

Molecular basis of inheritance & DNA Tools

Lecture – 6

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Outline

- Molecular basis of Inheritance
- Eukaryotic genome and chromosomal abnormalities
- DNA Tools & Biotechnology



Molecular basis of Inheritance

- Molecular basis of Inheritance
- Griffith, Hershey & Chase; Meselson & Stahl
- Genome & chromosomal abnormalities
- DNA Tools & Biotechnology

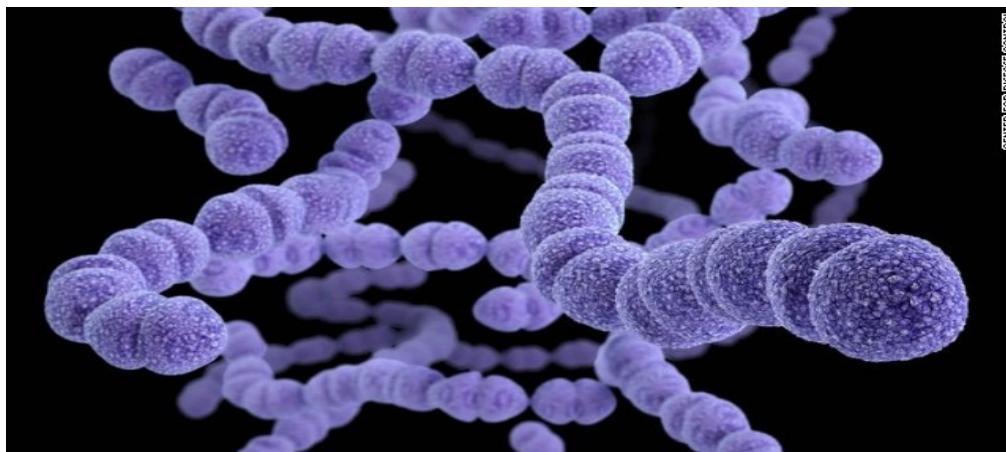
Can a genetic trait be transferred between different bacterial strains?

Griffith's Experiment (1928): The 'Transforming principle/factor'

In 1928 while developing a vaccine for pneumonia...



Frederick Griffith
(1879-1961)
British medical officer
and bacteriologist



- *Streptococcus pneumoniae* is a bacterium
- It causes pneumonia in mammals
- Griffith was trying to develop a vaccine
- By this time, some “biomolecules” were known: DNA, RNA, proteins
- **But which of them is the genetic material?**

Shown above is a digitally colorized image of Streptococci. <https://bioweb.uwlax.edu/> 5

Different strains of *S. pneumoniae*

What are strains? Same organism having different stable phenotypes

Strain S – appears smooth

- Has an outer capsule that protects from the defense system of mice
- Pathogenic strain
- Causes pneumonia in mice

Strain R – appears rough

- Lacks the outer capsule
- Non-pathogenic strain
- Does NOT cause pneumonia in mice



<http://www.planetsrk.com/>

Strain S CANNOT become Strain R and vice versa

Unlike Mendel, who showed inheritance between generations, Griffith showed transfer of a trait between bacteria

Can a genetic trait be transferred between different bacterial strains?

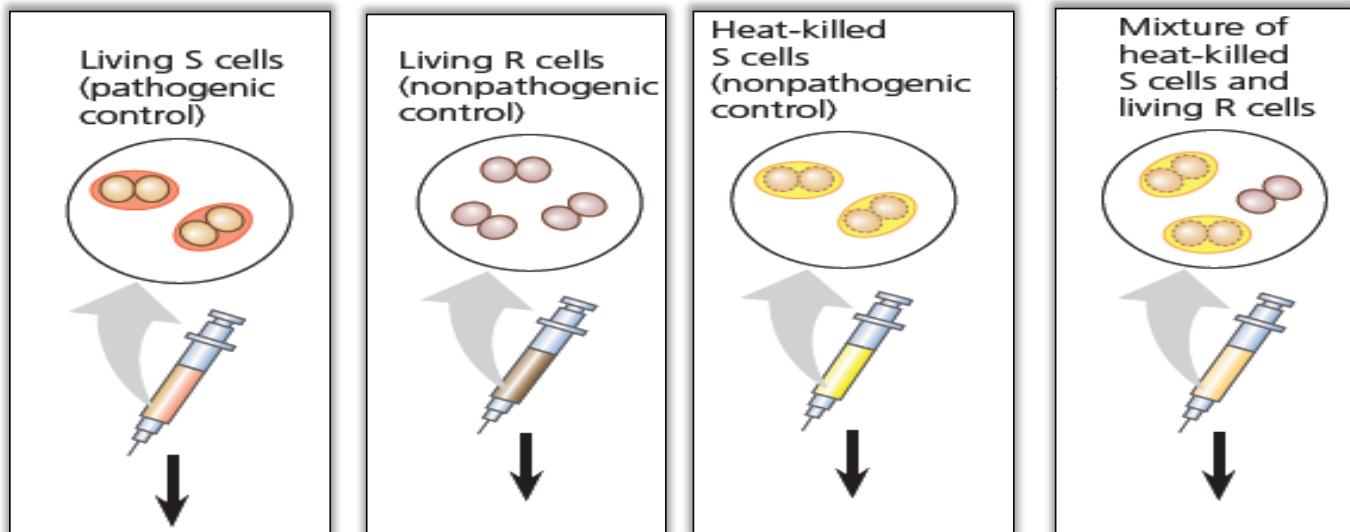
Streptococcus pneumoniae

S strain (Smooth strain - presence of a capsule) causing pneumonia

R strain (Rough strain - absence of a capsule) not causing pneumonia

Transformation of R cells by a heritable substance from S cells

Transformation - change in genotype and phenotype due to assimilation of external DNA by a cell

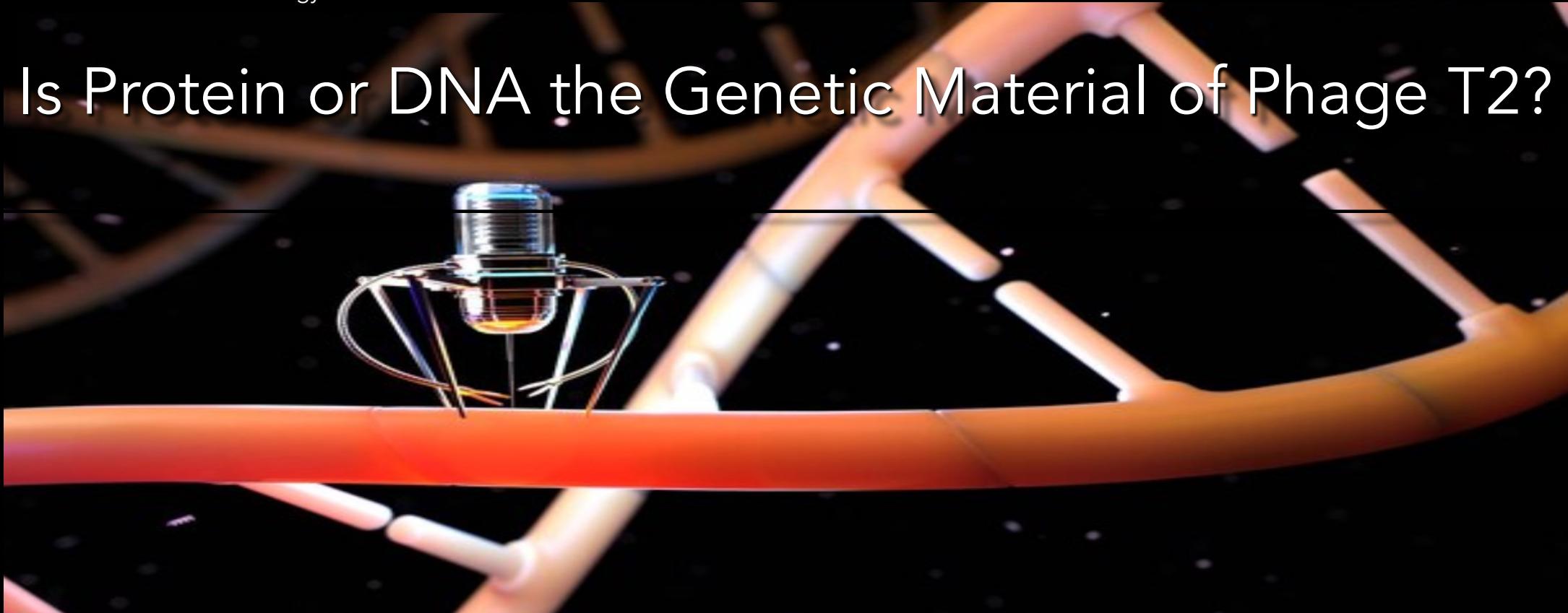


Living R bacteria was transformed into pathogenic S bacteria by a heritable substance from dead S cells that enabled R cells to make capsules

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Is Protein or DNA the Genetic Material of Phage T2?



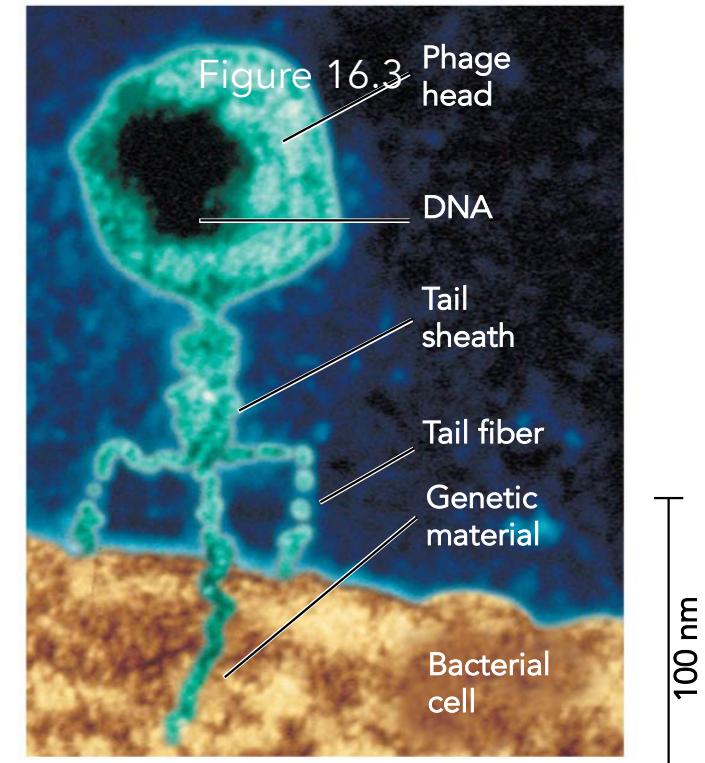
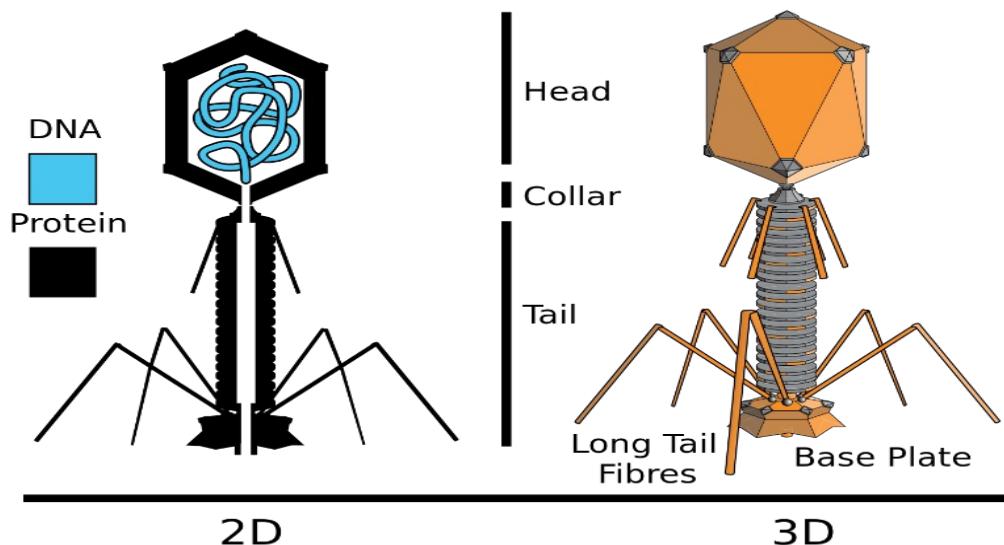
Alfred Hershey and Martha Chase used radioactive sulfur and phosphorus to trace the fates of protein and DNA, respectively, of T2 phages that infected bacterial cells

Passing on genetic information by a virus

Phages – viruses that infect bacteria

Phage T2

- Is attached to its host cell
- Is injecting the genetic material
- Head and tail parts remain outside the host



Colorized transmission
electron micrograph

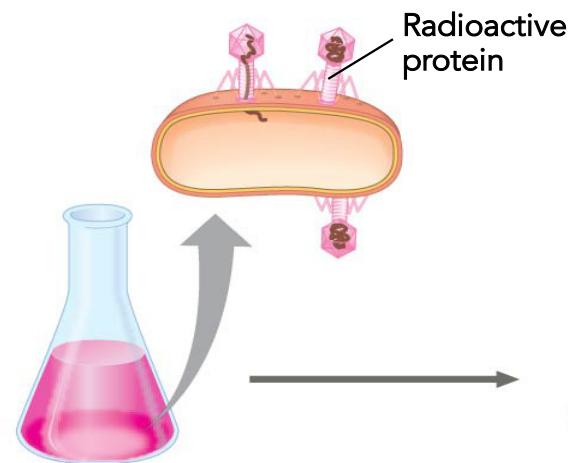
In 1952, Alfred Hershey & Martha Chase asked “What is the genetic material of T2 phage ?”

