Restriction Digest[1]:

Materials Needed:

2 plasmids (a vector and an insert)
1.5mL Eppendorf Tubes
Restriction Enzymes
Buffer for Digest (eg: BSA)
T4 DNA Ligase
DNA Ligase Buffer
Heat Block/Water Bath
Pipets
Competent *E. coli* DH5α
Equipment for DNA Agarose Gel Electrophoresis

Protocol (estimated time 4 hours):

1. Thaw both plasmids and digest buffer on ice. Obtain restriction enzyme(s) that give specific cut sites outside the gene of interest for the insert plasmid and with only one cut site for the vector plasmid. Keep these enzymes on an ice block.
    a. Add 1µg of the plasmid to a sterile eppendorf tube and fill the tube up with distilled water until the final volume is 42.5mL
2. Add 1µL of each restriction enzyme to the Eppendorf tubes and 5µL buffer for digest if necessary.
    a. Depending on the restriction enzyme, different types of buffer can be used to increase the efficiency.
3. Flick the mixture and incubate it at 37°C in either a water bath (a) or heat room (b) for 60 minutes.
    a. Ensure the entire volume of the mixture is under the water and periodically agitate.
    b. Place the mixture on a shaker or periodically agitate.
4. After the 60 minutes, heat inactivate the restriction enzymes by placing them either on a heat block or in a water bath set to 80°C for 20 minutes.
5. Run a DNA agarose Gel with the digest mixtures.
    a. Refer to our DNA agarose gel electrophoresis **protocol here.**
6. If the plasmids are of two different antibiotic cassettes ignore this step. Excise both the insert and vector DNA out of the gel and perform a DNA agarose gel clean up.
    a. Refer to our DNA agarose gel clean up **protocol here.**
7. Create a DNA ligase mixture using the ligation **calculator here.**
    a. As the DNA ligase buffer has ATP, it is sensitive to freeze thaw cycles.
    b. Ensure to keep the enzyme in an ice block to prolong its usage.
8. Incubate the mixture at room temperature for 60 - 120 minutes, while periodically agitating it to ensure proper mixing.
9. After 60 - 120 minutes, heat inactivate the T4 DNA ligase by placing the solution into a water bath or heat block set to 80°C.

10. Transform competent *E. coli* DH5α cells, refer to our competent *E. coli* DH5α heat shock **protocol here.**

Reference

1. New England Biolabs Inc. (2015). *Optimizing Restriction Endonuclease Reactions.* Retrieved from https://www.neb.com/protocols/2012/12/07/optimizing-restriction-endonuclease-reactions