Polymerase Chain Reaction (PCR)

- PCR = amplify a particular piece of DNA
    - *Amplify= making numerous copies of a segment of DNA*
- PCR can make ***billions*** of copies of a target sequence of DNA in a few hours
- PCR was invented in the 1984 as a way to make numerous copies of DNA fragments in the laboratory
- Its applications are vast and PCR is now an integral part of Molecular Biology

DNA Replication vs. PCR

- PCR is a laboratory version of DNA Replication in cells
  - The laboratory version is commonly called "*in vitro*" since it occurs in a test tube while "*in vivo*" signifies occurring in a living cell.

Key enzymes involved in DNA Replication

- DNA Polymerase
- DNA Ligase
- Primase
- Helicase
- Topoisomerase
- Single strand binding protein

PCR: the *in vitro* version of DNA Replication

The following components are needed to perform PCR in the laboratory:

1) DNA (your DNA of interest that contains the target sequence you wish to copy)
2) A heat-stable DNA Polymerase (like Taq Polymerase)
3) All four nucleotide triphosphates
4) Buffers +Mgcl2
5) Two short, single-stranded DNA molecules that serve as primers
6) Thin walled tubes
7) Thermal cycler (a device that can change temperatures dramatically in a very short period of time)

PCR

The DNA, DNA polymerase, buffer, nucleoside triphosphates, and primers are placed in a thin-walled tube and then these tubes are placed in the PCR thermal cycler

PCR Thermocycler

The three main steps of PCR

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- In a PCR reaction, the following series of steps is repeated 20-40 times

  *(*note: 25 cycles usually takes about 2 hours and amplifies the DNA fragment of interest 100,000 fold)

Step 1: **Denature** DNA

At 95°C, the DNA is denatured (i.e. the two strands are separated)

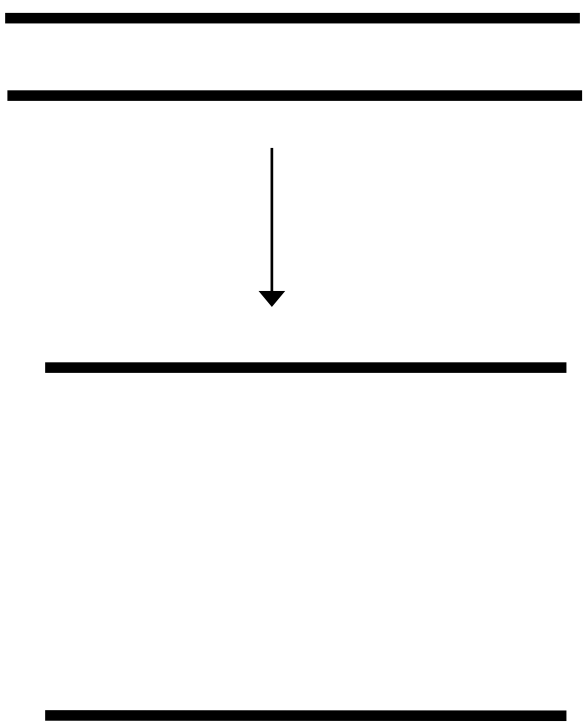
Step 2: Primers **Anneal**

At 40°C- 65°C, the primers anneal (or bind to) their complementary sequences on the single strands of DNA

Step 3: DNA polymerase **Extends** the DNA chain

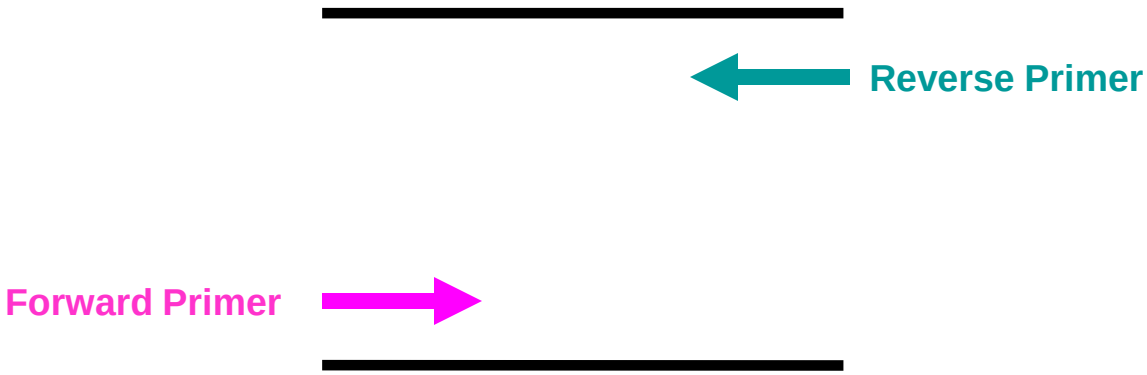
At 72°C, DNA Polymerase extends the DNA chain by adding nucleotides to the 3' ends of the primers.

