HC 70 AL
Lab Handout

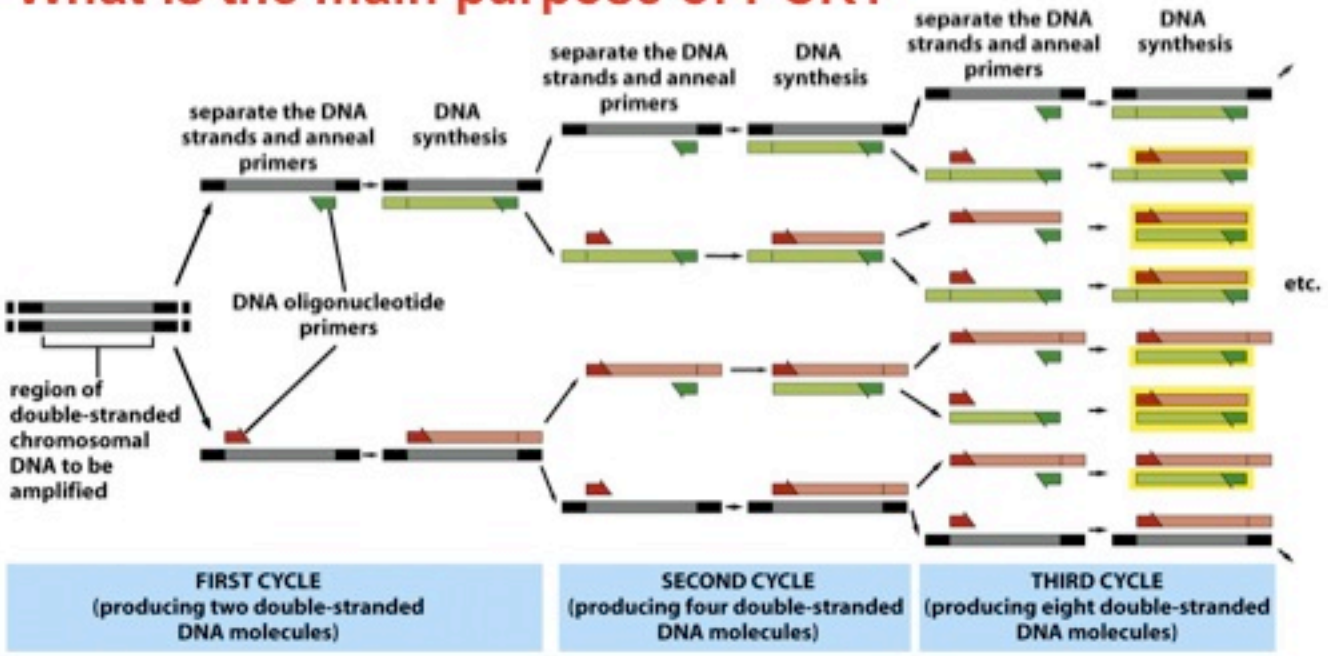
Polymerase **C**hain **R**eaction (PCR)

Week 1
Thursday
March 31, 2011

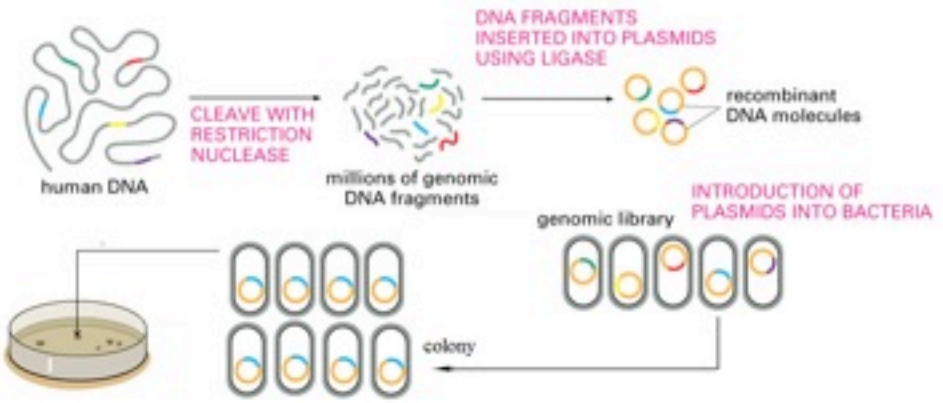
Eden Maloney

What is the Polymerase Chain Reaction?

