NEAR EAST UNIVERSITY
DESAM RESEARCH INSTITUTE

Third Generation Sequencing: From Wet Lab to Bioinformatics

"Sequence Specific Primer Design"

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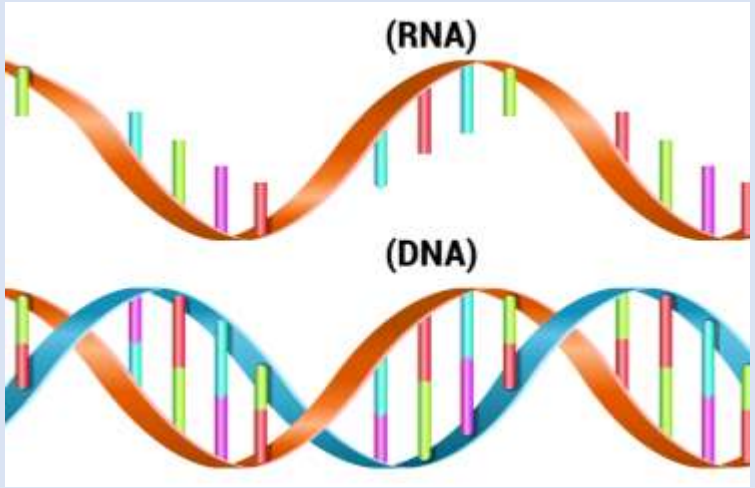
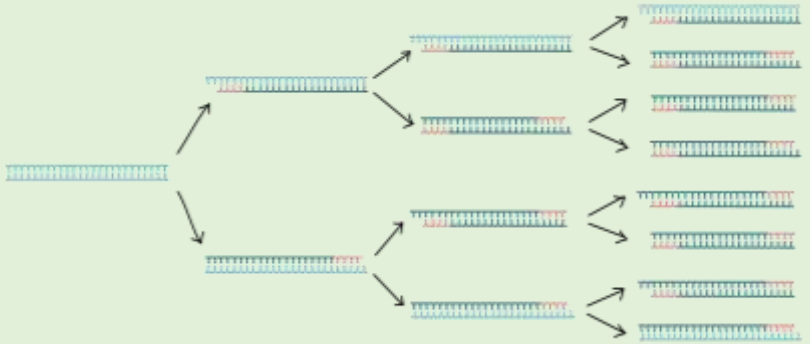
Step I

