGTTTAGTTAATCAAGGGATGTATAATAAACAAGAGGYCGGAGGCCCCGGGCCGCTC 217940
217941 CCGCCCACCTCCGCGGACGAGCGCCCMCTCCGACCCCATTCCCTGCGGTCTGGGCGTTC 218000
218001 AGGCCCTTCGGTCTGGGCKATCCCGGAGAACCACCCACGGGGCTTTAAAAATGTTGGTGC 218060
218061 CCACCACCTCCCCGGAACAGGGCCCGCTCTACCTCGGTCGGGGAGCGCGGRACCTCAGCG 218120
218121 TTCCCTTAACGCCACCGTCCGCGGGTCCGCTTTGCGCAGGCGCGGCGCCCCCACT CAGTA 218180  EX1
218181 CCCGCTCCGGGCGTGGCATGGTGCGCAGGCGCGATGTCCCCCACTGCAGCCCCGCTCGAC 218240
218241 YCCGGCGTGGTGCGCAGGCGCGGTATCCCCCCTCCCCCGCCAGCTCGACCCCGGTGTGGT 218300
218301 GCGCAGGCGCAGTCTGCGCAGGGACTGGCGGGACTGCGCGGCGGCRACAGCRGACVTGTC 218360
218361 GGGGGTCCGGGRCCTGTCGCGGCTGCYRAGCGCTCGGCGCCTGGCGCTGGCCAAGGCGGT 218420
218421 GAGTCCGTGCCGCGGACCGGGCGGGGCAGGCGGGGGCCGAGGCGGCGGTAGGAGCGGGA 218480
218481 CGRTCCCCAGCGGGTCCGAGCGGAGCGGGCGCCGGGTCCCTGCGCCCTCTGTCCC GGAT 218540
218541 CGKGAAGGGGCTGAGAGAGCCCTGGGCCGGTGCGAGGGGAAGCCGCGGGGCGGACTCGGG 218600
218601 GACCCGGGGAGCTCGGTCCTTAGTAGATAGTCCGCGTCCGGGTGAAGGTCACAACCCCGC 218660
218661 GGGCTTGCTGGGCGTCCCCTCCGCCGCYTTGGTCYGGGCCTGGGGTCCTGGGACCCCGCG 218720
218721 GGCTGAGGTAGCCCCTCGCCTCAGTGCCTGGCAGGTGGACTCGGGGAGGAGTCGTGTCTG 218780
218781 CCCAAGGTCACCCGGCGGATAGCGGCCGGTGGCCGCCCTGGCTGGGCTGGCCTCTGCC 218840
218841 GCCCTCTGTGCGGGTTGTCCTGAGGAGCAGCCCGCAGCCCGTGGGTGGGGCCGGCGGGGC 218900
```

Figure 4. Select the sequence of the exon and a wide range of the sequence both before and after the exon or select the entire sequence (**Exon** 1 will be presented as an example) ,place square brackets [   ] both before and after the beginning and end of the first fragment by 60 -100bp (whatever is between the square brackets is untouchable). Select the fragment and about 300-400bp before and after the brackets (the more area you select before and after the fragment, the more options you provide for the primer selection). Sometimes we have to deal with a relatively big exon, In this case the exon will be divided in to two fragments (1a &1b).

>chromosome:GRCh38:5:217641:257300:1
217641 GTGGGGAAGGTGCAAACAGTGCTAGAAGCTTCTGAGGCCTCCTCRTGGTGGTGATGAATG 217700
217701 CAGAGATTACRGAGYTCTCTGCTTCGGGCTCAGCATGCACCCTCCATAGCTAGGCTGGGG 217760
217761 GCTAACGTTAGTAAGAATTTACTGATTTACTGAGTACCTACACGARGTTTCACATTTTCA 217820
217821 CAAAGTAGAGTCACTTTAAGTTTGTTTACATGTGAMCTCCACAAACACACACRATTACAA 217880
217881 AATAGTTTAGTTAATCAAGGGATGTATAATAAACAAGAGGYCGGAGGCCCCGGGCCGCTC 217940
217941 CCGCCCACCTCCGCGGACGAGCGCCCMCTCCGACCCCATTCCCTGCGGTCTGGGCGTTC 218000
218001 AGGCCCTTCGGTCTGGGCKATCCCGGAGAACCACCCACGGGGCTTTAAAAATGTTGGTGC 218060
218061 CCACCACCTCCCCGGAACAGGGCCCGCTCTACCTCGGTCGGGGAGCGCGGRACCTCAGCG 218120
218121 TTCCCTTAACGCCACCGTCCGCGGGTCCGCTTTGCGCAGGCGCGGCGCCCCCACTICAGTA 218180
218181 CCCGCTCCGGGCGTGGCATGGTGCGCAGGCGCGATGTCCCCCACTGCAGCCCCGCTCGAC 218240
218241 YCCGGCGTGGTGCGCAGGCGCGGTATCCCCCCTCCCCCGCCAGCTCGACCCCGGTGTGGT 218300
218301 GCGCAGGCGCAGTCTGCGCAGGGACTGGCGGGACTGCGCGGCGGCRACAGCRGACVTGTC 218360
218361 GGGGGTCCGGGRCCTGTCGCGGCTGCYRAGCGCTCGGCGCCTGGCGCTGGCCAAGGCGGT 218420
218421 GAGTCCGTGCCGCGGACCGGGGCGGGGCAGGCGGGGCCGAGGCGGCGGTAGGAGCGGGA 218480
218481 CGRTCCCCAGCGGGTCCGAGCGGAGCGGGCGCCGGGTCCCTGCGCCCTCTGTCCCIGGGAT 218540
218541 CGKGAAGGGGCTGAGAGAGCCCTGGGCCGGTGCGAGGGGAAGCCGCGGGGCGGACTCGGG 218600
218601 GACCCGGGGAGCTCGGTCCTTAGTAGATAGTCCGCGTCCGGGTGAAGGTCACAACCCCGC 218660
218661 GGGCTTGCTGGGCGTCCCCTCCGCCGCYTTGGTCYGGGCCTGGGGTCCTGGGACCCCGCG 218720
218721 GGCTGAGGTAGCCCCTCGCCTCAGTGCCTGGCAGGTGGACTCGGGGAGGAGTCGTGTCTG 218780
218781 CCCAAGGTCACCCGGGCGGATAGCGGCCGGTGGCCGCCCTGGCTGGGCTGGGCCTCTGCC 218840

Primer3web version 4.0.0 - Pick primers from a DNA sequence.

disclaimer   code
cautions

Select the Task for primer selection [generic   ▼]

Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library) [NONE   ▼]

