DNA ISOLATION

You will use the Qiagen DNeasy kit to isolate your DNA. Qiagen, Inc. DNeasy DNA isolation Tissue kit. Valencia, CA. This kit contains chemicals to denature proteins, precipitate out the DNA from solution and filter the extract to allow the collection of DNA in buffer. Unfortunately, when purchasing kits, the manufacturer does not include information on what the buffers contain, to prevent laboratories from replicating independently their kits. More than likely, the kit contains SDS (sodium dodecyl sulfate) to denature the proteins, ethanol to precipitate ou the DNA, and TE buffer as the DNA eluate in the last step.

Materials:

70°C heat block, microfuge, vortexor, yellow & blue pipette tips, micropipettors, biohazard bags, 1.5 ml tubes, microtube racks & marker, spin columns, collecting tubes, PBS buffer, proteinase K, 100% EtOH, AW1 buffer, AW2 buffer, AE buffer.

Procedure:

1) Add 180 μL PBS and homogenize each sample using a yellow micropipettor tip, push down hard in tube to bend the end of the tip first. Then homogenize your sample using a fast up and down motion.

2) Place buffer AL in a 55°C water bath for 10 min to warm it up. Add 20 μL proteinase K to the sample first, then 200 μL buffer AL to the sample. Vortex well for about 1 min, and incubate at 55°C in the water bath for 2-4 hours or overnight.

3) Label screw caps: date, initials, sample #, “DNA”

DAY II (DNA Isolation Continued) ~45 min procedure

4) Add 200 μL 100% EtOH to the sample and vortex thoroughly. In your rack, set up three (3) sets of collecting tubes.

5) Pipet all of the mixture into the spin column which is sitting in a 2.0 mL collection tube. Include all that has precipitated as well.

6) Centrifuge at 8,000 rpm, for 1 min. Discard the collecting tube in the waste beaker.

7) Place the spin column in a new 2.0 ml collecting tube in the rack. Add 500.0 μL buffer AW1, and centrifuge at 8000 RPM for 1 min. Discard collecting tube in waste beaker. (Keep spin column).

8) Place the spin column in a new 2.0 ml collection tube in rack, add 500 μl buffer AW2, and centrifuge at 13,000 RPM for 3 min. to dry the membrane. Discard collecting tube. If the membrane is not fully dry, then empty the collecting tube, place the column back in the tube and spin at 13,000 RPM for 1 more min.

9) Place the spin column in a new collecting tube, and pipet 50 μL buffer AE directly onto the spin column membrane without touching the tip to the membrane. Incubate at room temp. for 1 min. Centrifuge at 8,000 RPM for 1 min. to elute. Do NOT discard LIQUID from tube!!! That's your DNA.

10) Place DNA in appropriate screw cap tubes.