



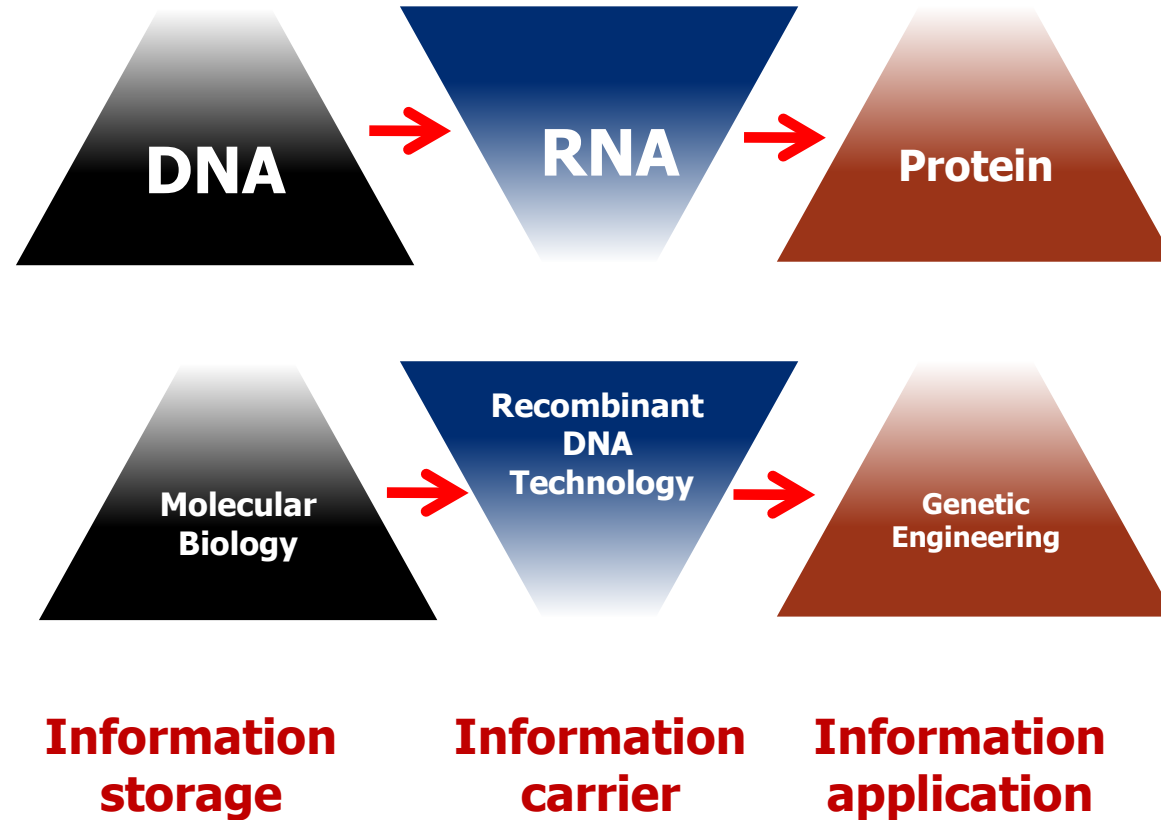
JOHNS HOPKINS

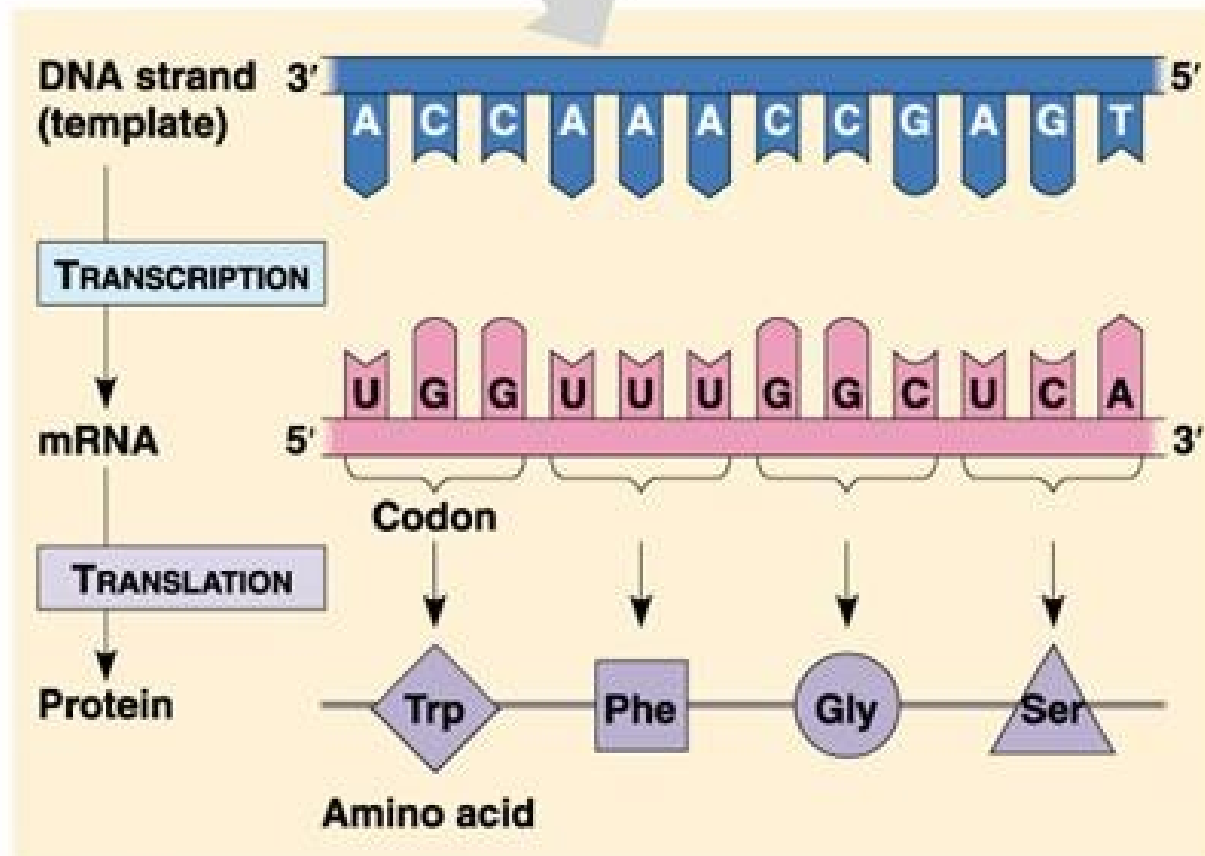
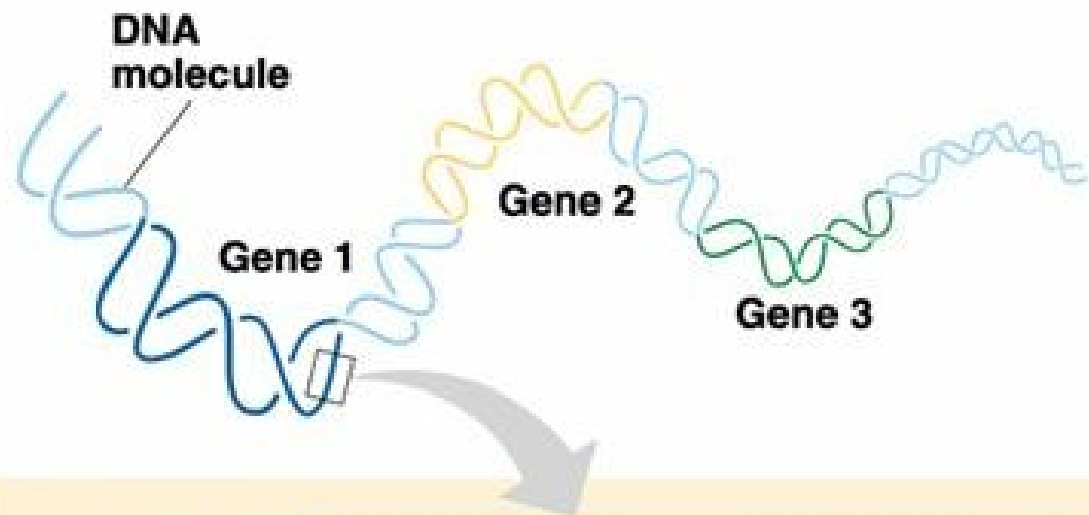
WHITING SCHOOL
of ENGINEERING

Cell and Tissue Engineering

Quantum Information Processing and Genetic Engineering, Part 2

Central dogma of Molecular Biology





Genotype



Phenotype

Levels of gene regulation



- Evolutionary or Genomic Level (species)
- Individual level (genotype)
- Chromosomal level (open or closed, transcription factors)