What is the main purpose of PCR?



Who developed this technique?



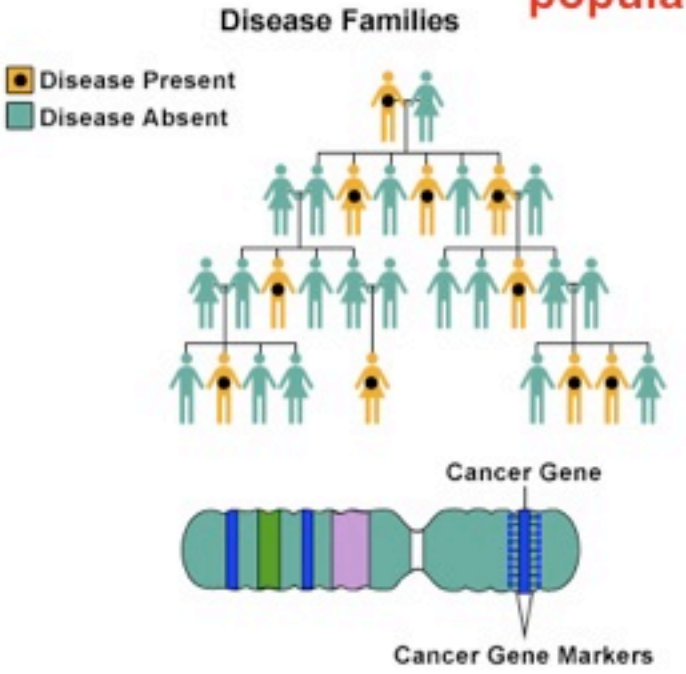
How was DNA amplified before PCR?

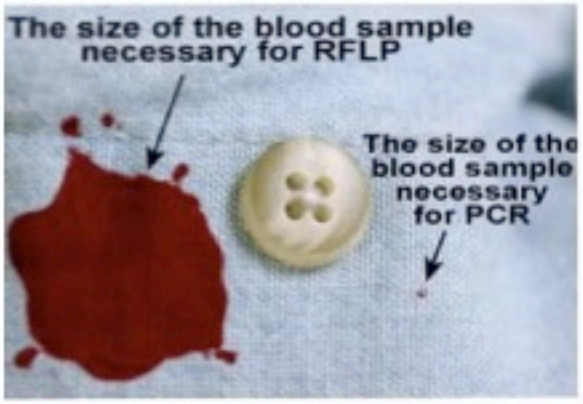
What is PCR used for?

Long-Locked Genome of Ancient Man Sequenced

Preserved in hair and bone samples for 4,000 years, the DNA of an early Greenlander reveals new clues about everything from skin color to migration patterns February 10, 2010

How has PCR helped scientists understand ancient populations?





How has PCR helped criminologists and doctors?

What are the requirements for PCR?

1. **Knowledge of DNA sequence**

2. **PCR tubes**
   - *What conditions do they have to withstand?*

3. **Ex Taq Buffer and Water**
   - *What environment do these reagents mimic?*

4. **Specific Primers**
   - *What is a primer?*
   - *Why does DNA polymerase need them?*

5. **DNA template containing sequence of interest**

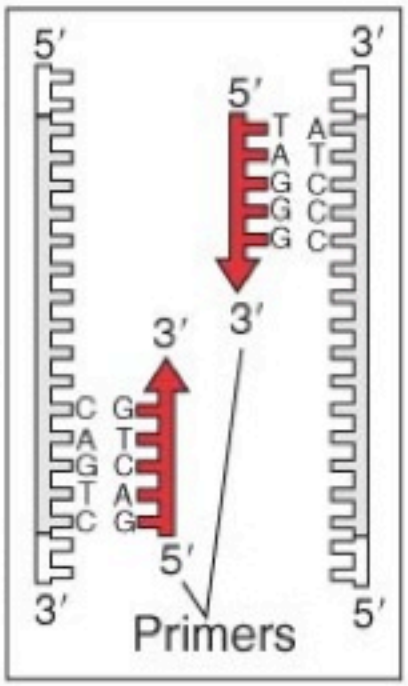
6. **dNTPs (deoxyribonucleotide triphosphates)**
   - *What is the purpose of these?*

7. **DNA polymerase (Ex Taq)**
   - *What kind of polymerase is used in PCR?*
   - *Where did this polymerase come from?*
   - *What order does the DNA polymerase lay down nucleotides?*

8. **Thermocycler**
   - *How does this regulate PCR?*

Where do the primers come from?