Taking advantage of S in proteins, P in DNA

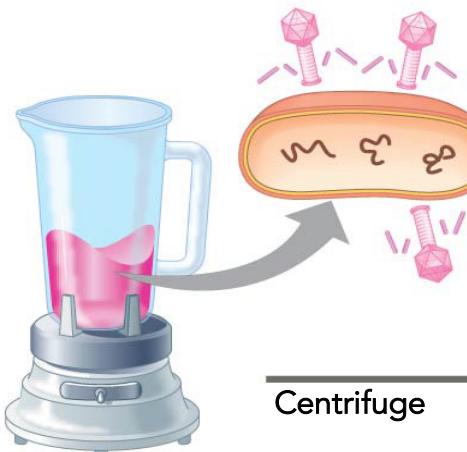
Expectation: genetic material will be found in the host cells

Batch 1: Radioactive sulfur (^{35}S) in phage protein

- 1 Labeled phages infect bacterial cells.

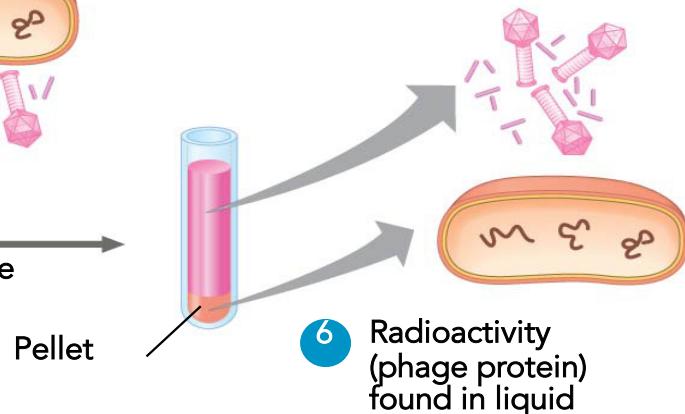


- 2 Agitation frees outside phage parts from bacterial cells.



- 3 Centrifuged bacterial cells form a pellet.

Radioactive sulfur was not found in the bacterial pellet

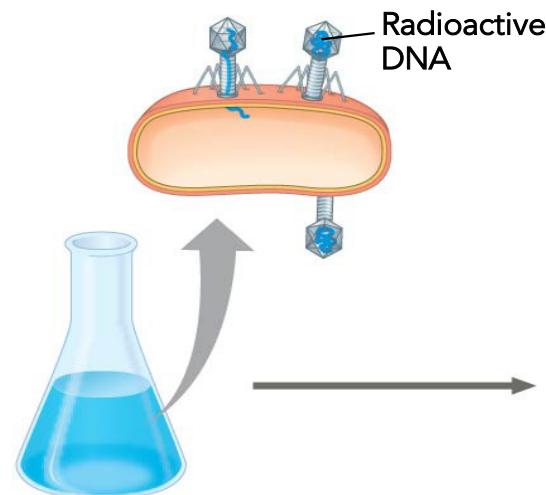


- 6 Radioactivity (phage protein) found in liquid

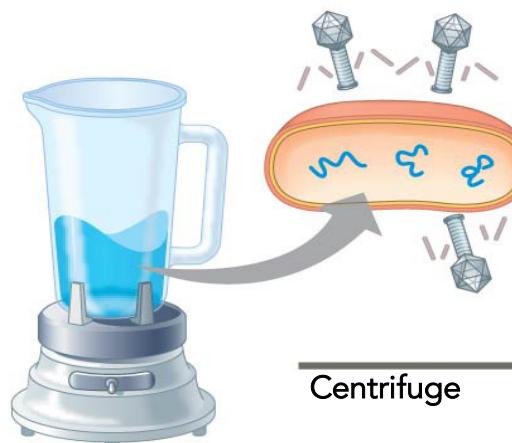
DNA is the genetic material

Batch 2: Radioactive phosphorus (^{32}P) in phage DNA

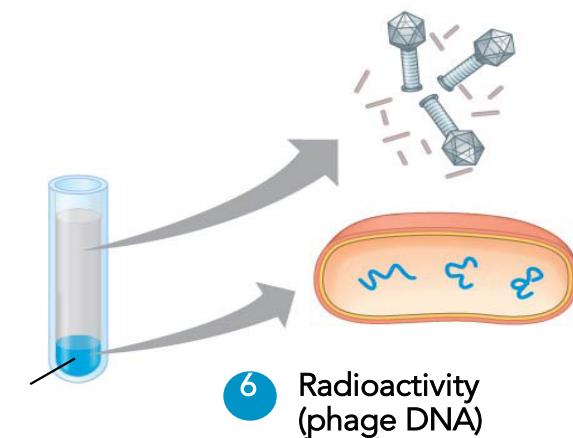
1 Labeled phages infect bacterial cells.



2 Agitation frees outside phage parts from bacterial cells.



3 Centrifuged bacterial cells form a pellet.



Radioactive phosphorus was found in the bacterial pellet

6 Radioactivity (phage DNA)

- When proteins were labeled (batch 1), radioactivity remained outside cells, when DNA was labeled (batch 2), radioactivity was found inside cells.
- Bacterial cells containing radioactive phage DNA released new phages with some radioactive phosphorus.
- Conclusion - Phage DNA entered bacterial cells, but phage proteins did not

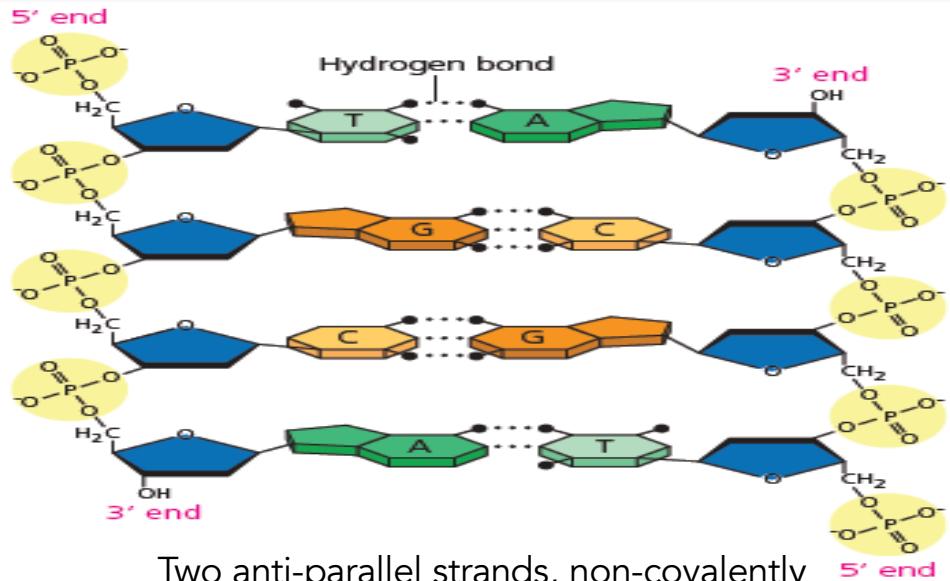
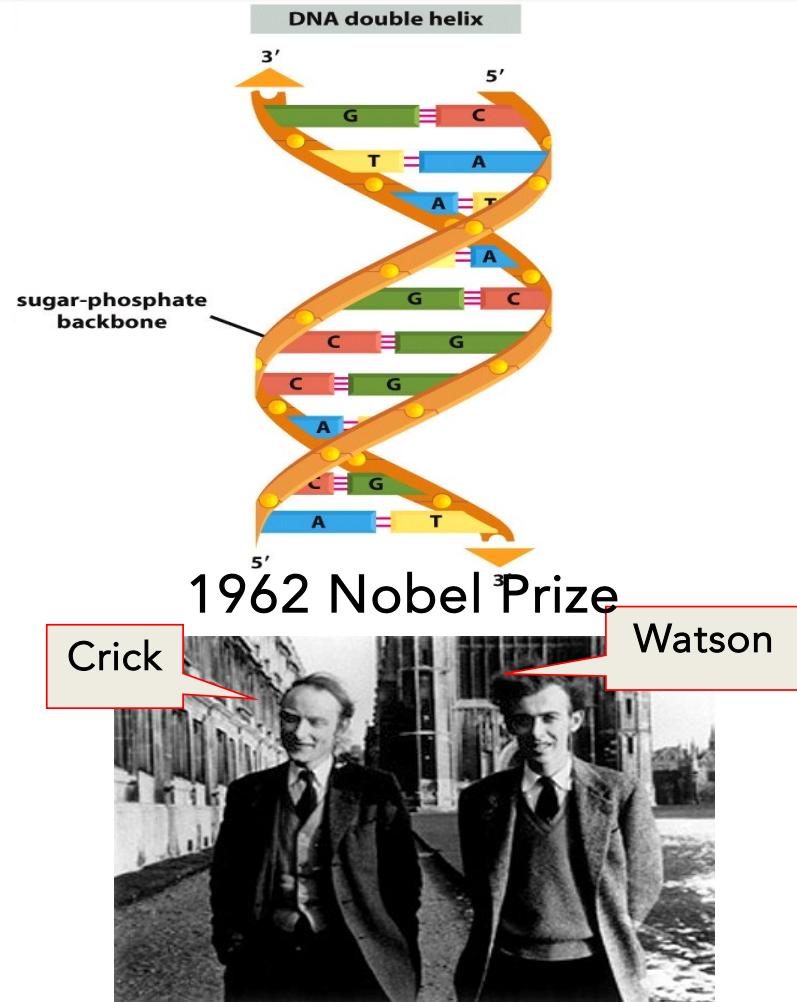
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Chemical Composition of DNA & DNA Replication

D N A
deoxyribonucleic acid



Chemical Composition of DNA



Two anti-parallel strands, non-covalently attached to each other

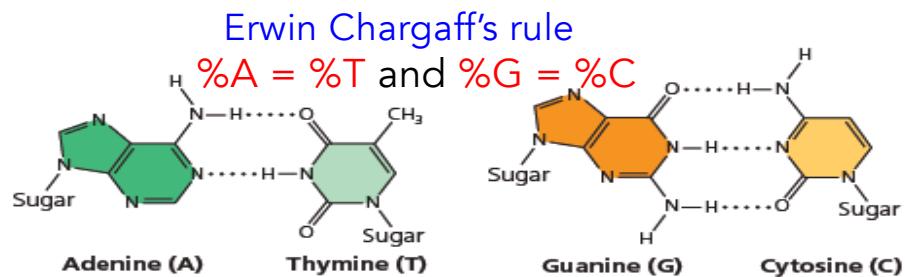


Figure 16.8

Meselson and Stahl: Experimental evidence for DNA replication

Matt Meselson and Frank Stahl



www.netxplica.com/

Designed an innovative experiment

Question: DNA replication follows what mechanism?

Novelty: exploiting the availability of a heavy isotope of nitrogen

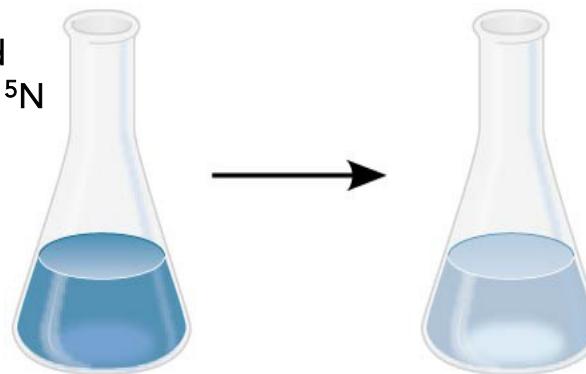


Does DNA replication follow the Conservative,
Semi-conservative or Dispersive model?

