

Molecular and Cellular Biology (MCB)

BB101



LECTURE-6

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Molecular Basis of Inheritance & DNA Tools

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Outline

1. Molecular Basis of Inheritance
2. DNA Tools and Biotechnology
 - Gene Cloning
 - PCR
 - Electrophoresis

Can a genetic trait be transferred between different bacterial strains?

*Two strains of the bacterium **Streptococcus pneumoniae***

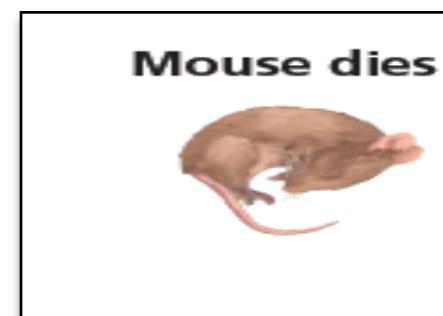
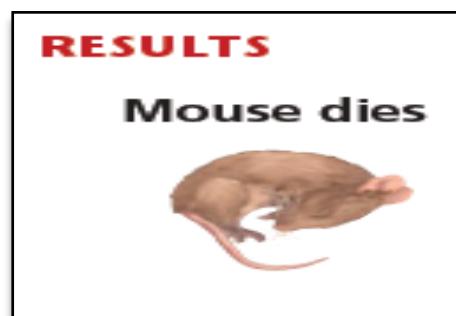
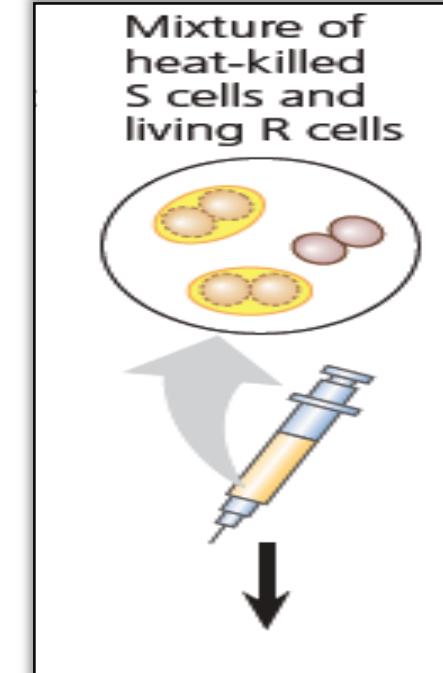
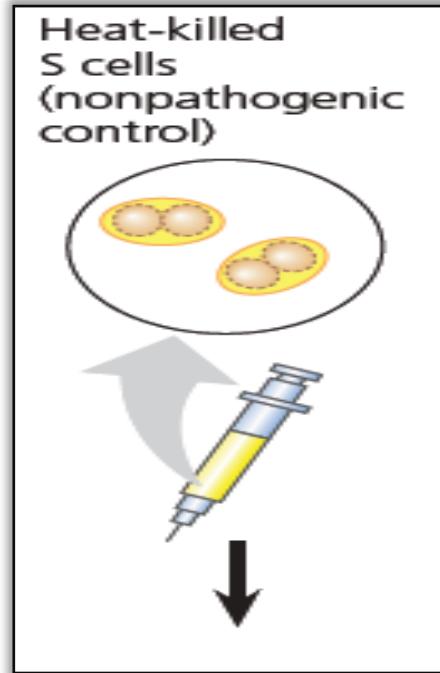
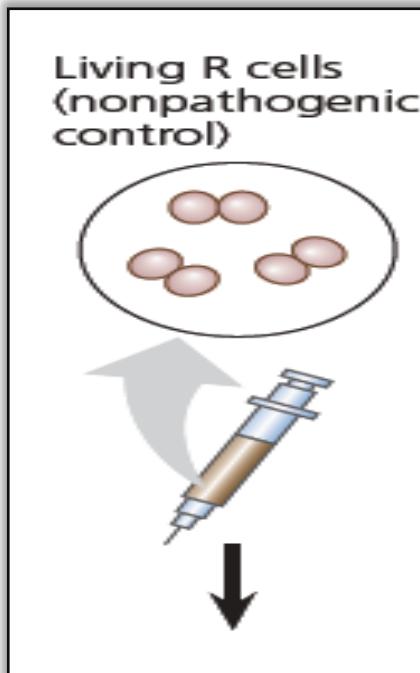
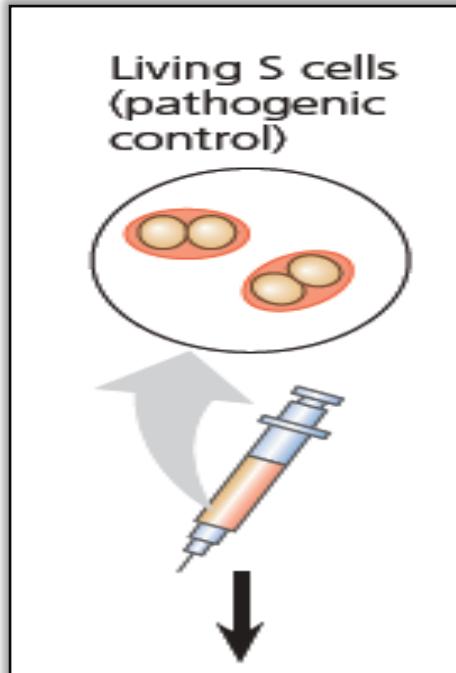
S strain (Smooth strain - presence of a capsule) causing pneumonia

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

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

Griffith's Experiment (1928)

The 'Transforming principle/factor'



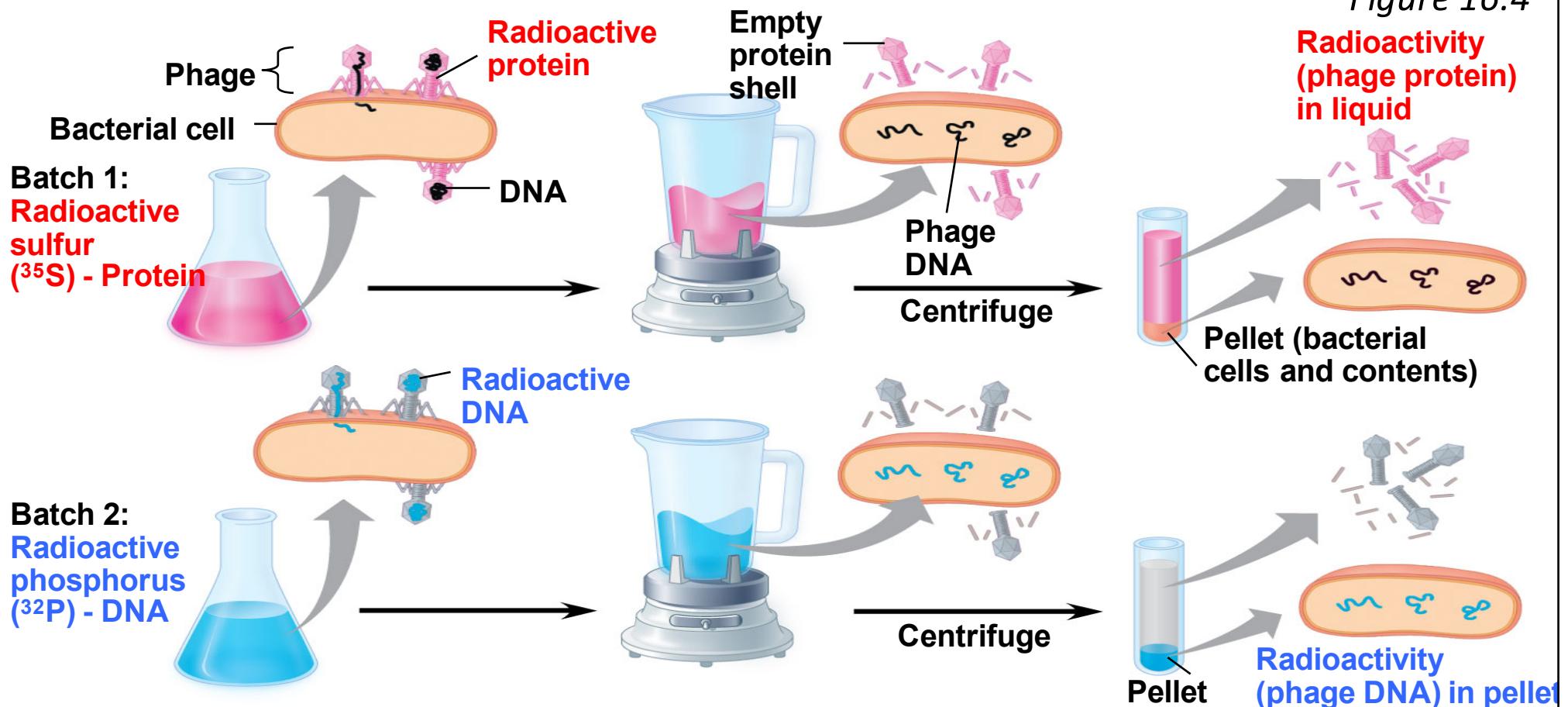
The living R bacteria was transformed into pathogenic S bacteria by a **heritable substance from dead S cells that enabled R cells to make capsules**

Figure 16.2

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

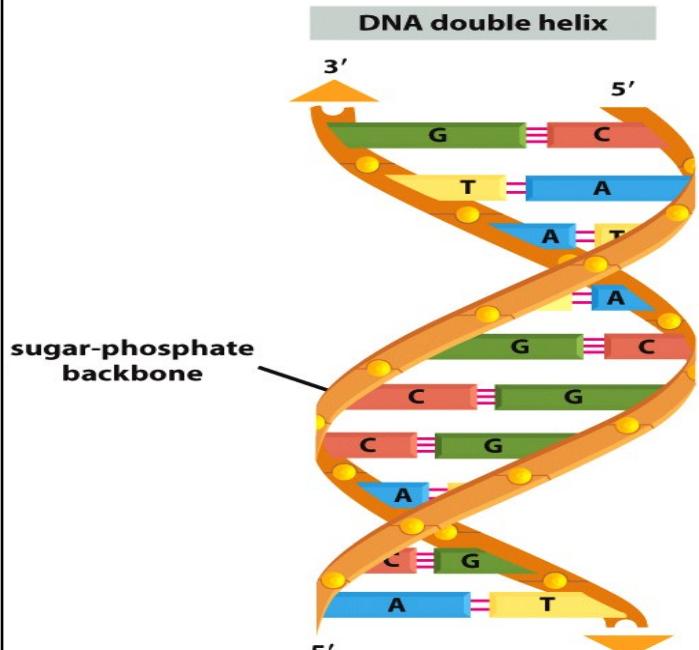
Discovering the Transforming Factor



- 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.