Step 1: Denaturation of DNA



This occurs at 95 ºC mimicking the function of helicase in the cell.

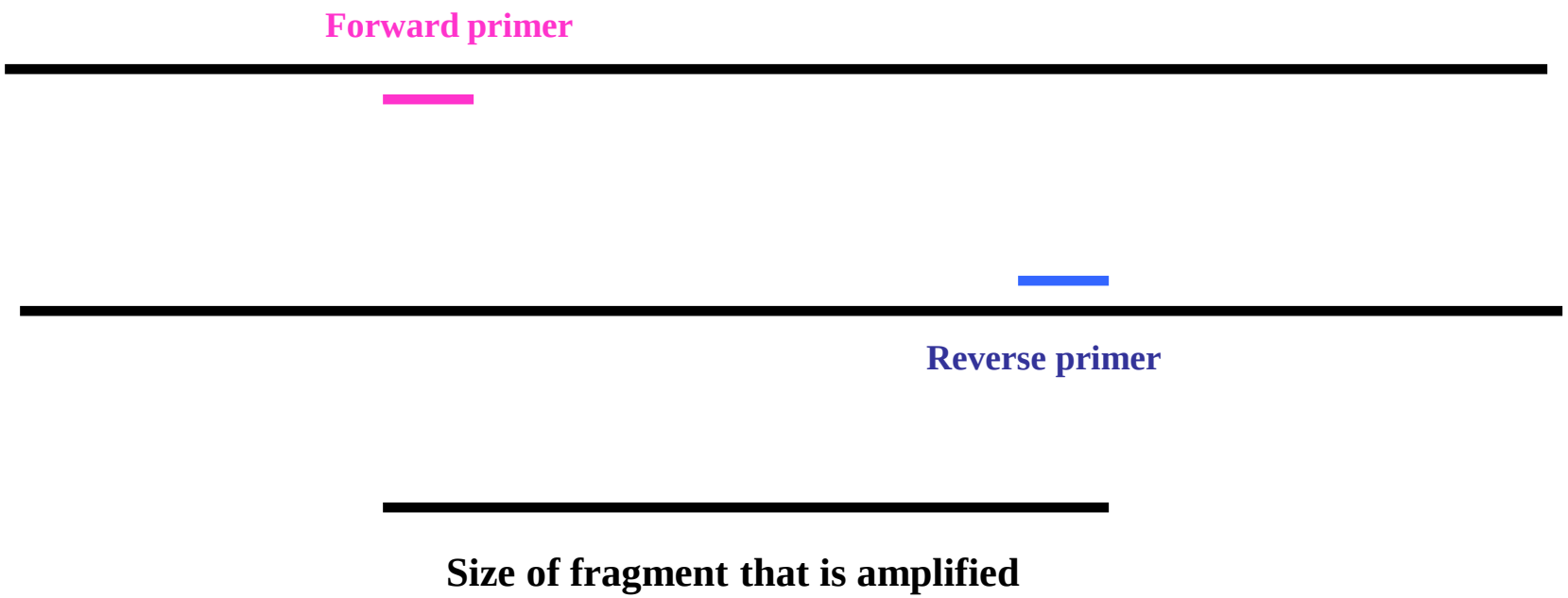
Step 2 Annealing or Primers Binding



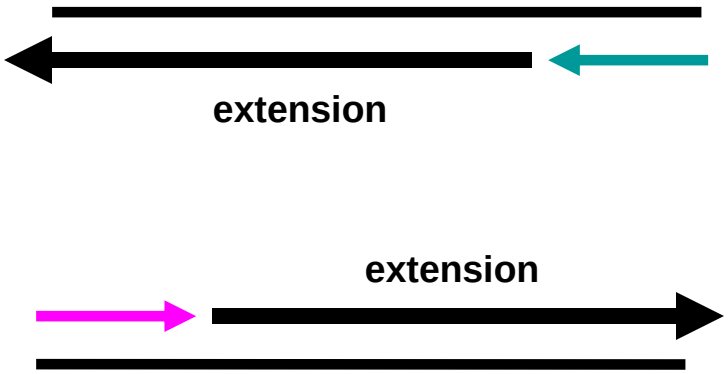
Primers bind to the complimentary sequence on the target DNA. Primers are chosen such that one is complimentary to the one strand at one end of the target sequence and that the *other* is complimentary to the *other* strand at the other end of the target sequence.

