HC 70 AL
Lab Handout

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

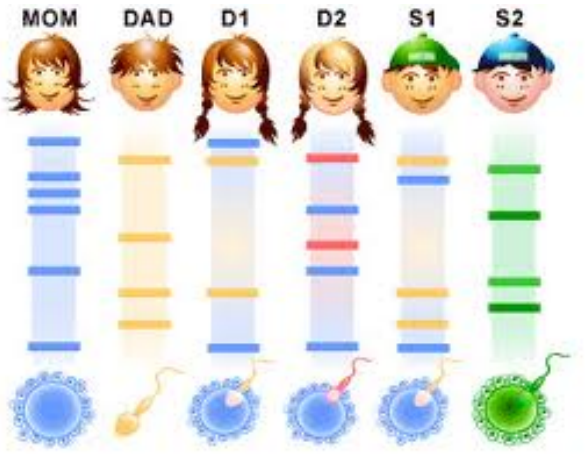
Week 1
Thursday
8/7/14

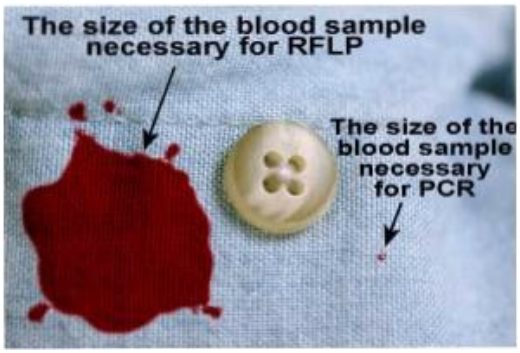
Mike Lyons

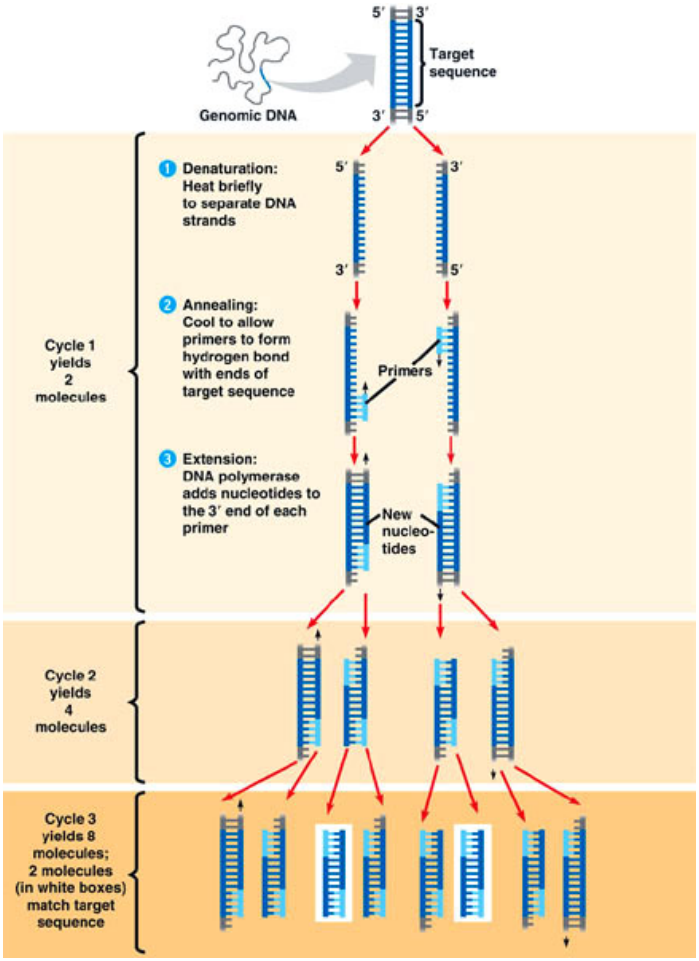
What is the Polymerase Chain Reaction?

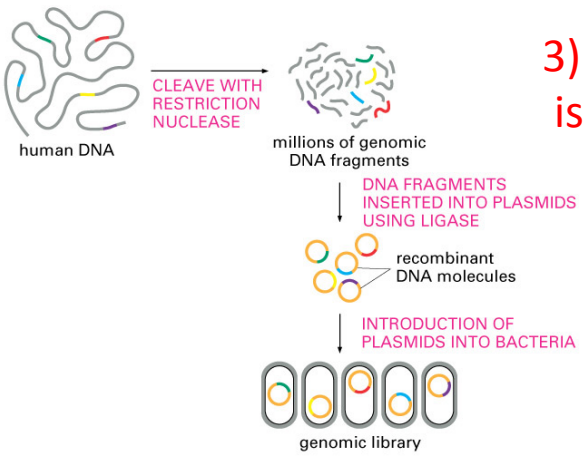
1) What is the purpose of PCR?

