BIOTECHNOLOGICAL TOOLS AND TECHNIQUES

- Recombinant DNA technology employs cutting and splicing together DNA from different species, inserting them into bacteria or cells that are capable of rapid replication.
- the cells then make copies of the foreign DNA along with their own

Restriction Endonucleases

- are restriction enzymes that cut double-stranded DNA at a specific base sequence.
- a recognition site is a sequence of nucleotides (4 to 8 base pairs) that the enzyme recognizes so the cut can be made.
- Recognition sites are usually characterized by a complementary palindromic sequence
- See Fig. 1 on pg. 278 and Table 1 on pg. 279
- a cut at two identical sequences in the same molecule produces a fragment
- enzymes can make staggered cuts leaving a single-stranded portion of the molecule at both ends (sticky ends).
- these ends can base pair with other DNA molecule that have been cut by the same restriction enzyme.
- Some enzymes produce fragments with ends that are fully based paired (blunt ends)
- Restriction endonucleases that produce sticky ends are more useful than ones that produce blunt ends, because sticky-end fragments can be easily joined to other fragments cut by the same restriction endonuclease through complementary base pairing

- One of the roles of restriction enzymes in bacteria is to provide a crude immune system
- When a virus (bacteriophage) enters the bacteria, the restriction enzyme cleaves it into fragments so it is no longer capable of transcription or translation. (See Fig. 2 on pg. 280)
- Restriction enzymes are named from the bacteria they came from.

- Ex. HindII:

H	Haemophilus	Genus name
in	influenzae	Species name
d	Rd strain	Strain
II	Second endonuclease isolated from this strain.	

Methylases

- Restriction enzymes must be able to distinguish foreign DNA from their own genetic material,
- To do this, prokaryotes use enzymes called methylases.
- This enzyme adds a methyl group to the recognition site for a restriction endonuclease, preventing the enzyme from cutting the DNA.
- See Fig. 3 on pg. 281

DNA Ligase

- In order to be useful to molecular biologists, genes cut out of source DNA must be joined to foreign target DNA

- If two fragments have been created using the same restriction enzyme, they will be attracted to each other at their complimentary sticky ends
- H-bonds will join the complimentary base pairs, but this is not stable
- The sugar-phosphate backbone of DNA must be sealed, joining the cut strands of DNA together
- DNA ligase does this using a condensation reaction, and it is only efficient for joining sticky ends together
- T4 DNA ligase is used instead to joins the blunt ends together.
- See Fig. 4 on pg. 282.

Seatwork
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