```
218181 CCCGCTCCGGGCGTGGCATGGTGCGCAGGCGCGATGTCCCCCACTGCAGCCCCGCTCGAC 218240
218241 YCCGGCGTGGTGCGCAGGCGCGGTATCCCCCCTCCCCCGCCAGCTCGACCCCGGTGTGGT 218300
218301 GCGCAGGCGCAGTCTGCGCAGGGACTGGCGGGACTGCGCGGCGGCRACAGCRGACVTGTC 218360
218361 GGGGGTCCGGGRCCTGTCGCGGCTGCYRAGCGCTCGGCGCCTGGCGCTGGCCAAGGCGGT 218420
218421 GAGTCCGTGCCGCGGACCGGGGCGGGGCAGGCGGGGCCGAGGCGGCGGTAGGAGCGGGA 218480
218481 CGRTCCCCAGCGGGTCCGAGCGGAGCGGGCGCCGGGTCCCTGCGCCCTCTGTCCC]GGGAT 218540
218541 CGKGAAGGGGCTGAGAGAGCCCTGGGCCGGTGCGAGGGGAAGCCGCGGGGCGGACTCGGG 218600
```

☑ Pick left primer, or use left primer below	☐ Pick hybridization probe (internal oligo), or use oligo below	☑ Pick right primer, or use right primer below (5' to 3' on opposite strand)

[Pick Primers] [Download Settings] [Reset Form]

Figure 5. Go to primer 3 data base, and paste in the blank empty window and select pick primers. (At this point, in computer language, you are ordering primer 3 to select primers for the selected fragment anywhere out the square brackets).

Primer3 Output

No mispriming library specified
Using 1-based sequence positions
WARNING: Unrecognized base in input sequence