Nucleic acid isolation



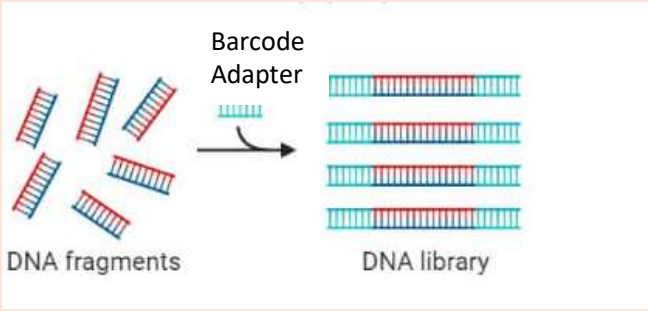
Step II



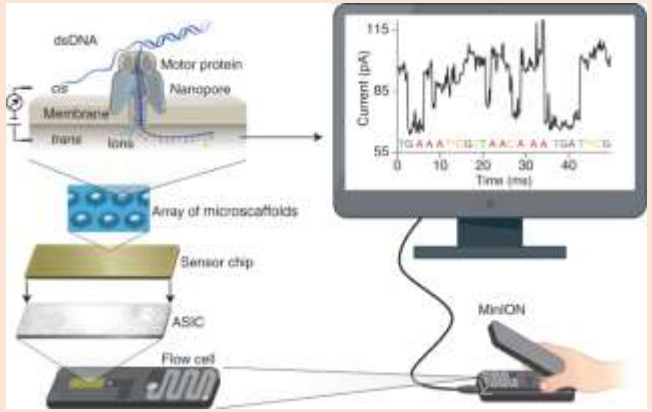
Amplification

Step III

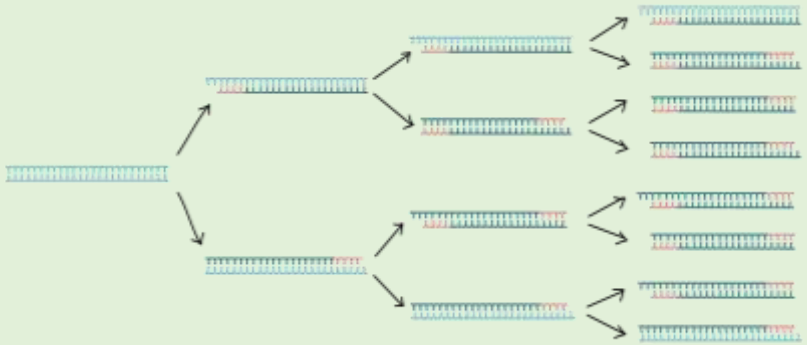
Library Preparation



Sequencing



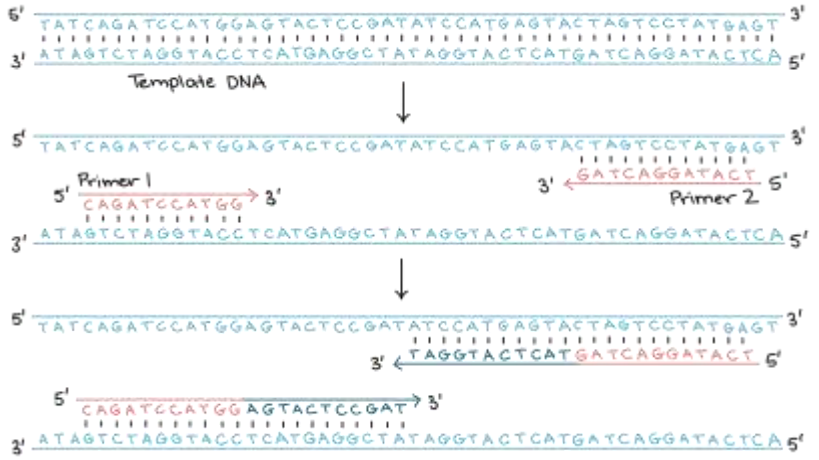
Step II

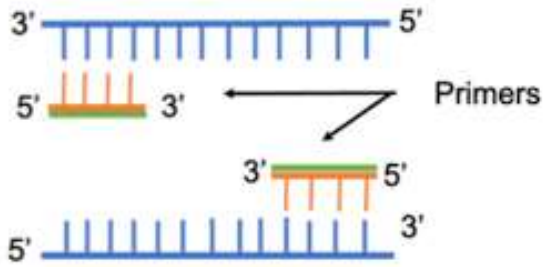


Amplification

- Primers are used for targeted nucleic acid amplification through polymerase chain reaction (PCR).

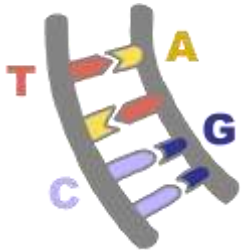
- Short pieces of single-stranded DNA that are complementary to the target sequence.





Primers

- To amplify any DNA sequence, two primers are necessary. One is called '**forward primer**' and the other one is called '**reverse primer**'. The forward primer initiates synthesis of the upper strand using the bottom strand as a template. Whereas reverse primer uses the upper strand as a template and initiate synthesis of the lower strand.
- The main property of primers is that they must correspond to sequences on the template molecule (must be complementary to template strand). Especially 3' ends.
- Usually a guanine or cytosine is used at the 3' end, and the 5' end of the primer usually has stretches of several nucleotides.



