Gel Purification

December 10, 2010

Description: After DNA is isolated from running the gel, the relevant band is cut out using a razor blade, and then purified using a DNA purification kit.

Time: 45min

1. Cutting out bands from gel

- a. Label large epi tubes for each band
- b. Take gel, along with razor blad, forceps, goggles, labeled large epi tubes, and lane info to UV camera (in common room)
- c. Take a quick UV picture of the gel. Keep this picture up this will tell you if your gel ran correctly and which bands to cut
- d. Open door, and with GOGGLES ON and gel near the door, turn on UV by pressing UV button on inside (tip-near on inside ceiling). Bands will glow. (Alternatively, you may use the UV box in the 4th floor common room).
- e. Carefully and quickly cut bands out with a razor blade, and use forceps to pull them out.
- f. Place each band into appropriate labeled tube.

2. DNA purification from gel

- a. Take out UltraClean DNA purification kit (bottom right drawer of Zeller's bench).
 - *The following steps apply to each tube
- b. Pre-heat 65C dry bath
- c. Thaw glass milk (should be stored in freezer box)
- d. Add ~0.5ml of iodide salt (smaller brown bottle)
- e. Place tubes in dry bath at 65C for 5-10min. Vortex if necessary. This will melt gel. Make sure gel is entirely melted!
- f. Add 6ul of dissolved (vortexed) glass milk. Suspend for 5-10min (vortex). DNA will stick to glass beads in milk.
- g. Centrifuge for 5sec. Remove supernatant
- h. Repeat Ultra Wash
- i. Resuspend in 25ul of ultrapure H2O. Pipette up and down to mix. **Do** not vortex!
- j. Incubate in H20 for 5-10min
- k. Supernatant contains DNA. Transfer supernatant to new tube. **DO NOT THROW AWAY SUPERNATANT!** Store in -20C.