- mRNA Level (regulated initiation, splicing, degradation)
- Protein Level (regulated initiation, modifications, sorting, degradation)
- Composite Outcome + environment (phenotype)

Levels of gene regulation

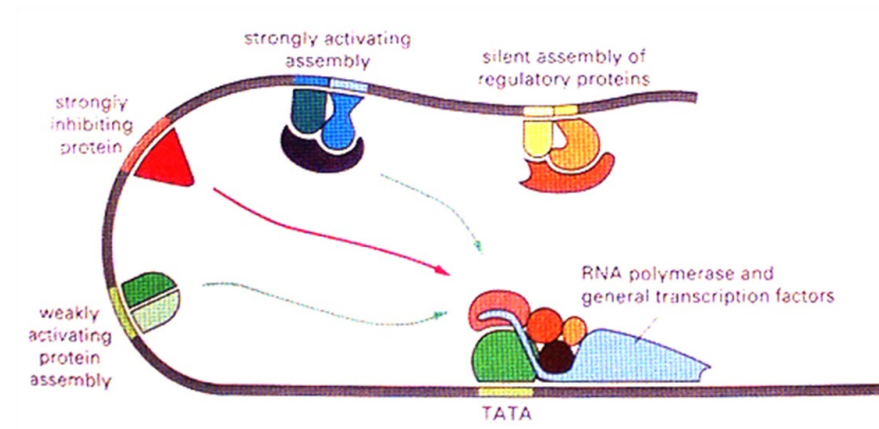
- Evolutionary or Genomic Level (species)
- Individual level (genotype)

Complex diseases are more likely to be due to genetic variation in regions that control gene activity than regions that encode proteins