2) What are some applications of PCR?









3) How would genes be isolated and amplified before PCR?

What do you need to perform PCR?

1. **What is going to be copied?**

2. **What enzyme do you need to replicate DNA?**
   - *What kind of polymerase is used in PCR?*

3. **How is the polymerase going to bind to the target DNA?**

4. **What do we need to know to design the primers to replicate our desired region?**

5. **What building blocks need to be supplied for DNA polymerase to make new strands of DNA?**

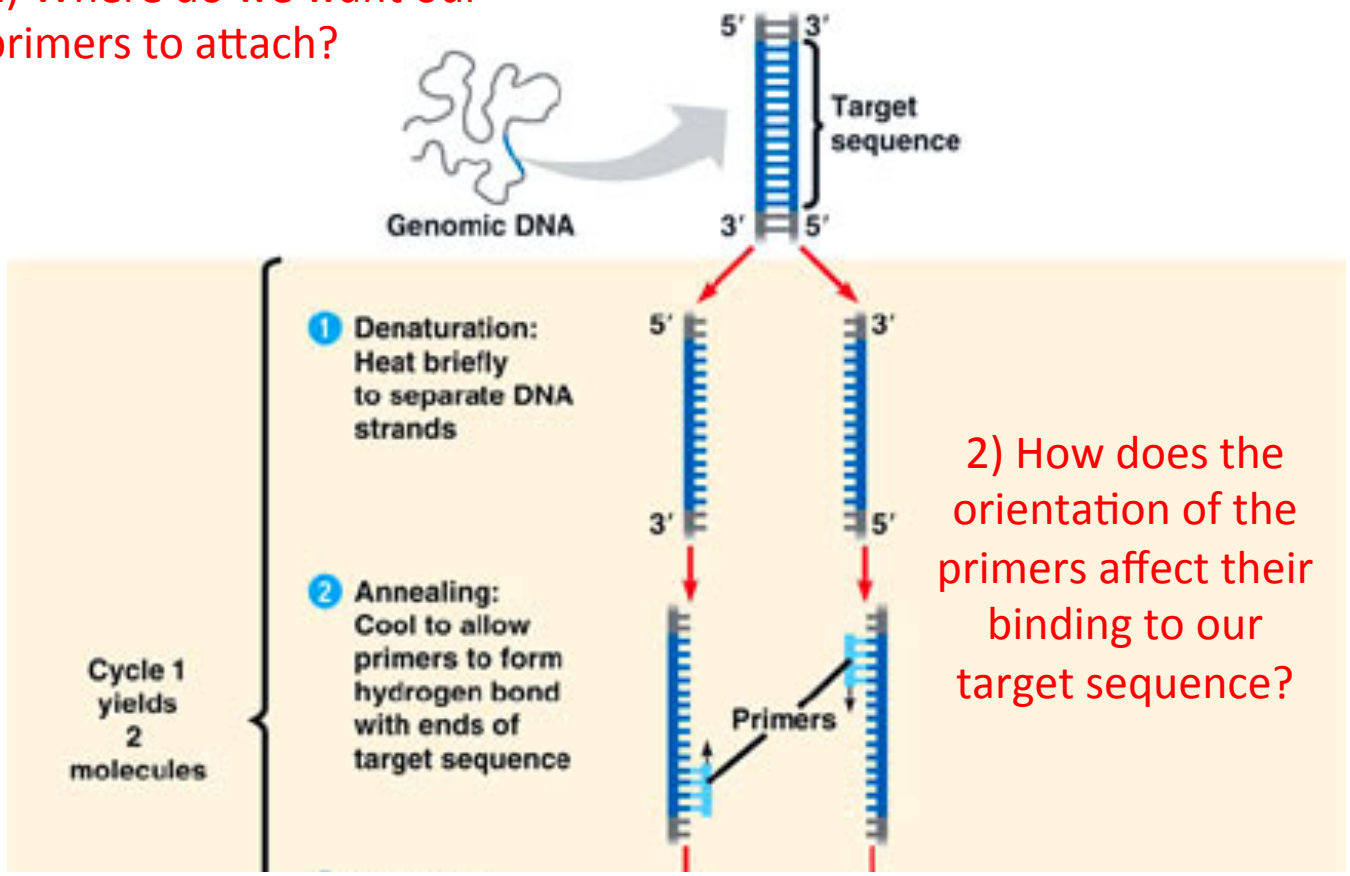
6. **How can we mimic the environment that the polymerase is active in?**

