Copying DNA – PCR (Polymerase Chain Reaction)

Why we need so many copies

- Biologists needed to find a way to read DNA codes.
- How do you read base pairs that are angstroms in size?
  - It is not possible to directly look at it due to DNA's small size.
  - Need to use chemical techniques to detect what you are looking for.
  - To read something so small, you need a lot of it, so that you can actually detect the chemistry.
- Need a way to make many copies of the base pairs, and a method for reading the pairs.

Molecular Biology Laboratory Techniques :Polymerase Chain Reaction (PCR)

- Restriction enzymes and plasmid cloning techniques are used routinely in many laboratory experiments.
- The discovery of PCR has replaced these techniques for large scale sequencing of genomes.
- Without PCR automated and fast sequencing technology would not have been possible.

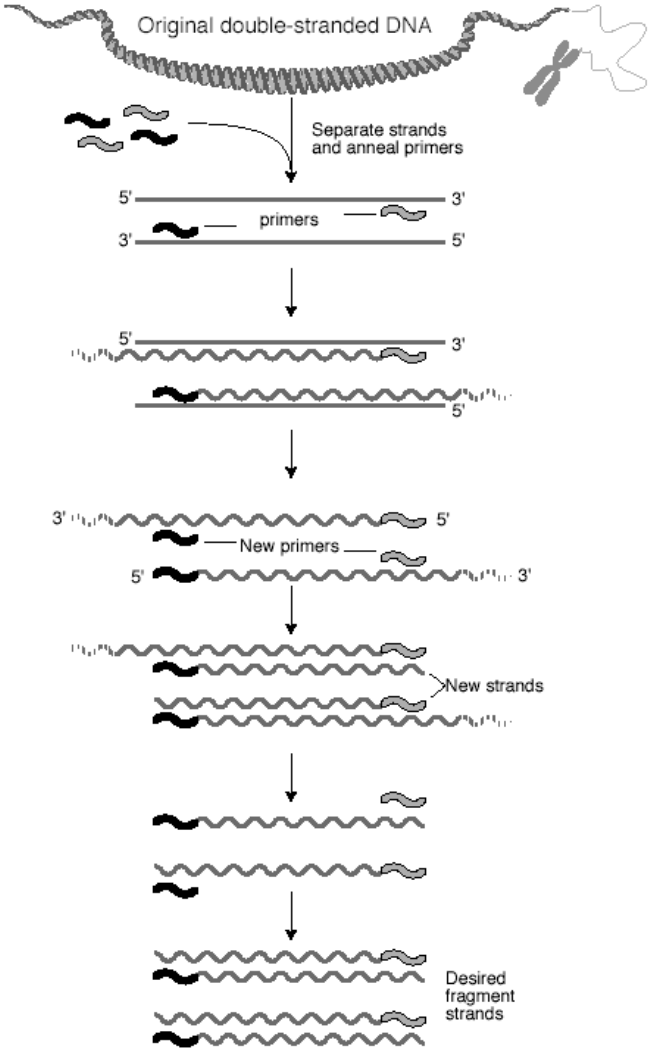
- PCR is a cell-free method of amplifying a short (<15kb) fragment of a target DNA in large quantities.
- People have compared PCR with the Gutenberg printing press of DNA and Kary Mullis who invented PCR in 1983 got Nobel Prize.
- He thought it was a good idea because he “had been spending a lot of time writing computer programs”.
- PCR is a laboratory application of the concept of “recursion” in computer science.

- PCR technique depends on the existence of a ***primer sequence*** of 15-30 nucleotides long at the end of a target DNA .
- When added to a ***denatured*** DNA (single stranded DNA at temperature >91^0 C), the primers will bind to complementary sequences if the temperature is now cooled to =50^0 C.
- This process is called ***annealing***.

- Under the presence of a DNA polymerase at =72^0 C, the synthesis of new DNA strands complementary to both strands of the target DNA will start.
- PCR is called a “chain reaction” because both the newly synthesized DNA strands now act as templates for future iterations, doubling the number if DNA fragments at every cycle.
- This results in a huge quantity of the DNA fragments in a short time.
- For further details, see http://web.utk.edu/~khughes/main.htm.