Meselson and Stahl experiment: Design

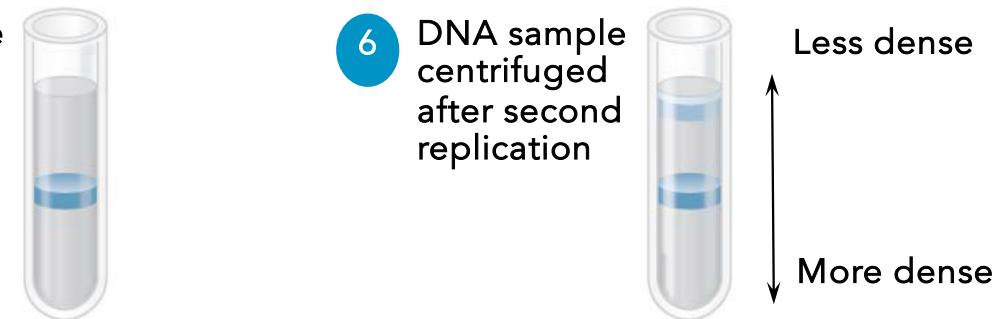
Experiment

- 1 Bacteria cultured in medium with ^{15}N (heavy isotope)
- 2 Bacteria transferred to medium with ^{14}N (lighter isotope)



Results

- 3 DNA sample centrifuged after first replication
- 6 DNA sample centrifuged after second replication



Meselson and Stahl experiment: Expectations and Results

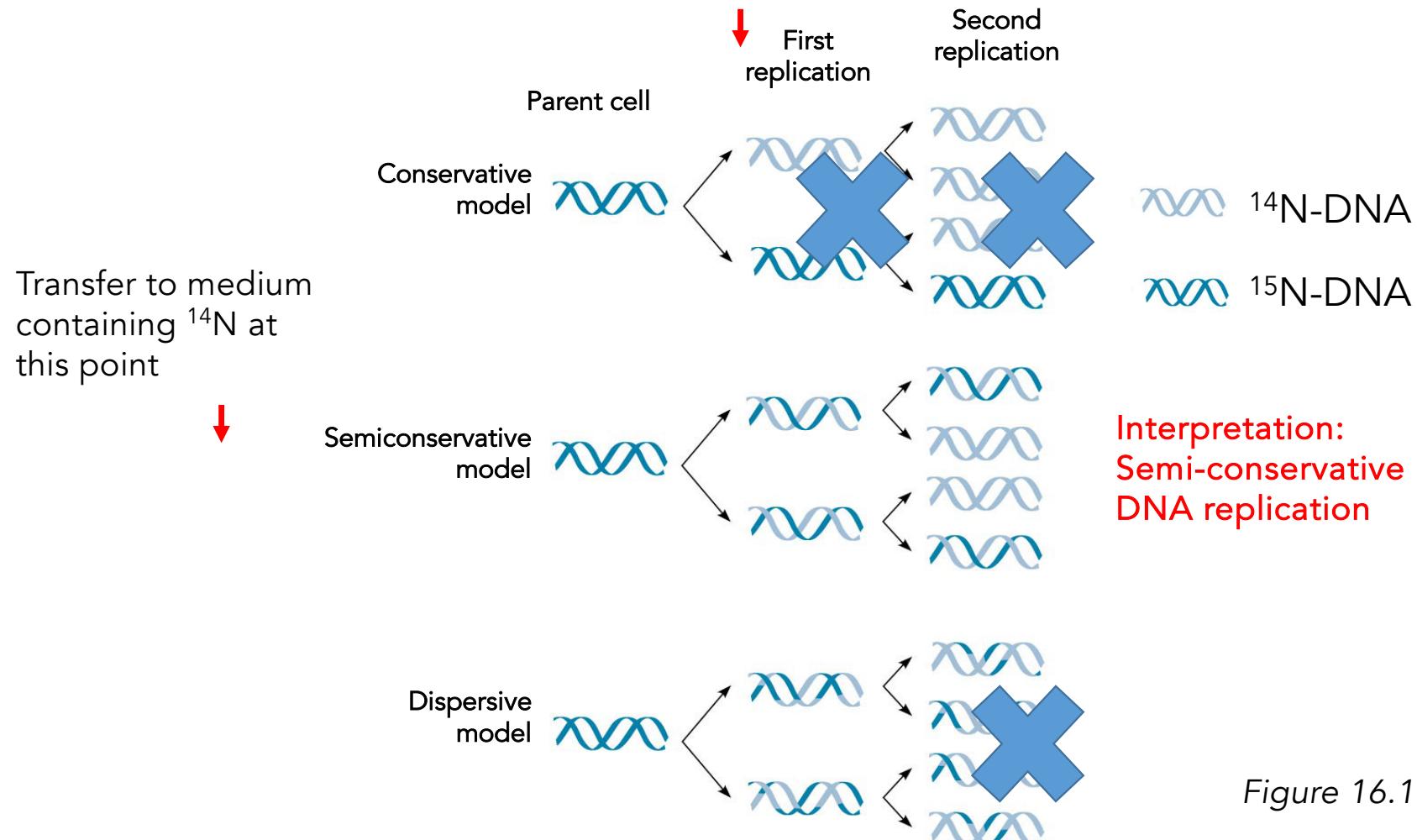
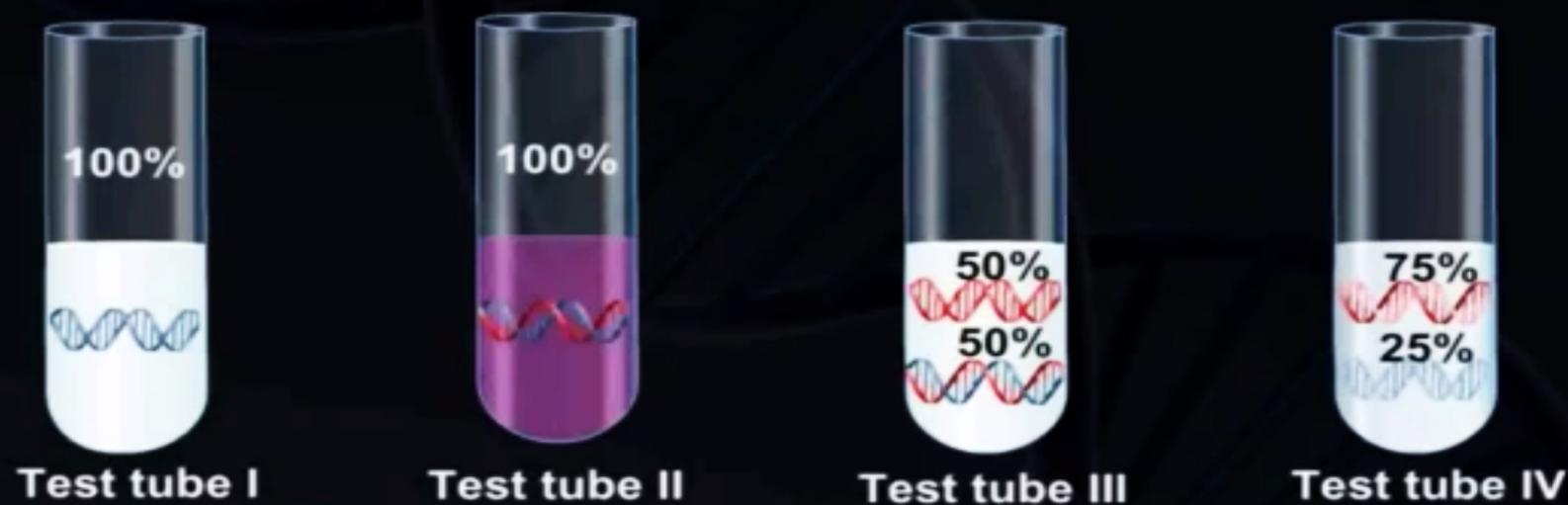


Figure 16.11

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So, on the basis of evidence provided by this experiment it was concluded that the process of replication is semiconservative.

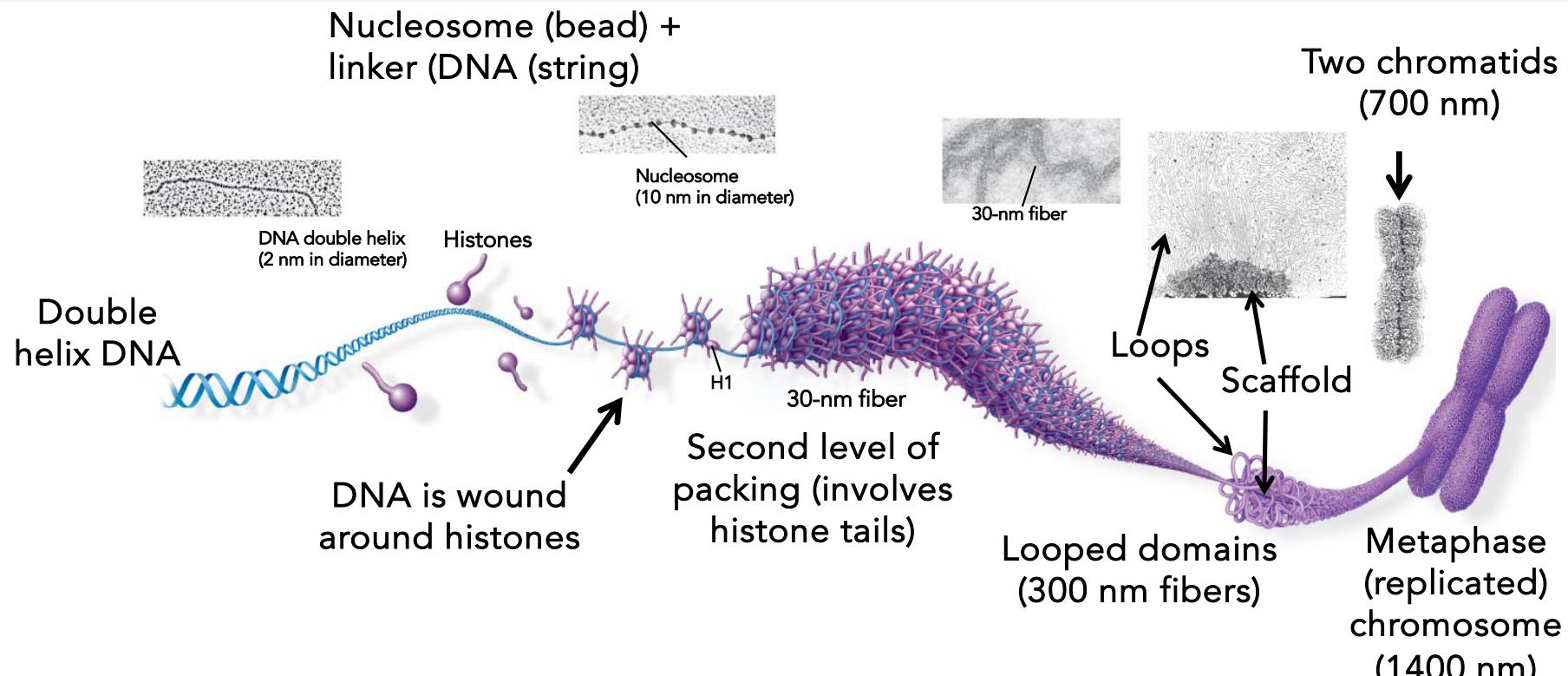


DNA Replication

- Molecular basis of Inheritance
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- **Genome & chromosomal abnormalities**
- DNA Tools & Biotechnology



