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# PCR

## What is it?

### Polymerase Chain Reaction

### A method to amplify a particular piece of DNA

### *In vitro* procedure that simulates DNA replication in cells

### It amplifies the specific DNA sequence millions of times in just hours

### Necessary for several other genetic uses of DNA such as Sanger sequencing

### Developed by Kary Mullis in 1983

## What is needed?

### DNA sequence of interest

### Heat stable DNA polymerase (ex: Taq from *Thermus aquaticus*)

#### Extends the primers in the 5’-3’ direction

#### Must be heat stable so it does not denature during the extreme range of temperatures used throughout the reaction

### All 4 deoxynucleotides (dNTPs): A, T, C, and G

#### Used by the polymerase enzyme to extend the primers

### Forward and reverse DNA primers

#### Primers: short single-stranded DNA sequences (15-40 base pairs long) that bind to either side of the DNA of interest. This allows the specific sequence to be amplified. They are made commercially and can be ordered to match the DNA sequence of interest.

#### Provide a starting point for the polymerase to begin adding nucleotides

### Buffer

#### A salt solution buffer with a regulated pH is used to stabilize the reaction by providing the optimal environmental conditions for the polymerase enzyme (enzymes are most effective in their optimal conditions)

### Thermal cycler

#### A device programmed to change the temperatures for the various steps of the reaction

## What are the steps?

### Denaturation

#### 94 °C to separate the double-stranded DNA helix

#### Breaks hydrogen bonds between base pairs

#### Similar to what helicase does in DNA replication

### Annealing

#### 50°C to allow the primers to bind to their complementary sequence on the target DNA

#### One primer binds upstream and the other binds downstream, thus flanking the DNA segment of interest

### Extension

#### 72°C to allow a heat-stable polymerase (like Taq) to extend the primers 5’-3’ at its optimal temperature

### Process then repeats to create many copies of the desired sequence

## How much DNA does it replicate?

### PCR amplifies a DNA sequence exponentially

### 2n is a formula that can be used to determine how many identical copies will be produced, where n equals the number of rounds of replication

### Ex: After 15 rounds, there will be 32768 copies of the desired DNA sequence because 215=32768

## What is it used for?

### Disease

#### PCR can be used in genetic counseling to test for carriers of certain alleles or mutations, such as in prenatal testing for different diseases

#### PCR can be used to study the genes that cause cancer

#### PCR can be used to study infectious diseases like HIV

### Forensic Science

#### PCR can be used for paternity testing

#### PCR can be used to amplify DNA found at a crime scene and allow that DNA to be compared to suspects’ DNA

#### PCR can be used to identify dead bodies or body parts

#### PCR can be used in rape kits to help find the perpetrator

### Research

#### Mapping out of the human genome

#### Sequencing of DNA

## What are the benefits of PCR?

### Makes billions of copies of target DNA sequence in hours

### Cheap and relatively easy to perform

### Can use PCR products in a variety of fields in science

# Multiple Choice Questions

### Which of the following components is NOT needed in PCR?

#### A heat-stable polymerase

#### RNA primers

#### Buffer solution

#### The nucleotide adenine (A)

#### DNA primers

### What is the optimal temperature for the heat-stable polymerase?

#### 50°C

#### 100°C

#### 94°C

#### 72°C

#### 74°F

### How many PCR products will you have after 20 rounds of replication?

#### 400

#### Over 1 billion

#### Over 1 million

#### 4 million

#### 10,000

### Which of the following primers would you use if you wanted to amplify this segment of DNA: 5’ GGACTCGTAACGTTA 3’?

#### 5’ TAACGTTACGAGTCC 3’

#### 5’ CCTGAGCATTGCAAT 3’

#### 3’ GGACTC 5’

#### 3’ CCTGAG 5’

#### Both C and D

### What step of PCR is being shown in this picture?

#### Extension

#### Polymerization

#### Denaturing

#### Addition of nucleotides

#### Primer annealing

# Essay Questions

### What happens at each step of PCR? Please explain how PCR can be applied in science today and why it is useful.

### Compare and contrast PCR with *in vivo* DNA replication.

# Helpful Resources and Videos

## <http://highered.mheducation.com/olc/dl/120078/micro15.swf>

### PCR animation with explanations

## <http://scienceblogs.com/insolence/wp-content/blogs.dir/445/files/2012/04/i-2b771ce908cdcfd17c0a348b0076e511-PCR.jpg>

### PCR step-wise picture set with captions

## <http://www.sumanasinc.com/webcontent/animations/content/pcr.html>

### Another PCR animation

## <http://highered.mheducation.com/sites/0072556781/student_view0/chapter14/animation_quiz_6.html>

### The same animation as A, but with an online quiz

# Answer Key

## Multiple Choice

### B; DNA primers are used not RNA primers

### D; 72°C is the temperature used during extension and is optimal for Taq polymerase

### C; over 1 million because 220 = 1,048,576

### D; the primer should be a short sequence complementary (not identical) to the DNA sequence of interest

### E; primers are attaching (annealing) via hydrogen bonds to their complementary sequences on the target DNA

## Essay

### 1st step- denaturation at 94°C; break hydrogen bonds between double helix to produce a single-stranded DNA sequence

### 2nd step- primers annealing at 50°C; requires forward and reverse primers to flank the DNA sequence of interest; gives the polymerase enzyme a place to begin extension

### 3rd step- extension at 72°C; a heat-stable DNA polymerase enzyme (like Taq) adds nucleotides to the growing 3’ end of the primers; produces a strand of DNA complementary to the sequence of interest

### PCR is useful today because it allows a specific DNA sequence to be amplified, producing over 1 billion copies after mere hours; PCR is cheap, quick, and effective

### PCR can be used in forensics to amplify DNA evidence at a crime scene

### PCR can be used in genetics to analyze certain mutations that cause disease; can also be used to diagnose these diseases in genetic counseling

### PCR can be used in research studies to understand more about diseases such as cancer and HIV

### PCR occurs *in vitro*; DNA replication occurs *in vivo*

### PCR uses heat to separate the double-stranded DNA; DNA replication relies on the helicase enzyme to separate the double-stranded DNA

### PCR uses DNA primers; DNA replication uses RNA primers

### PCR uses a heat-stable polymerase enzyme to extend the primers; DNA replication relies on DNA polymerase to extend the primers

### Both PCR and DNA replication extend the primers with DNA nucleotides

### Both PCR and DNA elongate in the 5’-3’ direction

### Both PCR and DNA require a template strand of DNA