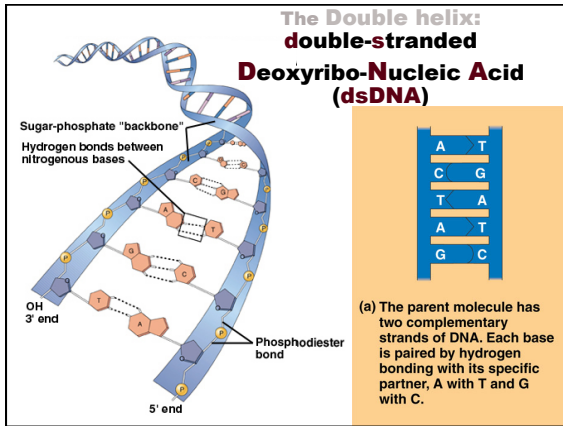


DNA Digestion & Ligation Lab



RESTRICTION ENZYMES

- Bacteria produce special enzymes to chop up viral DNA.
- Biotechnologist use these "restriction enzymes" to cut DNA in specific places (restriction sites).
- Many restriction enzymes cut the DNA polymer in a staggered pattern that produce "sticky" single-stranded ends to the DNA fragments.

RESTRICTION ENZYMES

- Since a particular restriction enzyme only cuts DNA at a specific DNA sequence (restriction site):
- Any DNA cut by that enzyme will have the same "sticky ends".

Eco RI bound to DNA

Homodimer. Pink circles are manganese ions.

Restriction Sites

HinDIII **EcoRI**

TAAGCTTGTCAAATGAATTCCTAC
ATTCGAACAGTTTACTTAAGAGATG

Restriction Digests

with HinDIII

TA AGCTTGTCAAATGAATTCCTAC
ATTCGA ACAGTTTACTTAAGAGATG

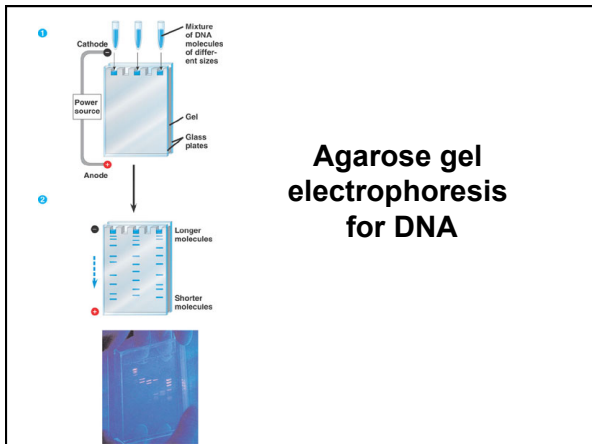
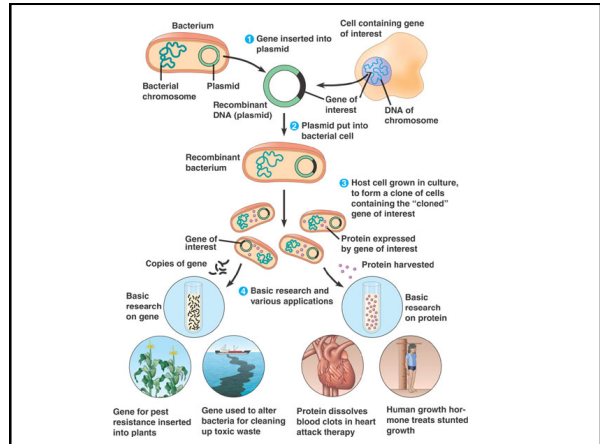
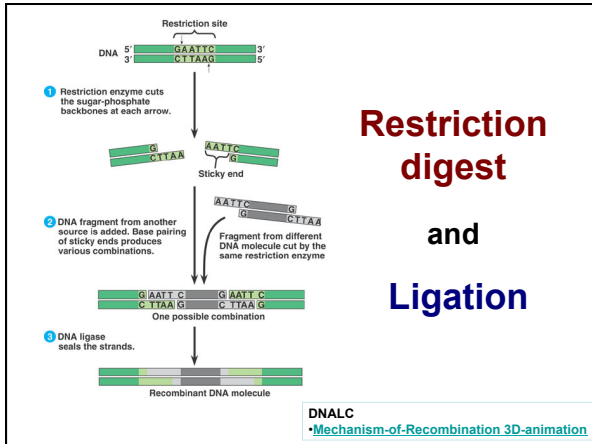
with EcoRI

TAAGCTTGTCAAATG AATTCCTAC
ATTCGAACAGTTTACTTAA GAGATG

with both HinDIII & EcoRI

TA AGCTTGTCAAATG AATTCCTAC
ATTCGA ACAGTTTACTTAA GAGATG

DNA Digestion & Ligation Lab



- ## Stock Reagents for DNA Digest & Ligation Lab
- Lambda DNA: 250 ng/ul [nanograms per microliter]
 - Eco RI: 20 Units/ul
 - Hin DIII: 20,000 Units/ml
 - Ligase: 3 Units/ul
- Subject to verification before use.
 - Use the above to calculate your working volumes, concentrations and dilutions.
 - Pay attention to the units!
 - Keep all solutions cold (on ice).