The DNA polymer is long and needs to be compacted



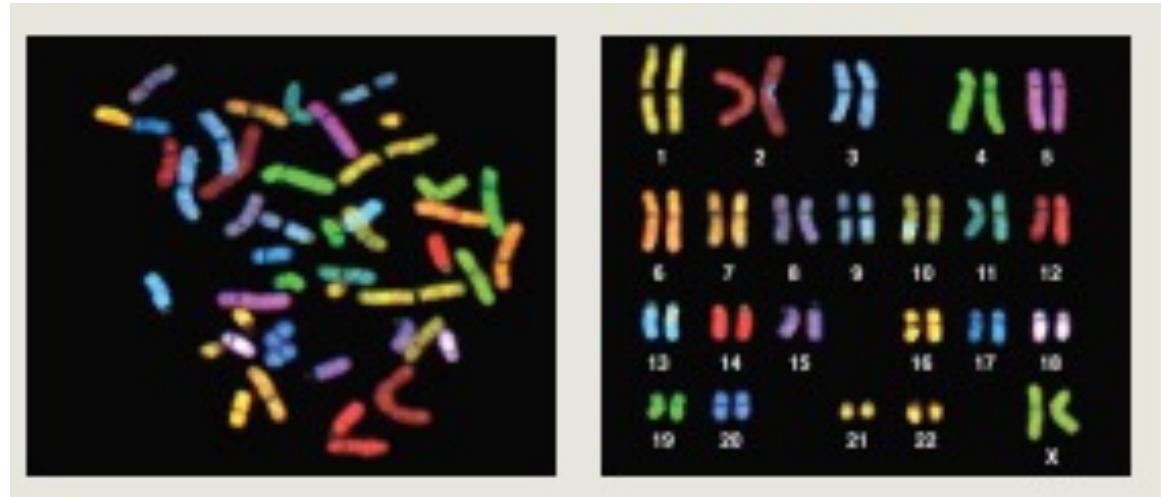
Human chromosomes: end to end they are ~ 2 meters long

3 billion base pairs with 0.34 nm between each base = 1 meter per single genome
(2 copies of each chromosome)

Eukaryotic genomes are organized into chromosomes

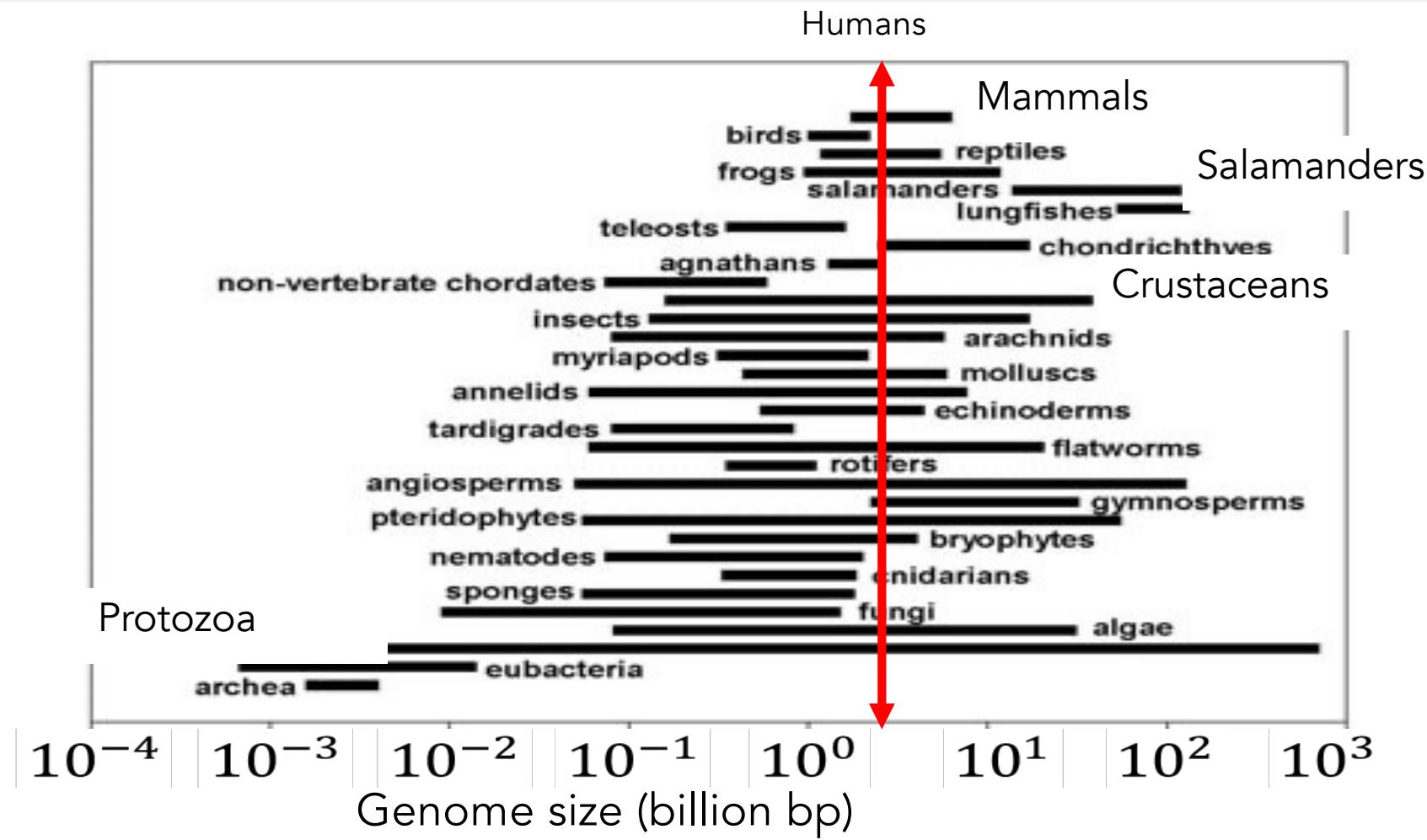
Humans have 23 pairs of chromosomes: 22 pairs of autosomes and one pair of sex chromosomes (X and Y) [similar to Mendel's pea plants]

The image below shows a spread of human chromosomes, each 'painted' with a different color; on the right these are organized into a karyotype



Karyotypes can tell us about diseases such as cancer (chromosome aberrations/translocations), disorders such as Down's syndrome (Trisomy 21) and sex determination (XX vs XY)

Do humans have the largest genome?



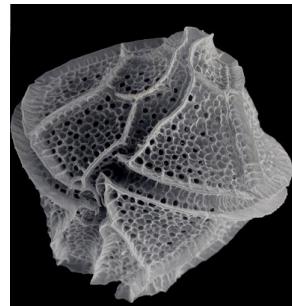
Does genome size correlate with "complexity"?



Human
3 billion bp
(base pairs)



Onion
~16 billion bp



An alga
~98 billion bp



Marbled lungfish
~130 billion bp

Onion: genome size is for *Allium cepa*; may be different for other onion varieties
Can. J. Genet. Cytol. (1983) 25:554 (PMID 6671147)

Can we make an organism with a synthetic instruction manual (genome)?

Science (2016) 351:1414

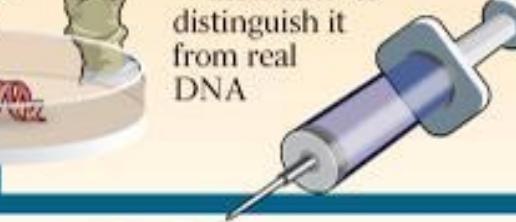
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How scientists created the first artificial life

1. Decode DNA from a bacterium (single-celled organism), in this case *Mycoplasma mycoides*



2. Synthetically create the DNA of the bacterium in the lab and add a "watermark" to distinguish it from real DNA



6. Allow the artificial bacteria to produce proteins



5. Add an antibiotic that kills the bacteria with authentic DNA, but not the bacteria with artificial DNA

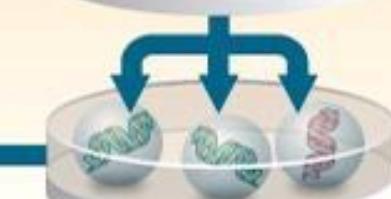


The synthetic genome in each cell contained only 473 key genes thought to be essential for life (half the size of the original genome..)

living bacterium (in this case *Mycoplasma capricolum*) with its own authentic DNA



4. Allow the bacterium, which now contains artificial and authentic DNA, to divide and create "daughter" bacteria, some of which contain artificial DNA and others that contain authentic DNA

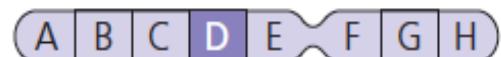


RESULT: The artificial DNA produce proteins from the original bacterium, the *Mycoplasma mycoides*, qualifying as the world's first artificial cell

Graphic: Edi Sizgoric

Alterations of Chromosome Structure

DELETION

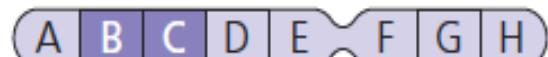


INVERSION



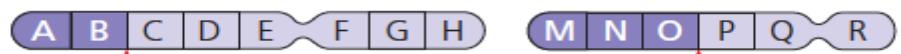
An **inversion** reverses a segment within a chromosome.

DUPLICATION



A **duplication** repeats a segment.

TRANSLOCATION



A **translocation** moves a segment from one chromosome to a nonhomologous chromosome. In a reciprocal translocation, the most common type, nonhomologous chromosomes exchange fragments.



Less often, a nonreciprocal translocation occurs: A chromosome transfers a fragment but receives none in return (not shown).

Figure 15.14

Aneuploidy: Abnormalities in Chromosome Number



Down's Syndrome:
Trisomy 21



Edwards Syndrome:
Trisomy 18

Down's syndrome is usually result of an extra chromosome 21, so that each body cell has a total of 47 chromosomes. Cells are trisomic for chromosome 21, Down syndrome is often called *trisomy 21*



Patau syndrome:
Trisomy 13



Turners Syndrome: Absence of one or both copies of X chromosome

Figure 15.15

Food for thought



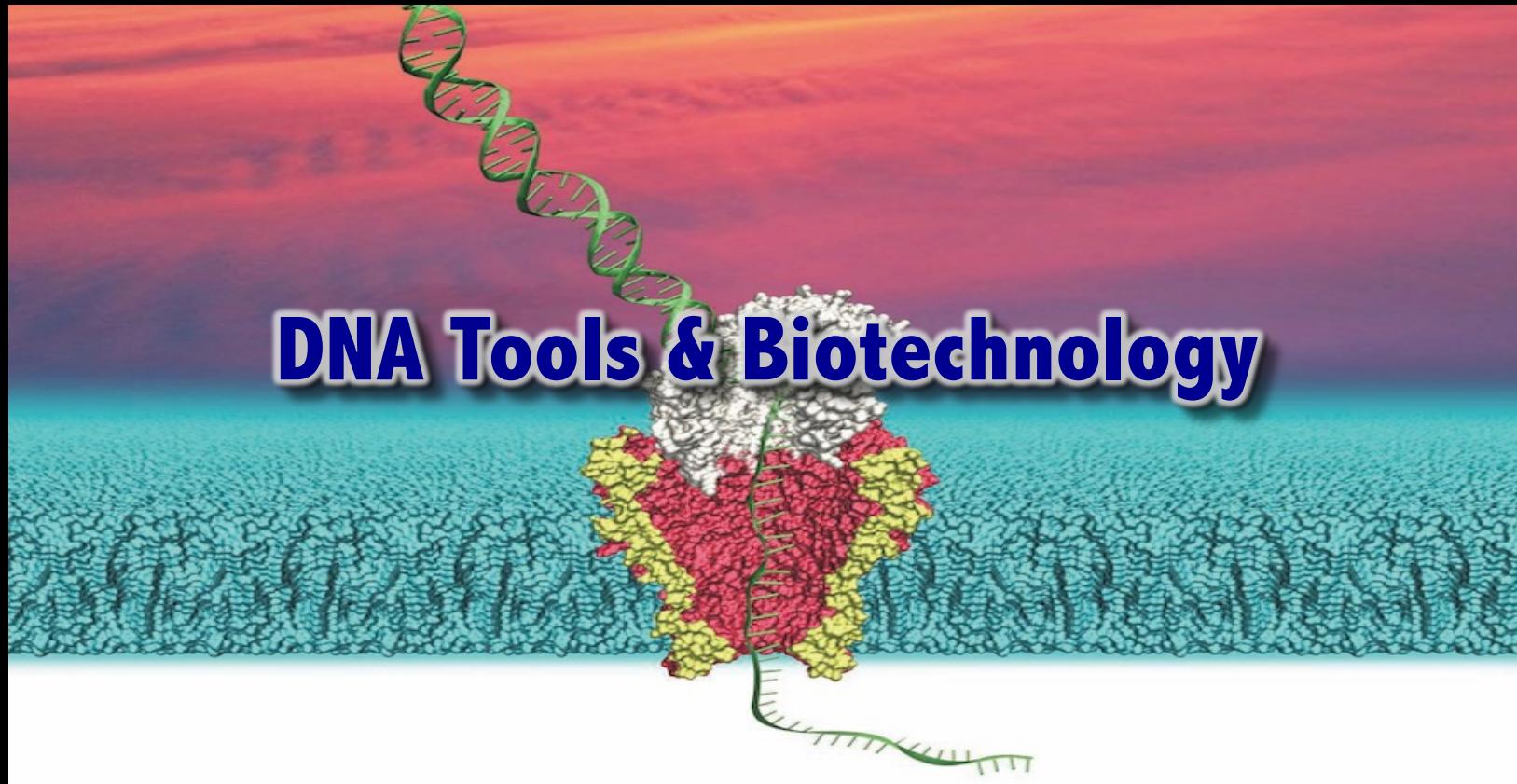
Progeria or Hutchinson-Gilford Progeria Syndrome caused by gene LMNA (or Lamin A).

