

1. What does PCR stand for?

Polymerase Chain Reaction

2. What is the purpose of PCR? Name at least 3 examples of what PCR can be used for.

PCR is a technique in which specific DNA sequences are replicated/amplified.

1. DNA cloning for sequencing
2. DNA-based phylogeny
3. Functional analysis of genes
4. Diagnosis of hereditary diseases
5. Identification of genetic fingerprints
6. Detection and diagnosis of infectious diseases

3. Indicate the 3 steps of PCR and the approximate temperatures in which each step occurs.

1. Denaturation- 95 °C (DNA melting, breaking of the Hydrogen bonds creating sstDNA)
2. Annealing- 65 °C (annealing of primers to sstDNA)
3. Elongation- 70-80 °C (temp. depends on DNA polymerase used/Taq polymerase synthesizes a new strand of DNA complementary to the original strand) Under optimum conditions the amount of DNA target is doubled.

3 steps equal 1 cycle. PCR usually consist of 20-40 cycles.

4. How many molecules will be present after 12 cycles of PCR?

2^n therefore $n=12$ and $2^{12}= 8192$.

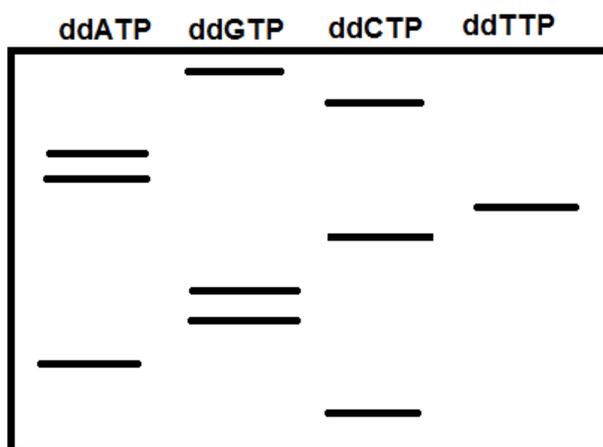
5. What is needed in order to do PCR?

Taq Polymerase-thermostable DNA polymerase

Primers-bind to sample DNA and initiate elongation

dNTPs

6. Read the sequence of DNA that the gel below represents from top to bottom. What type of sequencing method does this gel most likely represent?



3'GCAATCGGAC5' ← complementary sequence read via Sanger sequencing

5'CGTTAGCCTG3' ← original DNA sequence amplified.

Sanger sequencing is the method displayed above by gel electrophoresis.

7. What is special about ddNTP?
ddNTP= dideoxynucleosidetriphosphate. It lacks a 3'-OH group which terminates DNA synthesis.
8. What is the difference between a genomic library and a cDNA library?
Genomic library- collection of clones containing all the DNA fragments from one source
cDNA library-consist only of those DNA sequences that are reverse transcribed from mRNA.
9. Briefly describe the steps needed to find a specific gene in a genomic library?
A probe is a short single stranded chain of nucleotides that is complementary to the target sequence and has labeled ^{32}P tag. The probe anneals with the target DNA sequence and can then be filtered out.