The Size of the DNA Fragment Produced in PCR is Dependent on the Primers

- The PCR reaction will amplify the DNA section between the two primers.
- If the DNA sequence is known, primers can be developed to amplify any piece of an organism's DNA.

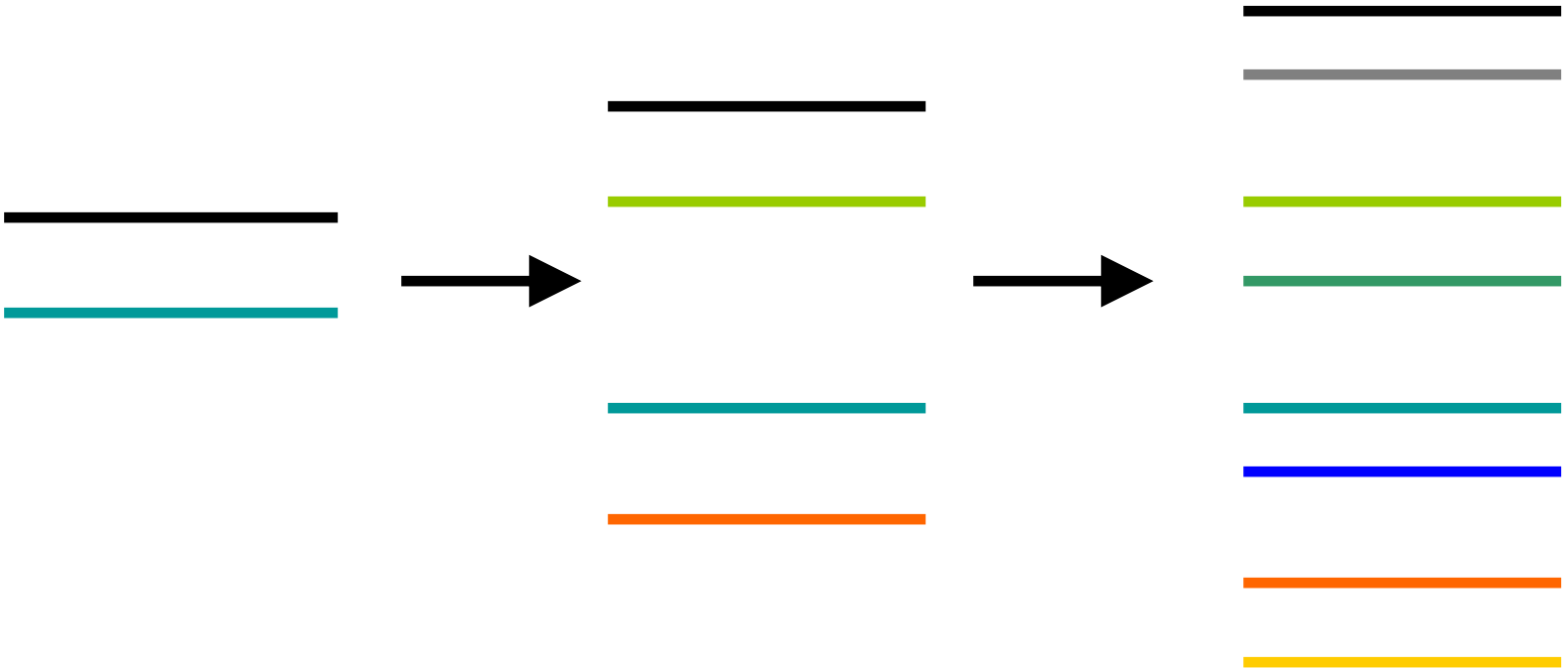


Step 3 Extension or Primer Extension



DNA polymerase catalyzes the extension of the strand in the 5-3 direction, starting at the primers, attaching the appropriate nucleotide (A-T, C-G)

- The next cycle will begin by denaturing the new DNA strands formed in the previous cycle



The DNA of interest is amplified by a power of 2 for each PCR cycle

For example, if you subject your DNA of interest to 5 cycles of PCR, you will end up with 2^5 (or 64) copies of DNA.

