LABORATORY TECHNIQUES

Section I - PCR

I. Polymerase chain reaction (PCR) (Figure 2.3.1)

A. A method of amplifying DNA

B. Four elements are necessary for PCR

1. A source DNA template
2. Knowledge of the adjacent regions (flanking sequences) to the target that will be amplified (a DNA primer can then be utilized to bind to these regions)
3. A DNA polymerase that can withstand high temperature (thermostable)
4. Deoxynucleotide triphosphates

C. There are three steps in the process of amplification

1. Denaturing: this occurs by heating up the DNA template
2. Annealing: the sample is cooled and primers adhere to the flanking regions of the target.
3. Elongation: the sample is warmed and DNA polymerase copies the DNA

D. The intensity of an amplified band on a gel can provide 'semi-quantitative' results.

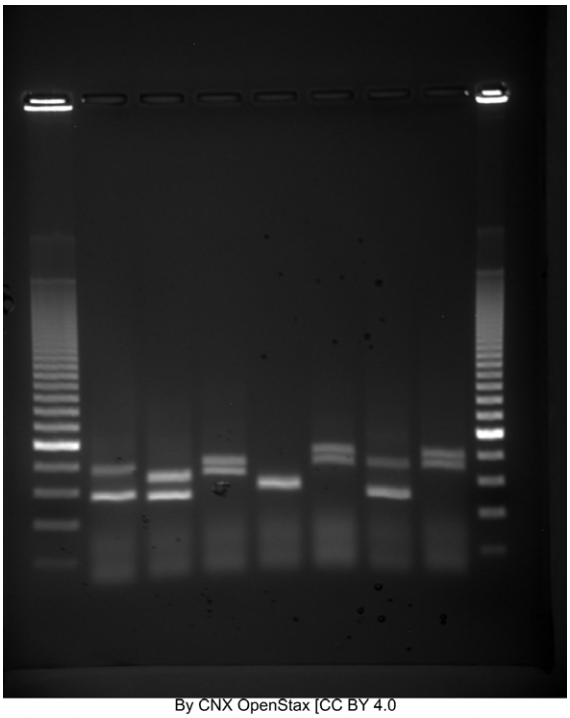

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Figure 2.3.2 - DNA bands from electrophoresis