```
OLIGO            start  len     tm     gc%   any th  3' th hairpin seq
LEFT PRIMER        195   18  59.81  66.67    0.00    0.00    0.00 GAACAGGGCCCGCTCTAC
RIGHT PRIMER       748   20  58.68  60.00    3.51    0.00    0.00 CTACTAAGGACCGAGCTCCC
SEQUENCE SIZE: 782
INCLUDED REGION SIZE: 782

PRODUCT SIZE: 554, PAIR ANY_TH COMPL: 4.74, PAIR 3'_TH COMPL: 0.00
TARGETS (start len

     1 AATAGTTTAGTTAATCAAGGGATGTATAATAAACAAGAGGYCGGAGGCCCCGGGCCGCTC

    61 CCGCCCACCTCCGCGGACGAGCGCCCMCTCCGACCCCATTCCCTGCGGTCTGGGCGTTC

   121 AGGCCCTTCGGTCTGGGCKATCCCGGAGAACCACCCACGGGGCTTTAAAAATGTTGGTGC

   181 CCACCACCTCCCCGGAACAGGGCCCGCTCTACCTCGGTCGGGGAGCGCGGRACCTCAGCG
                       >>>>>>>>>>>>>>>>>>

   241 TTCCCTTAACGCCACCGTCCGCGGGTCCGCTTTGCGCAGGCGCGGCGCCCCCACTCAGTA
                                                                *****

   301 EXCCCGCTCCGGGCGTGGCATGGTGCGCAGGCGCGATGTCCCCCACTGCAGCCCCGCTCG
        *************************************************************

   361 ACYCCGGCGTGGTGCGCAGGCGCGGTATCCCCCCTCCCCCGCCAGCTCGACCCCGGTGTG
        *************************************************************

   421 GTGCGCAGGCGCAGTCTGCGCAGGGACTGGCGGGACTGCGCGGCGGCRACAGCRGACVTG
        *************************************************************

   481 TCGGGGGTCCGGGRCCTGTCGCGGCTGCYRAGCGCTCGGCGCCTGGCGCTGGCCAAGGCG
        *************************************************************

   541 GTGAGTCCGTGCCGCGGACCGGGGCGGGGCAGGCGGGGGCCGAGGCGGCGGTAGGAGCGG
        *************************************************************

   601 GACGRTCCCCAGCGGGTCCGAGCGGAGCGGGCGCCGGGTCCCTGCGCCCTCTGTCCCGGG
        *************************************************************

   661 ATCGKGAAGGGGCTGAGAGAGCCCTGGGCCGGTGCGAGGGGAAGCCGCGGGGCGGACTCG

   721 GGGACCCGGGGAGCTCGGTCCTTAGTAGATAGTCCGCGTCCGGGTGAAGGTCACAACCCC
               <<<<<<<<<<<<<<<<<<<<
```

Figure 6. Primer3 will provide you with many choices. Select the pair that has a similar melting temperature, the product size should not go beyond 700bp (the temperature variation shod not exceed 2 degrees).