Similarly, if you subject your DNA of interest to 40 cycles of PCR, you will end up with 2^{40} (or) copies of DNA!

Heat-stable DNA Polymerase

- Given that PCR involves very high temperatures, it is imperative that a heat-stable DNA polymerase be used in the reaction.
    - Most DNA polymerases would denature (and thus not function properly) at the high temperatures of PCR.
- Taq DNA polymerase was purified from the hot springs bacterium *Thermus aquaticus* in 1976
- Taq has maximal enzymatic activity at 75 °C to 80 °C, and substantially reduced activities at lower temperatures.

Watch this!!

https://www.youtube.com/watch?v=iQsu3Kz9NYo

RT-PCR

Dr. Suheir Ereqat 2019/2012

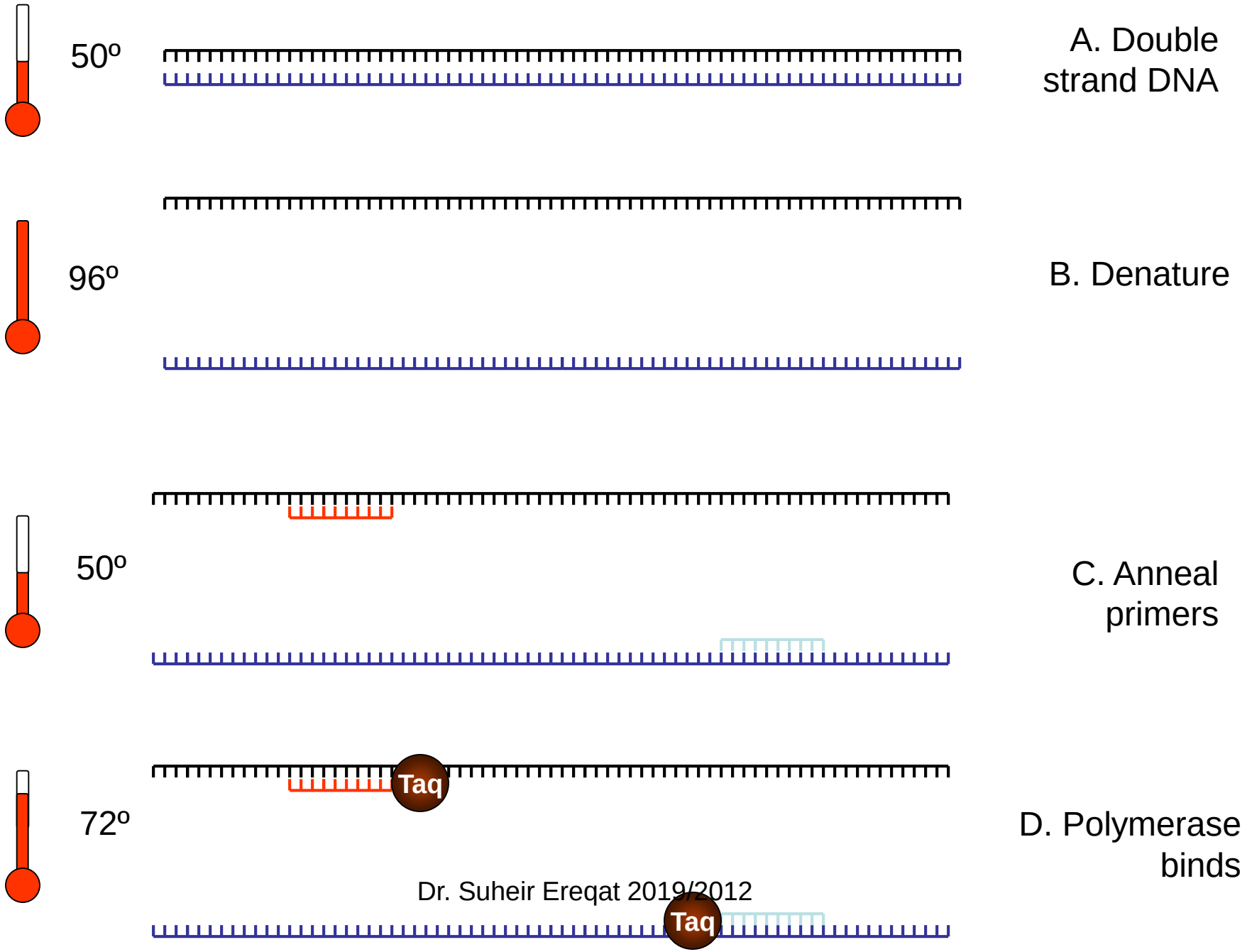
Oligo dT primer is bound to mRNA

Reverse transcriptase (RT) copies first cDNA strand

Reverse transcriptase digests and displaces mRNA and copies second strand of cDNA

