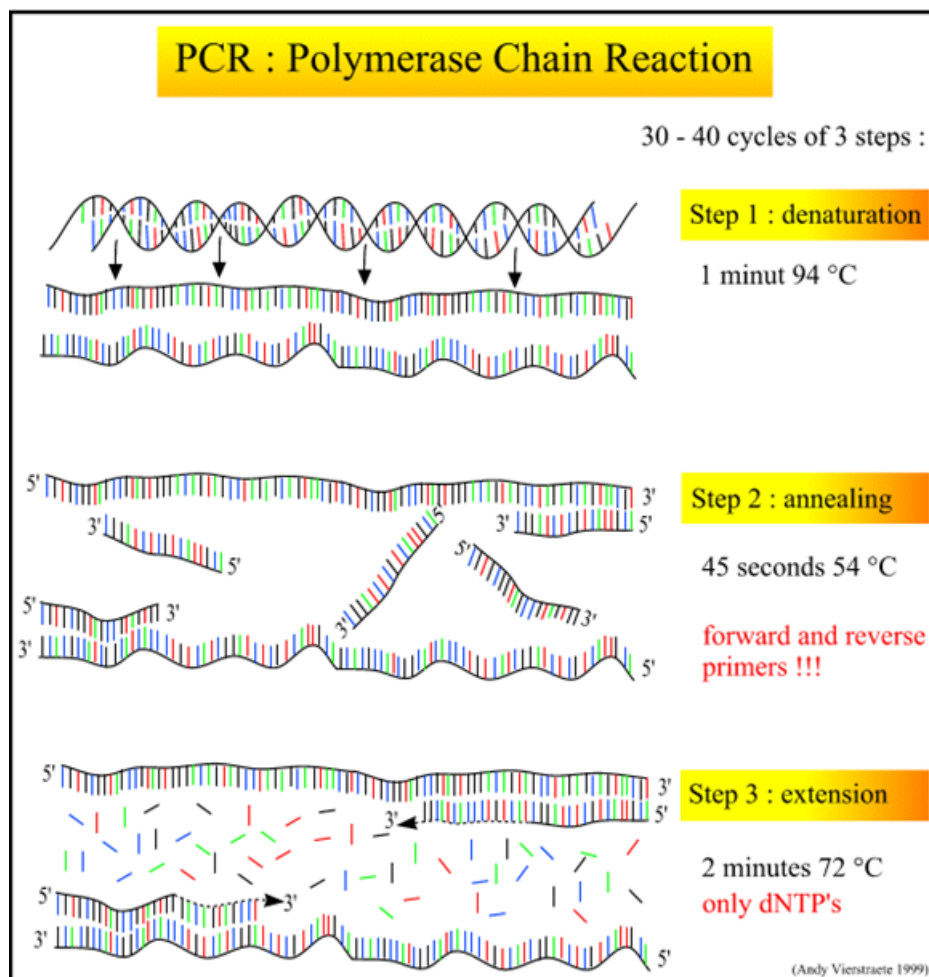


## Designing PCR Primers

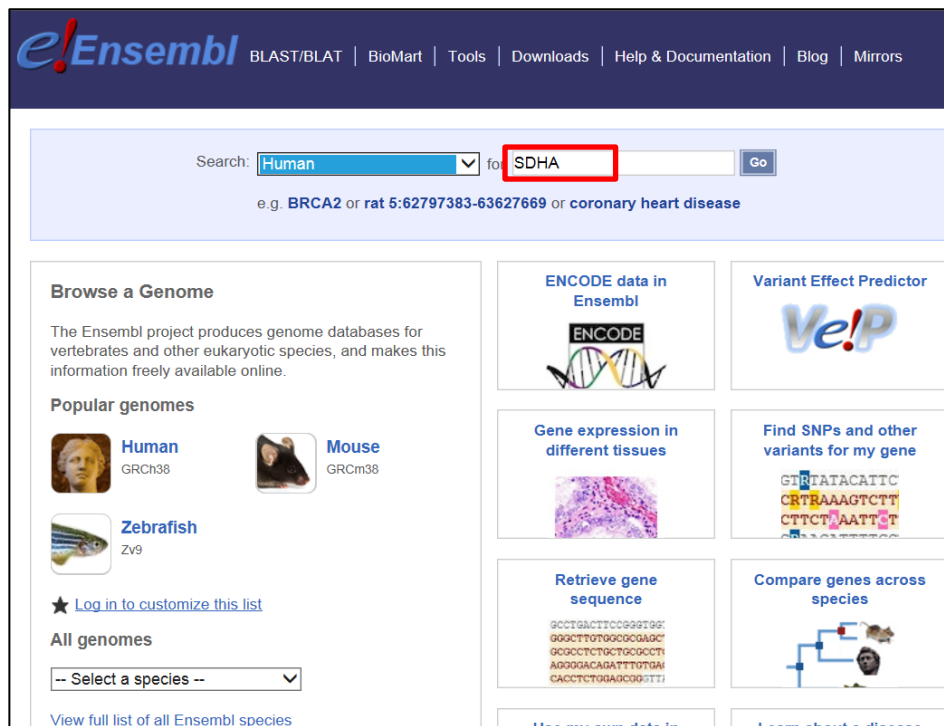
**A primer** is a short synthetic oligonucleotide which is designed to have a reverse sequence that is complement to a region of interest in a target DNA