Chemical Composition of DNA & DNA Replication

Chemical Composition of DNA

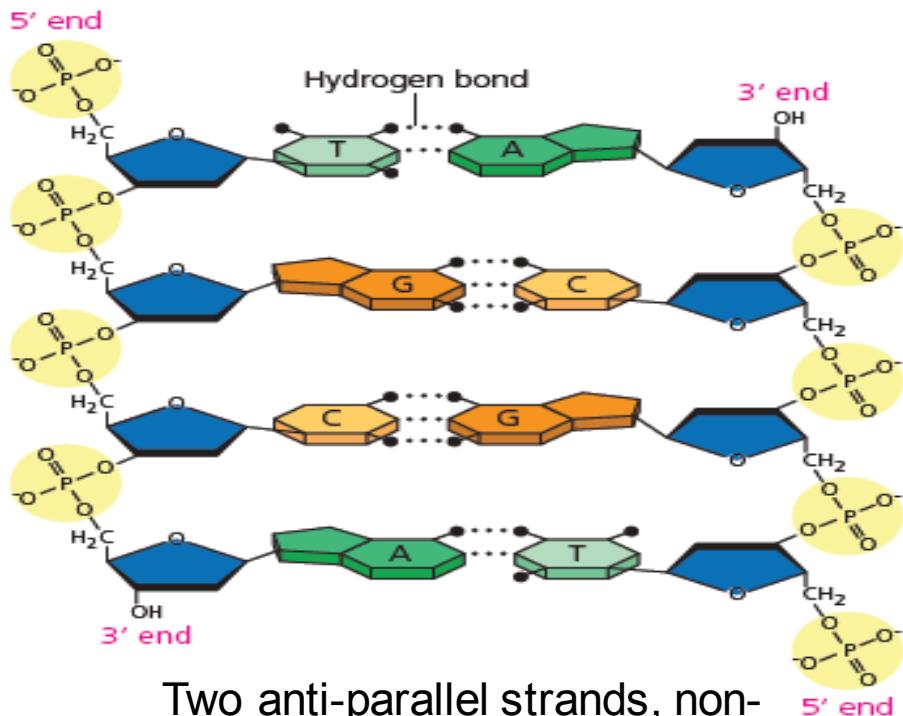


1962 Nobel Prize

Crick



Watson



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

Erwin Chargaff's rule

$\%A = \%T$ and $\%G = \%C$

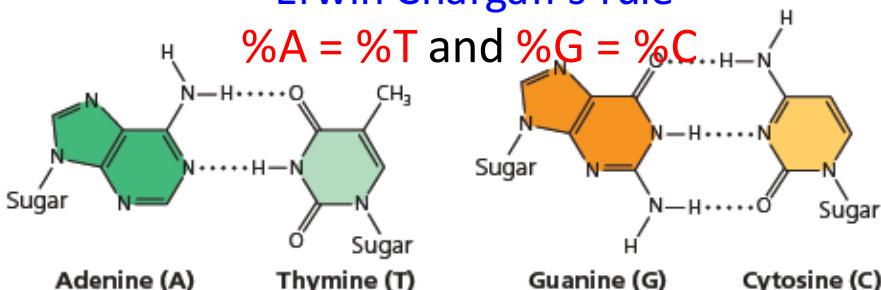


Figure 16.8

DNA Replication

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

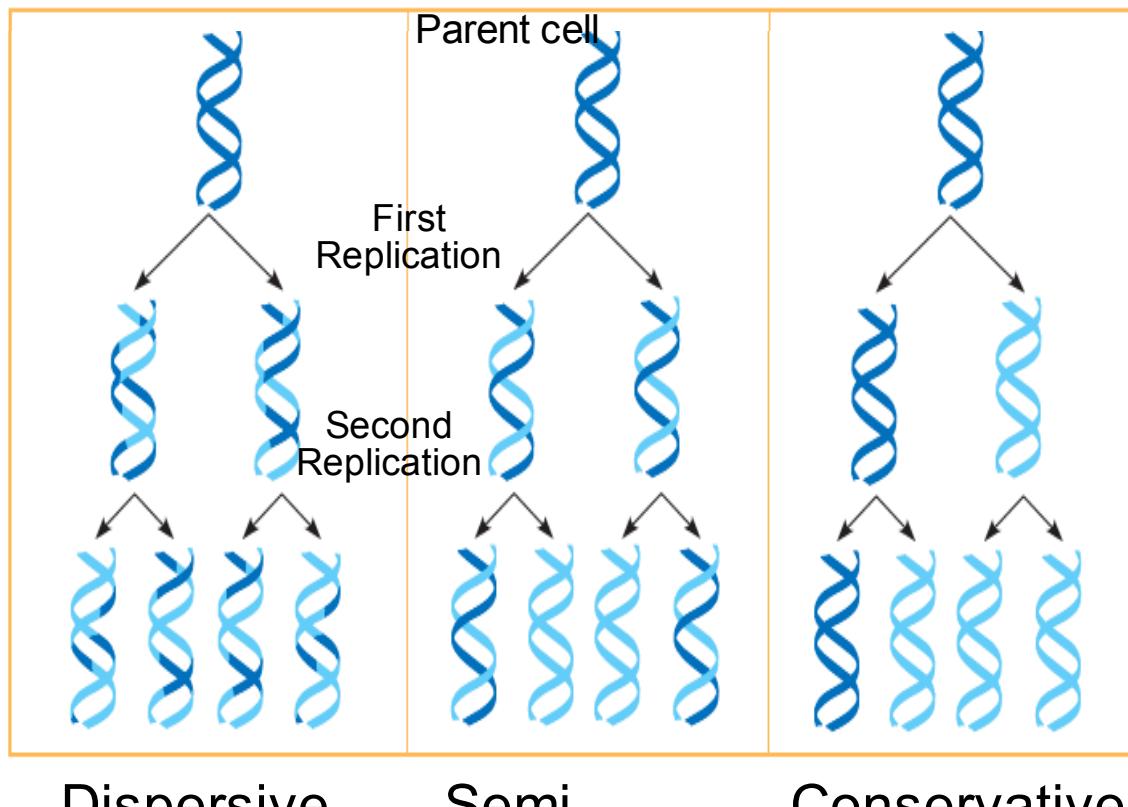


Figure 16.10

Meselson and Stahl's Experiment

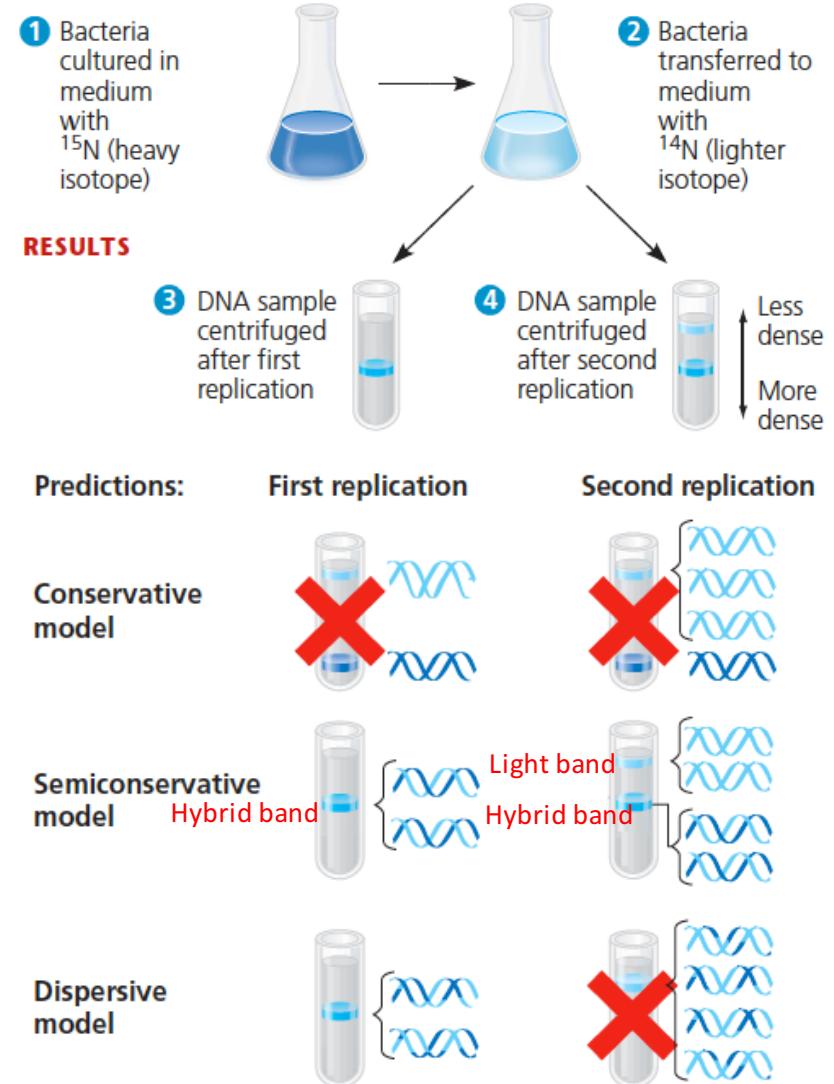
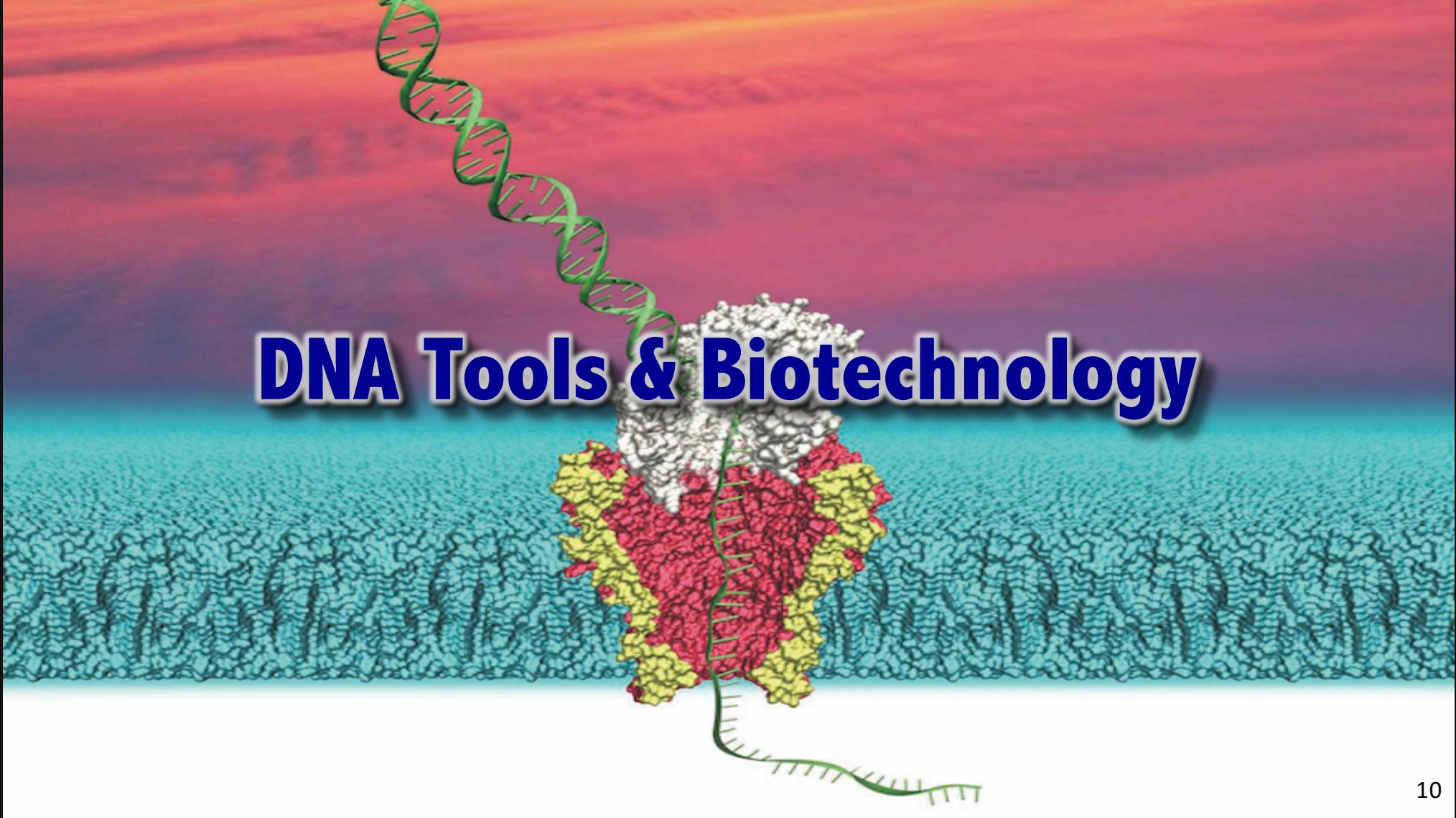
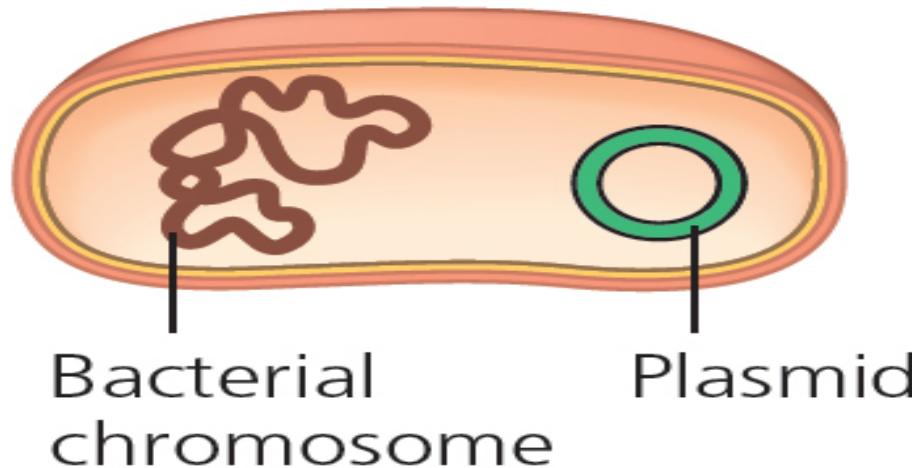