Primers

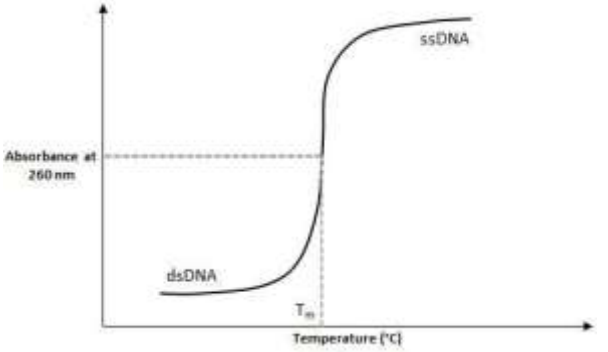
- The structure of the primer should be relatively simple and contain no internal secondary structure to avoid internal folding- hairpin formation.
- One also needs to avoid self or cross dimerization, which disrupts the amplification process.

Unwanted secondary structures

Primer Designing Parameters

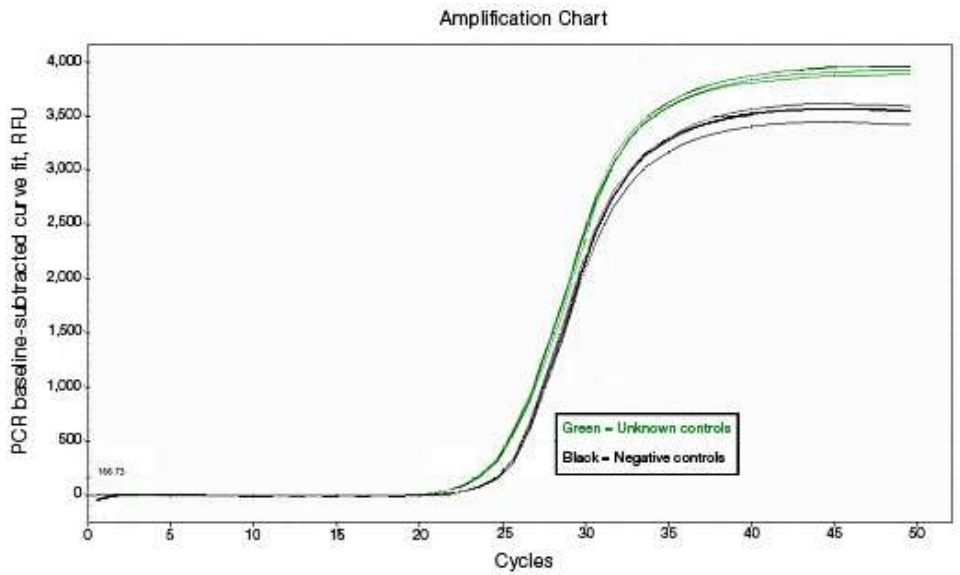
- Length of 18-24 bases
- 40-60% G/C content
- Start and end with 1-2 G/C pairs
- Melting temperature (Tm) of 50-60°C
- Primer pairs should have a Tm within 5°C of each other
- Primer pairs should not have complementary regions

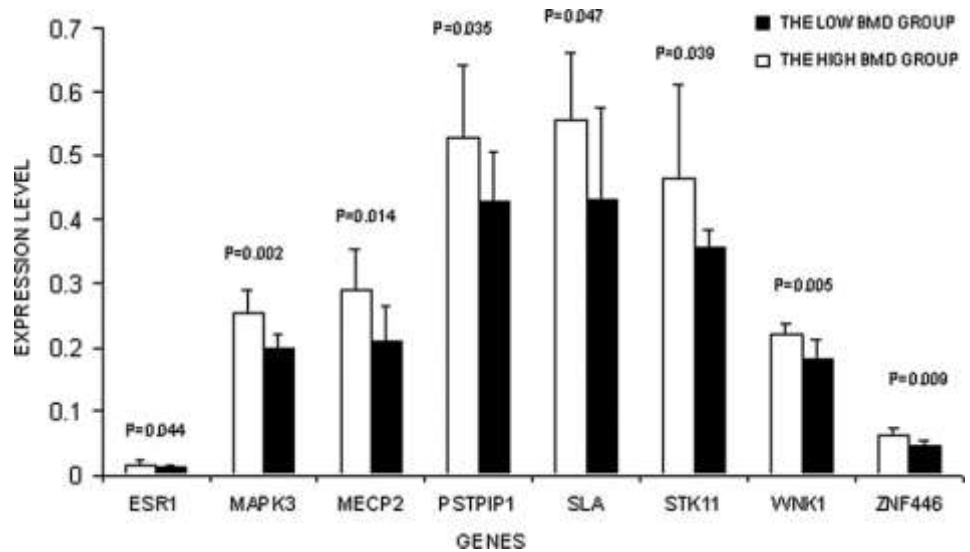
"the temperature at which one-half of the DNA duplex will dissociate to become single stranded"