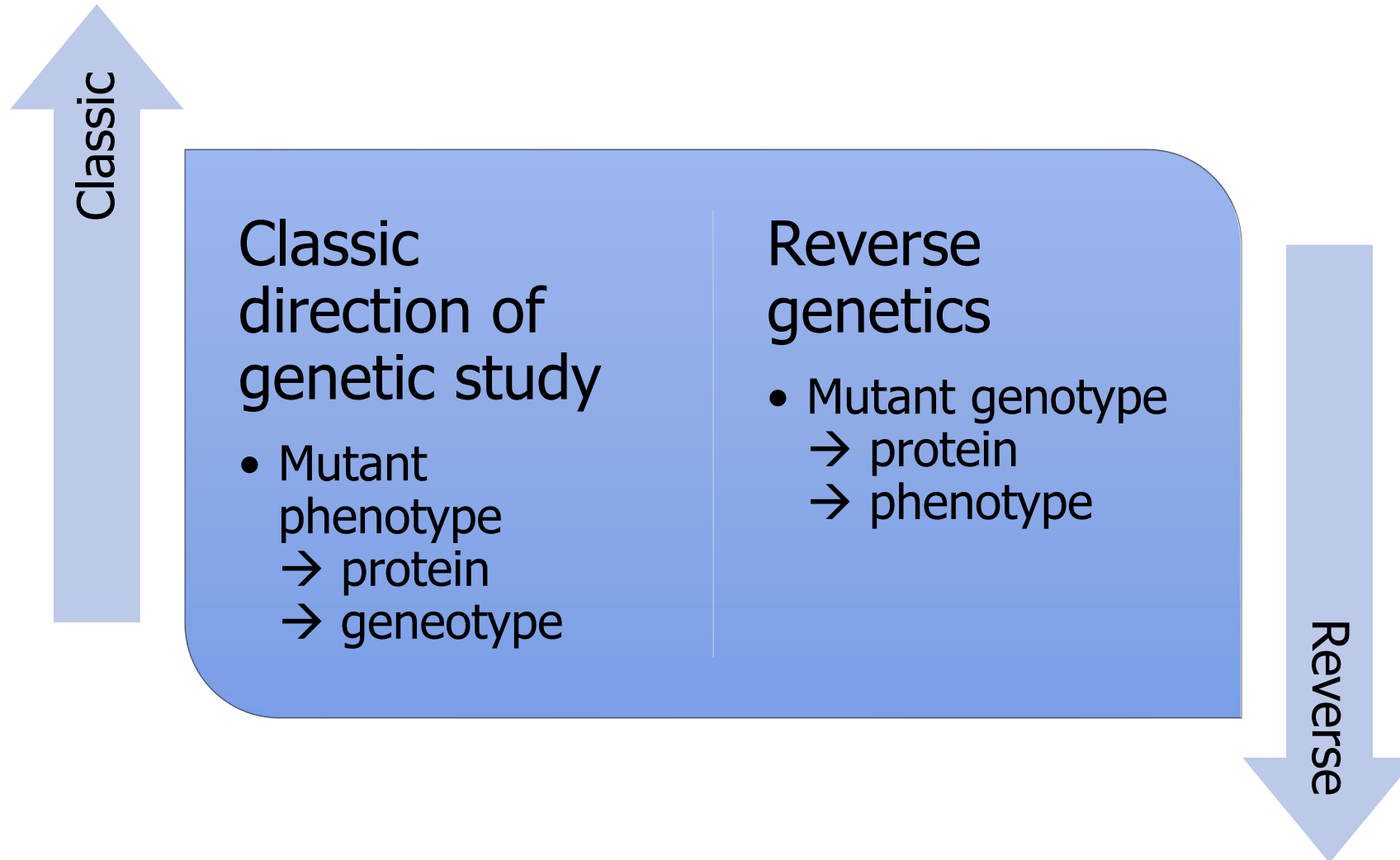
Control of gene expression is important!

Levels of regulation

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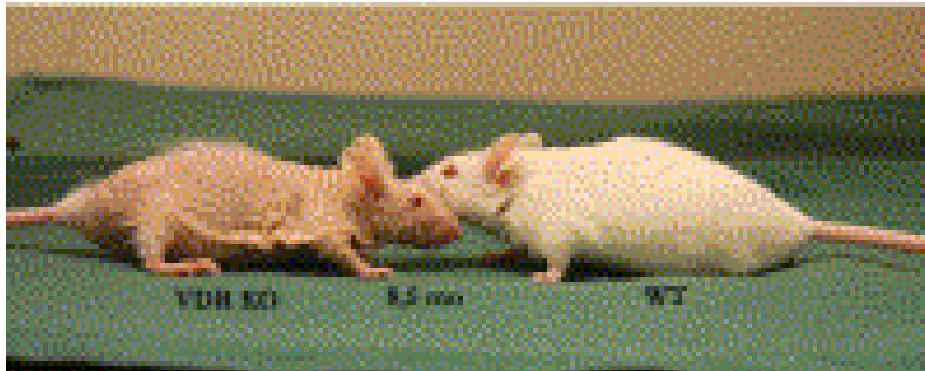