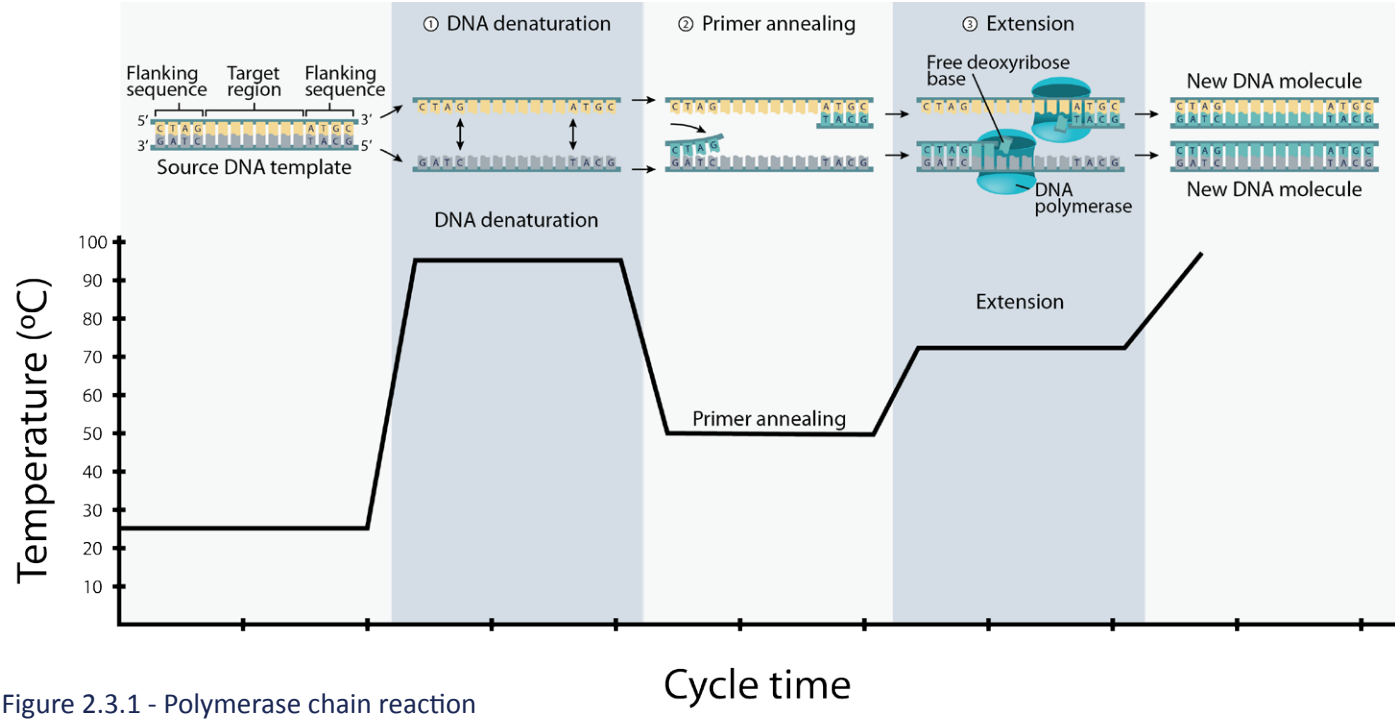

Figure 2.3.1 - Polymerase chain reaction

II. Real time PCR (Figure 2.3.3)

A. Single stranded DNA fluorescent used

B. As DNA polymerase replicates the DNA, the probe is removed and activated → light emitted

C. Fluorescence intensity reflects the quantity of DNA

D. Curve which crosses threshold line first had the highest concentration of DNA in the initial sample

III. Reverse transcription PCR

A. A template of cDNA is produced from a mRNA by reverse transcriptase.

B. The cDNA doesn't contain introns because it is made from mRNA.

C. This test is useful in measuring mRNA.

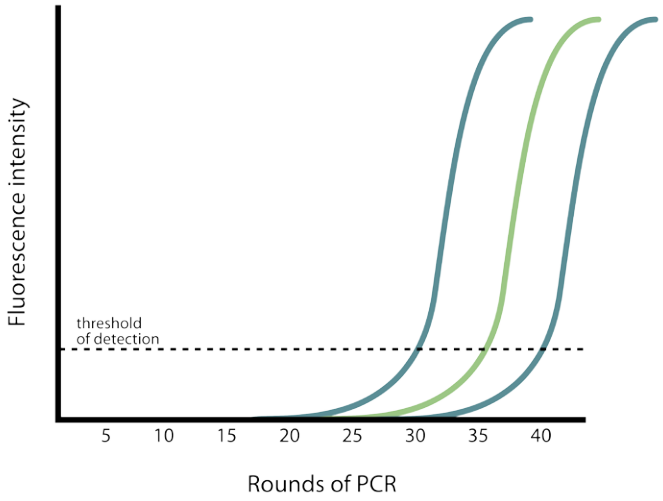

Figure 2.3.3 - Real time PCR

REVIEW QUESTIONS

1. A 37-year-old female has been working with an infertility specialist in an attempt to become pregnant. During *in vitro* fertilization, an egg is retrieved from the ovaries. The egg is then artificially inseminated and prepared for uterine implantation. However, the patient requests that the embryo be screened for genetic mutations prior to uterine implantation. A sample of DNA is obtained from the embryo and PCR is performed to screen for several genetic abnormalities including Huntington disease. A gene on chromosome 4 is amplified and the embryo produces a PCR product that is much larger than expected. What does the large PCR product most likely indicate about the embryo?

   - **Huntington disease → CAG trinucleotide repeat disorder on chromosome 4**
   - **Amplification of this region → product that was much larger than expected → patient must have CAG repeats resulting in a longer amplified region → embryo most likely has Huntington disease**

2. A 14-year-old boy is diagnosed with acute lymphoblastic leukemia. He is started on an aggressive chemotherapeutic regimen and receives regular follow up care. During one of these visits his treatment response is analyzed by using reverse transcription PCR. A bone marrow aspirate is obtained and mRNA created by a BCR/ABL translocation is then used as the template for real time PCR. How will the PCR product most likely differ from the BCR/ABL gene?

   - **BCR/ABL is usually associated with CML but can sometimes be associated with ALL**
   - **The mRNA produced by BCR/ABL represents malignancy and can be quantified to asses the treatment response**
   - **If the chemotherapeutic regimen is effective → ↓ mRNA**
   - **If the regimen is ineffective → ↑ mRNA**
   - **In order to quantify the mRNA it must be converted into cDNA to be amplified by real time PCR**
   - **The PCR product will lack introns (derived from mRNA) and the BCR/ABL gene will contain introns**