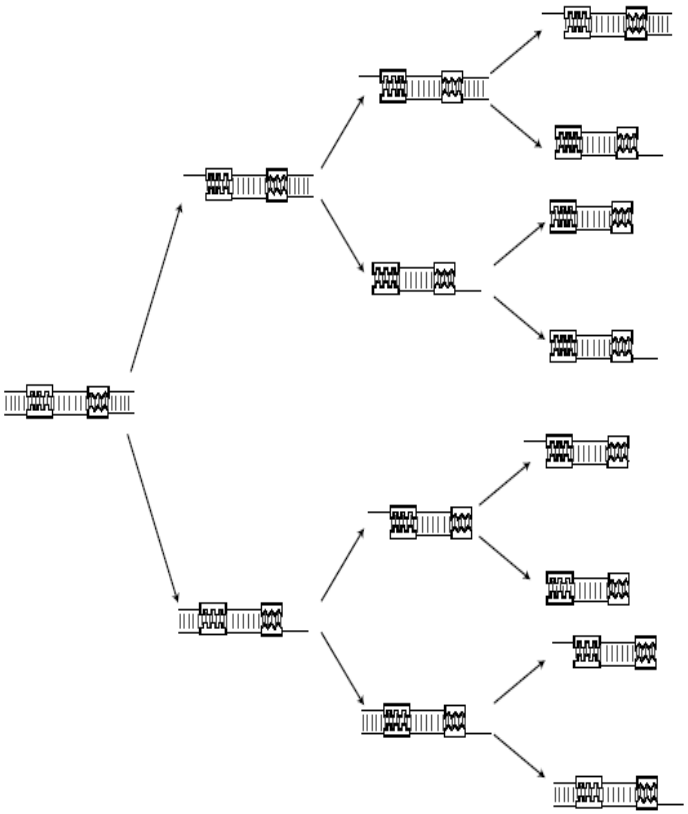
Polymerase Chain Reaction (PCR)

- Polymerase Chain Reaction (PCR)
  - Used to massively replicate DNA sequences.
- How it works:
  - Separate the two strands with low heat
  - Add some base pairs, primer sequences, and DNA Polymerase
    - Creates double stranded DNA from a single strand.
    - Primer sequences create a seed from which double stranded DNA grows.
  - Now you have two copies.
  - Repeat. Amount of DNA grows exponentially.
    - 1→2→4→8→16→32→64→128→256…



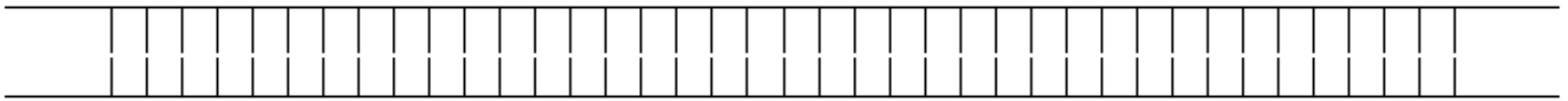
Polymerase Chain Reaction

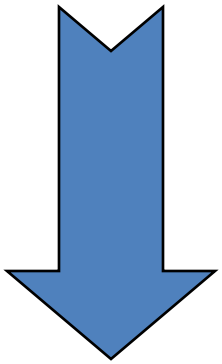
- **Problem**: Modern instrumentation cannot easily detect single molecules of DNA, making amplification a prerequisite for further analysis
- **Solution**: PCR doubles the number of DNA fragments at every iteration



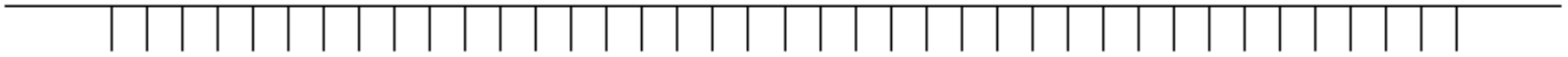
1… 2… 4… 8…

Denaturation



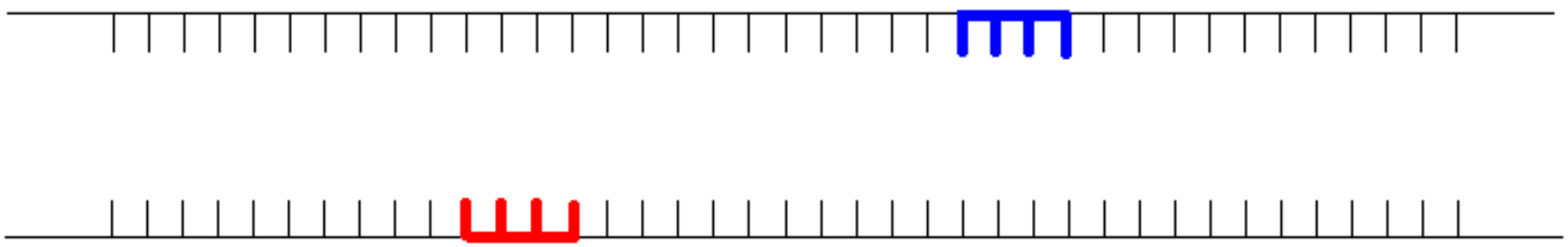


Raise temperature to 94^{o}C to separate the duplex form of DNA into single strands