**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°C are preferred

## Primer design steps:

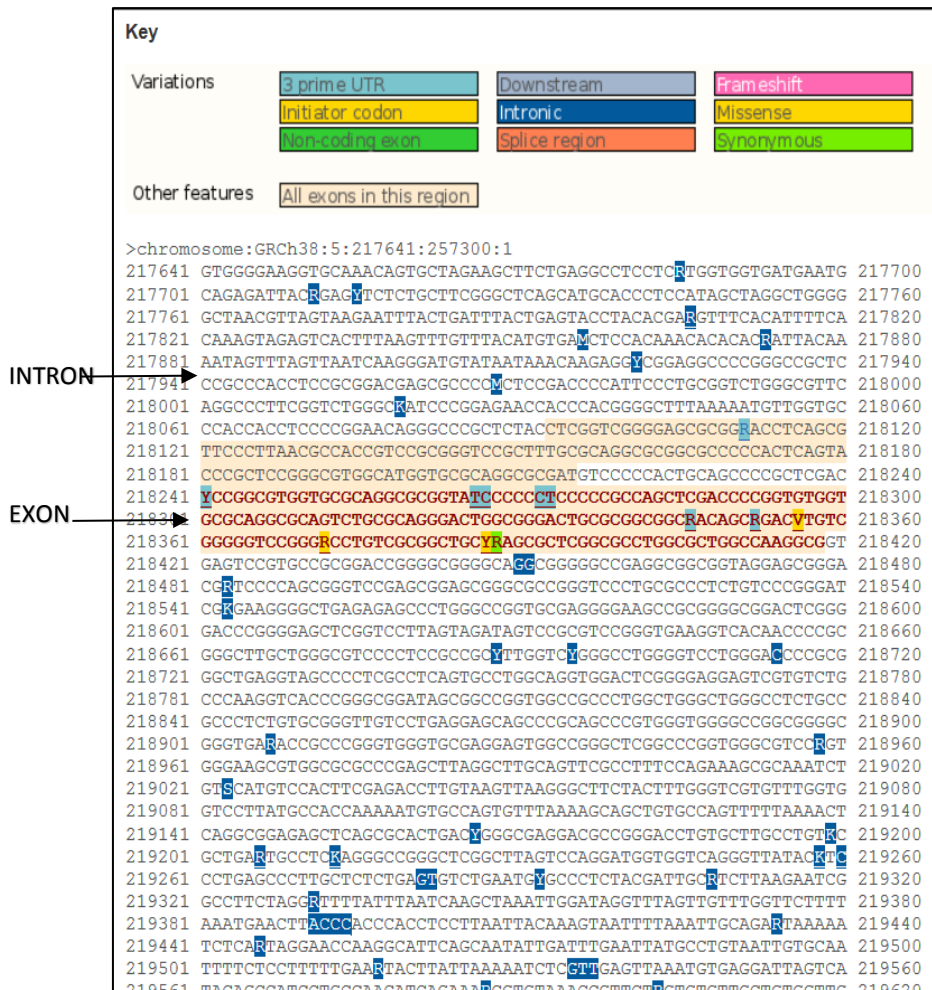


The screenshot shows the Ensembl genome browser search interface. At the top, the Ensembl logo is followed by navigation links: BLAST/BLAT, BioMart, Tools, Downloads, Help & Documentation, Blog, and Mirrors. Below this is a search bar with a dropdown menu set to 'Human' and a text input field containing 'SDHA'. A 'Go' button is to the right of the text field. Below the search bar, there is a hint: 'e.g. BRCA2 or rat 5:62797383-63627669 or coronary heart disease'. The main content area is divided into several sections: 'Browse a Genome' with a description and 'Popular genomes' (Human, Mouse, Zebrafish); 'ENCODE data in Ensembl'; 'Variant Effect Predictor (VeP)'; 'Gene expression in different tissues'; 'Find SNPs and other variants for my gene'; 'Retrieve gene sequence'; and 'Compare genes across species'.