Uses/Types of Primers

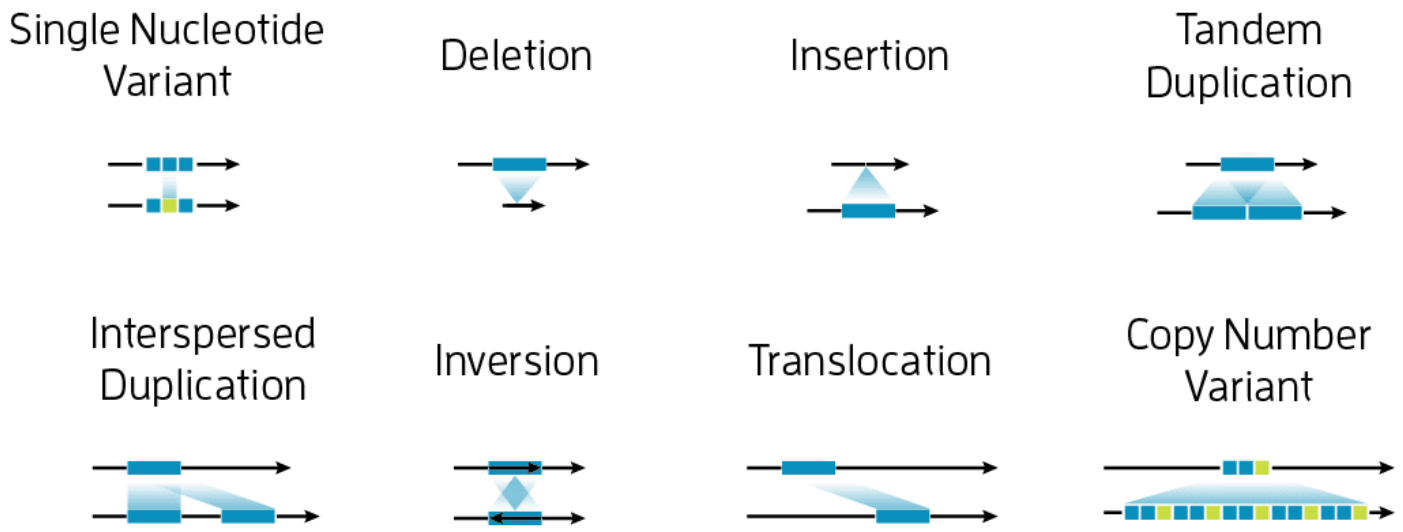
- Gene expression





Uses/Types of Primers

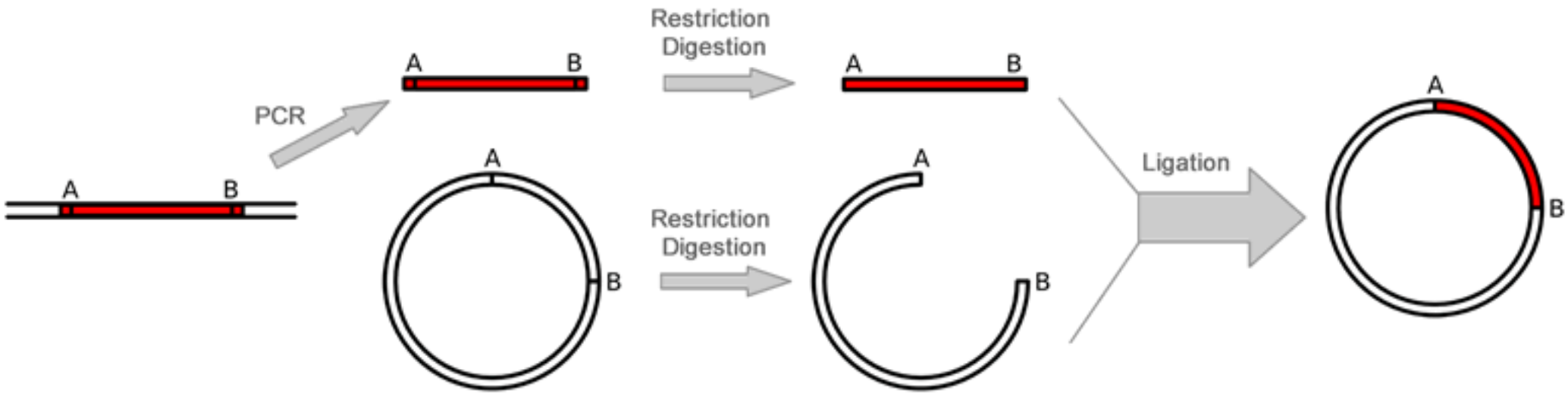
- Variant detection (present/absent)