Figure 16.11



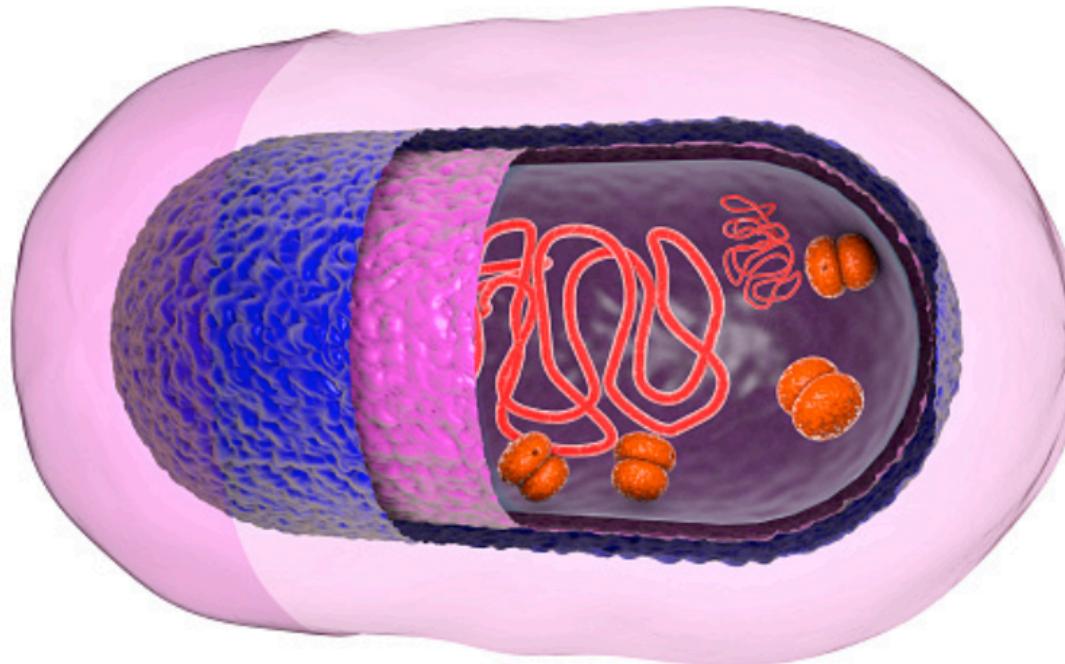
DNA Tools & Biotechnology

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.

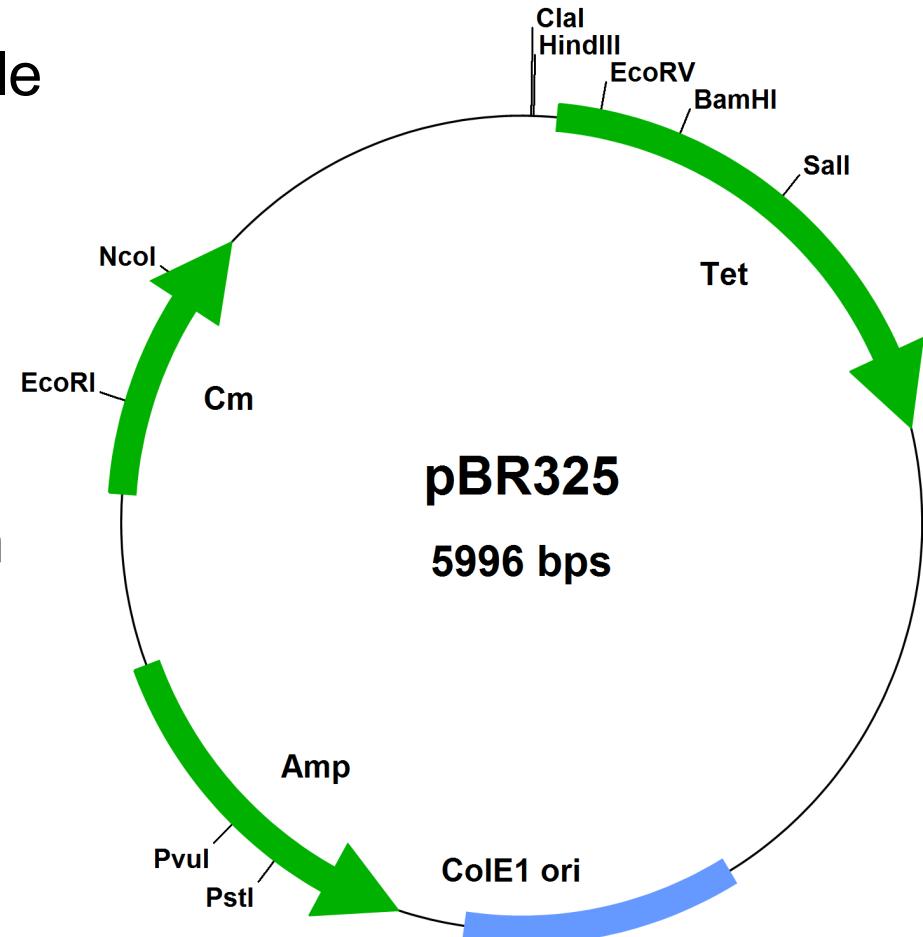
Plasmids have very few Genes



- A plasmid has only a small number of genes.
- These genes may be useful when the bacterium is in a particular environment.
- However, these genes may not be required for survival or reproduction under most conditions.

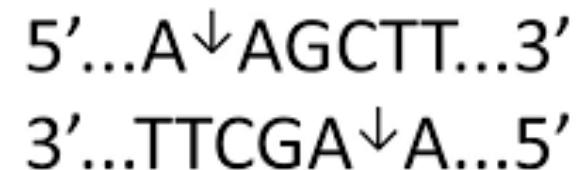
Plasmid as Vector

- 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.



