General Biology II (101-HTK) Recombinant DNA Technology & Biotechnology – Concepts and Learning Outcomes

Topic	Chapters 19, 20 pages*	Concept	Learning Outcomes
Overview of recombinant DNA technology	p. 368-369	1. Genetic engineering refers to the manipulation of DNA sequences or genes in living organisms using a variety of different techniques most important of which is recombinant DNA technology. 2. In recombinant DNA technology, biologists insert DNA sequences from one organism into the DNA of another organism.	1. Define genetic engineering and recombinant DNA technology
Tools used in recombinant DNA technology: restriction endonucleases	p. 369-371	3. Manipulation of DNA sequences was made possible after the discovery of restriction endonucleases in bacteria, DNA ligases, and plasmids. 4. Restriction endonucleases allow biologists to cut DNA at specific sequences (usually palindromic) and insert them into plasmids or other vectors with the help of DNA ligase.	2. Define restriction endonuclease and explain how a typical restriction enzyme cuts DNA molecules 3. Describe how restriction endonucleases are used in recombinant DNA technology
Using recombinant DNA technology to produce proteins: human growth hormone example	p. 369-374; 384-385	5. Recombinant DNA strategy for producing human growth hormone (GH) involves: (1) cloning of human *GH* (gene), (2) inserting (transfecting) the cloned gene into a host cell (eg, *E. coli* or yeast), thus producing transgenic cells, and (3) having host cells express the cloned gene to produce GH. 6. Cloning of human *GH* involves the following steps: a. Isolating mRNAs from pituitary b. Producing complimentary DNAs (cDNAs) from the isolated mRNAs using reverse	4. Using human growth hormone (GH) as an example, describe how biologists use recombinant DNA technology to mass produce GH 5. Distinguish between genomic library and cDNA library 6. List and describe the use of the different molecular tools used in recombinant DNA technology, transfection of host cells, and isolation of transfected cells that contain the target gene

transcriptase

c. Attaching restriction recognition sites to cDNAs

d. Digesting cDNAs and plasmid with restriction endonuclease

e. Mixing cut cDNAs and plasmids (sticky ends join cDNA and plasmid together and ligase ligate the 2 DNA sequences together) to produce recombinant plasmids

7. Transfecting *E. coli* with recombinant plasmids using transformation techniques, thus resulting in the formation of cDNA library
8. To have host *E. coli* cells express the cloned *GH*, the cells that got transfected with the recombinant plasmid containing the *GH* cDNA are 1st isolated (using a combination of marker gene and DNA hybridization technique) and then mass cultured.
9. When mass cultured, transfected *E. coli* cells can produce large quantities of GH, which can be purified and used to treat patients requiring GH therapy.

* Textbook: Biological Science, Freeman, S. *et al., 2nd Canadian* ed., 2014