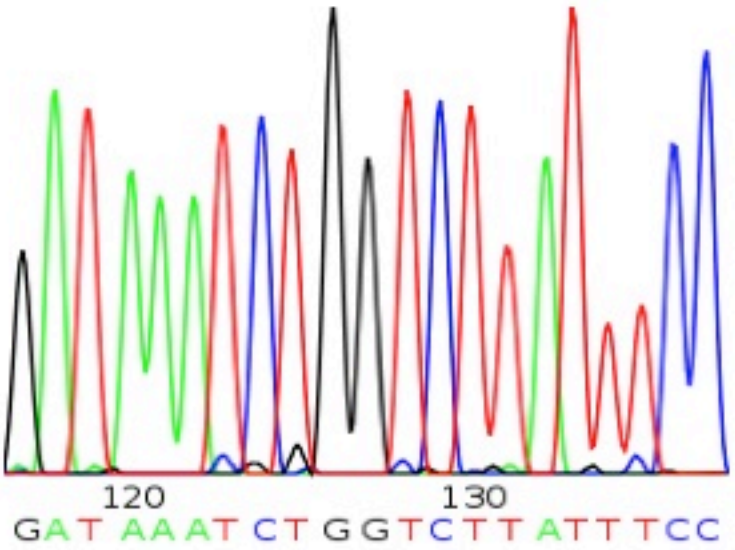
How are these primers made?



Where will these primers bind?

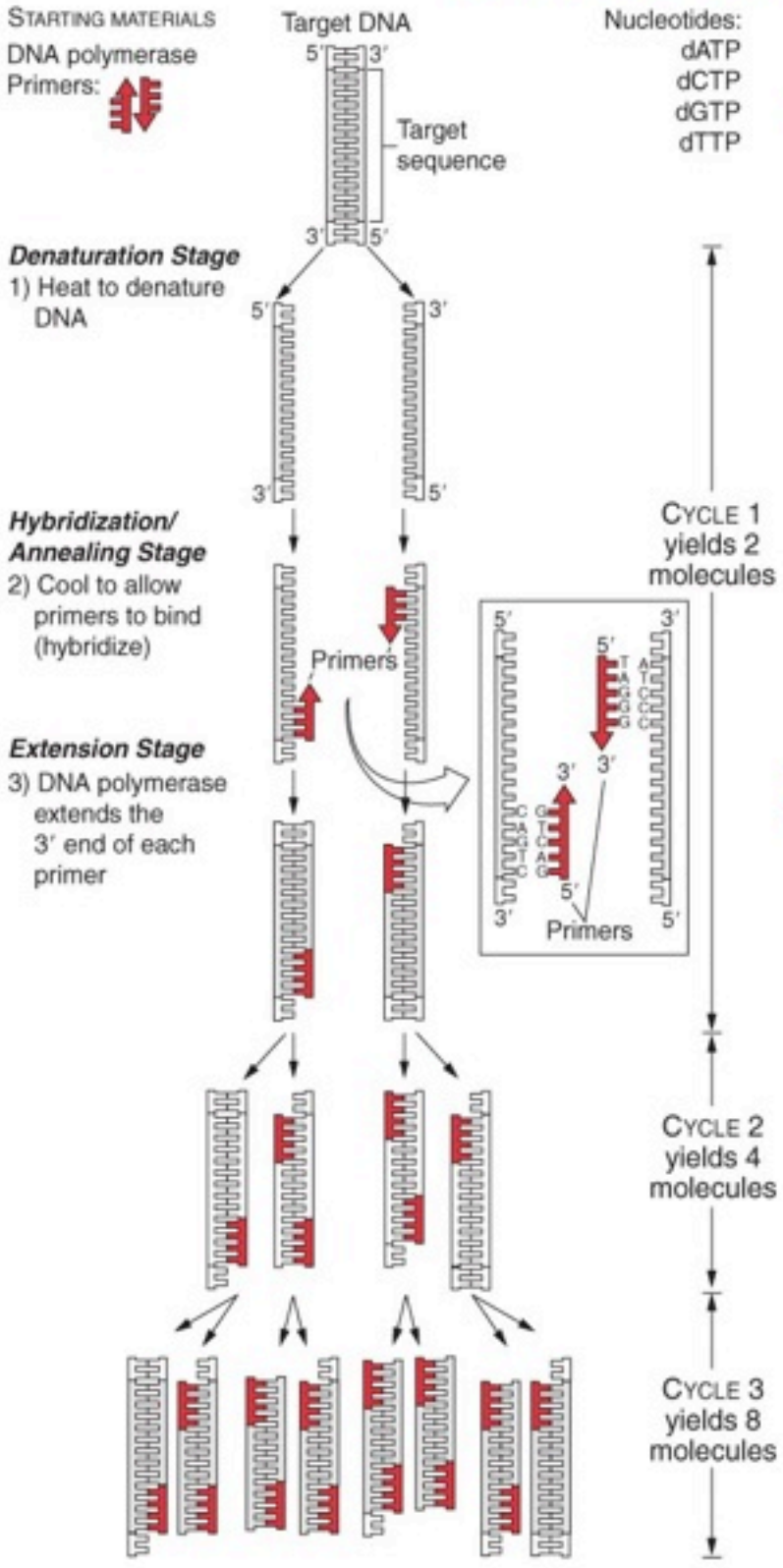
How many primers do we need?