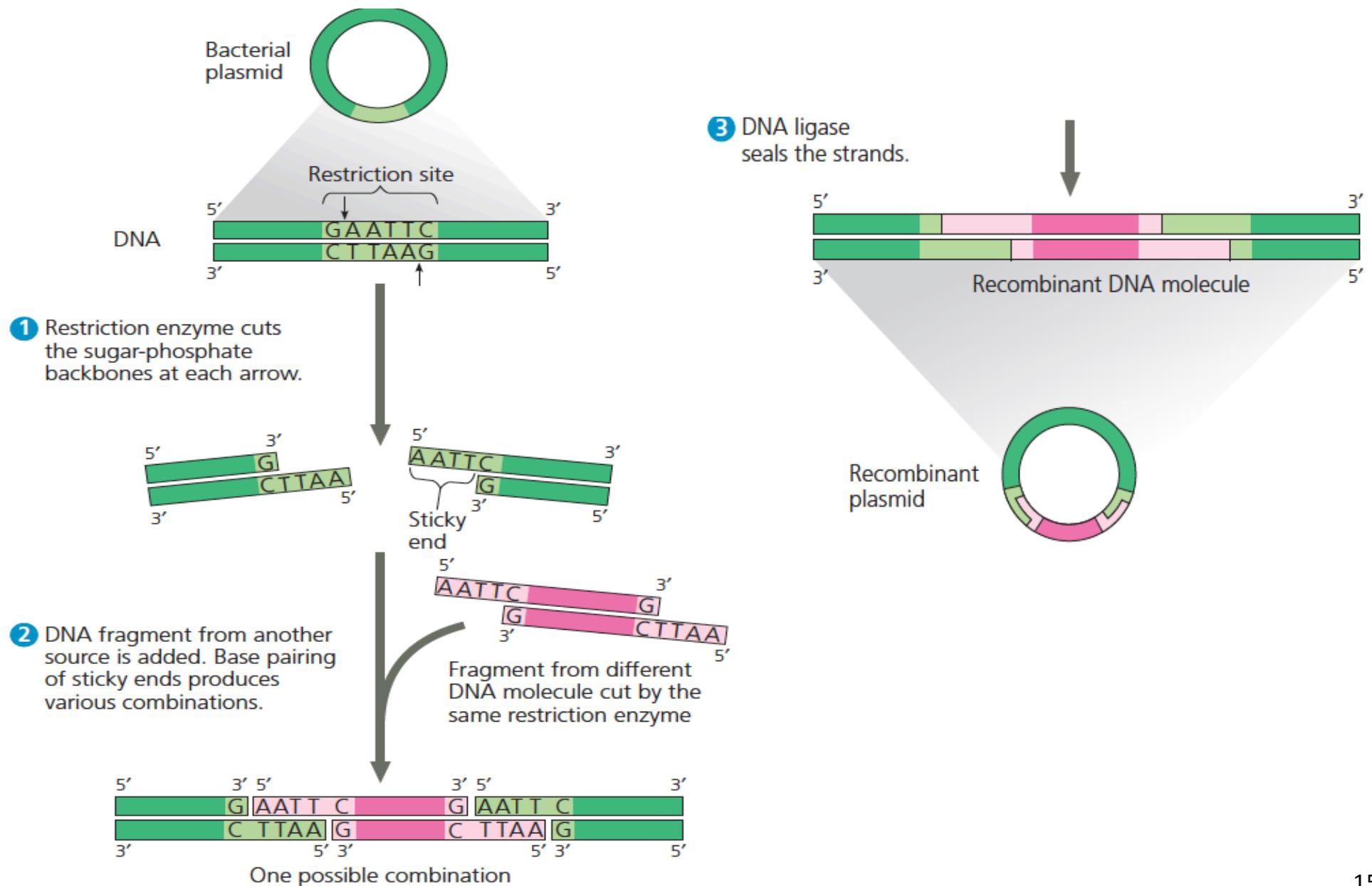
Restriction Enzymes to Make Recombinant DNA Plasmid

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



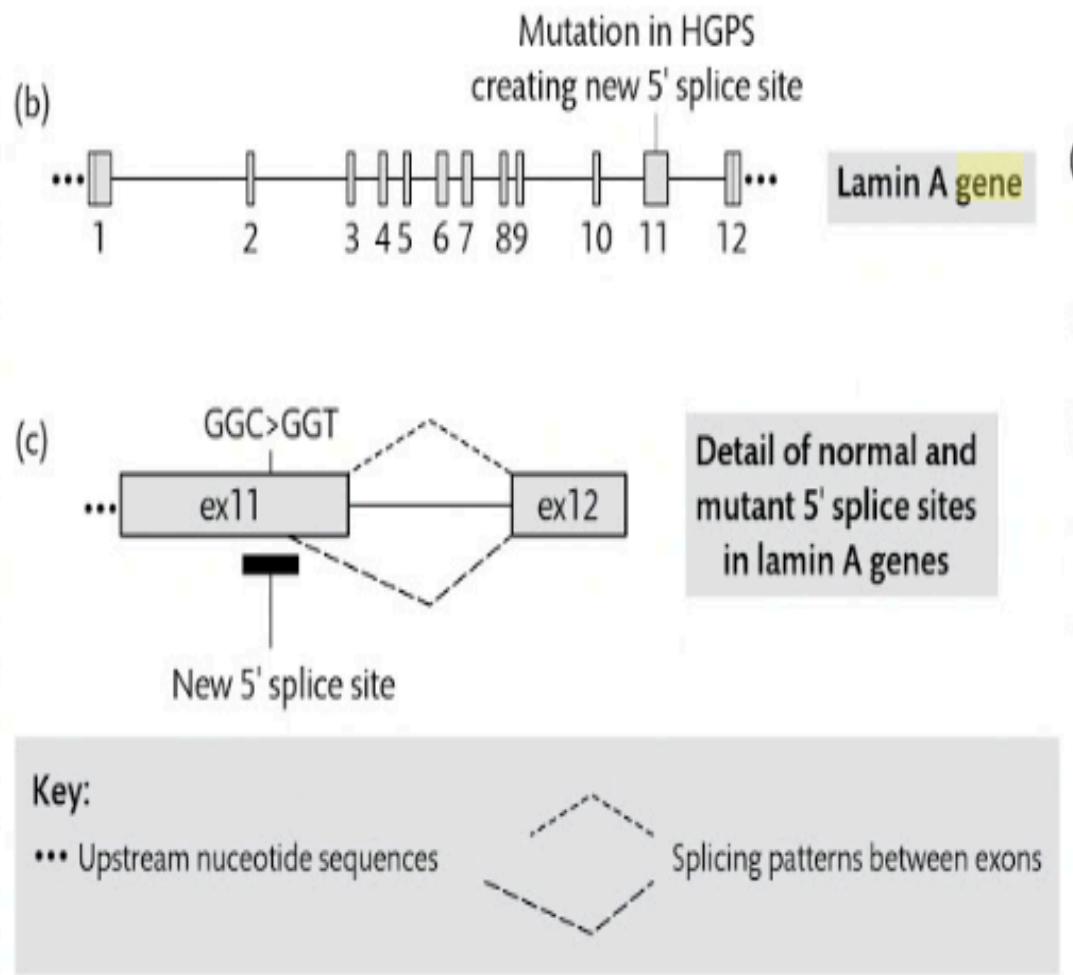
Restriction enzyme HindIII

Restriction Enzyme and Recombinant DNA Plasmid

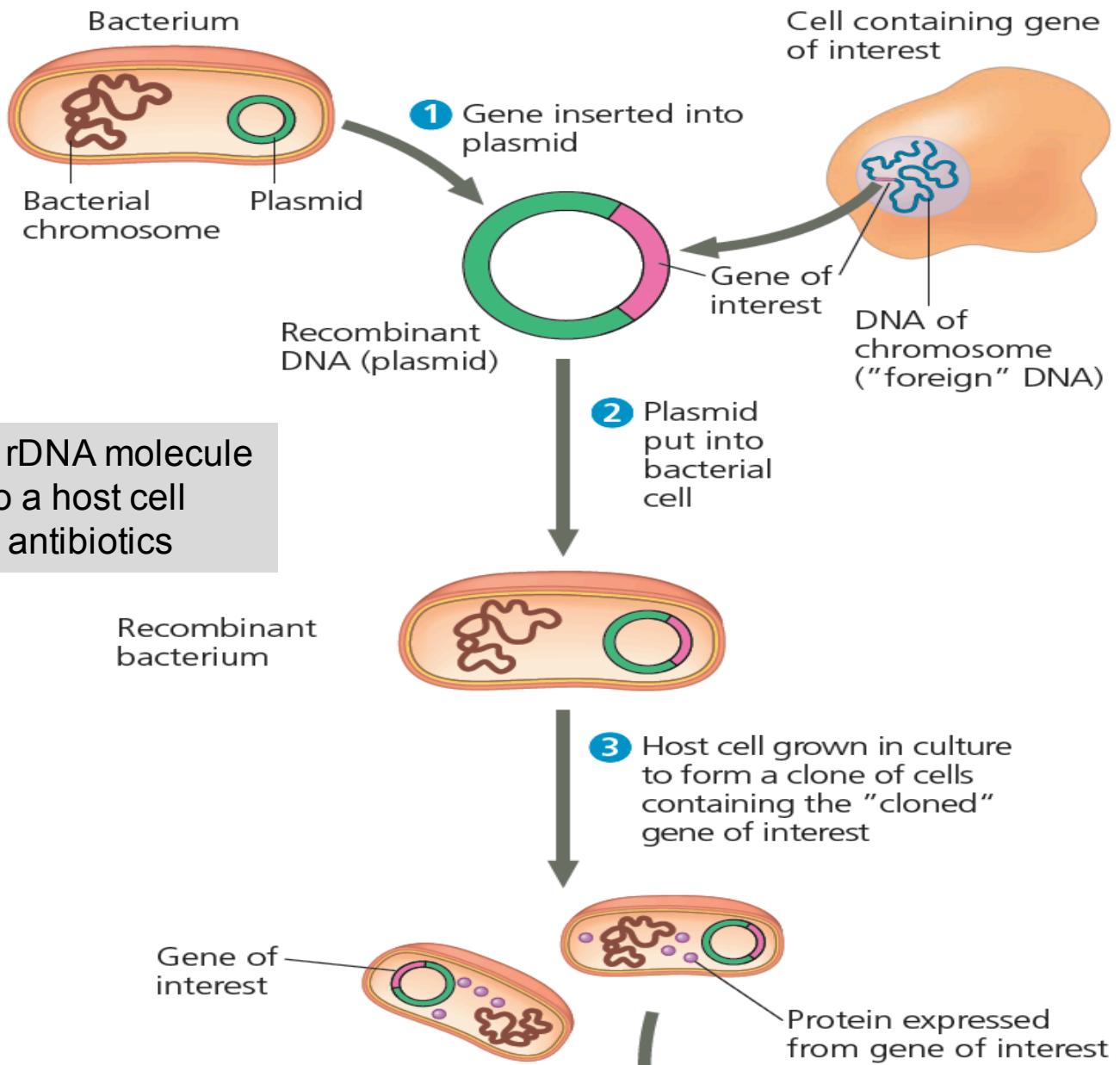


Why DNA Cloning?

