Chem 431C Lecture 10b

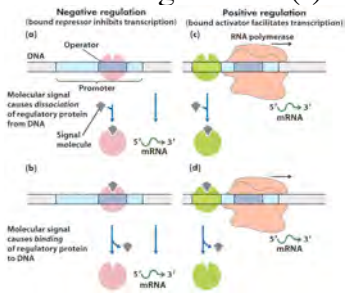
Today:

a) Finish Trp gene regulation (skip rest of chapt 28)

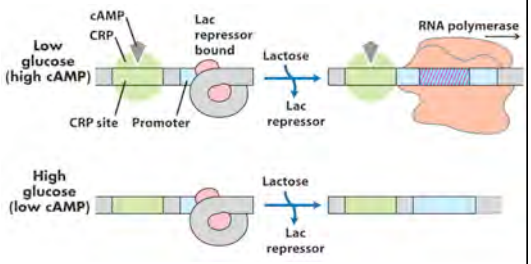
b) Student Survey

Friday: Quiz 8 + finish ch 9 + review

Patterns of regulation: (-) & (+)



Catabolite repression of lac op.



Trp operon regulation:2 mechs.

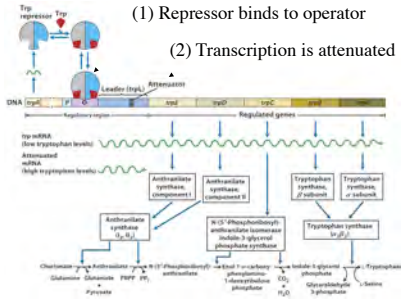
Trp operon is regulated by 2 mechanisms:

When Trp high:

1) repressor binds to operator

2) *Transcription attenuation*

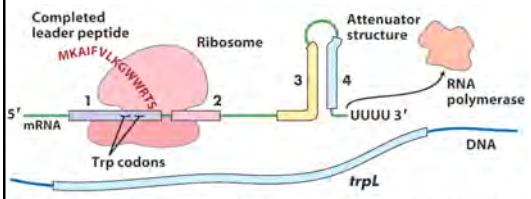
Trp operon regulation by 2 mechs.



(1) Repressor binds to operator

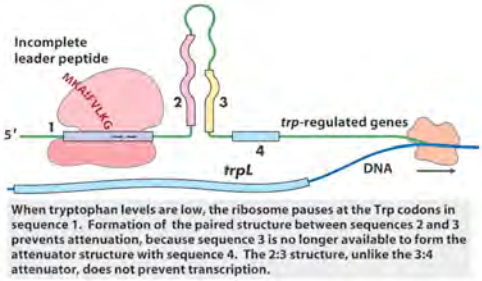
(2) Transcription is attenuated

Transcriptional attenuation of Trp

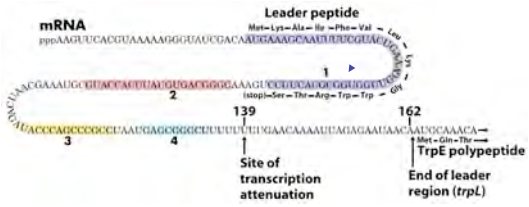


When tryptophan levels are high, the ribosome quickly translates sequence 1 (open reading frame encoding leader peptide) and blocks sequence 2 before sequence 3 is transcribed. Continued transcription leads to attenuation at the terminator-like attenuator structure formed by sequences 3 and 4.

When [Trp] is low, 2-3 structure forms, preventing 3-4 struc



When tryptophan levels are low, the ribosome pauses at the Trp codons in sequence 1. Formation of the paired structure between sequences 2 and 3 prevents attenuation, because sequence 3 is no longer available to form the attenuator structure with sequence 4. The 2:3 structure, unlike the 3:4 attenuator, does not prevent transcription.



DNA based information technologies Chapt 9

Cloning techniques have given rise to modern fields on the scale of whole cells:

Genomics - study of genomes of organisms

Proteomics - large scale study of proteins in a cell as it undergoes distinct changes

Clone = an identical copy

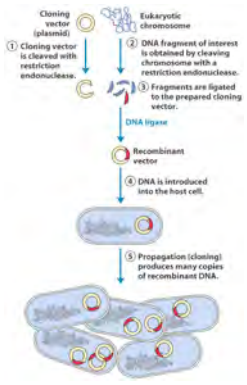
DNA cloning = selecting specific gene or sequence from chromosome, attaching it to small carrier DNA, replicating this DNA millions of times , resulting in selective amplification of this particular gene.