Why are primers usually only 20 bp?



How have genome projects made primer designing easier?

What are the three stages of PCR?



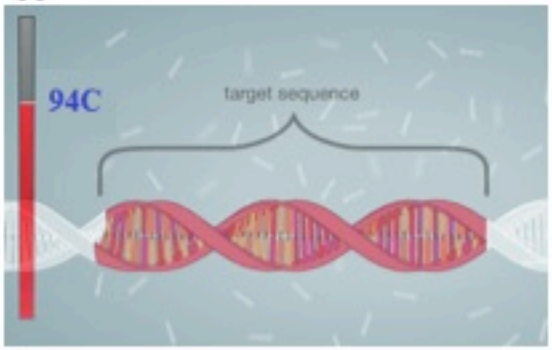
What bonds are broken during DENATURATION?

What will the orientation of the primers be relative to the template DNA?

What occurs during EXTENSION?

What are the optimal PCR conditions for Ex Taq DNA Polymerase?

1.



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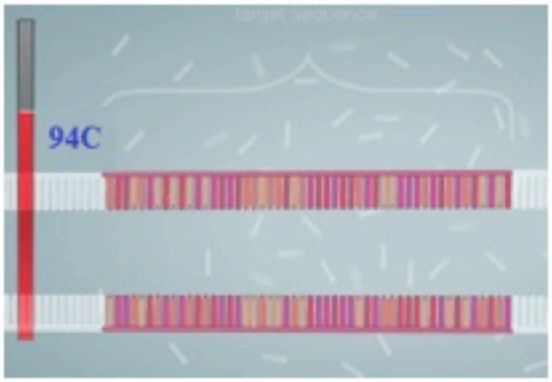

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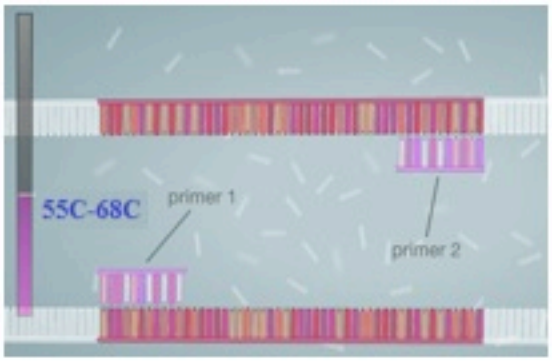

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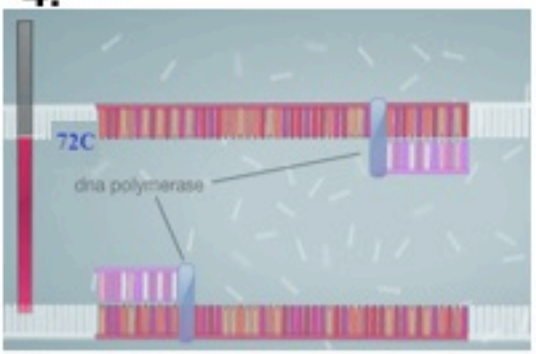