A shift in the way we examine gene function



Knockout mouse example

- Vitamin D₃ implicated in premature aging
- Vitamin D₃ receptor (VDR) KO mouse



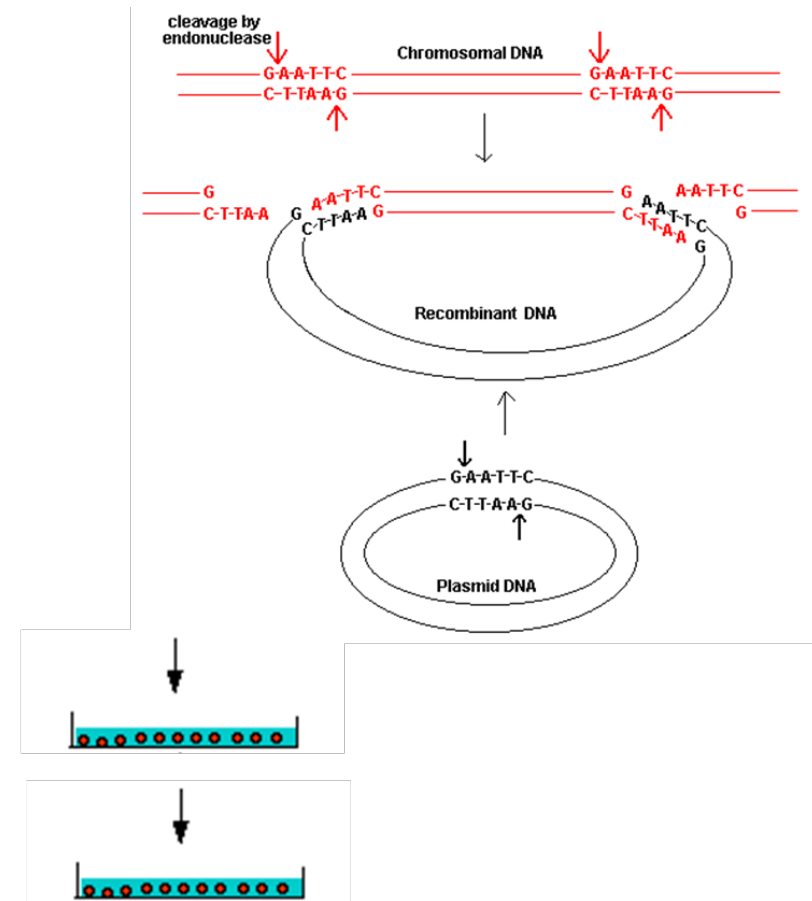


What is recombinant DNA technology?



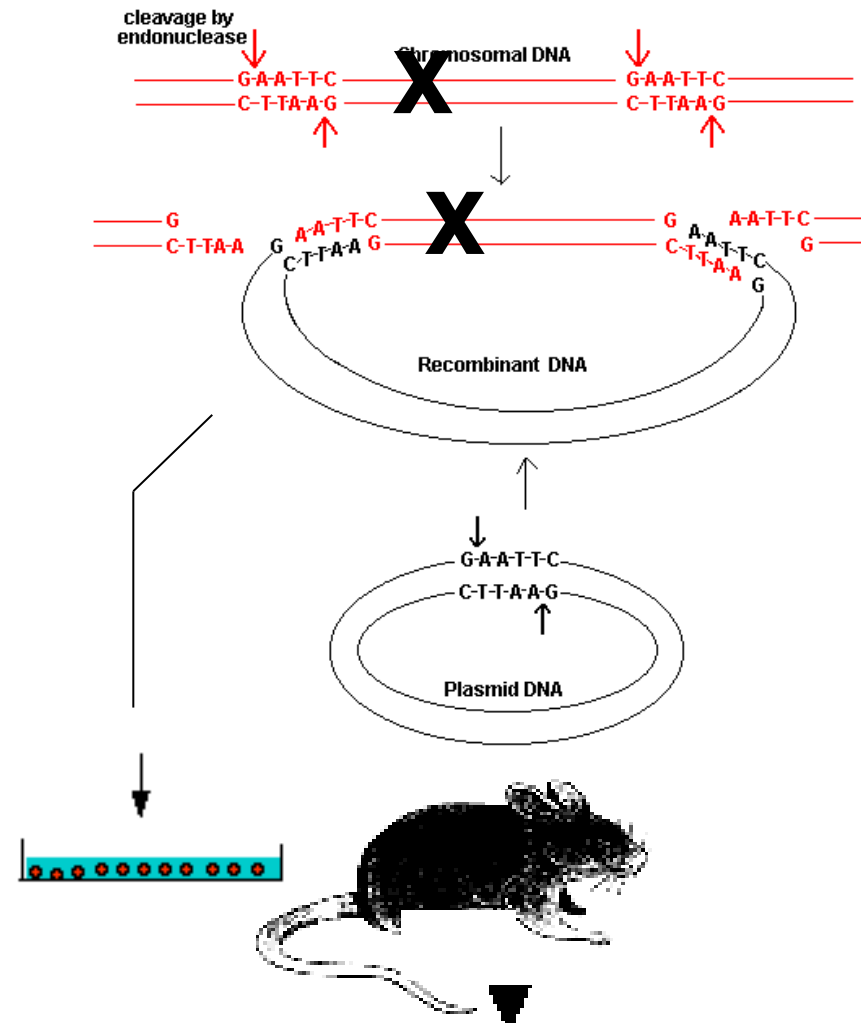
Recombinant DNA technology

- Cloned gene or protein of interest
- Mutagenesis
- Transformed into a cell
- Integrated into genome
- Expressed in daughter cells (whole organism)



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Recombinant DNA technology (cont.)



Why make a transgenic animal?

- To study gene function
- To produce human proteins for therapy or study
- To create a disease model to test therapeutics
- To improve organism traits



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Making a transgenic animal



5 major recombinant DNA techniques

Restriction	cutting at a specific site using restriction enzymes
Hybridization	using a specific fragment of DNA or RNA to identify related sequences
Sequencing	determining the nucleotide sequence of a DNA fragment
DNA cloning	taking a single DNA molecule and making billions of identical copies
DNA engineering	altering DNA sequences to modify gene function
DNA microarray	Monitoring the expression of every gene using nucleic acid microarrays