Double strand cDNA

Conversion of mRNA to cDNA by Reverse Transcription


50º
A. Double strand DNA
96º
B. Denature
50º
C. Anneal primers
72º
Taq
D. Polymerase binds
Taq

Restriction Enzymes

Dr. Suheir Ereqat 2019/2012

Restriction Endonucleases

Also called restriction enzymes

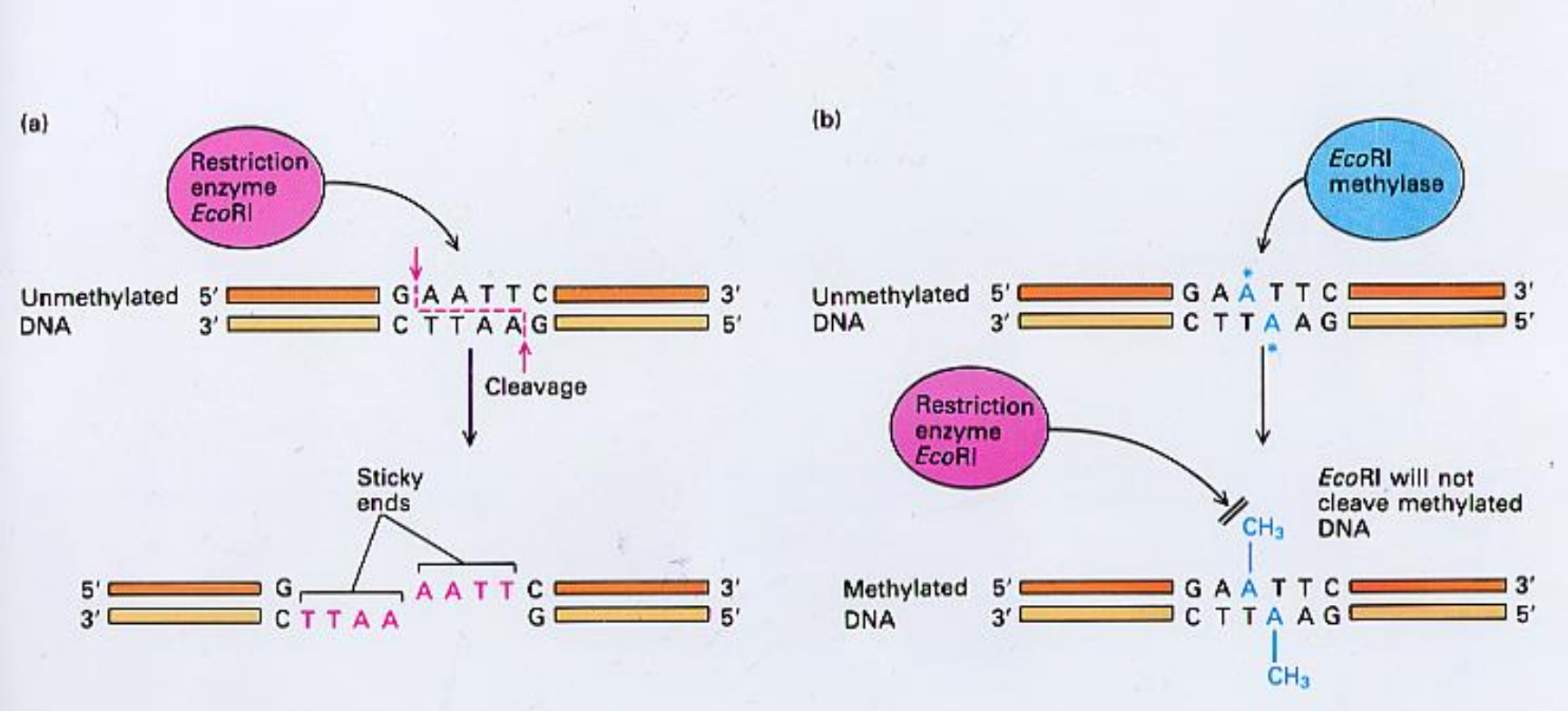
1962: “molecular scissors” discovered in in bacteria