```
>chromosome:GRCh38:5:217641:257300:1
217641 GTGGGGAAGGTGCAAACAGTGCTAGAAGCTTCTGAGGCCTCCTCRTGGTGGTGATGAATG 217700
217701 CAGAGATTACRGAGYTCTCTGCTTCGGGCTCAGCATGCACCCTCCATAGCTAGGCTGGGG 217760
217761 GCTAACGTTAGTAAGAATTTACTGATTTACTGAGTACCTACACGARGTTTCACATTTTCA 217820
217821 CAAAGTAGAGTCACTTTAAGTTTGTTTACATGTGAMCTCCACAAACACACACRATTACAA 217880
217881 AATAGTTTAGTTAATCAAGGGATGTATAATAAACAAGAGGYCGGAGGCCCCGGGCCGCTC 217940
217941 CCGCCCACCTCCGCGGACGAGCGCCCMCTCCGACCCCATTCCCTGCGGTCTGGGCGTTC 218000
218001 AGGCCCTTCGGTCTGGGCKATCCCGGAGAACCACCCACGGGGCTTTAAAAATGTTGGTGC 218060
218061 CCACCACCTCCCCGGAACAGGGCCCGCTCTACCTCGGTCGGGGAGCGCGGRACCTCAGCG 218120
218121 TTCCCTTAACGCCACCGTCCGCGGGTCCGCTTTGCGCAGGCGCGGCGCCCCCACT[CAGTA 218180 EX1
218181 CCCGCTCCGGGCGTGGCATGGTGCGCAGGCGCGATGTCCCCCACTGCAGCCCCGCTCGAC 218240
218241 YCCGGCGTGGTGCGCAGGCGCGGTATCCCCCCTCCCCCGCCAGCTCGACCCCGGTGTGGT 218300
218301 GCGCAGGCGCAGTCTGCGCAGGGACTGGCGGGACTGCGCGGCGGCRACAGCRGACVTGTC 218360
218361 GGGGGTCCGGGRCCTGTCGCGGCTGCYRAGCGCTCGGCGCCTGGCGCTGGCCAAGGCGGT 218420
218421 GAGTCCGTGCCGCGGACCGGGGCGGGGCAGGCGGGGGCCGAGGCGGCGGTAGGAGCGGGA 218480
218481 CGRTCCCCAGCGGGTCCGAGCGGAGCGGGCGCCGGGTCCCTGCGCCCTCTGTCCC]GGGAT 218540
218541 CGKGAAGGGGCTGAGAGAGCCCTGGGCCGGTGCGAGGGGAAGCCGCGGGGCGGACTCGGG 218600
218601 GACCCGGGGAGCTCGGTCCTTAGTAGATAGTCCGCGTCCGGGTGAAGGTCACAACCCCGC 218660
218661 GGGCTTGCTGGGCGTCCCCTCCGCCGCYTTGGTCYGGGCCTGGGGTCCTGGGACCCCGCG 218720
218721 GGCTGAGGTAGCCCCTCGCCTCAGTGCCTGGCAGGTGGACTCGGGGAGGAGTCGTGTCTG 218780
218781 CCCAAGGTCACCCGGGCGGATAGCGGCCGGTGGCCGCCCTGGCTGGGCTGGGCCTCTGCC 218840
218841 GCCCTCTGTGCGGGTTGTCCTGAGGAGCAGCCCGCAGCCCGTGGGTGGGGCCGGCGGGGC 218900
218901 GGGTGARACCGCCCGGGTGGGTGCGAGGAGTGGCCGGGCTCGGCCCGGTGGGCGTCCRGT 218960
218961 GGGAAGCGTGGCGCGCCCGAGCTTAGGCTTGCAGTTCGCCTTTCCAGAAAGCGCAAATCT 219020
```

Figure 7. Finally, highlight both forward and reveres primers to make sure that they cover the whole gene.

H.W

Order	Mapped genes	OMIM ID	OMIM Title	Gene Map Disorder	Expression in the nervous system and muscles
1	SDHA*(5p15)*	#252011	Succinate Dehydrogenase Complex, Subunit A	-Mitochondrial respiratory chain complex II -Leigh syndrome -Cardiomyopathy, dilated -Paragangliomas 5	↑
2	PDCD6*(5p15.33)*	*601057	Programmed Cell Death 6	-Neurodegenerative disorders -Apoptosis -Cancer -Autoimmune disease -Congenital malformations -Immunodeficiency	↑
3	AHRR*(5p15.3)*	*606517	Aryl-Hydrocarbon Receptor Repressor	-Ahh inducibility -Cancer lung -Liver diseases -Tumors -Cancer	↑
