Gel Extraction Protocol 8/9/2010

Running the Gel

1. Follow protocol the wiki protocols section for making a gel (50 mL TBE with .35 g or agarose) with following changes
    a. Use the larger comb to make the wells
    b. Mix the PCR product and blue juice in a 1:5 ratio
        i. 20 uL of PCR product (sample 14 flu operon PCR from today)
        ii. 5 uL of 10X blue juice
2. Before performing the electrophoresis make sure to use **FRESH TBE** so product does not become contaminated
3. Load gel with 25 ul of sample per well (ladder and sample)
    a. Make sure to separate the product from the ladder by a lane so not to cross contaminate
4. Set to 85 V and run for 1:15 to 1:30

Gel extraction

1. Preheat water bath to 50 C
2. Weigh and label 1 eppendorf tube for each sample
3. Use clean razor blade to cut sample from gel and clean tweezers to transfer the gel slice into the eppendorf tube
    a. **BE CONSCIENCE OF ETHIDIUM BROMIDE CONTAMINATION!!**
        i. Do not touch the outside of the eppendorf tube with anything that has contacted the gel
        ii. Everything in this process should be disposed of in the ethidium bromide waste (column from gel extraction, eppendorf tube, ext.)
    b. When the gel is done running, drain of all excess buffer, pat dry and place on the transilluminator. When working with the gel wear UV glasses and try to keep the shield down if possible.
    c. Turn on the transilluminator and score the gel on each side of the band with a razor blade
        i. Excess UV exposure can mutate DNA, only turn on UV for short amount of time to cut out the gel
    d. Turn off the transilluminator and cut out the band
    e. Place the band back on the transilluminator and make sure the right part of the gel was cut
    f. Place the band in the respective pre-weighed eppendorf tube

QIAgen kit

1. Cap and weight the eppendorf tubes
    a. Determine the weight of the band by subtracting the weight of the tube
    b. The maximum weight of the band is 300-400 mg
2. Add 3 volumes of buffer QG to 1 volume of gel
    a. 100 ug of gel is approximately 100 uL of QG buffer
3. Incubate at 50C in water bath for 10 minutes , or until completely dissolved. Every 2-3 minutes vortex to help the gel band dissolve
4. After the gel has dissolved the liquid should be yellow, if the solution is orange or purple the pH is too high. Add 10 uL of 3 M sodium acetate at a pH of 5
5. add 1 gel volume isopropanol to the mix only if the sample is less than 500 bp or more than 4 kb
6. Apply mixture to the spin column with 2 mL collection tube
7. Centrifuge at 13000 rpm for 1 minute
8. Discard flow through and place column back into the collection tube
9. Add 500 uL of QG buffer to wash off residual agarose, centrifuge at 13000 rpm for 1 minute
10. Add 750 uL of buffer PE and **LET SIT FOR 2-5 MINUTES.** Centrifuge at 13000 rpm for 1 minute
11. Discard flowthrough and centrifuge again at 13000 rpm for 1 minute
12. Place column into an eppendorf tube
13. Add 50 uL of EB buffer **DIRECTLY TO THE MEMBRANE** to elute the DNA, **LET SIT FOR 5 MINUTES** and centrifuge at 13000 for 1 minute