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

The screenshot shows the Ensembl genome browser interface for the SDHA gene. The top navigation bar includes the Ensembl logo, links for BLAST/BLAT, BioMart, Tools, Downloads, Help & Documentation, Blog, and Mirrors. A search bar is present on the right. The main header indicates the species is Human (GRCh38), the location is 5,218,241-256,700, and the gene is SDHA. The left-hand menu is titled 'Gene-based displays' and lists various options, with 'Sequence' highlighted in a red box. Below the menu are buttons for 'Configure this page' (also highlighted in a red box), 'Add your data', and 'Export data'. The main content area displays the gene's description, synonyms (FP, SDH2, SDHF), location (Chromosome 5: 218,241-256,700 forward strand), INSDC coordinates, and transcripts. A 'Marked-up sequence' section is visible, with buttons for 'Download sequence' and 'BLAST this sequence'. A 'Key' section shows a feature for 'All exons in this region'. The DNA sequence is displayed below, with exons highlighted in yellow.

**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 GTGGGGAAGGTGCAAACAGTGCTAGAAAGCTTCTGAGGCCCTCCTCCTGGTGGTGATGAATG 217700
217701 CAGAGATTACRAGAYTCTCTGCTTCGGGCTCAGCATGCACCCTCCATAGCTAGGCTGGGG 217760
217761 GCTAACGTTAGTAAGAATTTACTGATTTACTGAGTACCTACACGARGTTTCACATTTTCA 217820
217821 CAAAGTAGAGTCACTTTAAGTTTGTTTACATGTGAMCTCCACAAACACACACRATTACAA 217880
217881 AATAGTTTAGTTAATCAAGGGATGTATAATAAACAAGAGGYCGGAGGCCCGGGCCGCTC 217940
217941 CCGCCACCTCCGCGGACGAGCGCCCTCCGACCCATTCCCTGCGGTCTGGGCGTTC 218000
218001 AGGCCCTTCGGTCTGGGCKATCCCGGAGAACCACCACGGGGCTTTAAAAATGTTGGTGC 218060
218061 CCACCACCTCCCGGAACAGGGCCCGCTCTACCTCGGTCGGGGAGCGCGGRACCTCAGCG 218120
218121 TTCCTTAACGCCACCCTCCGCGGGTCCGCTTTGCGCAGGCGCGGCCCCCACTTCAGTA 218180 EX1
218181 CCCGCTCCGGGCGTGGCATGGTGCAGGCGCGATGTCCCCACTGCAGCCCCGCTCGAC 218240
218241 YCCGGCGTGGTGCAGGCGCGGTATCCCCCTCCCGCCAGCTCGACCCCGGTGTGGT 218300
218301 GCGCAGGCGCAGTCTGCGCAGGGACTGGCGGGACTGCGCGGCGGCRACAGCRGACVTGTC 218360
218361 GGGGGTCCGGGRCTGTGCGGCTGCYRAGCGCTCGGCGCTGGCGCTGGCCAAGGCGT 218420
218421 GAGTCCGTGCCGCGGACCGGGGCGGGGCAAGCGGGGCGGAGGCGGCGGTAGGAGCGGGA 218480
