Problem 1. Discuss the following questions with your group. *You do not have to write down your answers*, but if anything interesting comes up in your discussion, you can jot down quick notes in the box provided.

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

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

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

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

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

Notes:

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′-ATATTATATAATAC**T**TCTCGGAAATCT-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, and provide a step-by-step protocol in the space provided*. 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?*

Continued on next page.

(Problem 2 continued)