Example: Progeria



Gene Cloning: Steps



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

Figure 20.5

Gene Cloning: Applications

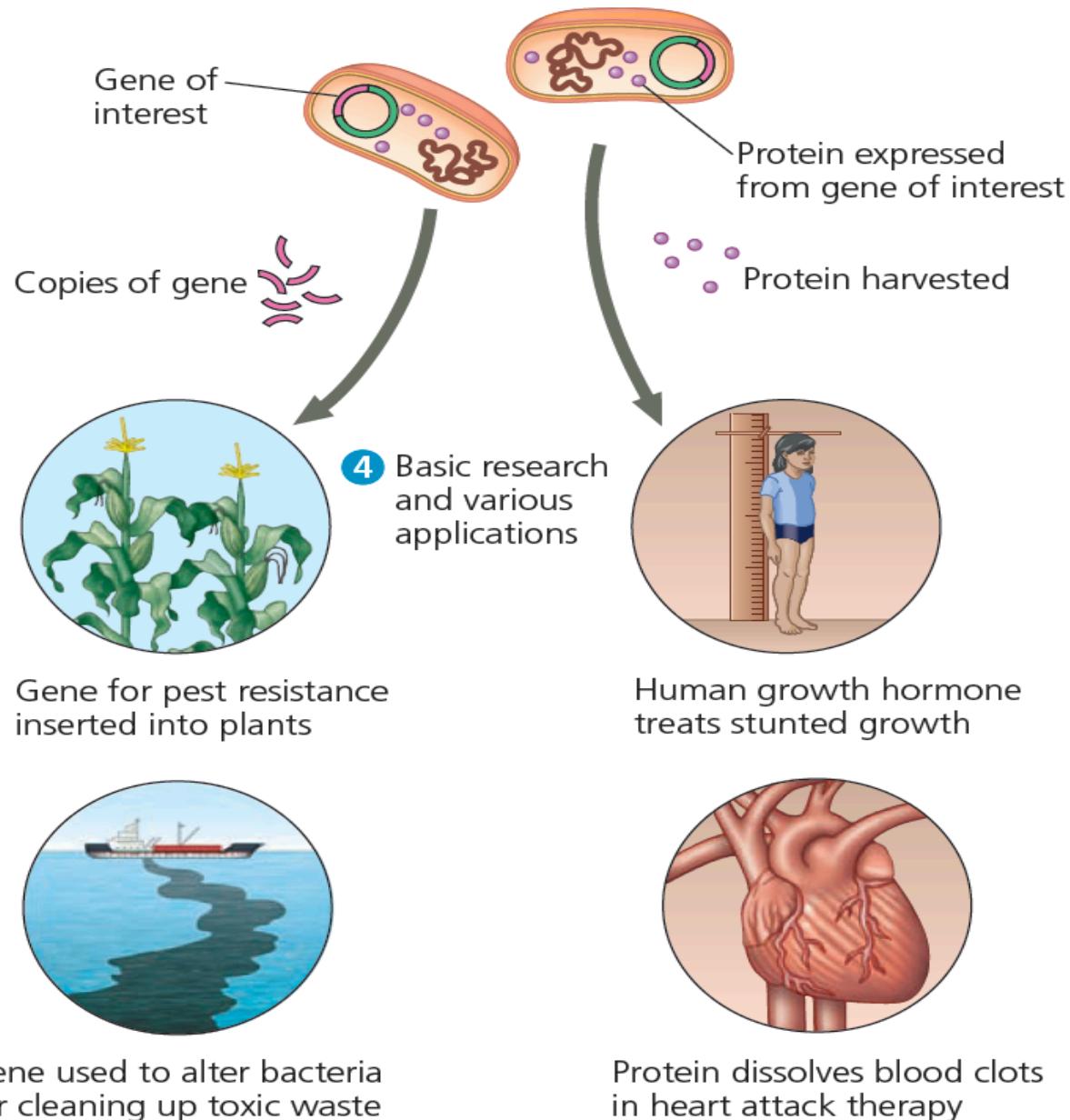
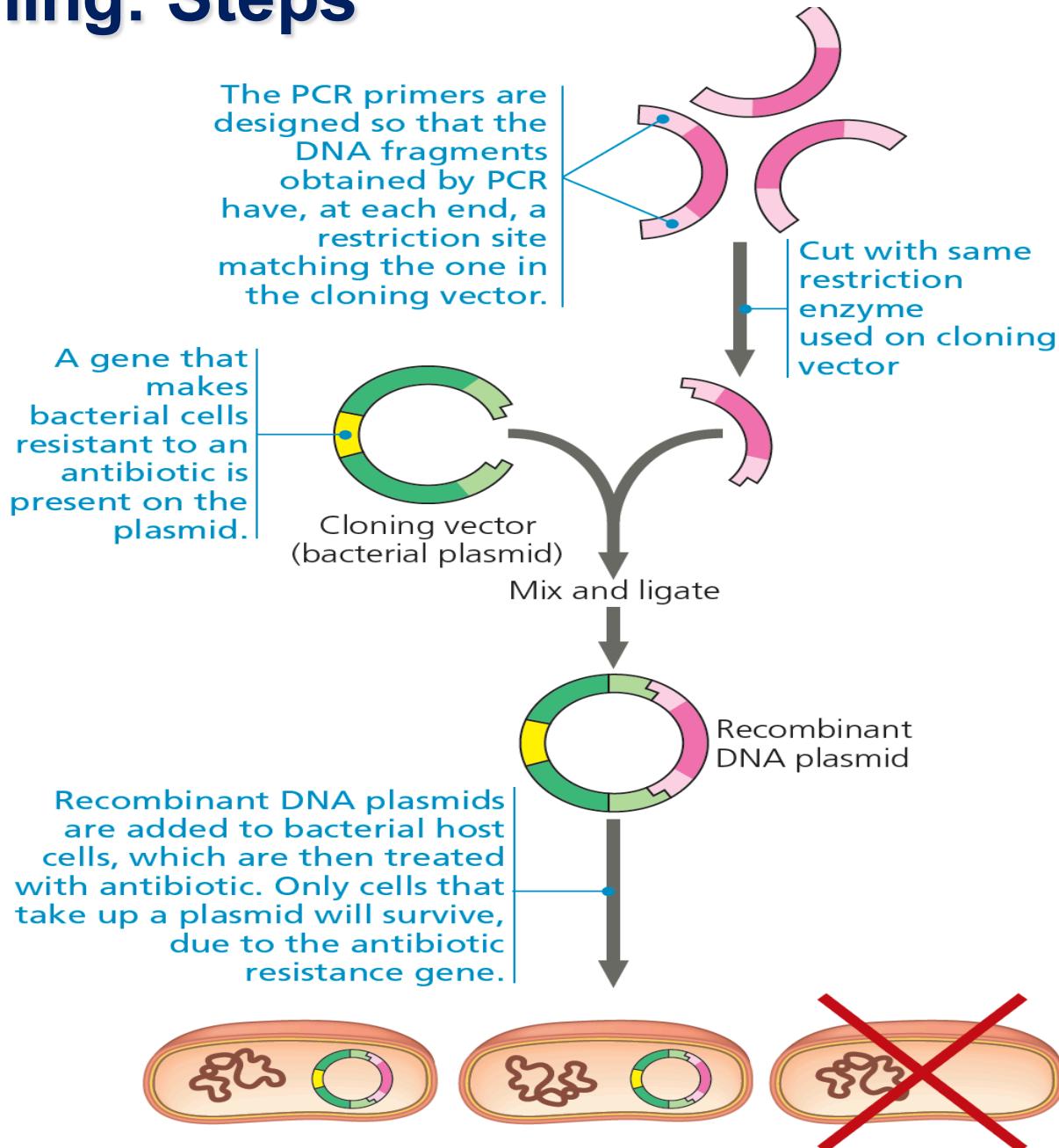


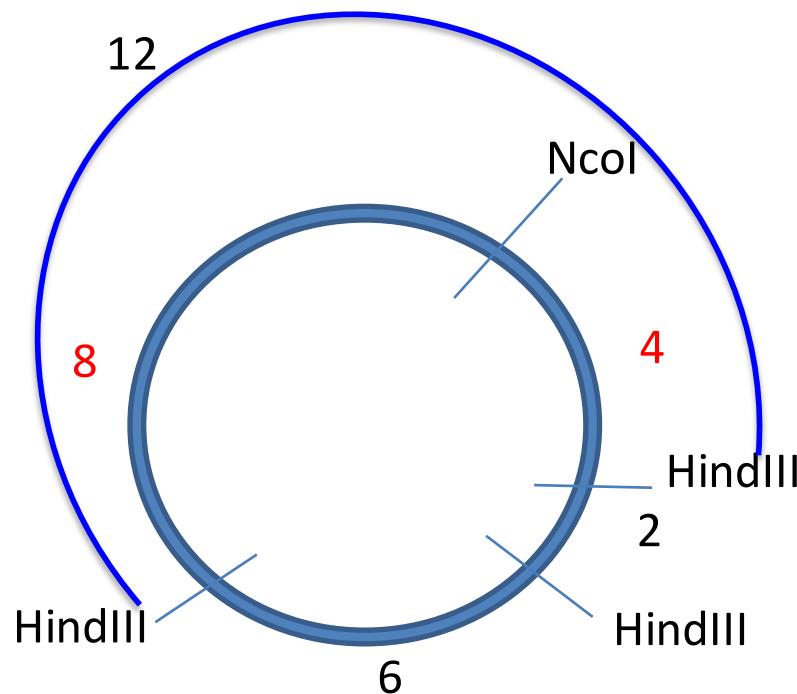
Figure 20.5

Gene Cloning: Steps



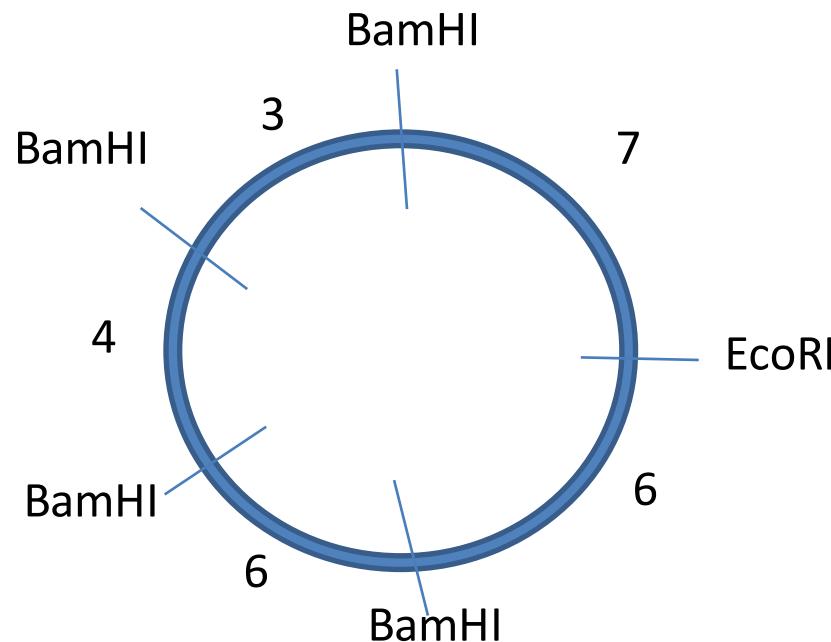
Plasmid map: Example-1

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.