218481 CGRTCCCCAGCGGGTCCGAGCGGAGCGGGCGCCGGGTCCCTGCGCCCTCTGTCCCGGAT 218540
218541 CGRGAAGGGGCTGAGAGAGCCCTGGGCCGTGCGAGGGGAAGCCGCGGGGCGGACTCGGG 218600
218601 GACCCGGGGAGCTCGGTCCCTTAGTAGATAGTCCGCGTCCGGGTGAAGGTCACAACCCCGC 218660
218661 GGGCTTGCTGGGCGTCCCCTCCGCGCYTTGGTCTYGGGCCTGGGGTCTGGGACCCCGCG 218720
218721 GGCTGAGGTAGCCCCTCGCCTCAGTGCTTGGCAGGTGGACTCGGGGAGGAGTCGTGTCTG 218780
218781 CCCAAGGTCACCCGGGCGGATAGCGGCGGTGGCCGCGCTGGCTGGGCTGGGCCTCTGCC 218840
218841 GCCCTCTGTGCGGGTTGTCCTGAGGAGCAGCCCGCAGCCCGTGGGTGGGGCCGGCGGGG 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 GTGGGGAAGGTGCAAAACAGTGCTAGAAGCTTCTGAGGCCTCCTCRTGGTGGTGATGAATG 217700
217701 CAGAGATTACRGAGYTCTCTGCTTCGGGCTCAGCATGCACCTCCATAGCTAGGCTGGG 217760
217761 GCTAACGTTAGTAAGAATTTACTGATTTACTGAGTACCTACACGARRGTTTCACATTTCA 217820
217821 CAAAGTAGAGTCACTTTAAGTTTGTTCATGTGAMCTCCACAAACACACACRATTACAA 217880
217881 AATAGTTTAGTTAATCAAGGGATGTATAATAAACAAGAGGYCGGAGGCCCGGGCCGCTC 217940
217941 CGGCCACCTCCGCGGACGAGGGCCCMCTCCGACCCATTCCCTGCGGTCTGGGGTTC 218000
218001 AGGCCCTTCGGTCTGGGCKATCCCGGAGAACCACCACGGGGCTTTAAAAATGTTGGTGC 218060
218061 CCACCACCTCCCGGAACAGGGCCCGCTCTACCTCGGTGGGGAGCGCGRACCTCAGCG 218120
218121 TTCCCTTAACGCCACCGTCCGCGGGTCCGCTTTGCGCAGGCGCGATGTCCCCCACTGCAGCCCGCTCGAC 218180 EX1
218181 CCGCTCCGGCGTGGCATGGTGCAGGCGCGATGTCCCCCACTGCAGCCCGCTCGAC 218240
218241 YCGGGCTGGTGGCGAGGCGGGTATCCCCCCTCCCCGCCAGCTCGACCCCGGTGTGGT 218300
218301 GCGCAGGCGCAGTCTGCGCAGGGACTGGCGGGACTGCGCGGGCGRACAGRGACYVTGTC 218360
218361 GGGGGTCCGGGRCCTGTGCGGGCTGCYRAGCGCTCGGCGCTGGCGCTGGCCAAGGCGGT 218420
218421 GAGTCCGTGCCGCGACCGGGGCGGGGCAGGCGGGGCCGAGGCGGGCGGTAGGAGCGGGA 218480
218481 CGRTCCCCAGCGGGTCCGAGCGGAGCGGGCGCGGGTCCCTGCGCCCTCTGTCCCGGGAT 218540
218541 CGKGAAGGGGCTGAGAGAGCCCTGGGCGGTGCGAGGGGAAGCCCGGGGCGGACTCGGG 218600
218601 GACCCGGGGAGCTCGGTCCTTAGTAGATAGTCCGCGTCCGGGTGAAGGTCACAACCCCGC 218660
218661 GGGCTTGCTGGGCGTCCCTCCGCGCTTTGGTCGGGCCTGGGGTCTGGGACCCCGCG 218720
218721 GGCTGAGGTAGCCCTCGCCTCAGTGCCTGGCAGGTGGACTCGGGGAGGAGTCTGTGTG 218780
218781 CCAAGGTCACCCGGCGGATAGCGCCGGTGGCGCCCTGGCTGGGCTGGGCCTCTGCC 218840

```

Primer3web

ersion 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