7. **How can we regulate temperatures to make them ideal for the different steps of PCR?**
   - *Why do we need to manipulate the temperature of the reaction?*

8. **What are we going to contain this reaction in?**

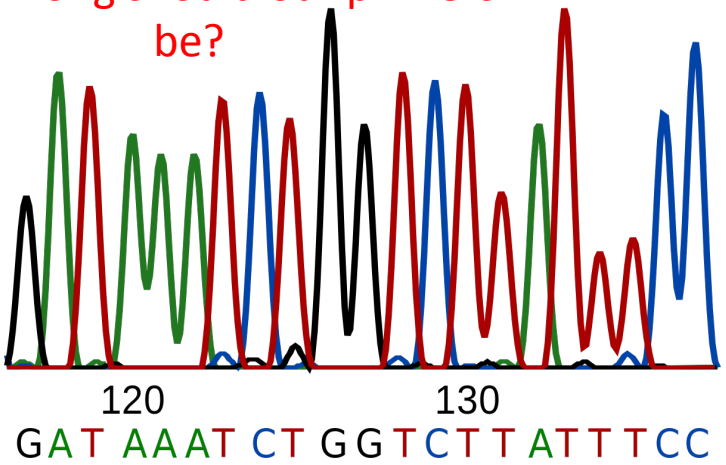
How do we design primers?

1) Where do we want our primers to attach?



2) How does the orientation of the primers affect their binding to our target sequence?

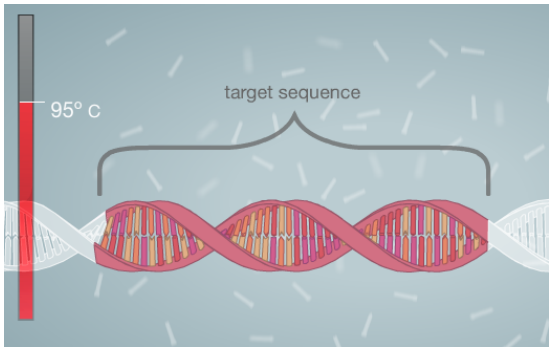
3) How long should our primers be?



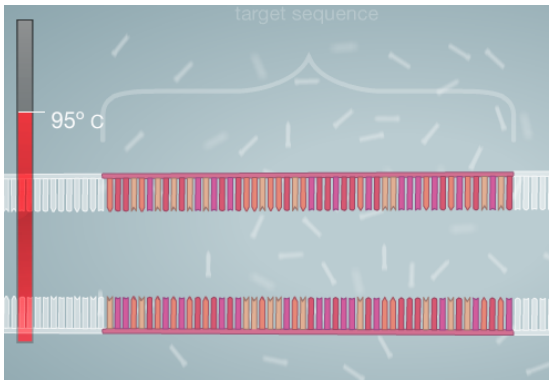
4) What is the probability of this sequence occurring randomly?

How does PCR lead to multiple copies of our target DNA?

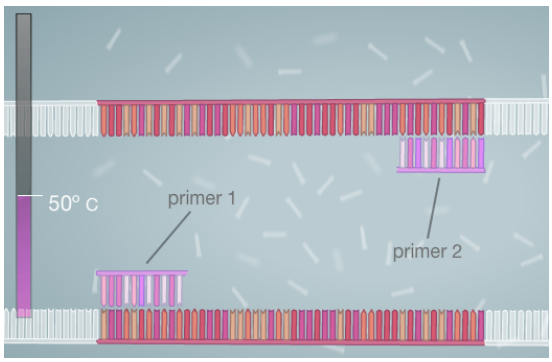
1) What template are we starting with?



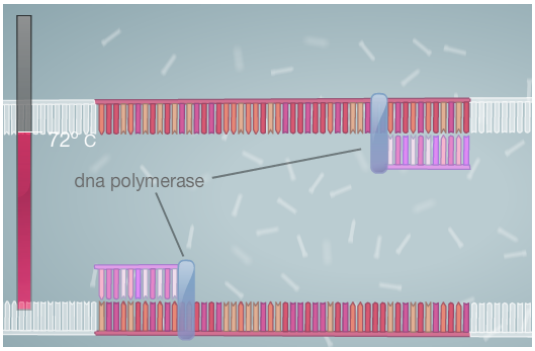
2) What bonds do we need to break to open the DNA?