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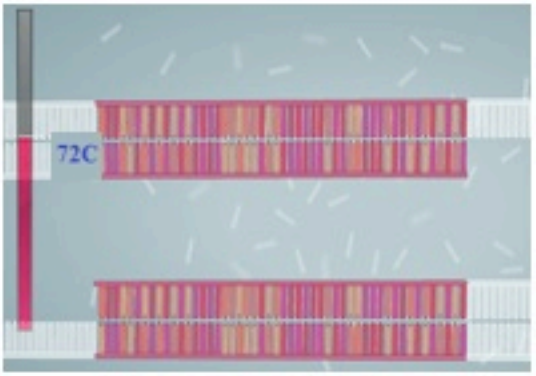

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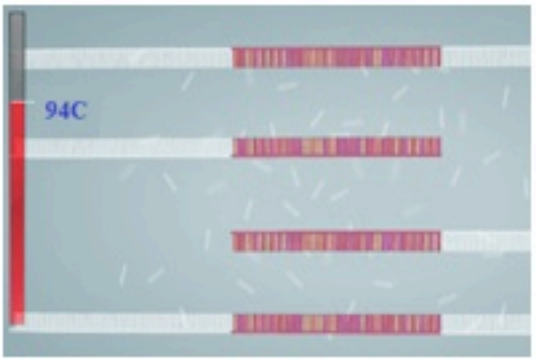
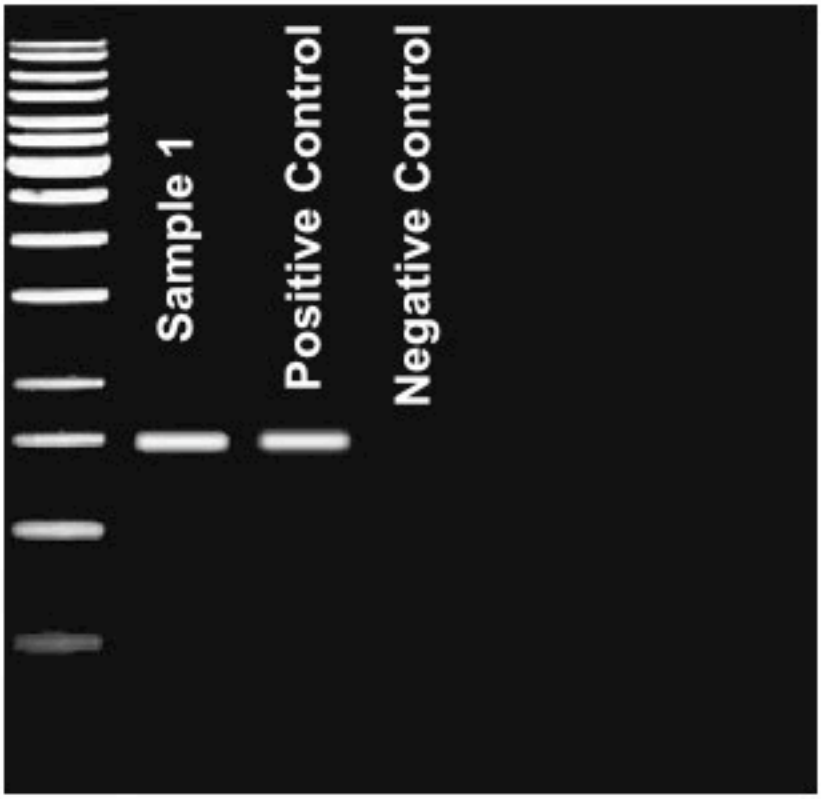


How does a Thermocycler regulate these stages?

How do you visualize PCR products?

How many bands would you expect to see after running a gel? WHY?



What are the positive and negative controls?

What does the ladder tell you?

What if you see more than one band in a lane?

What general considerations need to made when performing PCR?

PCR is very sensitive to contamination

Wear gloves.

Use filter **PCR tips**.

Ex Taq DNA Polymerase is very sensitive!

NEVER use the same filter tip in different solutions.

Use **NEW** solutions if you suspect they are contaminated.

Check off each solution as you pipette it into each tube.

The lid of the thermocycler is **hot**!

Stay focused (no chit-chat!)