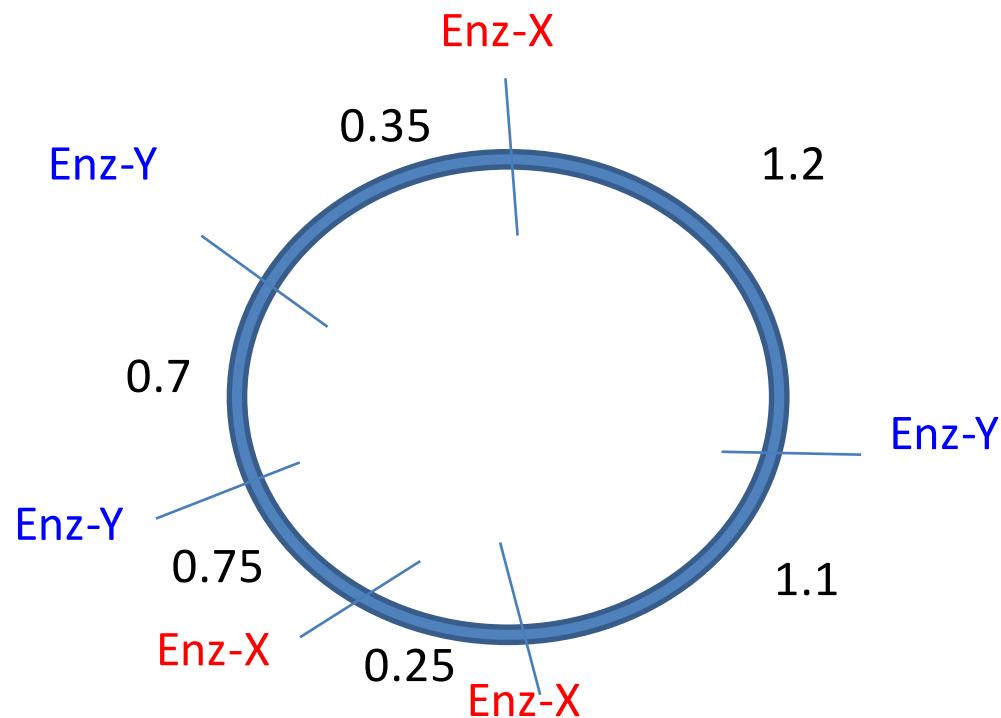
Plasmid map: Example-3

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



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.

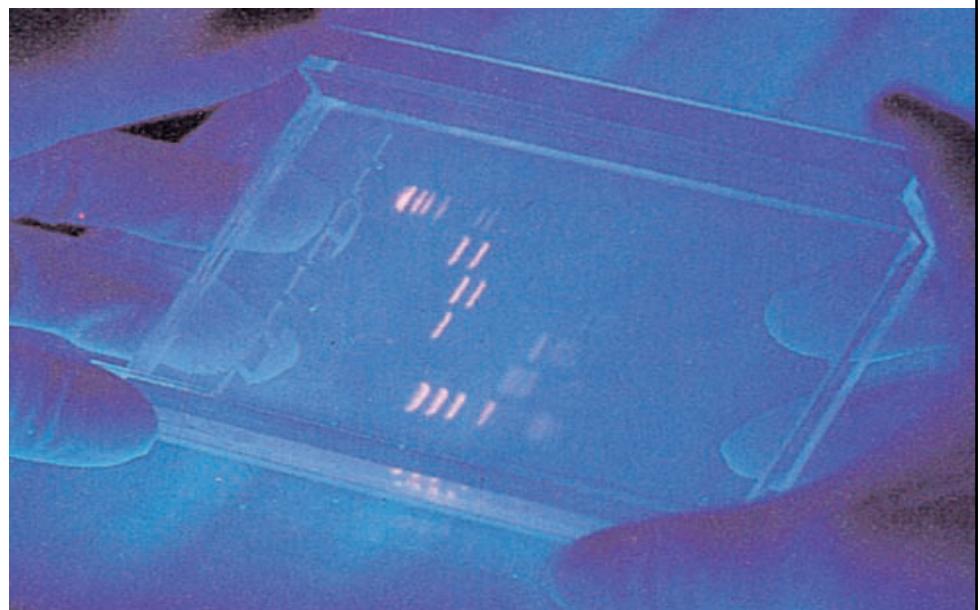
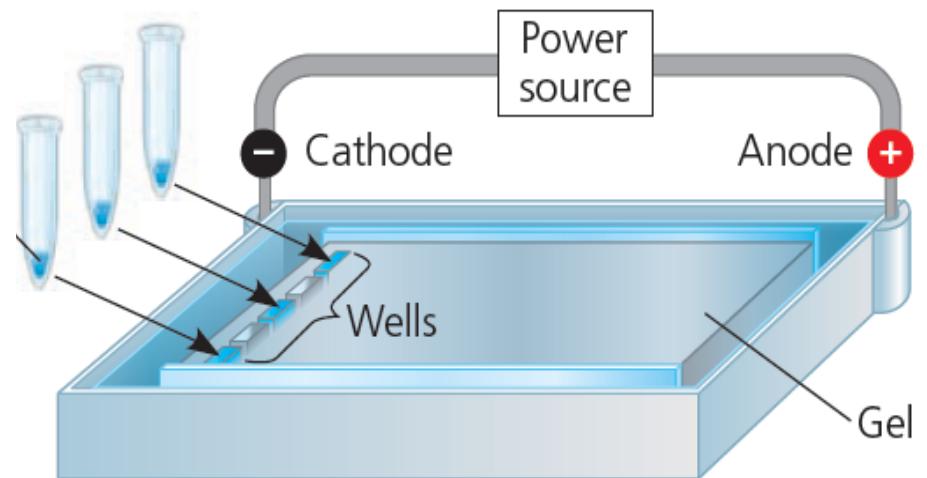
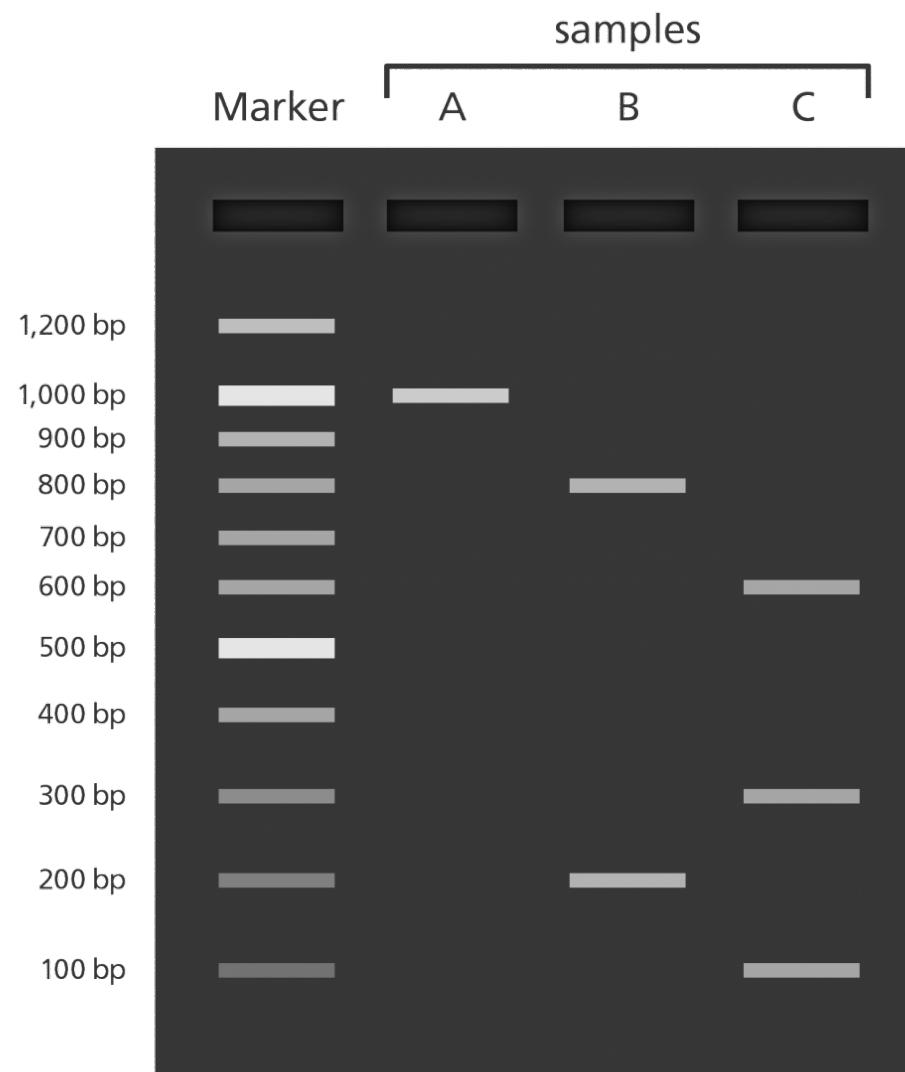


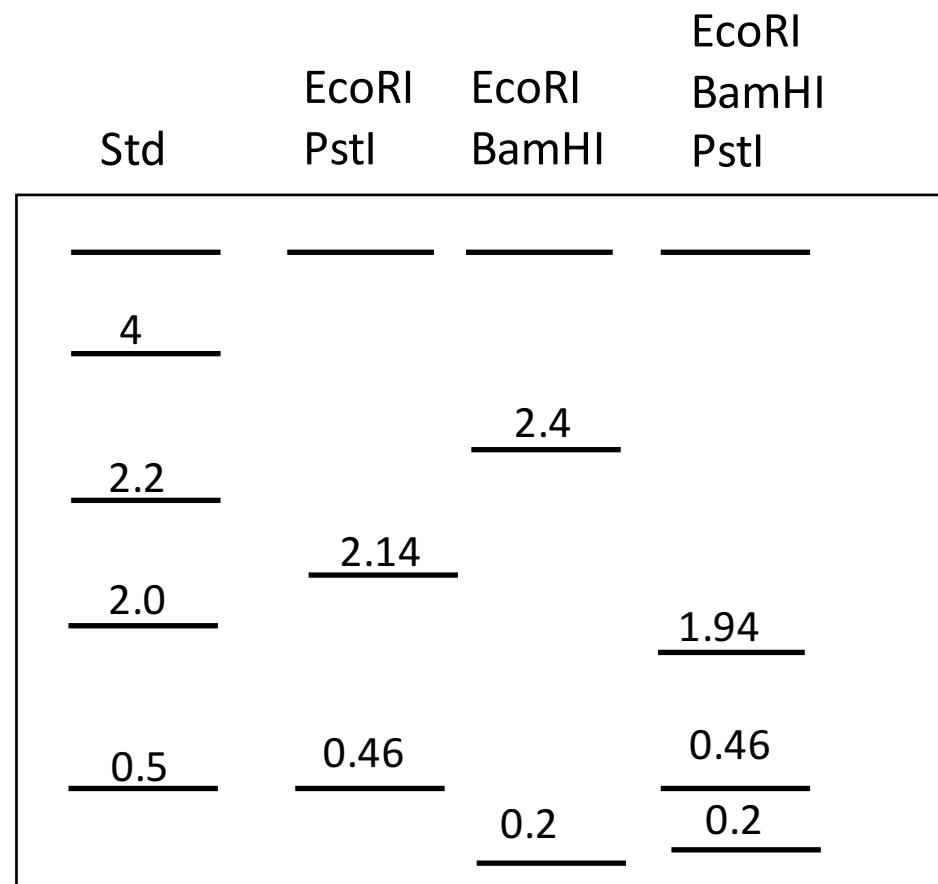
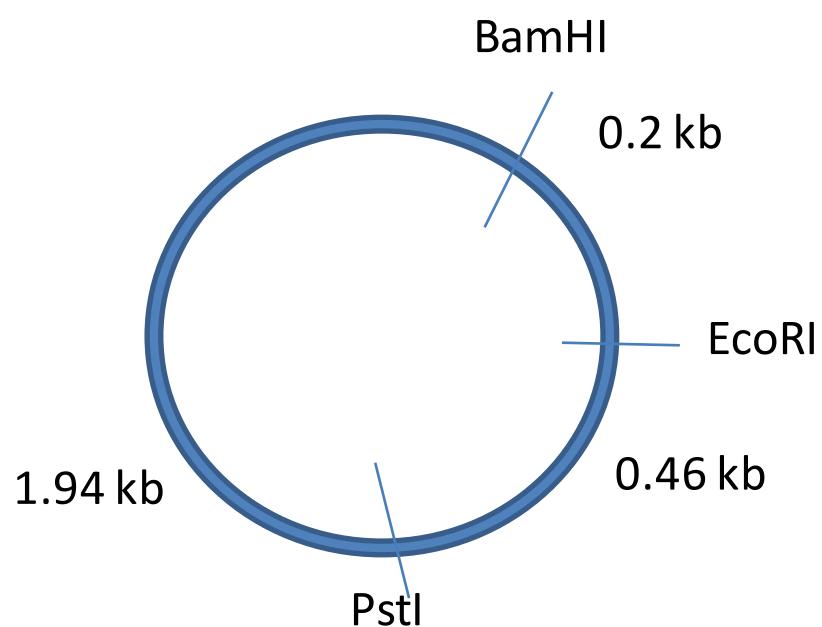
Figure 20.7