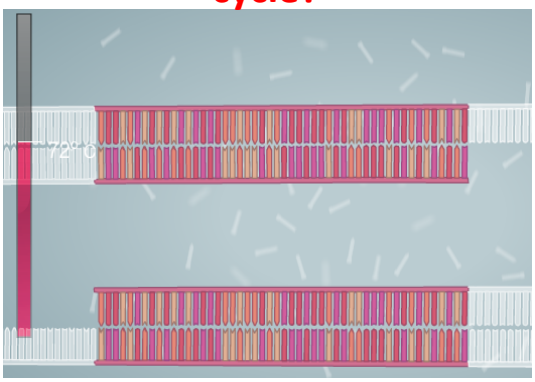
3) How can we attach our primers to the target DNA?



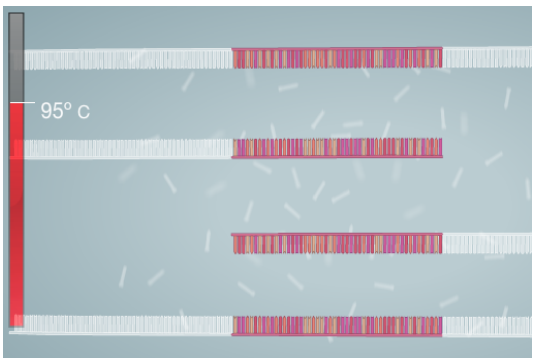
4) How are we going to make the DNA polymerase active?



5) What will our product look like after our first cycle?



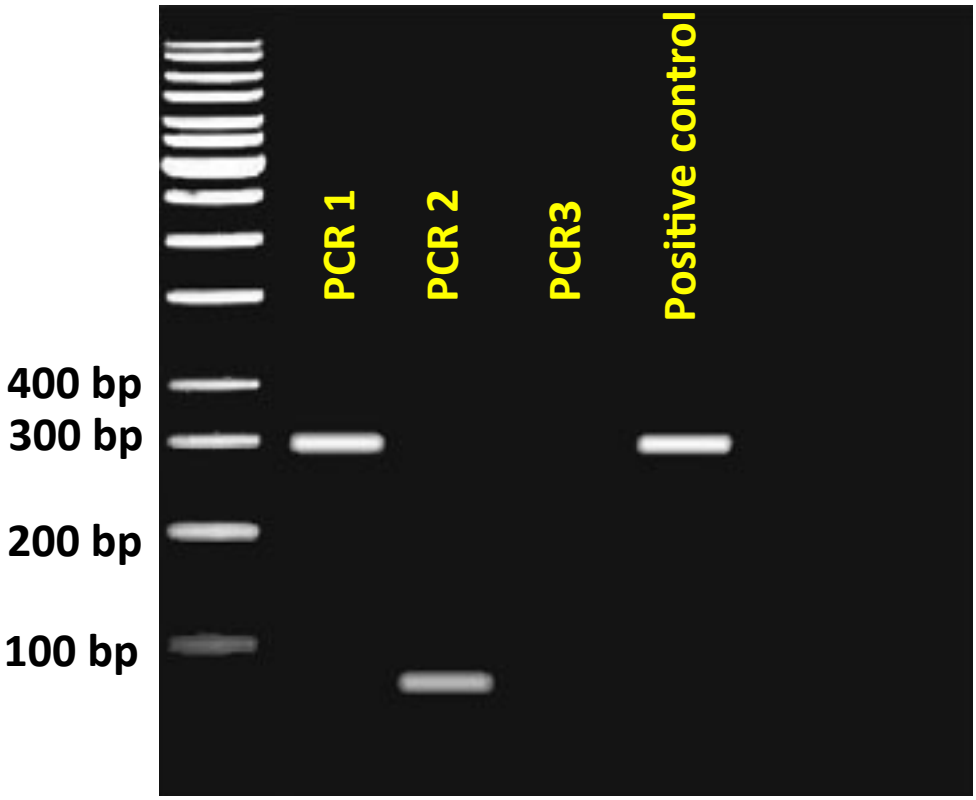
6) What will our product look like after multiple cycles?



How can we determine if our PCR reaction worked?

1) What experiment can we perform to visualize our DNA product?

Expected size: 300 bp



2) How many of the above PCR reactions were successful? What could have happened in the reactions that did not work?

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! Do not vortex vigorously. Keep it in the freezer until use.
Keep it on ice.

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!)