Restriction - isolating the DNA sequence

Restriction Endonucleases

BamH1

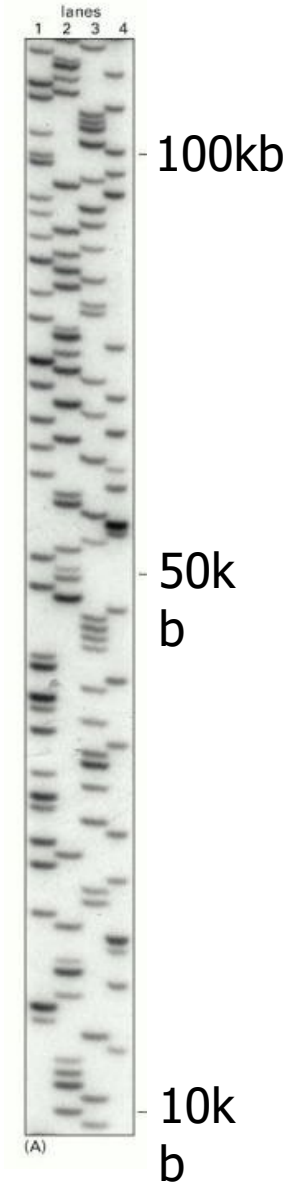
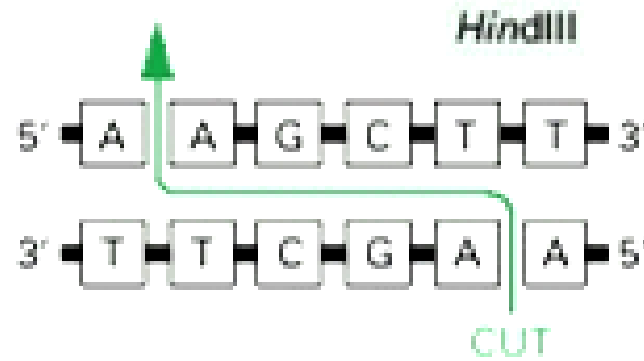
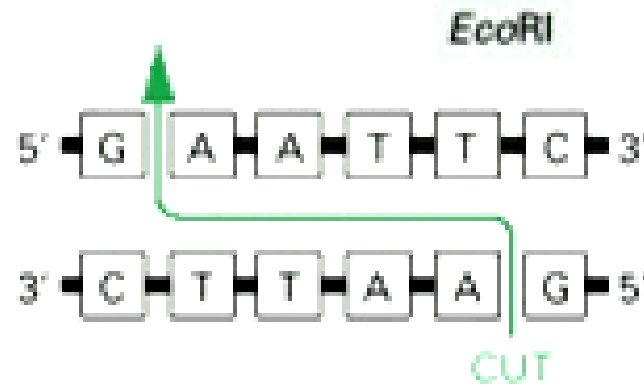
HindIII

EcoR1

...

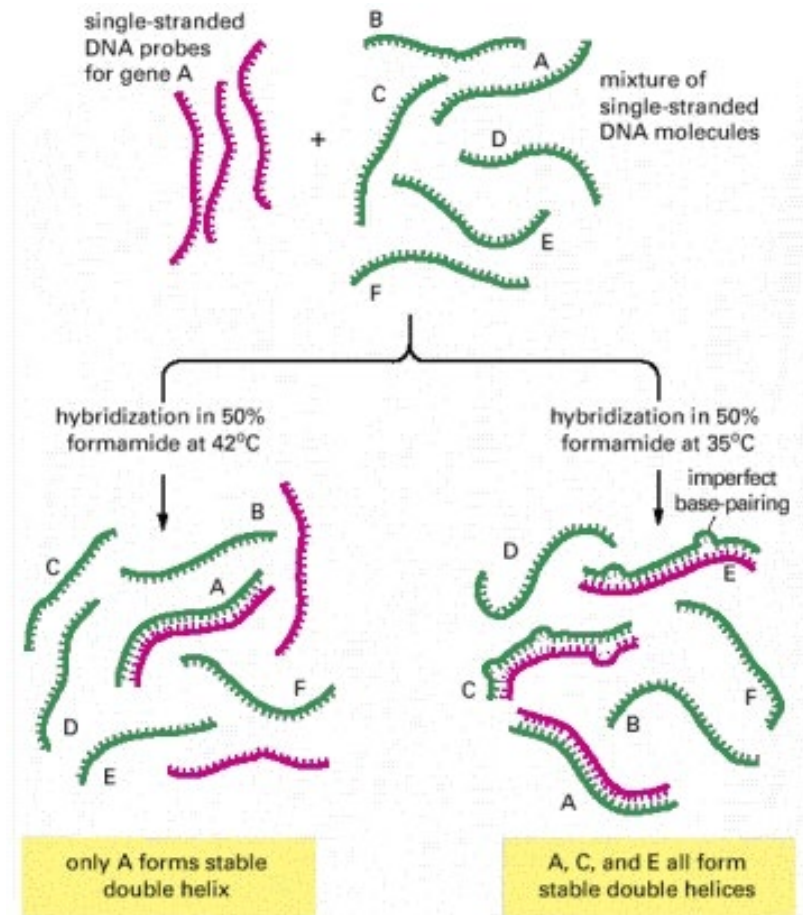
(AluI, HaeIII)

Ligase



Hybridization - detecting a sequence

Hybridization - detecting a sequence (cont.)



Hybridization - detecting a sequence (cont.)