Interpreting Gel Image



Interpreting Gel Image

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



Summary: Part-I

- DNA cloning produces multiple copies of a single gene
- Plasmid acts as a cloning vector to carry foreign DNA into a host cell
- Agarose gel electrophoresis can be used to separate DNA fragments
- Gene cloning could be useful for two basic purposes: to amplify a particular gene and to produce a protein product

- DNA Tools
 - Gene Cloning
 - Electrophoresis
 - **PCR**
 - Sequencing
 - Expression

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR)

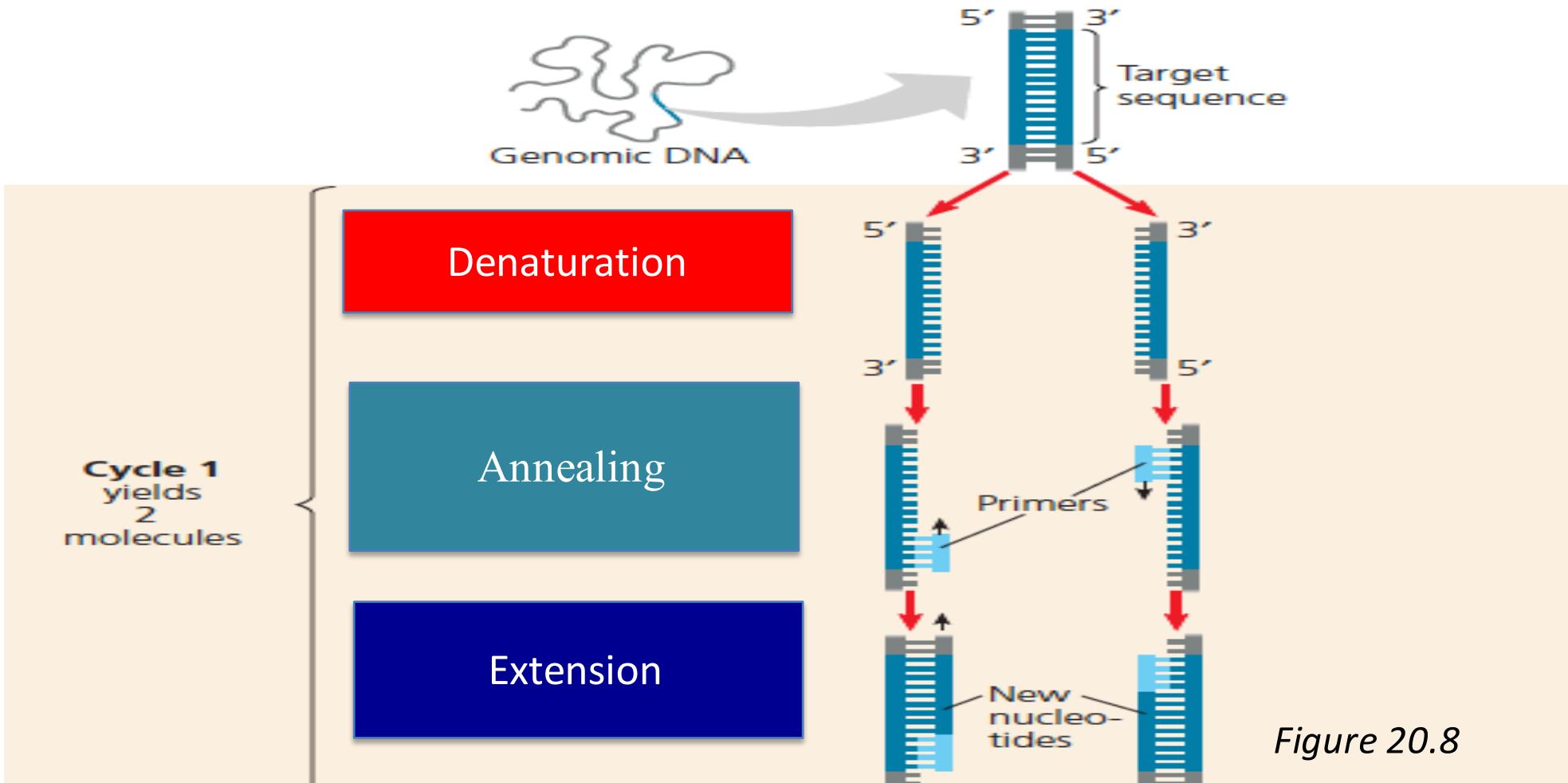
- 1984: Kary Mullis
- Amplification of a target sequence
- To obtain many copies of the desired gene by using a technique called the **Polymerase Chain Reaction**
- Three steps:
 - Strand separation
 - Annealing of primers
 - DNA synthesis

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.*

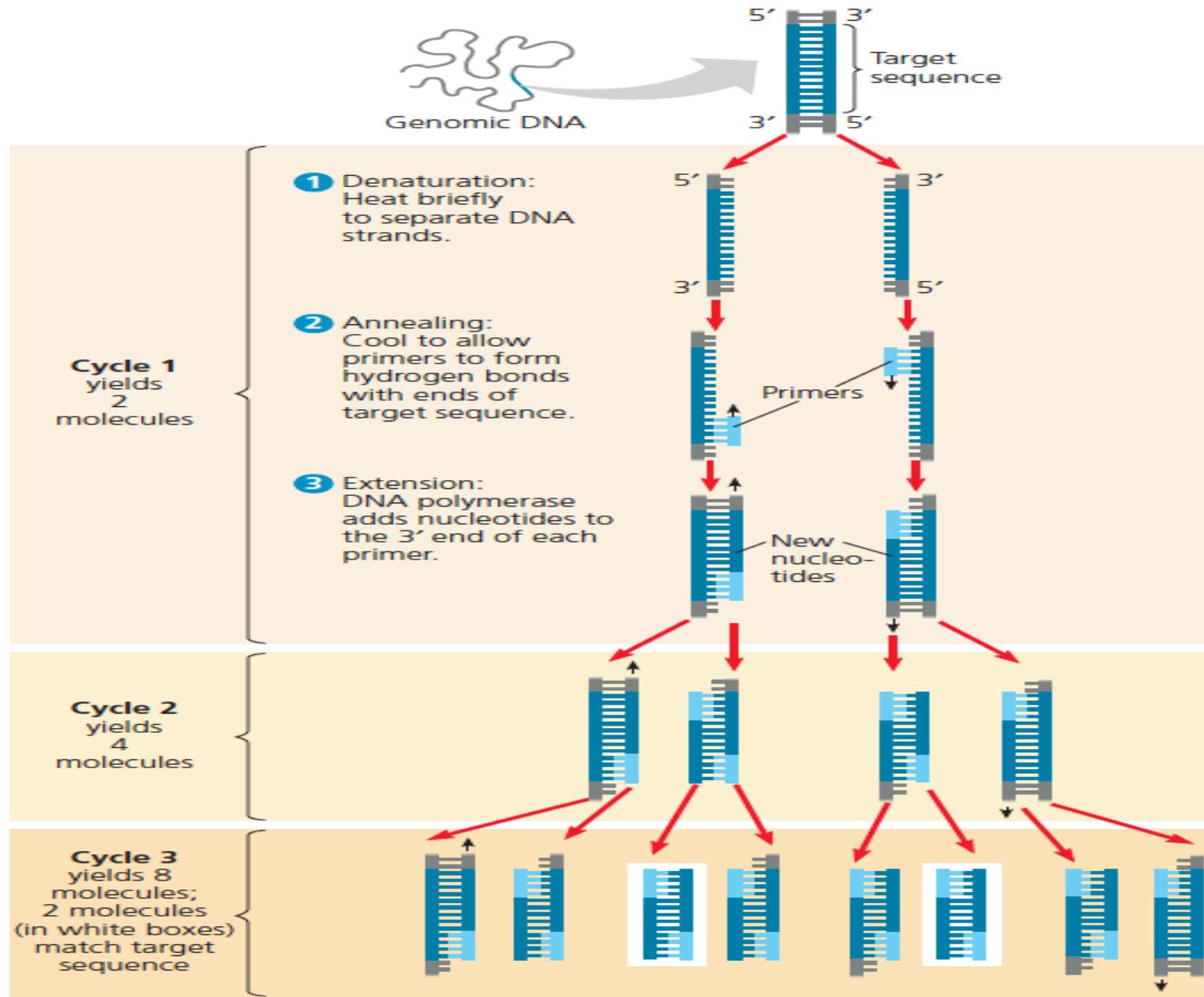
The stability of its DNA polymerase at high temperatures is an evolutionary adaptation that enables the bacterium to survive and reproduce at temperatures up to 95^oC.