4	EXOC3*(5p15.33)*	*608186	Exocyst Complex Component	NA	↑
5	SLC9A3*(5p15.3)*	*182307	Solute Carrier Family 9, Subfamily A (NHE3, Cation Proton Antiporter 3), Member 3	-Metabolic acidosis -Acidosis -Hypokalemia -Adenoma -Inflammatory bowel diseases -Diabetic nephropathies -Renal failure -Proteinuria -Colon cancer -Cystic fibrosis	↑
6	TPPP*(5p15.3)*	*608773	Tubulin Polymerization Promoting Protein	-Lewy body dementia -Multiple system atrophy	↑
7	TRIP13*(5p15.33)*	*604507	Thyroid Hormone Receptor Interactor 13	-Lung cancer	↑
8	NKD2*(5p15.3)*	*607852	Naked Cuticle Homolog 2 (Drosophila)	-Hyperopia	↑
9	SLC12A7*(5p15)*	*604879	Solute Carrier Family 12 (Potassium/Chloride Transporter), Member 7	-Renal tubular acidosis	↑
10	SLC6A19*(5p15.33)*	*608893	Solute Carrier Family 6 (Neutral Amino Acid Transporter), Member 19	-Hartnup disorder (HND) -Hyperglycinuria (HG) -Iminoglycinuria (IG) , digenic -Aminoaciduria	↑
11	SLC6A18*(5p15.33)*	*610300	Solute Carrier Family 6 (Neutral Amino Acid Transporter), Member 18	-Iminoglycinuria -Hyperglycinuria -Iminoglycinuria, digenic	↑

Order	Mapped genes	OMIM ID	OMIM Title	Gene Map Disorder	Expression in the nervous system and muscles
12	TERT*(5p15.33)*	+187270	Telomerase Reverse Transcriptase	-Aplastic anemia (AA) -Dyskeratosis congenita, autosomal dominant, 2 (DKCA2) -Pulmonary fibrosis, and/or bone marrow failure, telomere-related, 1 (PFBMFT1) -Dyskeratosis congenita, autosomal recessive, 4 (DKCB4) -Pulmonary fibrosis, idiopathic (IPF) -Melanoma, cutaneous malignant 9 (CMM9) -Laryngeal squamous cell carcinoma -Benign meningioma -Cancer, Tumors	↑
13	CLPTM1L*(5p15.33)*	*612585	CLPTM1-Like Protein	-Cleft lip -Lung cancer	↑
14	SLC6A3*(5p15.3)*	*126455	Solute Carrier Family 6 (Neurotransmitter Transporter), Member 3	Parkinsonism-dystonia infantile (PKDYS)	↑
15	LPCAT1*(5p15.33)*	*610472	Lysophosphatidylcholine Acyltransferase 1	NA	↑
16	MRPL36*(5p15.3)*	*611842	Mitochondrial Ribosomal Protein L36	NA	↑
17	NDUFS6*(5p15.33)*	*603848	NADH Dehydrogenase (Ubiquinone) Fe-S Protein 6, 13kDa (NADH-Coenzyme Q Reductase)	-Mitochondrial complex I deficiency -Leigh disease	↑
18	IRX4*(5p15.3)*	*606199	Iroquois Homeobox 4	NA	↑
19	IRX2*(5p15.33)*	*606198	Iroquois Homeobox 2	-Soft tissue sarcoma -Squamous cell carcinoma of the head and neck	↑
20	C5orf38*(5p15.33)*	*610522	Chromosome 5 Open Reading Frame 38 (Coordinated Expression To IRXA2 Protein)	-Hypertension	↑
21	IRX1*(5p15.3)*	*606197	Iroquois Homeobox 1	NA	↑