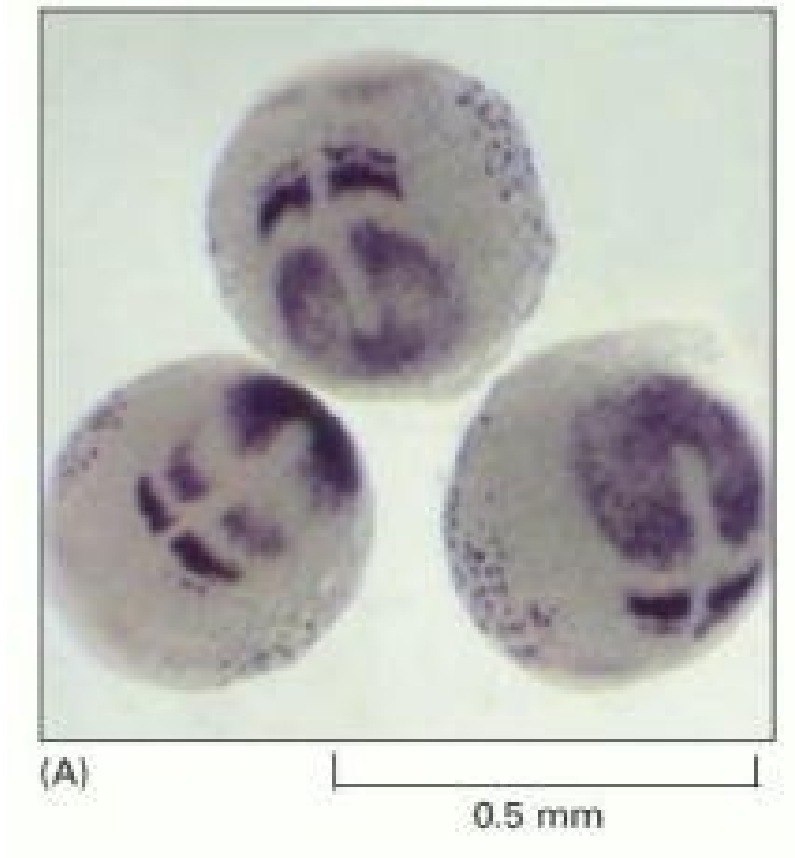


DNA – DNA

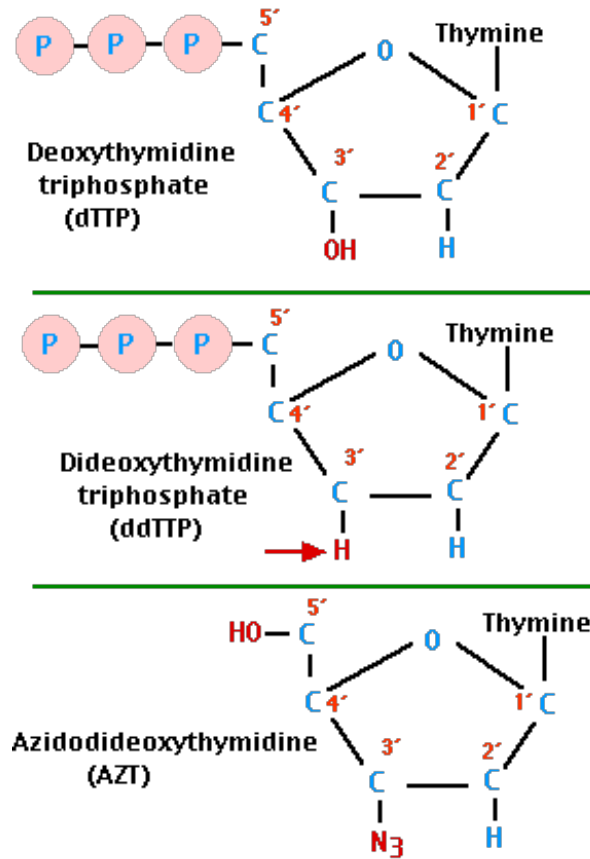
RNA – RNA

DNA – RNA

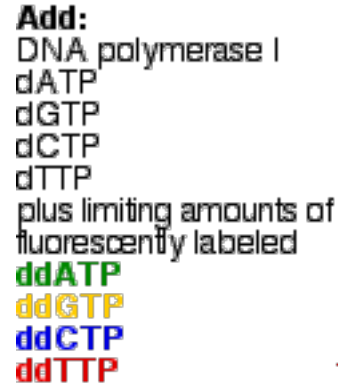
Hybridization - detecting a sequence (cont.)