```

218241 YCCGGCGTGGTGGCGCAGGCGGGTATCCCCCTCCCCGCCAGCTCGACCCCGGTGGT 218300
218301 GCGCAGGCGCAGTCTGCGCAGGGACTGGCGGGACTGCGCGGGCGCRACAGCGACTGTC 218360
218361 GGGGGTCCGGRCCTGTGCGGGCTGCTYRAGCGCTCGGCGCTGGCGCTGGCCAGGGCGGT 218420
218421 GAGTCCGTGCCGCGACCGGGGCGGGGCGAGCGGGGCCGAGGCGGGCGGTAGGAGCGGGA 218480
218481 CGRTCCCCAGCGGGTCCGAGCGGAGCGGGGCGCGGGTCCCTGGGCGCTCTGTCCCGGAT 218540
218541 CGKGAAGGGGCTGAGAGAGCCCTGGGCGGTGCGAGGGGAAGCCCGGGGCGGACTCGGG 218600

```

<input checked="" type="checkbox"/> Pick left primer, or use left primer below	<input type="checkbox"/> Pick hybridization probe (internal oligo), or use oligo below	<input checked="" type="checkbox"/> 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

WARNING: Numbers in input sequence were deleted.

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	GAACAGGGCCCCGCTCTAC
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 AATAGTTTGTGTTAATCAAGGGATGTATAATAAACAAGAGGYCGGAGGCCCGGGCGGCTC
61 CCGCCACCTCCGCGGACGAGCGCCCMCTCCGACCCATTCCTGCGGTCTGGCGTTC
121 AGGCCCTTCGGTCTGGGCKATCCCGGAGAACCACCCACGGGGCTTTAAAAATGTTGGTGC
181 CCACCACCTCCCGGAACAGGGCCCGCTCTACCTCGGTCCGGGAGCGCGGRACCTCAGCG
   >>>>>>>>>>>>>>>>>>>>
241 TTCCCTTAACGCCACCGTCCGCGGTCCGCTTTGCGCAGGCGCGGCAGGCCCACTCAGTA
   *****
301 EXCCCGCTCCGGGCGTGGCATGTTGCGCAGGCGGATGTCCCCACTGCAGCCCCGCTCG
   *****
361 ACYCCGGCGTGGTGGCAGGCGGATATCCCGCTCCCGCCAGCTCGACCCCGGTGTG
   *****
421 GTGCGCAGGCGAGTCTGCGCAGGACTGGCGGACTGCGGCGGCACAGCRGACVTG
   *****
481 TCGGGGGTCCGGGRCTGTGCGCGCTGCYRAGCGCTCGGCGCCTGGCGTGGCCAAGGCG
   *****
541 GTGAGTCCGTGCCCGGACCAGGGCGGGCAGCGGGGCGAGGCGCGGTAGGAGCGG
   *****
601 GACGRTCCCGAGGGTCCGAGCGGAGCGGGGCCCGGGTCCCTGCGCCCTCTGTCCCGGG
   *****
661 ATCGKGAAGGGGCTGAGAGAGCCCTGGGCGGTGCGAGGGGAAGCCGCGGGCGGACTCG
721 GGGACCCGCGGAGCTCGGTTCCTTAGTAGATAGTCGCGTCCGCGTGAAGGTCACAACCCC
   <<<<<<<<<<<<<<<<<<<<
  
```

**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 should not exceed 2 degrees).



```

>chromosome:GRCh38:5:217641:257300:1
217641 GTGGGGAAGGTGCAAACAGTGCTAGAAAGCTTCTGAGGCCCTCCTC 217700
217701 CAGAGATTACRAGYTCCTGCTTCGGGCTCAGCATGCACCCTCCATAGCTAGGCTGGGG 217760
217761 GCTAACGTTAGTAAGAATTTACTGATTTACTGAGTACCTACACGAR 217820
217821 CAAAGTAGAGTCACTTTAAGTTTGTTCATGTGAMCTCCACAAACACACACRATTACAA 217880
217881 AATAGTTTAGTTAATCAAGGGATGTATAATAAAACAAGAGGYCGGAGGCCCGGGCCGCTC 217940
217941 CCGCCACCTCCGCGGACGAGCGCCCTCCTCCGACCCCATTCCTGCGGTCTGGGCGTTC 218000
218001 AGGCCCTTCGGTCTGGGCKATCCCGGAGAACCACCCACGGGGCTTTAAAAATGTTGGTGC 218060
218061 CCACCACCTCCCCGGAACAGGGCCCGCTCTACTCGGTTCGGGGAGCGCGGRACCTCAGCG 218120
218121 TTCCCTTAACGCCACCGTCCGCGGGTCCGCTTTGCGCAGGCGCGCGCCCCACT [ CAGTA 218180 EX1
218181 CCCGCTCCGGGCGTGGCATGGTGCAGGCGCGATGTCCCCACTGCAGCCCCGCTCGAC 218240
218241 YCCGGCGTGGTGCAGGCGCGGTATCCCCCTCCCCCGCCAGCTCGACCCCGGTGTGGT 218300
218301 GCGCAGGCGCAGTCTGCGCAGGACTGGCGGGACTGCGCGGCGGCRACAGCRGACVTGTC 218360
218361 GGGGGTCCGGRCCTGTGCGGGCTGCYRAGCGCTCGGCGCCTGGCGCTGGCCAAGGCGGT 218420
218421 GAGTCCGTGCCGCGGACCGGGCGGGGCA 218480
218481 CGR 218540
218541 CGKGAAGGGCTGAGAGAGCCCTGGGCCGTGCGAGGGGAAGCCCGGGGCGGACTCGGG 218600
218601 GACCCGEGGAGCTCGGTCCTTAGTAGATAGTCCGCGTCCGGGTGAAGGTCAACCCCGC 218660
218661 GGGCTTGCTGGGCGTCCCCTCCGCCGYTTGGTCYGGGCTGGGGTCTGGGACCCCGCG 218720
218721 GGCTGAGGTAGCCCCTCGCCTCAGTGCTGGCAGGTGGACTCGGGGAGGAGTCGTGTCTG 218780
218781 CCCAAGGTCACCCGGGCGGATAGCGGCCGGTGGCCGCCCTGGCTGGGCTGGGCCTCTGCC 218840
218841 GCCCTCTGTGCGGGTTGTCCTGAGGAGCAGCCCGAGCCCGTGGGTGGGGCCGGCGGGGC 218900
218901 GGGTGARACCGCCCGGGTGGGTGCGAGGAGTGGCCGGGCTCGGCCGGTGGGCGTCCRGT 218960
218961 GGAAGCGTGGCGCGCCGAGCTTAGGCTGTCAGTTGCGCTTCCAGAAAGCGCAAATCT 219020

