Lab Protocols

DNA Extraction

Materials and Work Space Preparation:

- Turn on incubator to 56°C
- Clean the area before you start
- Get out a Bunsen burner and connect the hose to the table outlet. Clear the area of all flammable objects (including your hair and clothing)
- Get out razor blades and forceps. You will use one razor blade per sample and one forceps for cleaning solution (10% bleach and 70% ethanol).
- Use a glass surface (e.g., Petri dish) or autoclaved foil paper for cutting tissue samples. The glass surface can be sterilized with ethanol and then placed over the flame. Foil paper should be cut in small pieces (10x10 cm), autoclaved, and use one per sample.
- Place your samples in racks and label 1.5 – 2 ml tubes with the corresponding sample numbers (one tube per sample, if you re-label your samples keep a record), this will be used for incubating your tissue samples.
- Make sure that you have enough reagents for the number of extractions you plan to do. Remember that the first step for DNA extraction is incubation and after it is done you cannot stop. Also, ethanol and ddH₂O used after incubation are not included in the kit so fill a 15 ml sterile plastic tube with each of these solutions.

Cutting the tissue:

- With sterile forceps (first bleached and then rinsed with water and Ethanol) take the tissue sample to the sterilized surface and cut a small piece. Take the remaining of the tissue back to its original tube. Cut the sample of tissue into small pieces using a razor blade and forceps. Then, place the tissue pieces into a new labeled tube. If you work with muscle tissue static energy might disrupt the process, so you can use one drop of sterilize water for making the cutting process easier and then place the pieces into the tube. Let all tissue samples dry out (~ 25 minutes).
- After cutting and removing tissue soak the forceps in a solution of 10% bleach, then in ethanol and finally pass them over the flame.

Incubation (using Qiagen DNAesy tissue kit):

- Add 180 µl Buffer ATL to each sample
- Add 20 µl of proteinase K

Be careful and avoid contaminating the reagents or your samples! You can use the same pipette tip for adding the ATL Buffer to different samples only if the tip did not touch
any part of the tube containing your tissue sample. The same is true for proteinase K. Never use the same pipette tip for different solutions.

- Vortex and centrifuge
- Place tubes in racks and incubate at 56°C until tissue has been completely lysed. Set a shaker mode or vortex your samples occasionally.

After Incubation:

- Vortex and centrifuge samples for 15 sec.
- Add 200 µl Buffer AL
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- Add 200 µl ethanol (96-100%).
- Vortex and centrifuge samples for 15 sec.
- Pipette solution into spin column. Centrifuge for 1 min at 8000 rpm.
- Discard flow-through and collection tube
- Place spin column in a new collection tube.
- Add 500 µl Buffer AW1. Centrifuge for 1 min at 8000 rpm.
- Discard flow-through and collection tube
- Place spin column in a new collection tube.
- Add 500 µl Buffer AW2. Centrifuge for 2 min at 14000 rpm.
- Discard flow-through and place in same collection tube
- Centrifuge for 1 min at 14000 rpm.
- Discard flow-through and place in same collection tube and place spin column in a clean and label 1.5 ml tube
- Add 100 µl of ddH2O directly onto membrane
- Incubate at room temperature for 1 min
- Centrifuge for 1 min at 8000 rpm
- Add another 100 µl of ddH2O directly onto membrane
- Centrifuge for 1 min at 8000 rpm
- Through away your spin column and put your DNA samples in the freezer.

*You can use Buffer AE instead of water, however DNA samples in buffer will not allow further concentration if it is required.