Problem 1. Short answer.

(a) What are the functions of restriction enzymes and DNA ligase?

Restriction enzymes cleave DNA at a specific location, while DNA ligase joins parts of DNA together.

(b) Briefly describe the mechanisms behind CRISPR-Cas9.

A Cas9 protein complex attaches to a desired site in DNA, as determined by the sequence of the guide RNA. The Cas9 protein cleaves the DNA off, and a new edited piece of DNA is inserted. It is joined to the rest of the DNA by enzymes, usually DNA ligase.

(c) Besides those listed in the module slides, what is another application of genetic engineering?

Answers will vary.

(d) What is a liposome, and what is it made of?

A liposome is a cellular structure that holds and transports molecules, a process known as cellular trafficking. It is made up of phospholipids, similar to a cell membrane.

(e) What is a plasmid? What types of cells are plasmids typically found in?

A plasmid is a circular piece of DNA that replicates independently of the cell's chromosomes. Plasmids are typically found in bacteria (prokaryotic cells).

Problem 2. You are a scientist working for a biotech company that focuses on designing guide RNAs for CRISPR-Cas9. The sequence for the target gene, where the guide RNA attaches, was originally as follows

3′-ATATTATATAATACTTCTCGGAAATCT-5′

After accidentally leaving your DNA samples in UV light, you discover that the **T** in the sequence underwent a mutation and turned into a **A**.

Since it is expensive to redesign another set of guide RNAs from scratch, your job is to find a way to replace the nucleotide at the mutation point using the guide RNAs you currently have. *Propose a method of doing so*. Assume that you have Cas9 proteins, reverse transcriptase (which is an enzyme that converts RNA to DNA), transcription factors, RNA polymerase, and a smaller piece of guide RNA with sequence 5′-UGAA-3′. You also have an editing DNA with sequence:

3′-ACAT-5′
5′-TGTA-3′

Please show how the sequence changes throughout all steps in the process. Also, *what would be the final guide RNA sequence?*

Answers will vary, but here is one possible method.

Based on the target gene shown, the original guide RNA would appear as follows:

5′-UAUAAUAUAUUAUGAAGAGCCUUUAGA-3′

We can use reverse transcriptase to convert the RNA back to DNA.

3′-ATATTATATAATACTTCTCGGAAATCT-5′
5′-TATAATATATTATGAAGAGCCTTTAGA-3′

Now, we basically perform a mini CRISPR-Cas9 experiment with this new piece of DNA.

First, attach the Cas9 proteins to the guide RNA with sequence 5′-UGAA-3′. Mix the Cas9-gRNA complex with the DNA obtained from reverse transcriptase. This, will remove the portion that corresponds to

3′-ACTT-5′
5′-TGAA-3′

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(Problem 2 continued)

Then, mix the editing DNA into the sample. This will replace the above portion with the mutated form. Now, the DNA appears as follows:

3′-ATATTATATAATAC**A**TCTCGGAAATCT-5′
5′-TATAATATATTATG**T**AGAGCCTTTAGA-3′

To induce transcription, you can mix RNA polymerase and transcription factors into the solution. This will create a (guide) RNA with the new, desired sequence:

5′-UAUAAUAUAUUAUG**U**AGAGCCUUUAGA-3′

The sequence above is the answer to the second question of this problem.