Polymerase Chain Reaction (PCR)



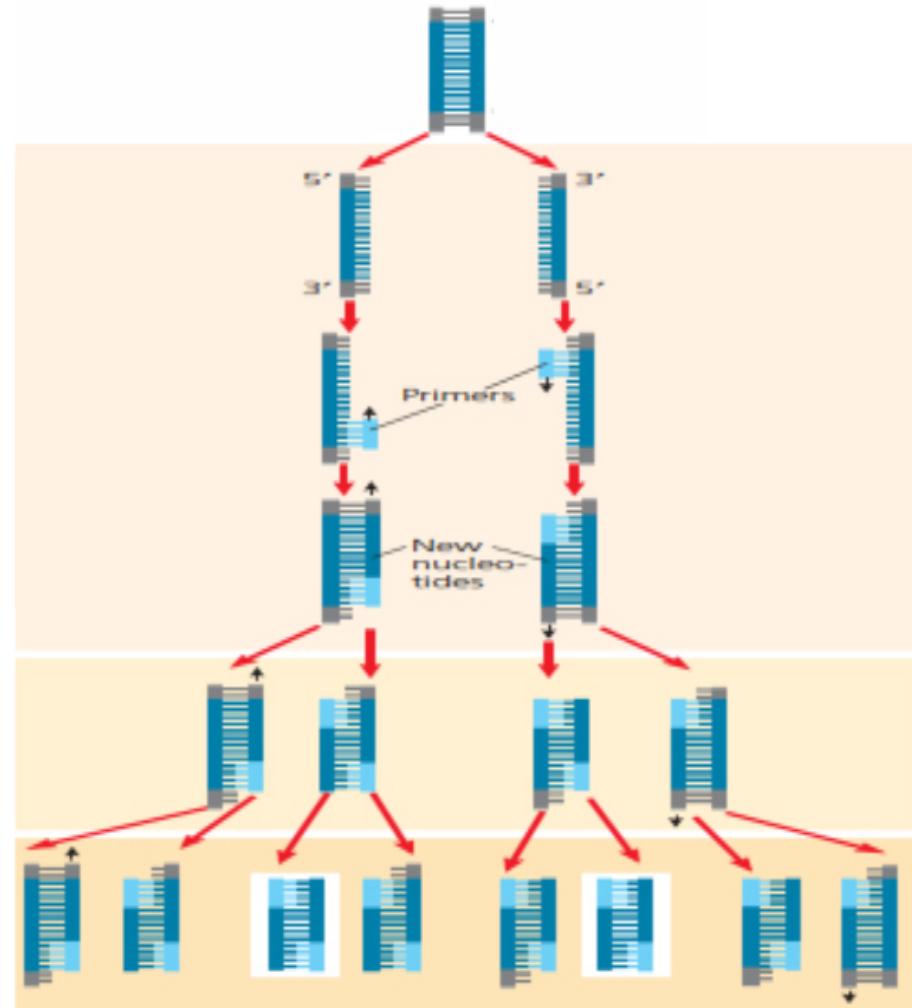
- **Primer** sequences are chosen so that they hybridize only to sequences at opposite ends of the target segment, one on the 3' end of each strand.

PCR: Amplification through Multiple Cycles



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



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)
- $Tm = (A+T) \times 2 + (G+C) \times 4$
- Primers should not be self complimentary

Primer Designing

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

CTGTCCACACAATCTGCCCTTCGAAACCATGGATCCAACGAAAAGAATTCCCACATGGTCCTT
GACAGGTGTGTTAGACGGAAAGCTTGGTACCCTAGGGTGCTTTCTTAAGGGTGTACCAAGGAA
>>>>>>>>>>
CTTGAATTCGAACAGCTGCTGGGATTACACATGGCATGGATGAACATAACAAATAA
GAACCTAACGATTGTCGACG**ACCCTAATGTGTACCGTACCTACTGATATGTTATT**
<<<<<<<<<<<

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

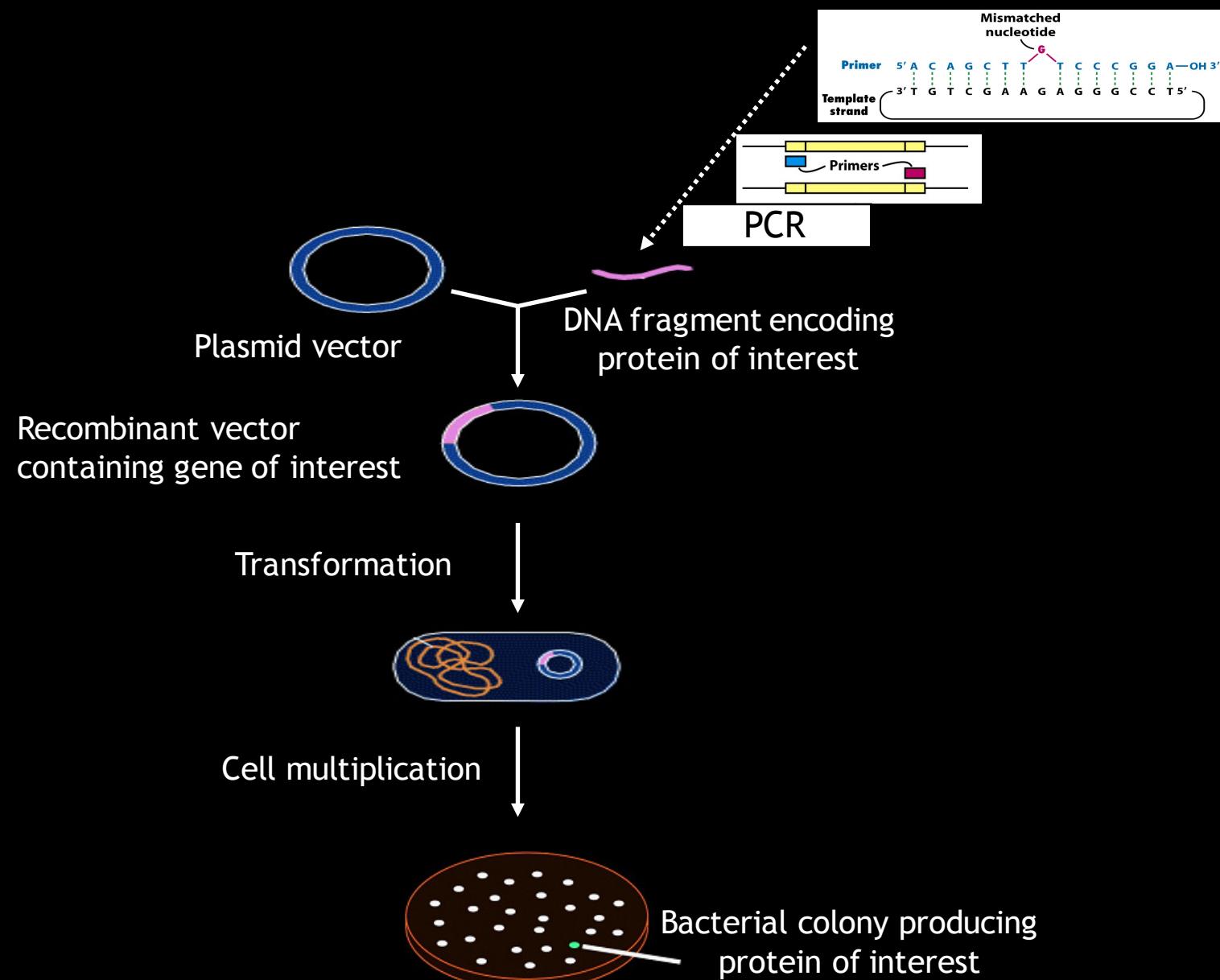
For the reverse primer, you will need to write the sequence of the other DNA strand. The reverse primer (<<<<) which will be complementary to the upper strand and must run 3'-5'. However, we always write DNA sequences in the 5'-3' direction so the reverse primer would be written: 5'-CATGCCATGTGTAATCCCAG-3'

Primers

1. For the primer 5' GATCCGATTGGACACTGTACTA 3'
calculate the Tm.
 - $Tm = (A+T) \times 2 + (G+C) \times 4$

Ans: 64 C

Want to Study a Mutant gene or Protein?



Summary: Part-II

- Primer designing: basic concepts
- PCR: amplification through multiple cycles - denaturation, annealing and extension
- By using genetic engineering tools we could introduce different mutant forms of the gene for that protein into eukaryotic cells

References

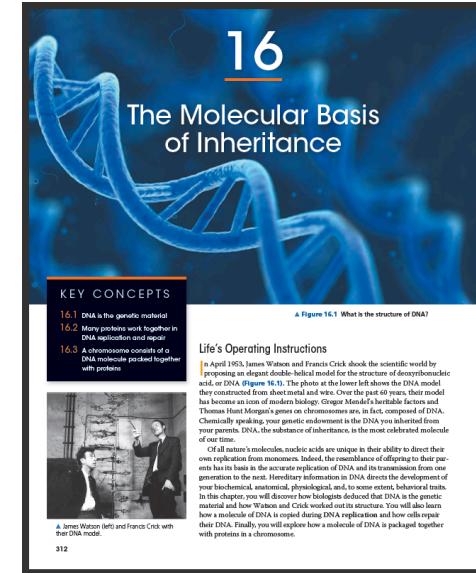
- Campbell Biology - Reece, Urry, Cain, Wasserman, Minorsky, Jackson 10th Edition, Cummings

- *Video contents*

- <https://www.youtube.com/watch?v=PbKpaLPV9A8>
- <https://www.youtube.com/watch?v=2KoLnIwoZKU>

- *Acknowledgment*

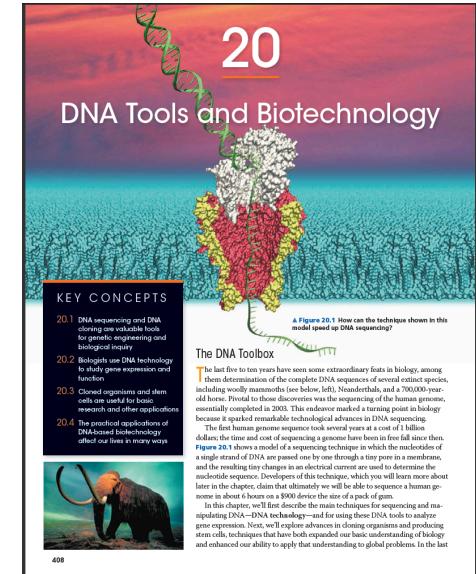
- Cover images – getty images



A Figure 16.1. What is the structure of DNA?

Life's Operating Instructions

In April 1953, James Watson and Francis Crick shook the scientific world by proposing an elegant double-helix model for the structure of deoxyribonucleic acid, or DNA (Figure 16.1). The photo in the lower left shows the DNA model they constructed in the Hertha Morrison Building at Cambridge University. This model has become an icon of modern biology. Gregor Mendel's heritable factors and Thomas Hunt Morgan's genes on chromosomes are, in fact, composed of DNA. Chromosomes may be microscopic, but the information in them is encoded in the molecules of our DNA. All of nature's molecules make use of their ability to direct their own replication from monomers. Indeed, the responsibility of offering to their parents has its basis in the accurate replication of DNA and its transmission from one generation to the next. Heredity is the process of passing traits from parents to offspring. These traits may be anatomical, physiological, and so on; or genetic, behavioral traits. In this chapter, you will discover how biologists deduced that DNA is the genetic material and how it can and does work as a template for its structure. You will also learn how a single DNA is used to direct DNA replication and how it directs the synthesis of other DNA. Finally, you will explore how a molecule of DNA is packaged together with proteins in a chromosome.



A Figure 20.1. How can the technique shown in this model speed up DNA sequencing?

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