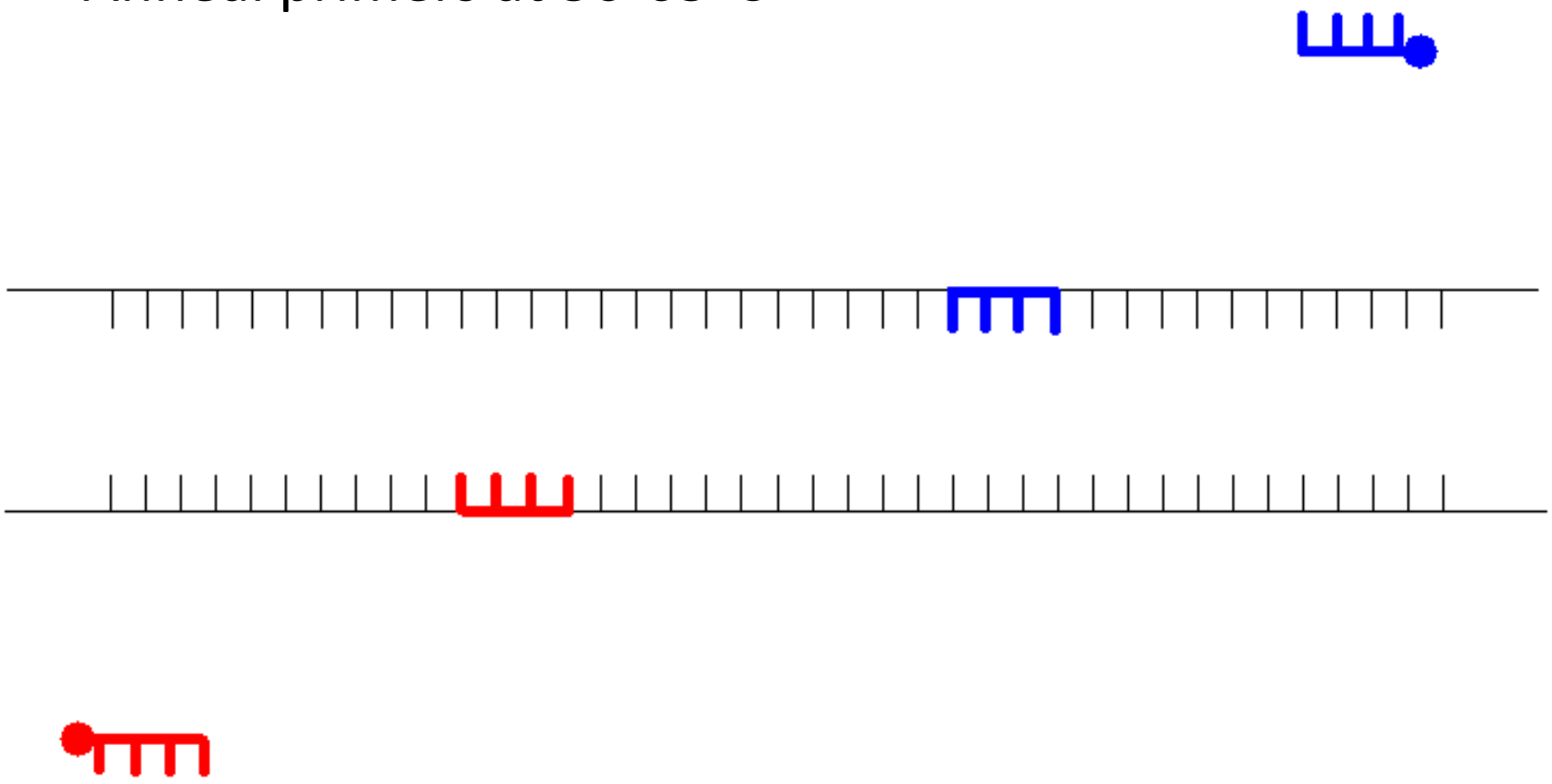
Design primers

- To perform PCR, a 10-20bp sequence on either side of the sequence to be amplified must be known because DNA polymerase requires a primer to synthesize a new strand of DNA