Restriction enzymes are found in bacteria. Bacteria use restriction enzymes to kill viruses – the enzymes attack the viral DNA and break it into useless fragments

3,000 enzymes have been identified, many are purified and available commercially

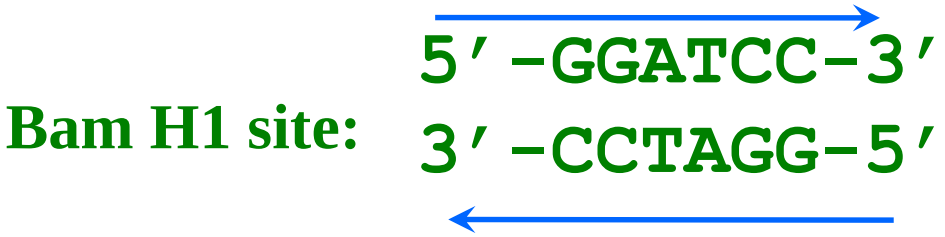
Why don’t bacteria destroy their own DNA with their restriction enzymes?

Methylation



Restriction Endonucleases

Recognition sites have symmetry (palindromic)

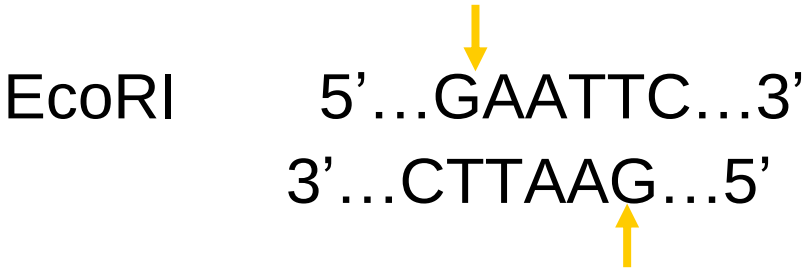


Restriction Endonucleases

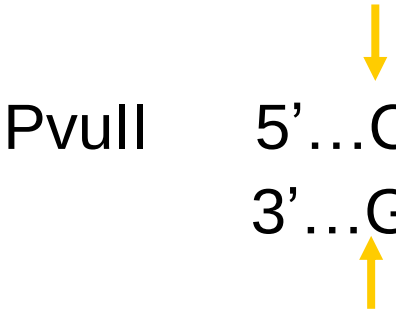
Enzymes recognize specific **4-8** bp sequences

Some enzymes cut in a staggered fashion - “sticky ends”

cohesive ends



Some enzymes cut in a direct fashion – “blunt ends”

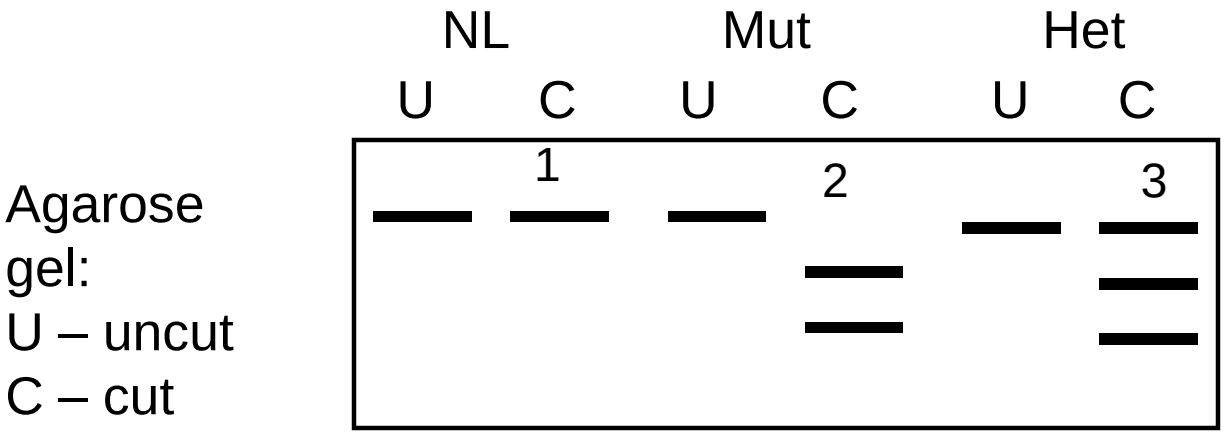


Restriction Fragment Length Polymorphism (RFLP)

- Restriction enzyme site recognition detects presence of sequence changes.