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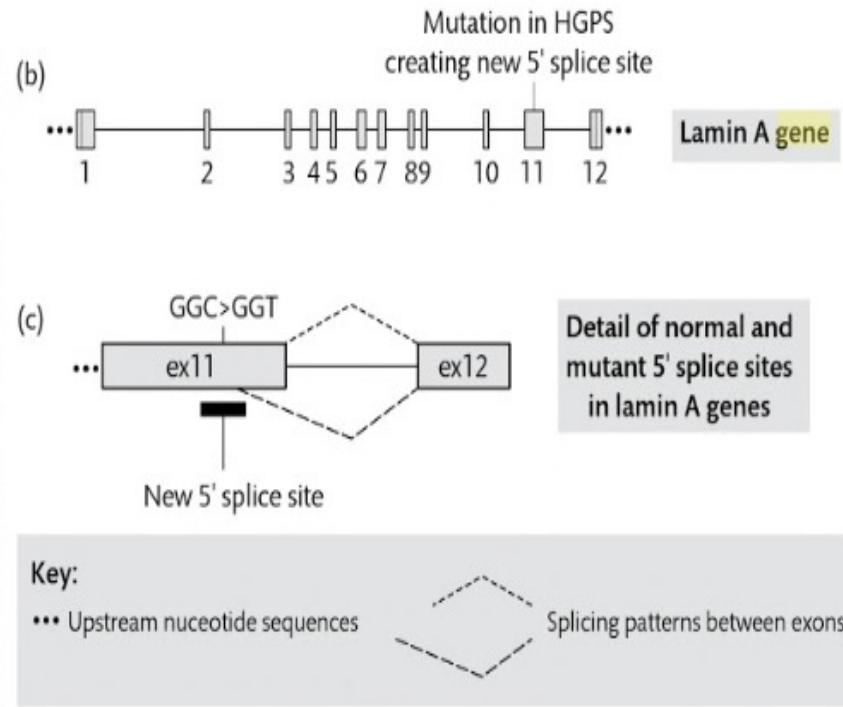
- Genome & chromosomal abnormalities

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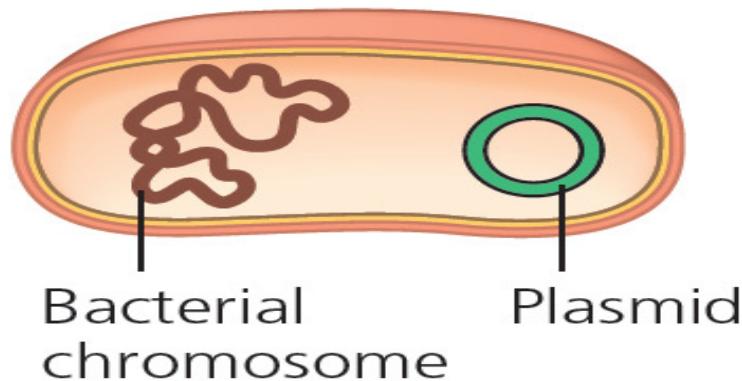
Why DNA Cloning?

Example: Progeria



Generating multiple identical copies of DNA is known as DNA cloning.

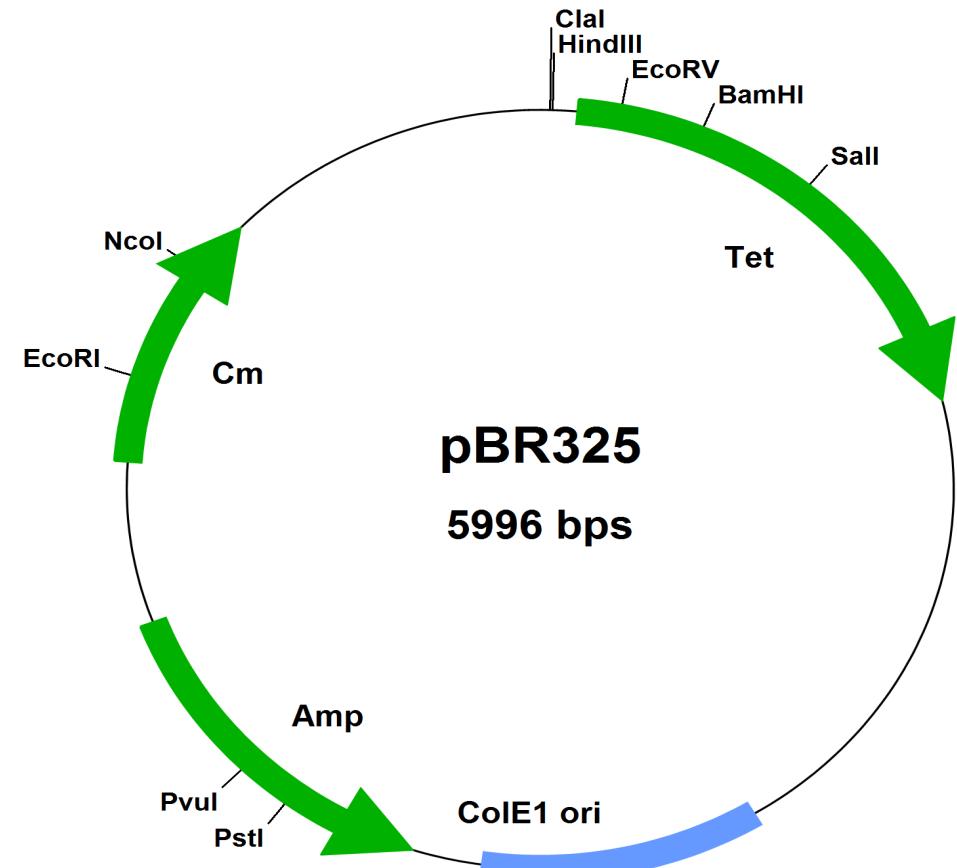
What is a Plasmid?



- Plasmids are extra-chromosomal molecules of DNA that vary in size from 1 kb to more than 200 kb.
- Double stranded, covalently closed, circular molecules found in wide variety of bacterial species.
- They behave as accessory genetic units that replicate and inherit independently of the bacterial chromosome.

Plasmid as Vector

- A cloning vector is a DNA molecule that can carry foreign DNA into a host cell and replicate there.
- pBR322 is a commonly used plasmid cloning vector in *E. coli*.
- It contains the origin of replication and unique restriction sites.
- It also contains the genes for resistance to ampicillin and tetracycline.



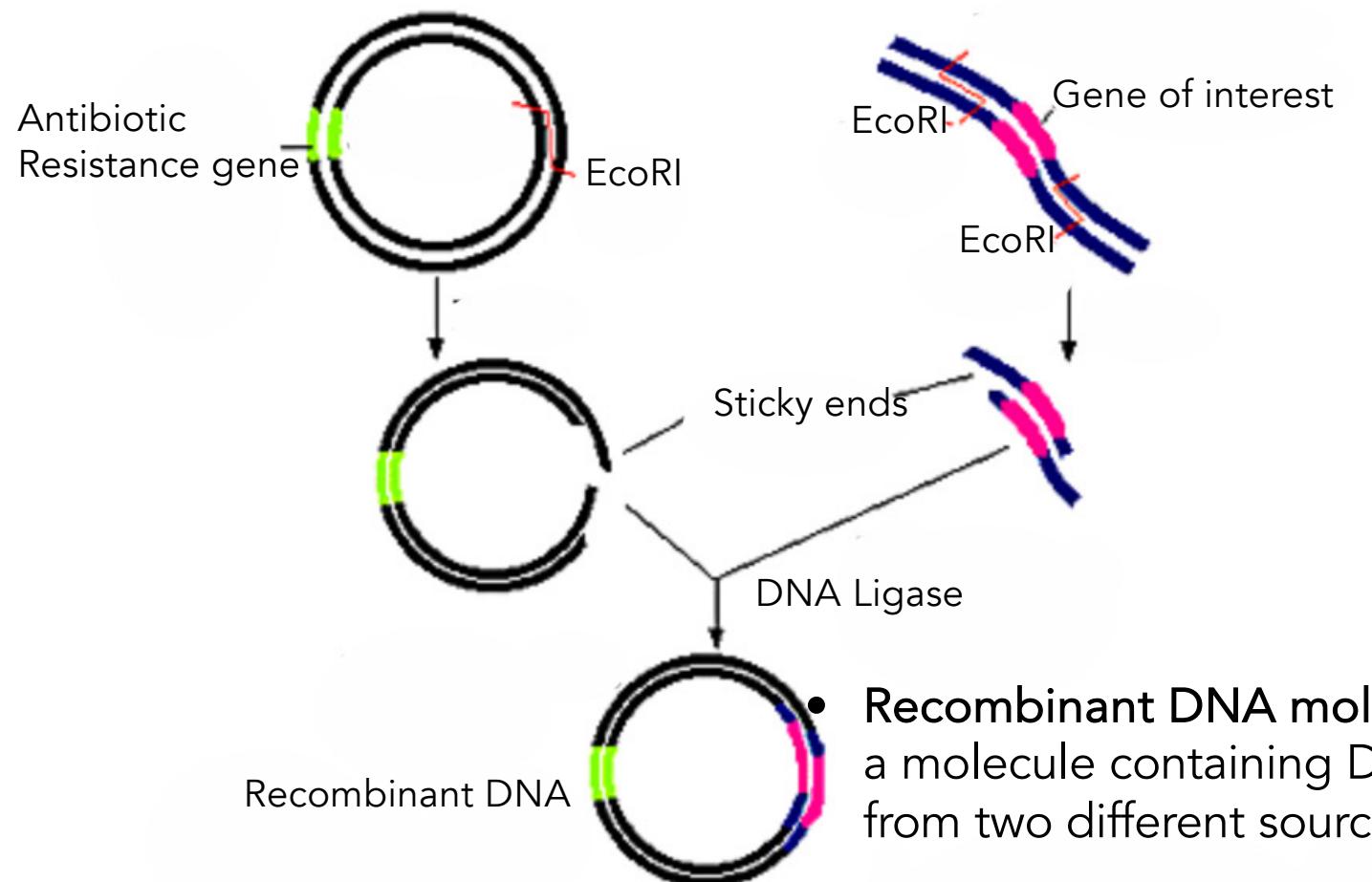
Restriction Enzymes to Make Recombinant DNA Plasmid

- A restriction enzymes or restriction endonuclease cut DNA molecules at a specific location.
- Each restriction enzyme recognizes a particular short DNA sequence, or restriction site, and cutting both DNA strands at precise points within this restriction site.
- The most commonly used restriction enzymes recognize sequences containing 4-8 nucleotide pairs, which yields a set of restriction fragments.