Annealing

- Anneal primers at 50-65^{o}C



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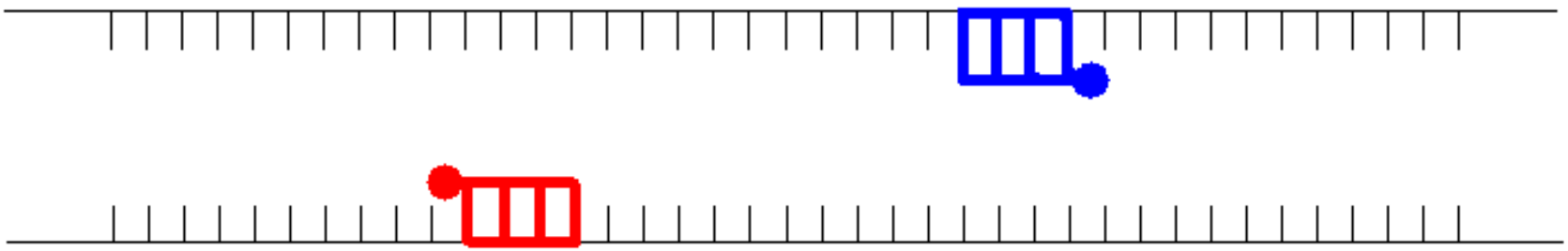
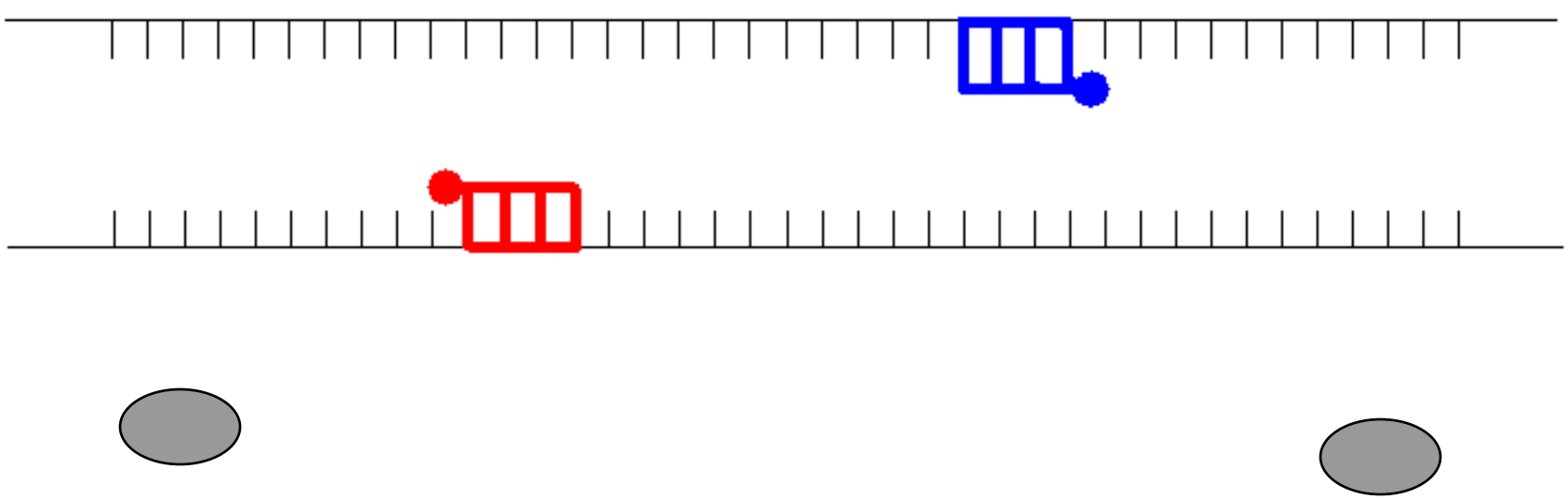


Extension

- Extend primers: raise temp to 72^{o}C, allowing Taq pol to attach at each priming site and extend a new DNA strand



