Purpose: To remove insert from plasmid and extract linearized plasmid

Gel Extraction

Qiagen QIAEX II Gel Extraction Kit

1. Run a restriction digest on the targeted DNA part using restriction enzymes and run 50 µL on an agarose gel for 1 hour
2. Cut the targeted DNA sequence out using a razor blade, making sure to get as much DNA while limiting the amount of agarose extracted
    a. Using a UV imager (**wear protective eye equipment!!**) will make DNA easier to see
3. Pre-weigh empty Eppendorf tubes before adding the gel exicisions.
4. Add the gel extracts to the Eppendorf tubes and weigh again.
5. Calculate the mass of the gel using the difference of the two measurements.
6. Multiply the mass by a factor of 3 to get the volume of Buffer QX1 needed.
7. Add the respective amounts of Buffer QX1 to each of the tubes
8. Add 30 µL of QIAEX II to the samples.
    a. Vortex QIAEX II before using
9. Incubate the tubes at 50° C for 10 minutes and vortex every 2 minutes to help dissolve the gel
    a. Check to make sure the color of the mixture is yellow
10. Once dissolved, centrifuge for 30 seconds and carefully remove the supernatant.
11. Add 500 µL of Buffer QX1 to the tubes and resuspend by vortexing
12. Resuspend the pellet in 500 µL of Buffer PE and centrifuge for 30 seconds. Remove supernatant and repeat this step.
13. Air dry pellet for 30 minutes
    a. Until it becomes white
14. Elute the DNA by adding 20 µL deionized water, vortex it, and incubate at room temperature for 5 minutes.
15. Centrifuge for 30 seconds and pipette the supernatant into a clean tube.
16. Measure and record the concentrations.