Sequencing - reading the sequence

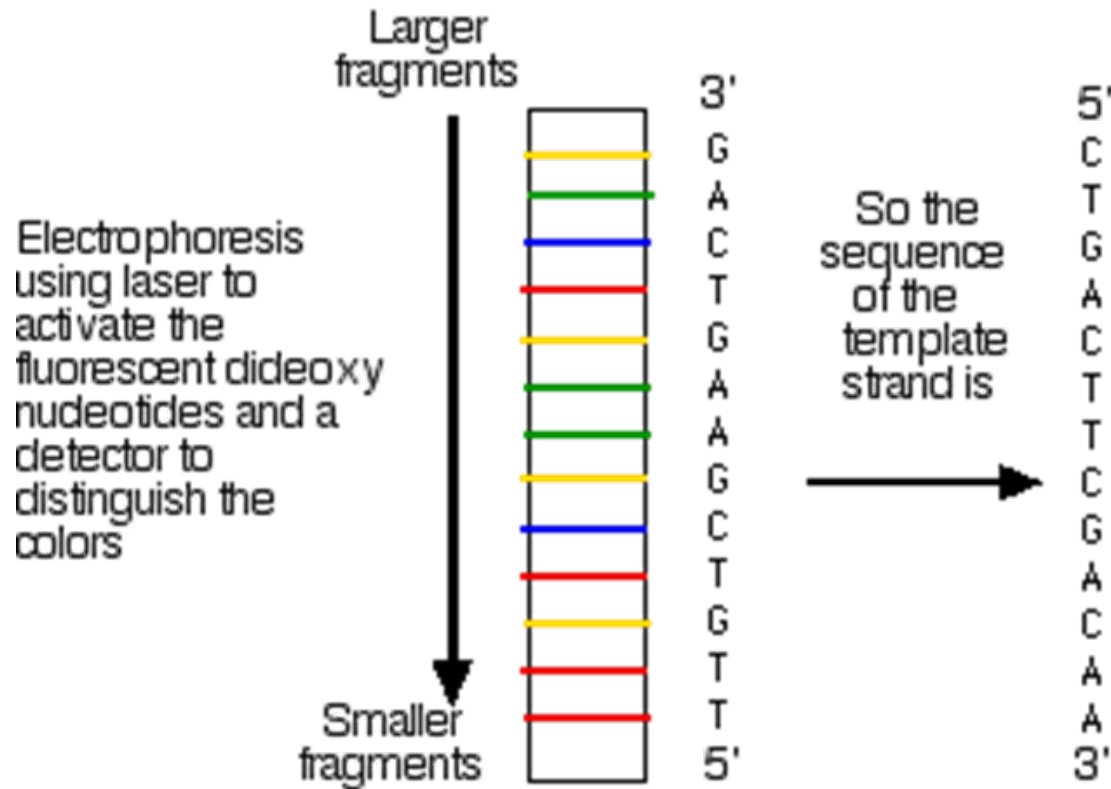


Sanger or chain termination method



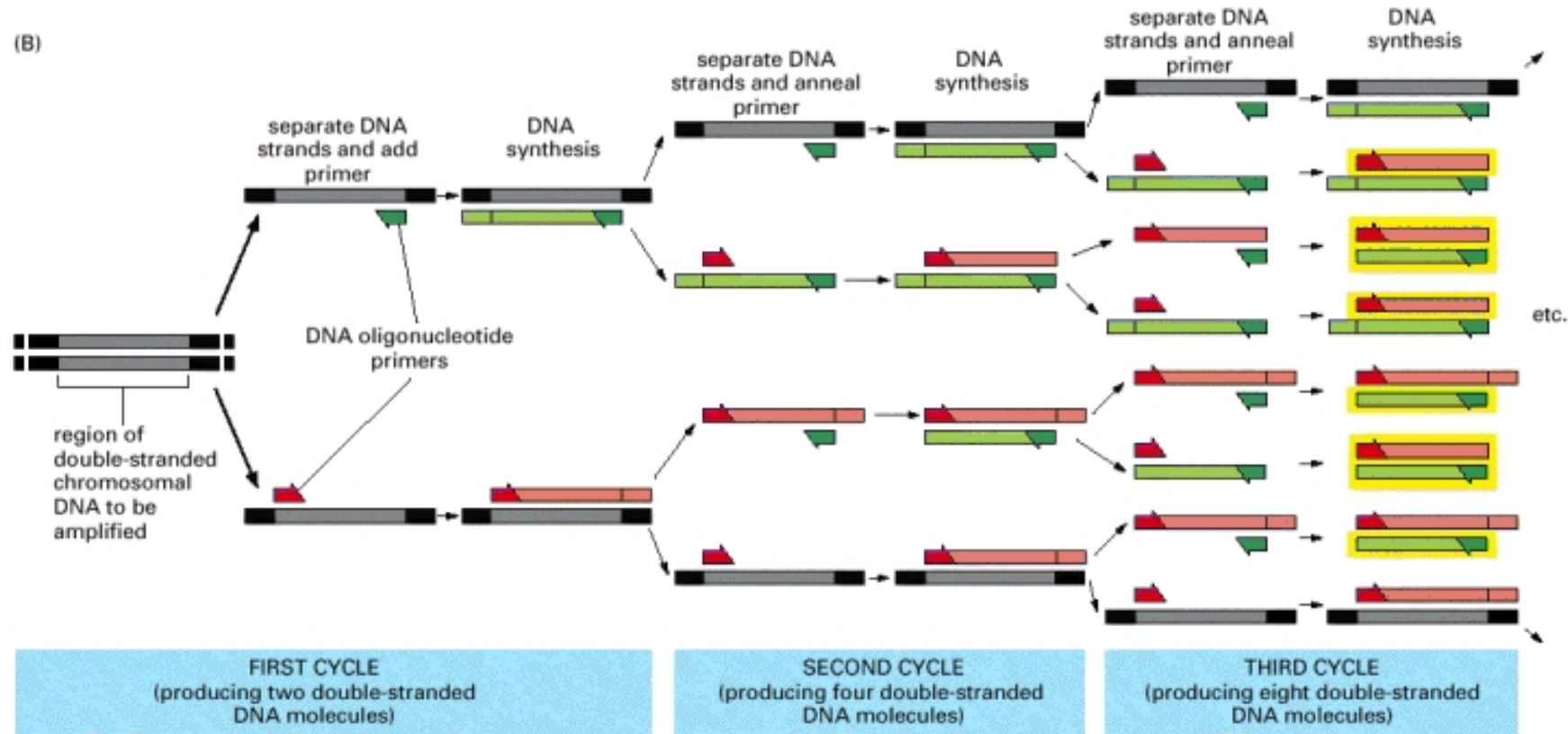
Sanger or chain termination method

Sequencing - reading the sequence (cont.)