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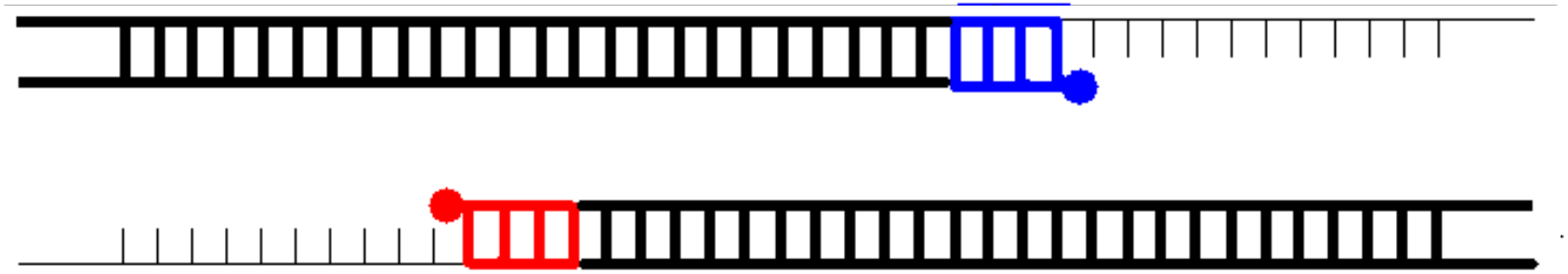
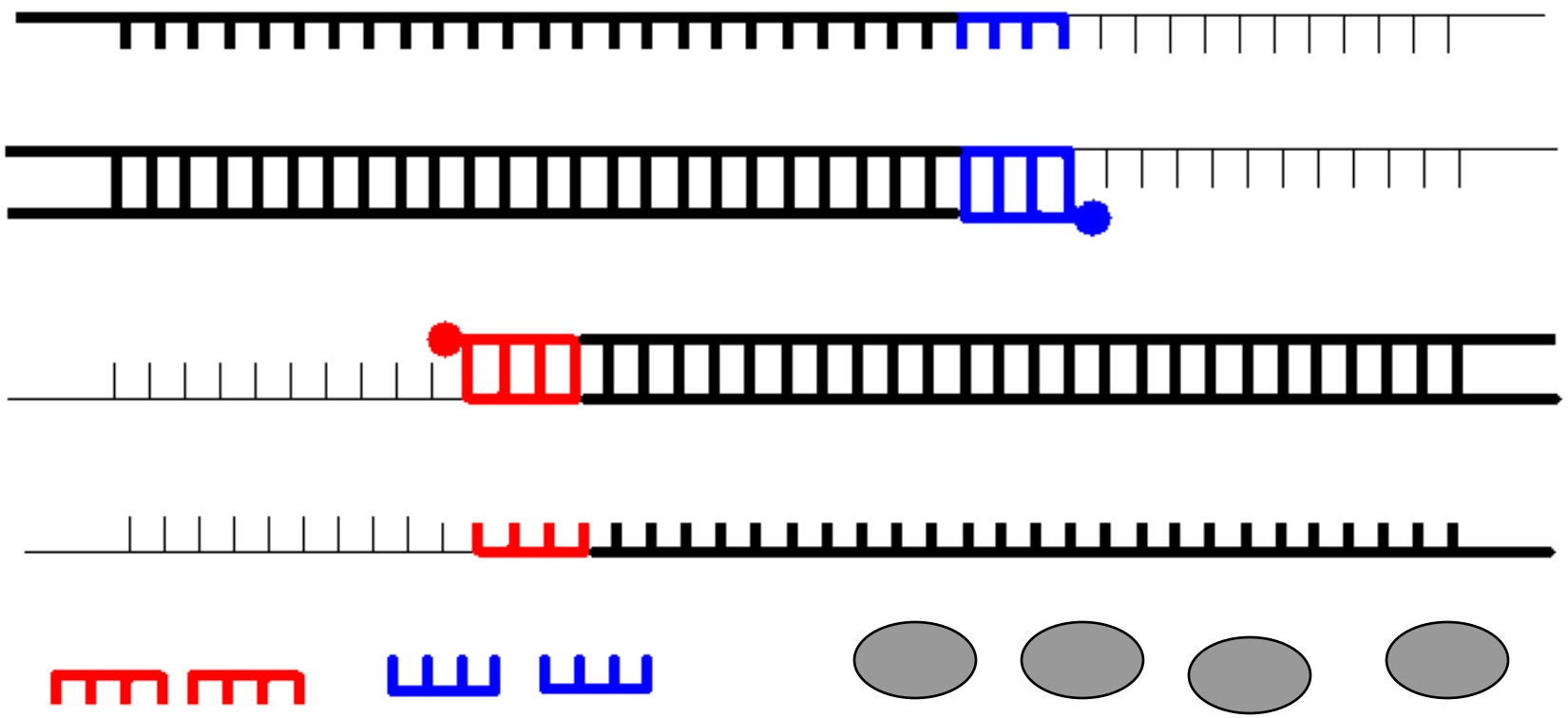


Repeat

- Repeat the Denature, Anneal, Extension steps at their respective temperatures…



Polymerase Chain Reaction