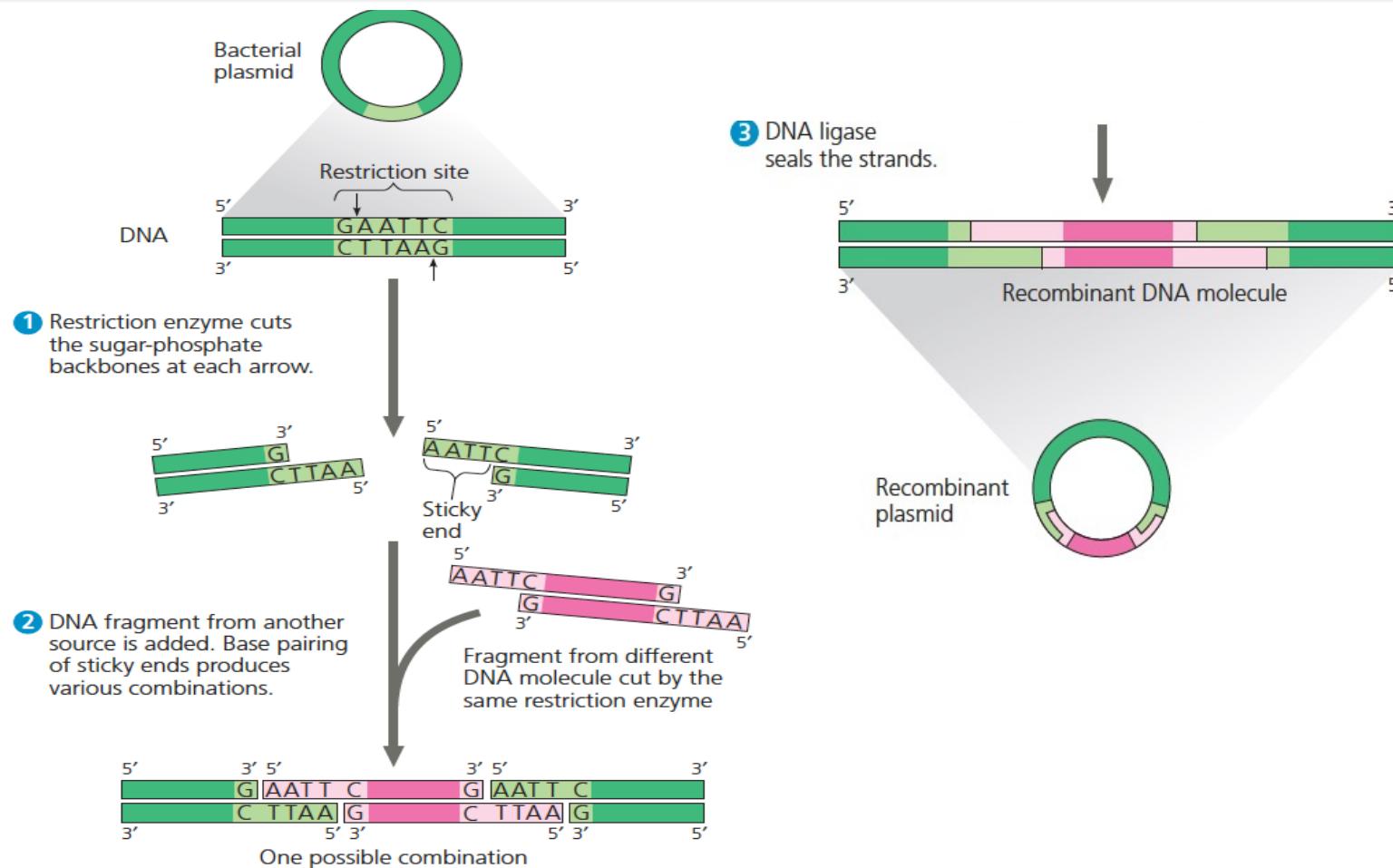
Restriction enzyme HindIII

Gene Cloning: Construction of rDNA Molecule



- **Recombinant DNA molecule:** a molecule containing DNA from two different sources

Restriction Enzyme and Recombinant DNA Plasmid



Gene Cloning Steps

- Construction of rDNA molecule
- Introduction into a host cell
- Selection using antibiotics

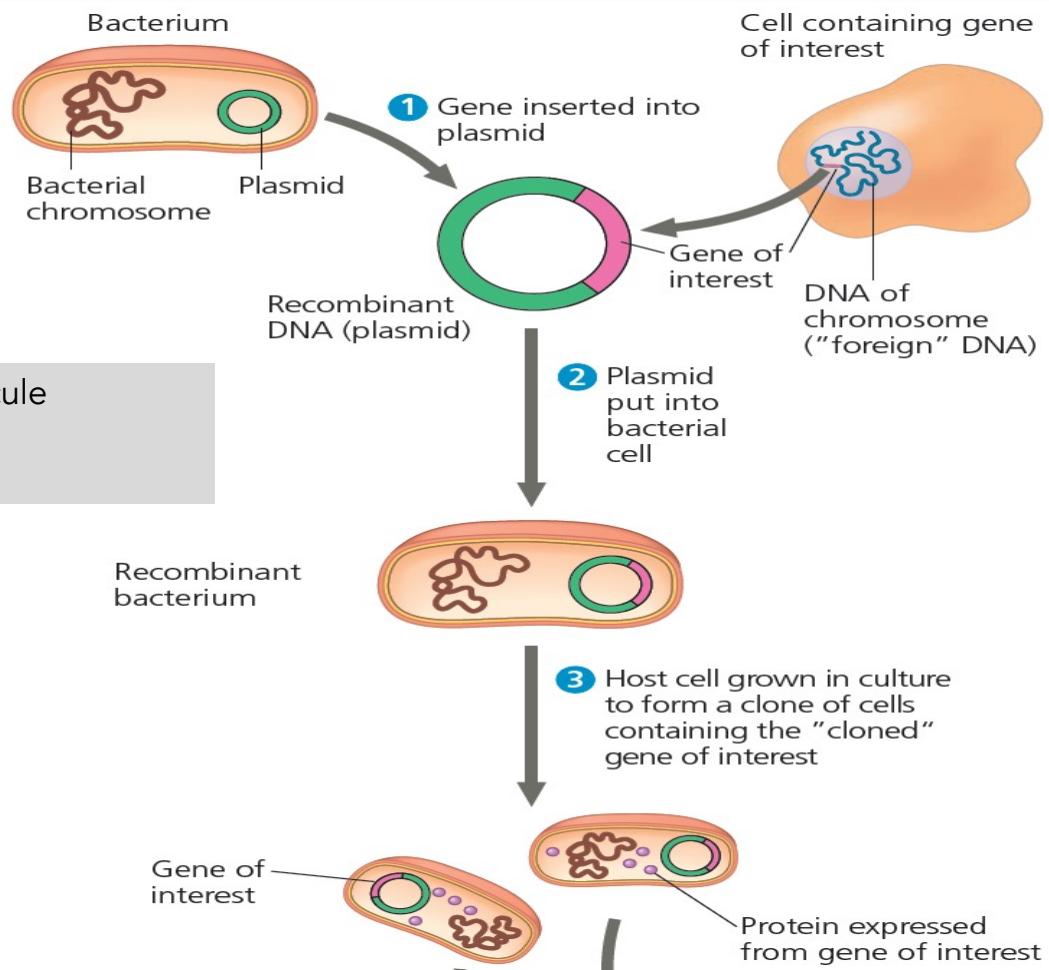


Figure 20.5

Gene Cloning Applications

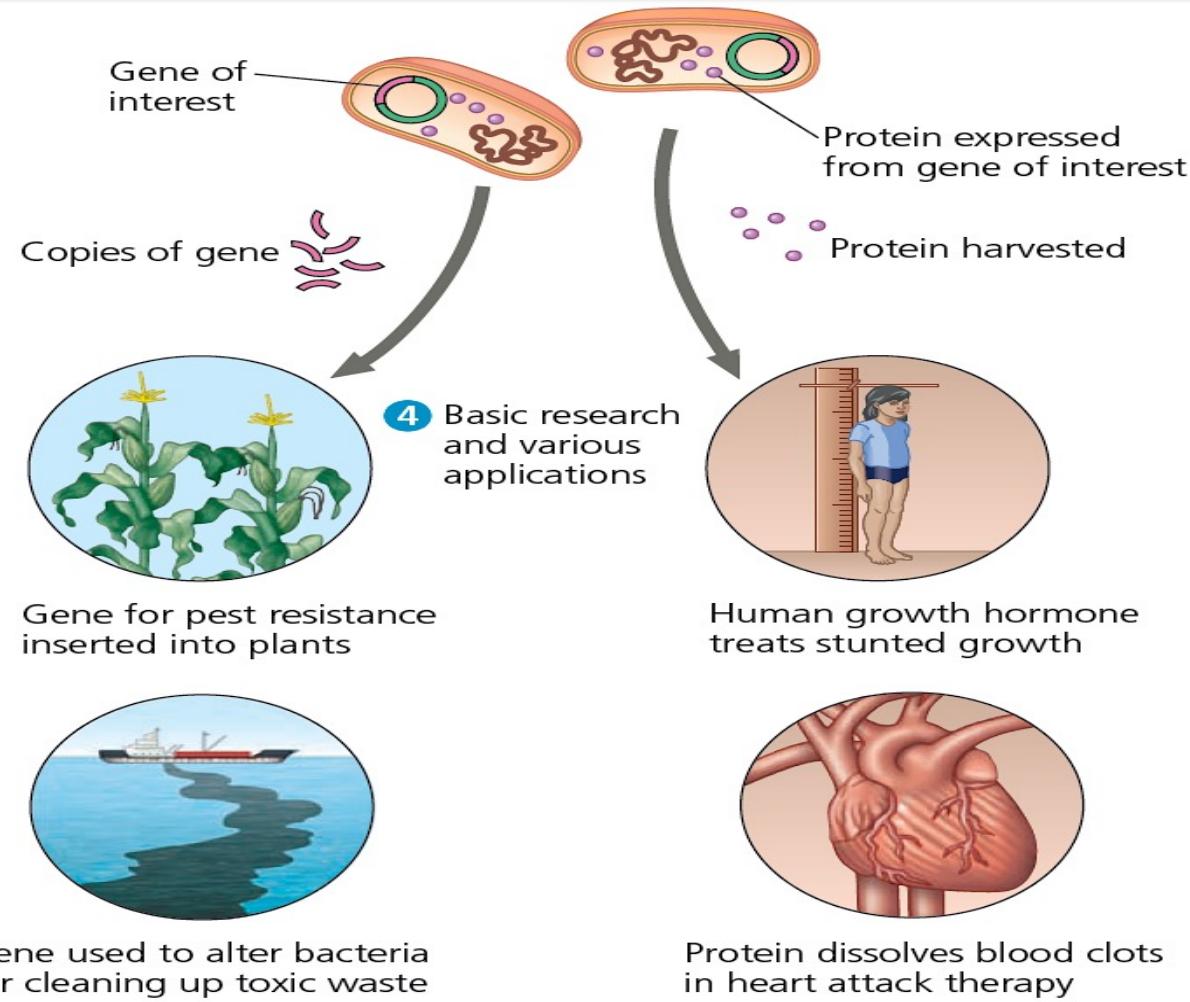
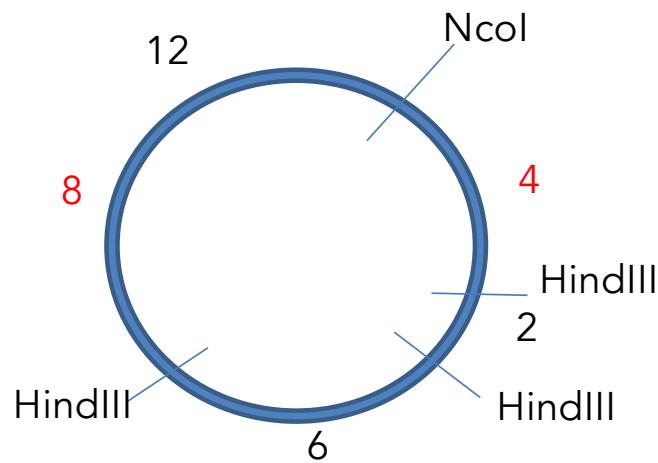


Figure 20.5

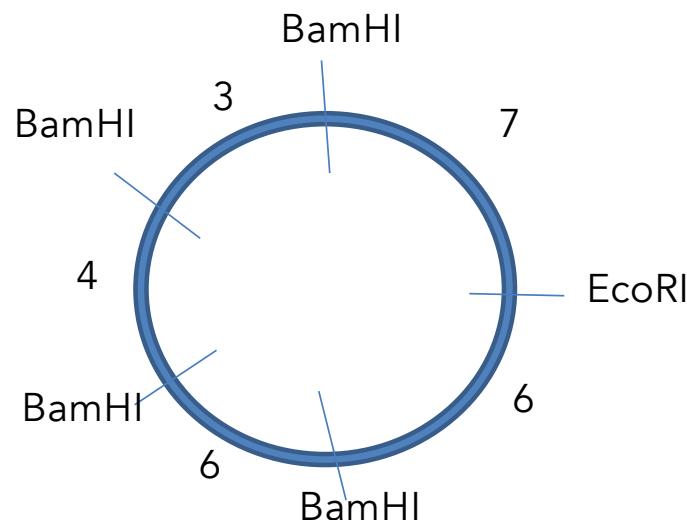
Plasmid Map

pANT7 is a plasmid of 20 kb. It was digested with Ncol, which resulted in one linear fragment. HindIII digestion results in 3 fragments – 12, 2, and 6 kb size. When both the enzymes were used, it resulted in 4 fragments of 8, 4, 2 and 6 kb. Develop a plasmid map and show restriction sites.



Plasmid Map: Example (2)

pUC1 vector is digested with EcoRI, which resulted in a band of 26 kb. BamHI enzyme digestion resulted into 4 bands of 13, 6, 4 and 3 kb. When both the enzymes were used it resulted in 4 bands of 7, 6, 4 and 3 kb. Develop a plasmid map and show restriction sites.