Basics of DNA cloning

Uses 5 General Procedures:

1. Cutting DNA at precise locations (restrictn endonucl)
2. Selecting a small molecule of DNA capable of self -replication (cloning vectors, plasmids)
3. Joining two DNA fragments covalently (ligases)
4. Moving recombinant DNA from test tube to a host cell
5. Selecting or identifying host cells that contain recombinant DNA

Overview of DNA cloning



Enzymes used in Recombinant DNA Technology

TABLE 9–1 Some Enzymes Used in Recombinant DNA Technology

Enzyme(s)	Function
Type II restriction endonucleases	Cleave DNAs at specific base sequences
DNA ligase	Joins two DNA molecules or fragments
DNA polymerase I (*E. coli*)	Fills gaps in duplexes by stepwise addition of nucleotides to 3′ ends
Reverse transcriptase	Makes a DNA copy of an RNA molecule
Polynucleotide kinase	Adds a phosphate to the 5′-OH end of a polynucleotide to label it or permit ligation
Terminal transferase	Adds homopolymer tails to the 3′-OH ends of a linear duplex
Exonuclease III	Removes nucleotide residues from the 3′ ends of a DNA strand
Bacteriophage λ exonuclease	Removes nucleotides from the 5′ ends of a duplex to expose single-stranded 3′ ends
Alkaline phosphatase	Removes terminal phosphates from either the 5′ or 3′ end (or both)

Restriction Endonucleases

Restriction endonucleases cleave foreign DNA duplex strands which are not properly methylated at restriction sites.

DNA methylases and restriction endonucleases come in pairs to make up the restriction modification system

(a kind of cell surveillance system)

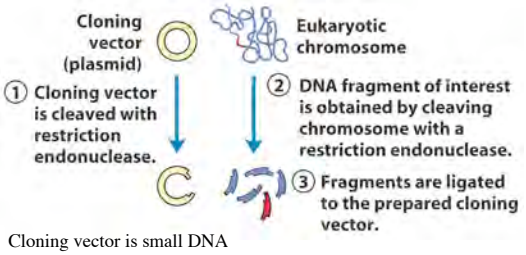
Essential for consistently cutting DNA at the same site.

Naming system:

HindII (**H**aemophillus **in**fluenza strain **dII**)

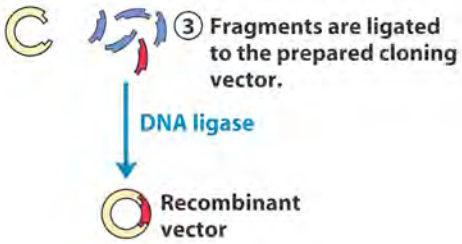
EcoRI (**E. co**li strain **R** (first recognized) **I**)

DNA cut precisely using Restriction endonuclease

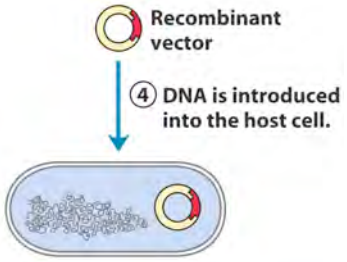


Cloning vector is small DNA molecule capable of self replication

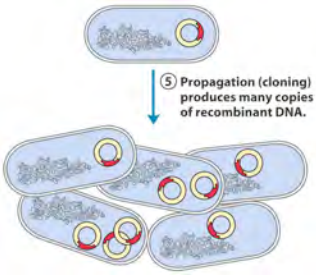
DNA ligase joins 2 DNA fragments covalently



Cloning vector enters cell



Propagation produces multiple copies



Recognition sequences for some Type II Restriction Endonucleases

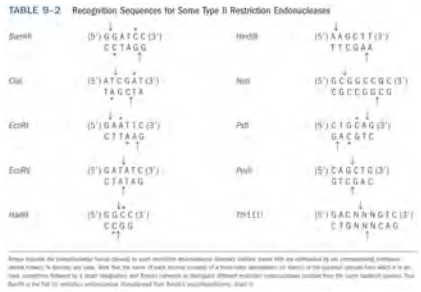


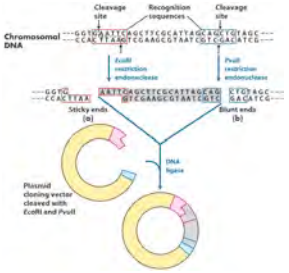
TABLE 9-2 Recognition Sequences for Some Type II Restriction Endonucleases

Cleavage of DNA by restriction endonucleases

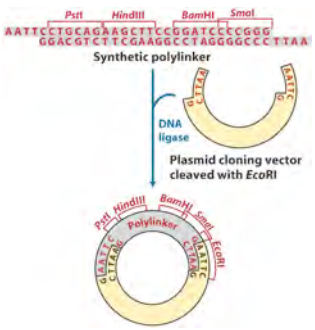
Two types of ends are formed:

Blunt ends and

Sticky ends



A synthetic polylinker can be incorporated into the plasmid



Constructed E.coli plasmid pBR322