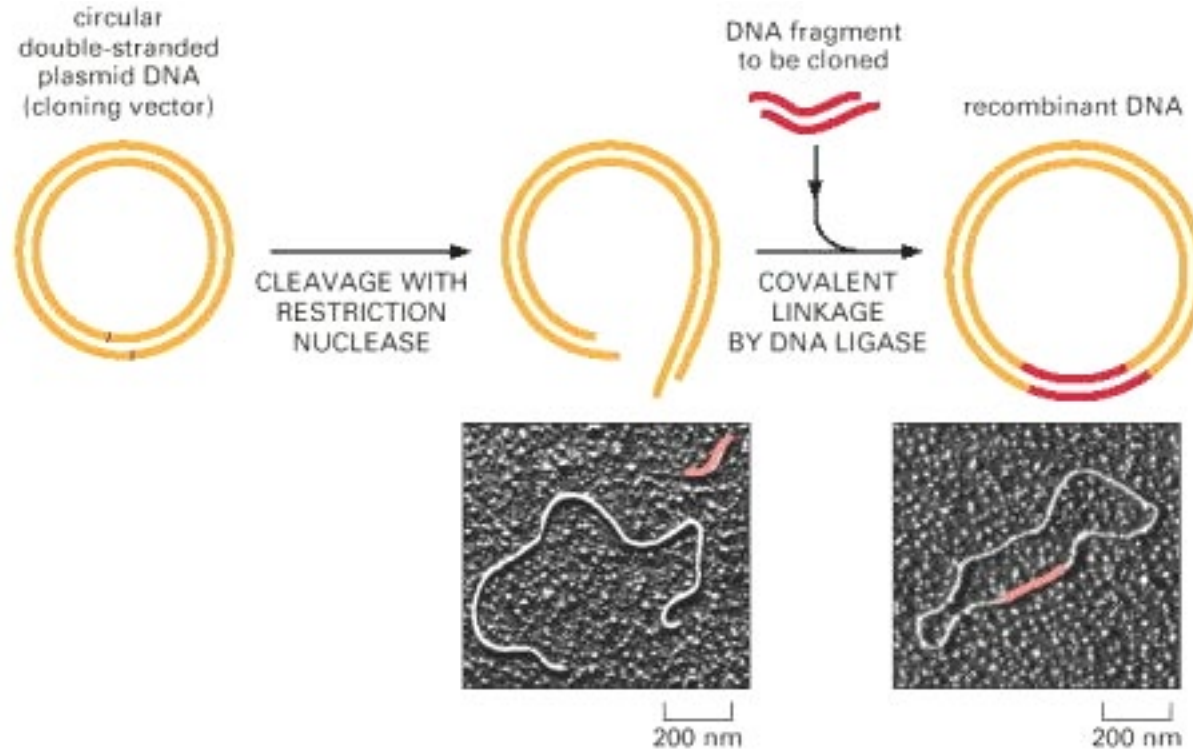
Sanger or chain termination method

Cloning - copying the DNA sequence



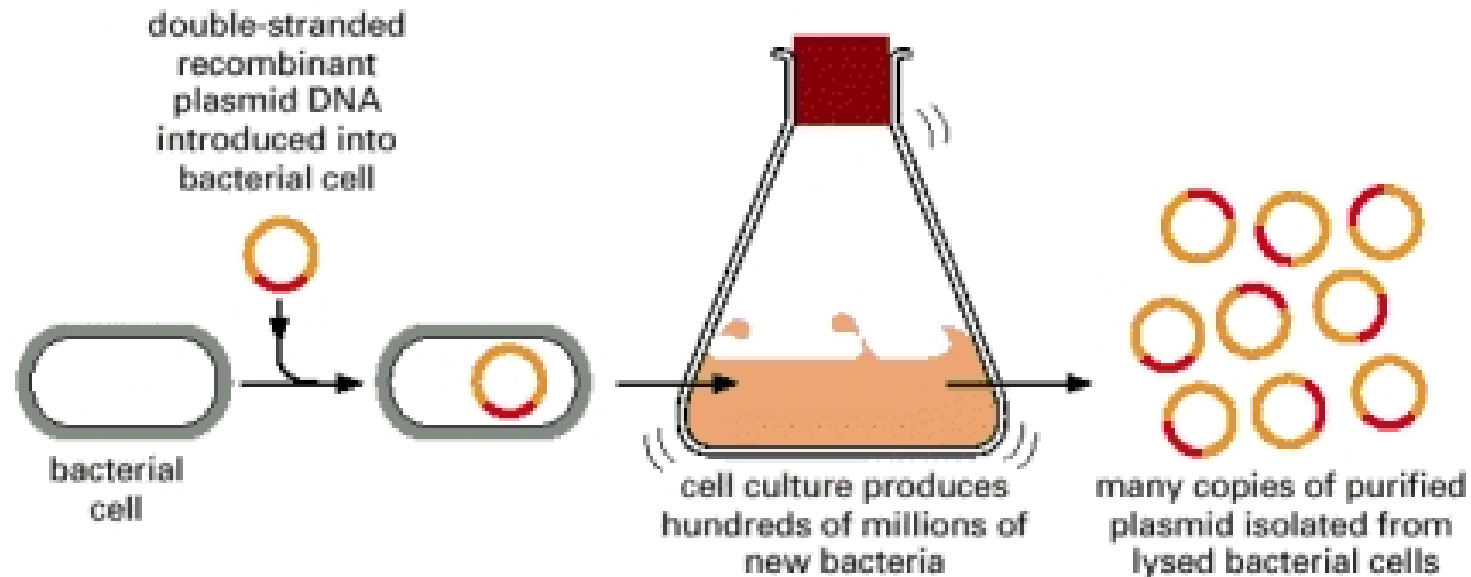
PCR Amplification Technique

Cloning - copying the DNA sequence



Cloning Vector Technique

Cloning - copying the DNA sequence



Cloning Vector Technique



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