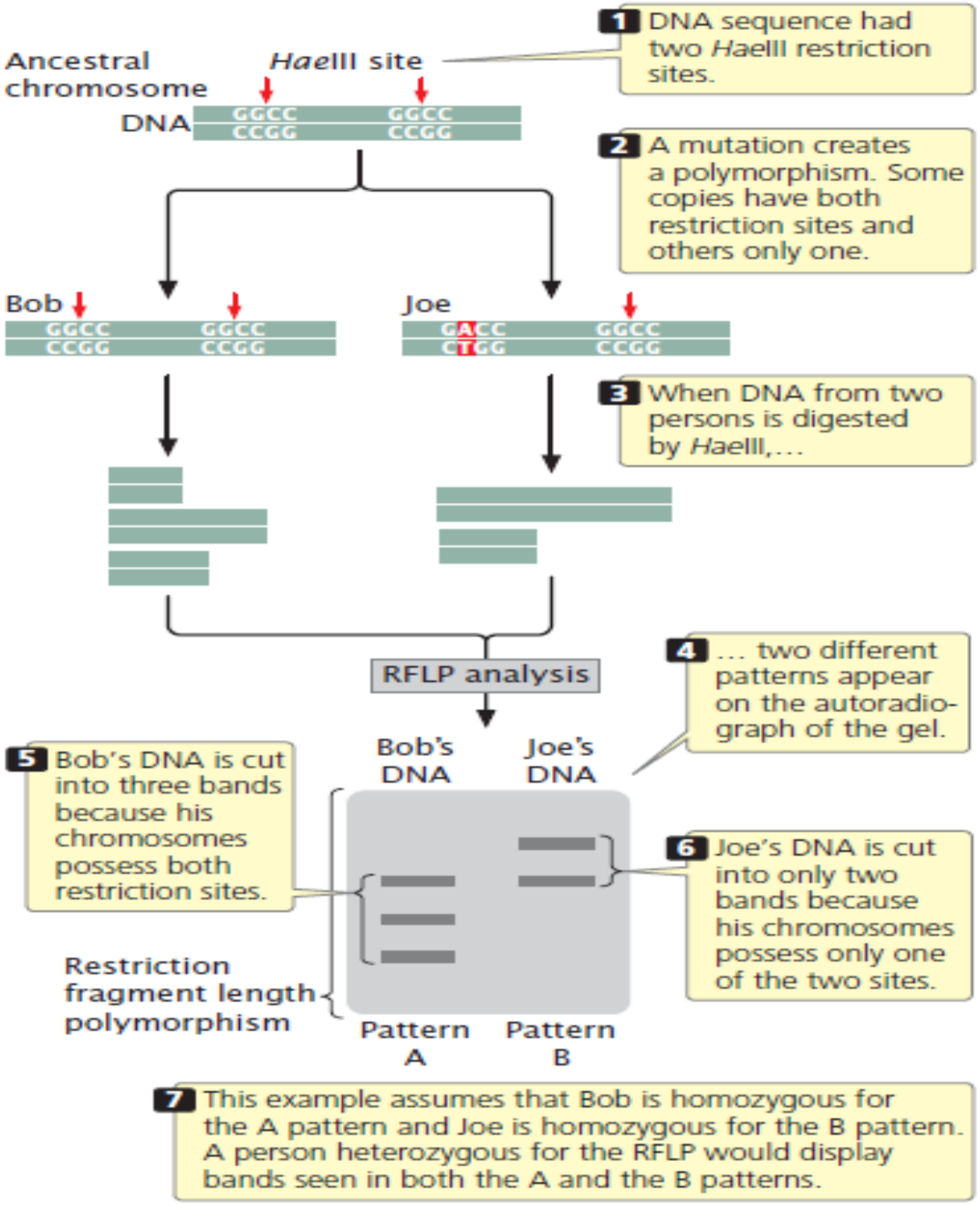
  e.g., G->A change creates *EcoR1* site:

NL: … GTCA GA**G**TTC GTGC…
Mut: … GTCA GAATTC CTGC…



Detection of mutation

RFLP



19.22 Restriction fragment length polymorphisms are genetic markers that can be used in mapping.