Types of Variants

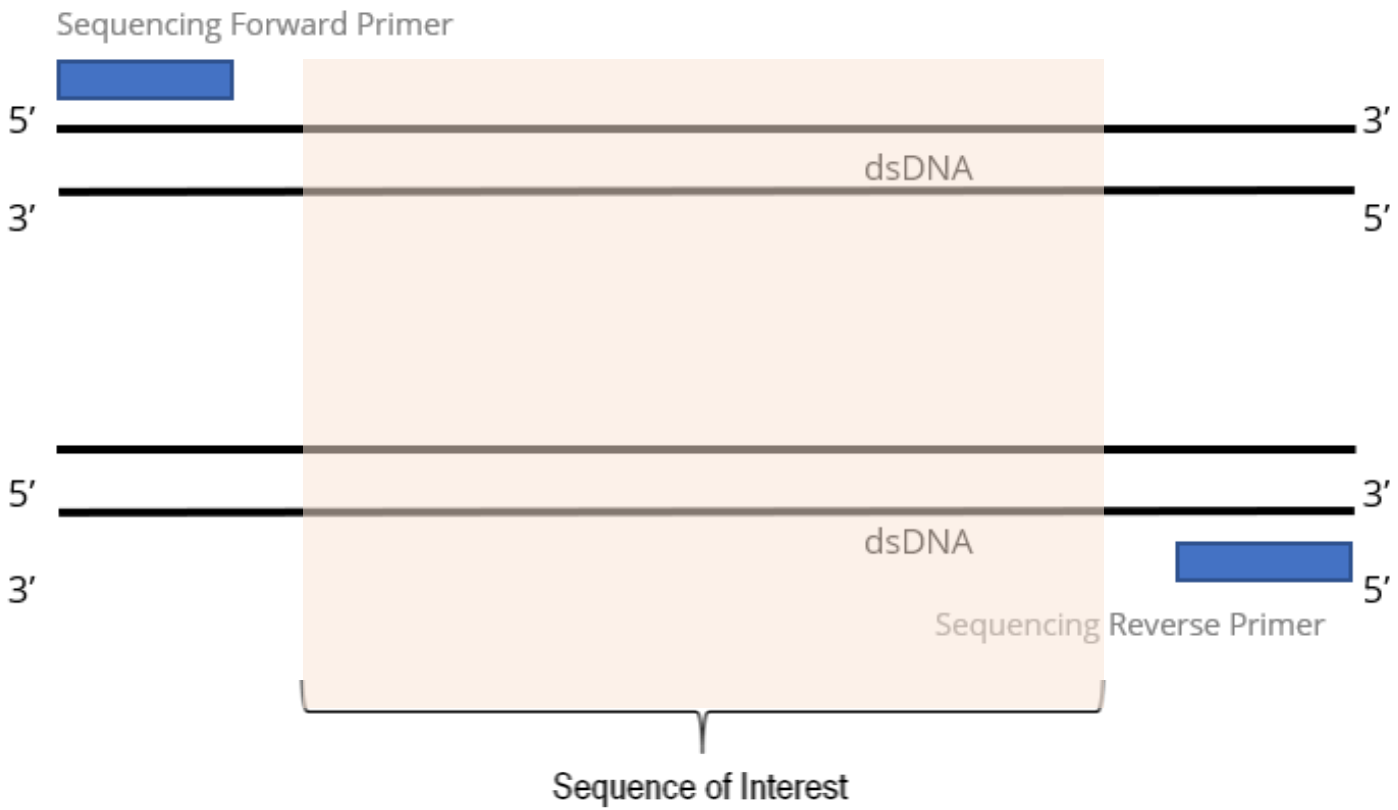
Uses/Types of Primers

- Cloning



Uses/Types of Primers

- Sequencing



Sequence Specific Primer Design
An Example: Phenylketonuria (PKU)

- Phenylketonuria also called PKU, is **a rare inherited disorder that causes an amino acid called phenylalanine to build up in the body**.
- Without treatment, PKU can damage the brain and nervous system, which can lead to learning disabilities.
- With early diagnosis and the correct treatment, most children with PKU are able to live healthy lives.

***AIM*: Design sequencing primers to screen for pathogenic variations in the gene responsible for PKU.**

Step 1: Find responsible gene

OMIM
Human Genetics Knowledge for the World

- Online Mendelian Inheritance in Man (*OMIM*)

https://www.omim.org/

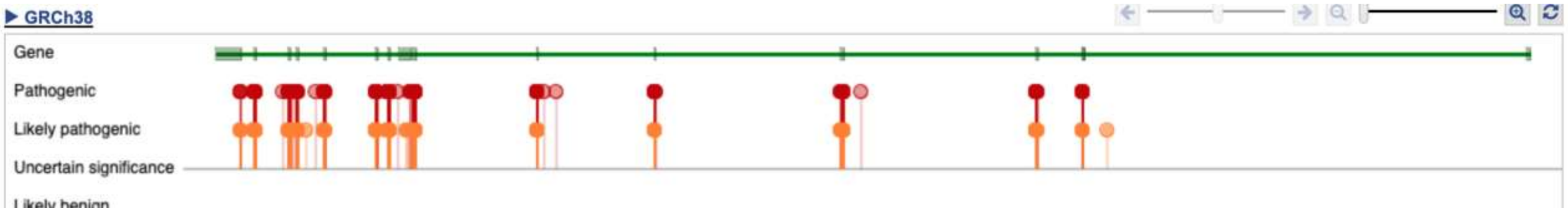
Phenotype-Gene Relationships

Location	Phenotype	Phenotype MIM number	Inheritance	Phenotype mapping key	Gene/Locus	Gene/Locus MIM number
12q23.2	[Hyperphenylalaninemia, non-PKU mild]	261600	AR	3	PAH	612349
12q23.2	Phenylketonuria	261600	AR	3	PAH	612349

Step 2: Find gene variations

- NCBI ClinVar aggregates information about genomic variations and their relationship to human health.