```

**Figure 7.** Finally, highlight both forward and reverse 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( <i>5p15</i> )	#252011	Succinate Dehydrogenase Complex, Subunit A	-Mitochondrial respiratory chain complex II -Leigh syndrome -Cardiomyopathy, dilated -Parangliomas 5	↑
2	PDCD6( <i>5p15.33</i> )	*601057	Programmed Cell Death 6	-Neurodegenerative disorders -Apoptosis -Cancer -Autoimmune disease -Congenital malformations -Immunodeficiency	↑
3	AHRR( <i>5p15.3</i> )	*606517	Aryl-Hydrocarbon Receptor Repressor	-Ahh inducibility -Cancer lung -Liver diseases -Tumors -Cancer	↑
4	EXOC3( <i>5p15.33</i> )	*608186	Exocyst Complex Component	NA	↑
5	SLC9A3( <i>5p15.3</i> )	*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( <i>5p15.3</i> )	*608773	Tubulin Polymerization Promoting Protein	-Lewy body dementia -Multiple system atrophy	↑
7	TRIP13( <i>5p15.33</i> )	*604507	Thyroid Hormone Receptor Interactor 13	-Lung cancer	↑
8	NKD2( <i>5p15.3</i> )	*607852	Naked Cuticle Homolog 2 ( <i>Drosophila</i> )	-Hyperopia	↑
9	SLC12A7( <i>5p15</i> )	*604879	Solute Carrier Family 12 (Potassium/Chloride Transporter), Member 7	-Renal tubular acidosis	↑
10	SLC6A19( <i>5p15.33</i> )	*608893	Solute Carrier Family 6 (Neutral Amino Acid Transporter), Member 19	-Hartnup disorder (HND) -Hyperglycinuria (HG) -Iminoglycinuria (IG) , digenic -Aminoaciduria	↑
11	SLC6A18( <i>5p15.33</i> )	*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( <i>5p15.33</i> )	+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( <i>5p15.33</i> )	*612585	CLPTM1-Like Protein	-Cleft lip -Lung cancer	↑
14	SLC6A3( <i>5p15.3</i> )	*126455	Solute Carrier Family 6 (Neurotransmitter Transporter), Member 3	Parkinsonism-dystonia infantile (PKDYS)	↑
15	LPCAT1( <i>5p15.33</i> )	*610472	Lysophosphatidylcholine Acyltransferase 1	NA	↑
16	MRPL36( <i>5p15.3</i> )	*611842	Mitochondrial Ribosomal Protein L36	NA	↑
17	NDUFS6( <i>5p15.33</i> )	*603848	NADH Dehydrogenase (Ubiquinone) Fe-S Protein 6, 13kDa (NADH-Coenzyme Q Reductase)	-Mitochondrial complex I deficiency -Leigh disease	↑
18	IRX4( <i>5p15.3</i> )	*606199	Iroquois Homeobox 4	NA	↑
19	IRX2( <i>5p15.33</i> )	*606198	Iroquois Homeobox 2	-Soft tissue sarcoma -Squamous cell carcinoma of the head and neck	↑
20	C5orf38( <i>5p15.33</i> )	*610522	Chromosome 5 Open Reading Frame 38 (Coordinated Expression To IRXA2 Protein)	-Hypertension	↑
21	IRX1( <i>5p15.3</i> )	*606197	Iroquois Homeobox 1	NA	↑