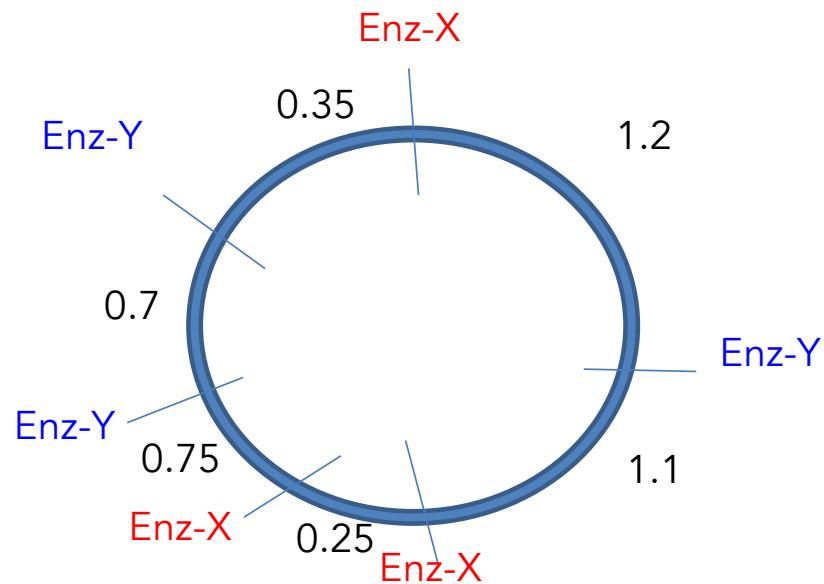
Plasmid Map: Example (3)

Develop a restriction map for pET28 (total size 4.35 kb).

Enz-X: 2.3, 0.25, 1.8

Enz-Y: 2.1, 1.55, 0.7

Enz-X + Enz-Y: 1.2, 1.1, 0.75, 0.7, 0.35, 0.25



Polymerase Chain Reaction

The key success for PCR came from the discovery of an unusual heat-stable DNA polymerase called Taq polymerase.

It was isolated from bacteria *Thermus aquaticus*, which lives in hot springs.

The stability of its DNA polymerase at high temperatures is an evolutionary adaptation that enables the bacterium to survive and reproduce at temperatures up to 95°C.

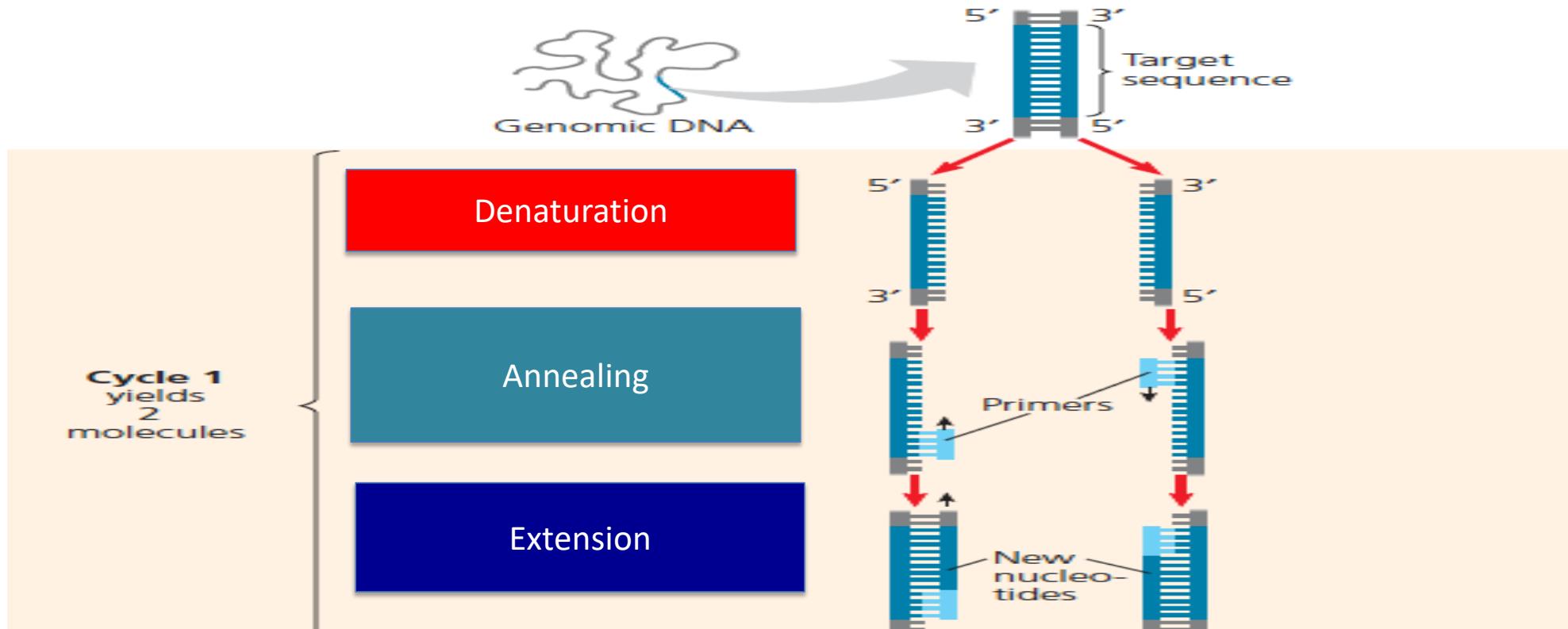
Polymerase Chain Reaction (PCR)

- 1984: Kary Mullis
- Amplification of a target sequence
- To obtain many copies of the desired gene by using a technique called the **Polymerase Chain Reaction**
- Three steps:
 - Strand separation
 - Annealing of primers
 - DNA synthesis



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Polymerase Chain Reaction (PCR)



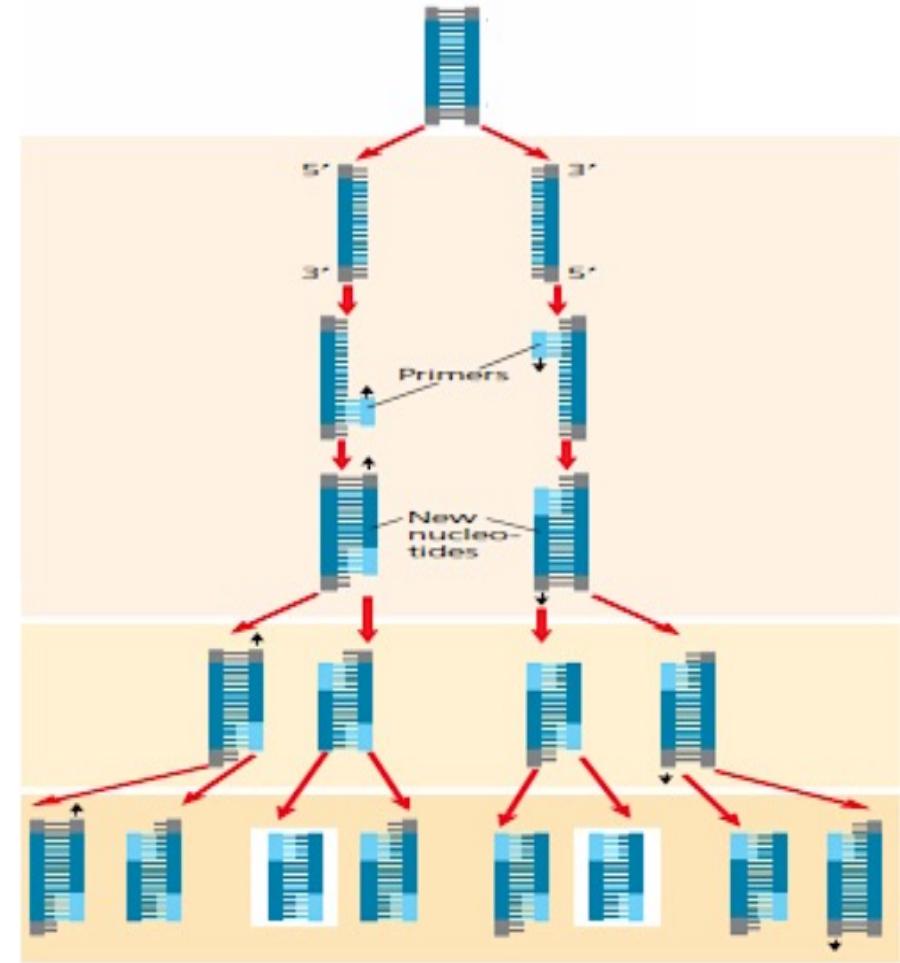
- Primer sequences are chosen so that they hybridize only to sequences at opposite ends of the target segment, one on the 3' end of each strand.

Figure 20.8

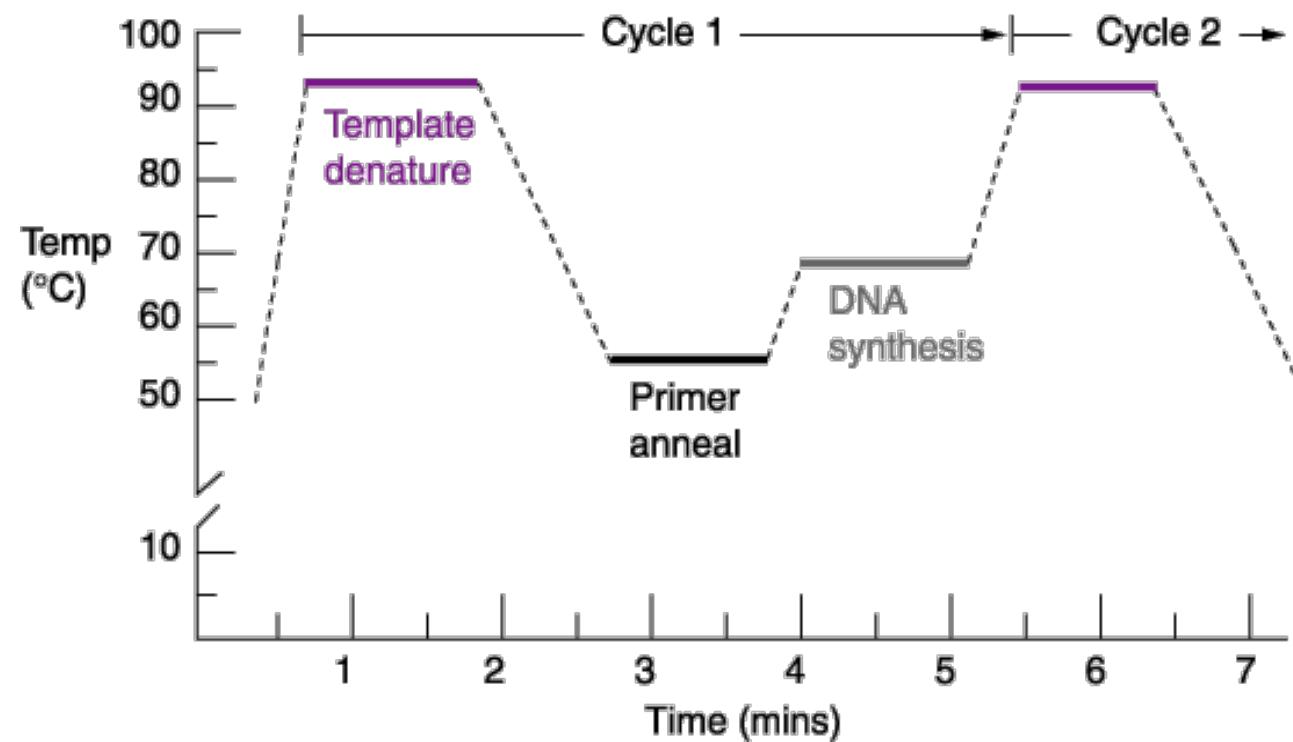
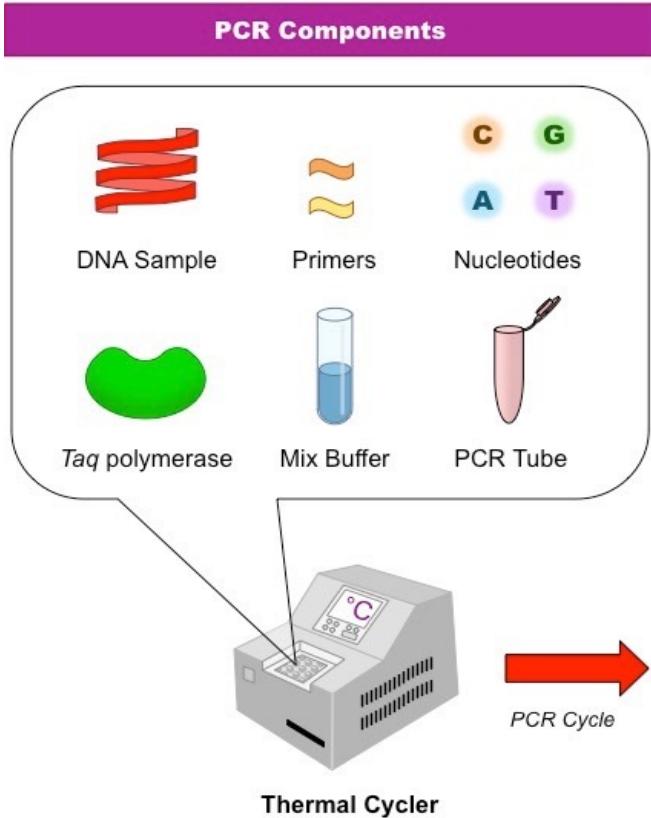
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PCR: Amplification through Multiple Cycles