https://www.ncbi.nlm.nih.gov/clinvar/



Step 3: Obtain sequence

- Ensembl annotate genes, computes multiple alignments, predicts regulatory function and collects disease data.

https://www.ensembl.org/index.html

Step 4 (*optional*): View and annotate sequences

- SnapGene Viewer - free
- View, annotate and share sequence files.

https://www.snapgene.com/snapgene-viewer

Step 5: Primer design

- A tool for finding specific primers. Finding primers specific to your PCR template (using Primer3 and BLAST).

https://www.ncbi.nlm.nih.gov/tools/primer-blast/

***AIM*: Design sequencing primers to screen for pathogenic variations in the gene responsible for PKU.**

***RESULT*:**

Name	Sequence (5'->3')
PAH_1_F	ACCACCCTCTTTTCCGAGCTTCAGG
PAH_1_R	TGCCCAGCAAACACCCAAATCAACG

Next step?

***AIM*: Design sequencing primers to screen for pathogenic variations in the gene responsible for PKU.**

***RESULT*:**

Name	Sequence (5'->3')
PAH_1_F	ACCACCCTCTTTTCCGAGCTTCAGG
PAH_1_R	TGCCCAGCAAACACCCAAATCAACG

→ Place the order to the company

↓

Receive primers

↘

PCR

Thank you for listening…

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