- A three-step cycle brings about a chain reaction that produces an exponentially growing population of identical DNA molecules.
- After each successive cycle, the number of target sequence doubles.
- Number of molecules equals 2^n where n is the number of cycles

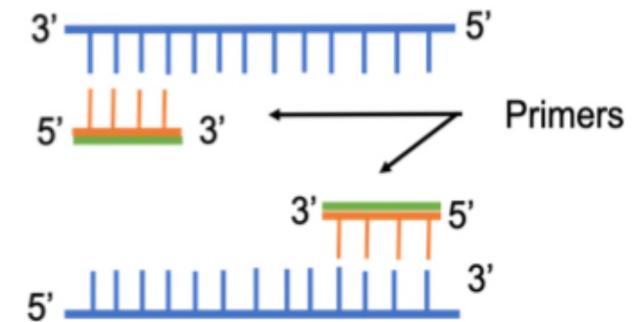


PCR Overview



Primers

- Short strand of nucleotides (about 18-28 nucleotides in length) that serves as a starting point for DNA synthesis
- 50-60% GC composition
- Have a balanced distribution of G/C and A/T domains
- No long strings of a single base (<4)
- $T_m = (A+T) \times 2 + (G+C) \times 4$
- Primers should not be self complimentary



Q. For the primer 5' GATCCGATTGGACACTGTACTA 3' calculate the Tm.

Ans: 64 C

Primer designing

Design primers for the following sequence. Location of 2 primers is indicated by >>'s.
(Remember, that when both strands of DNA are shown the top strand runs 5'-3')

CTGTCCACACAATCTGCCTTCGAAACCATGGGATCCCACGAAAAGAATTCCCACATGGTCCTT -upper strand

GACAGGTGTGTTAGACGGGAAAGCTTGGTACCCTAGGGTTGCTTTCTTAAGGGTGTACCAGGAA –lower strand

>>>>>>>>>>

CTTGAATTCTAACAGCTGCTGGGATTACACATGGCATGGATGAACATACAAATAA

GAACCTAACGATTGTCGACGACCCTAATGTGTACCGTACCTACTTGATATGTTATT

<<<<<<<<<<<<

The forward primer (>>>>) will be complementary to the lower strand and must run 5'-3'
5'-CTGTCCACACAATCTGCC -3'

For the reverse primer, you will need to write the sequence of the other DNA strand. The reverse primer (<<<<) which will be complementary to the upper strand and must run 3'-5'. However, we always write DNA sequences in the 5'-3' direction so the reverse primer would be written: 5'-CATGCCATGTGTAATCCCAG-3'

Primer 3 : <https://bioinfo.ut.ee/primer3-0.4.0/>

Primer- BLAST : <https://www.ncbi.nlm.nih.gov/tools/primer-blast/>

Agarose Gel Electrophoresis

- A gel made of polymer acts as a molecular sieve which separates nucleic acids based on electrical charge
- -ve charged DNA molecules move toward +ve electrode.
- DNA-binding dye is mixed, which fluoresces pink in UV light.
- Each pink band corresponds to DNA molecules.

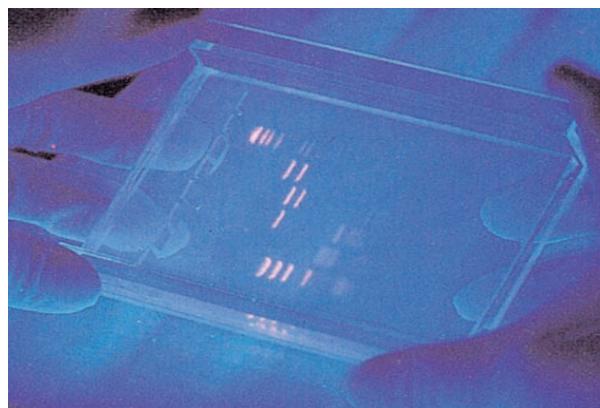
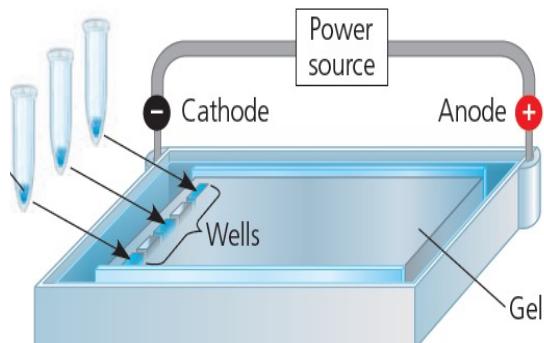
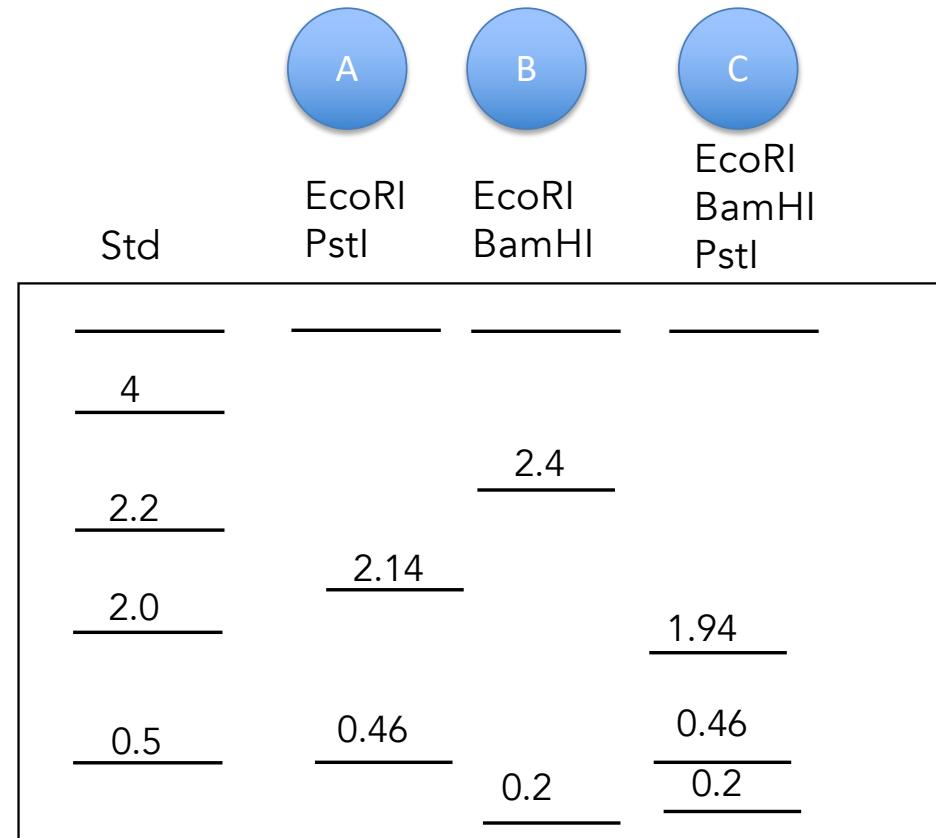
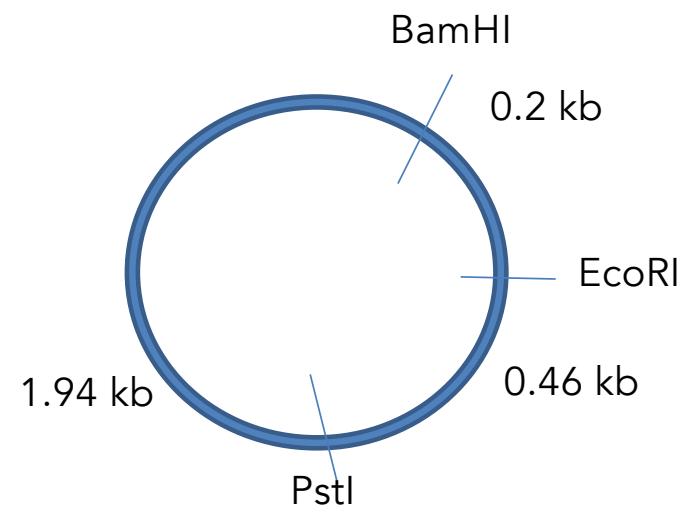


Figure 20.7



Interpreting Gel Image

Restriction map for pET28 is shown below. Draw the location of bands on gel.

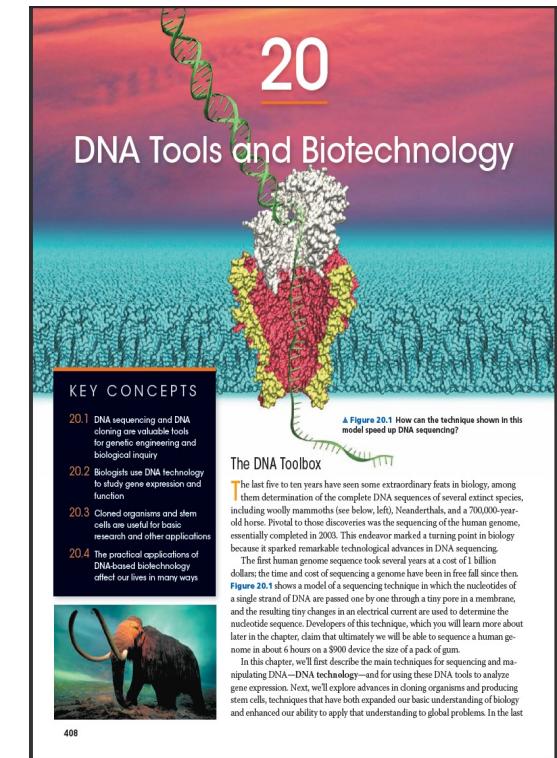
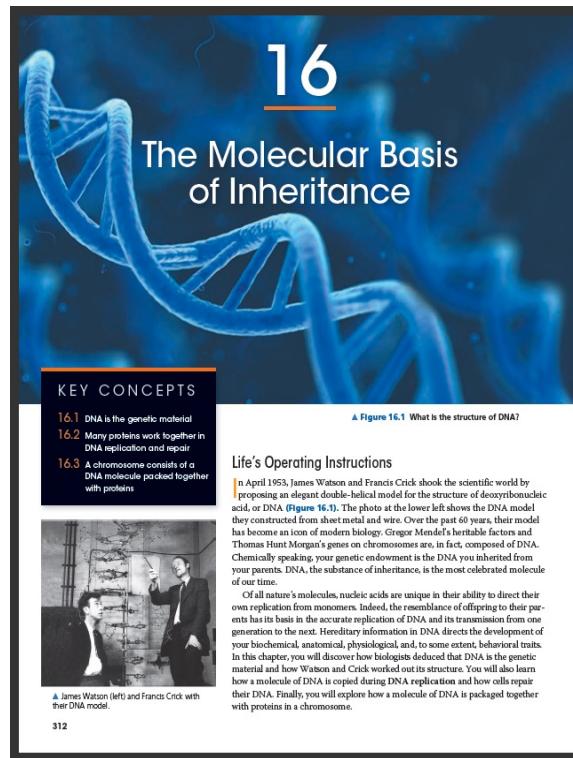


Summary

- Molecular basis of inheritance & discussed classical experiments - Griffith, Hershey & Chase; Meselson & Stahl
- Eukaryotic genome and chromosomal abnormalities
- Basic molecular biology tools are crucial for biotechnology
- Techniques such as PCR has transformed clinical assays

References

- Campbell Biology - Reece, Urry, Cain, Wasserman, Minorsky, Jackson 10th Edition, Cummings
- Acknowledgment
 - Cover images – getty images



Next Lecture...

Flow of information & Gene Regulation