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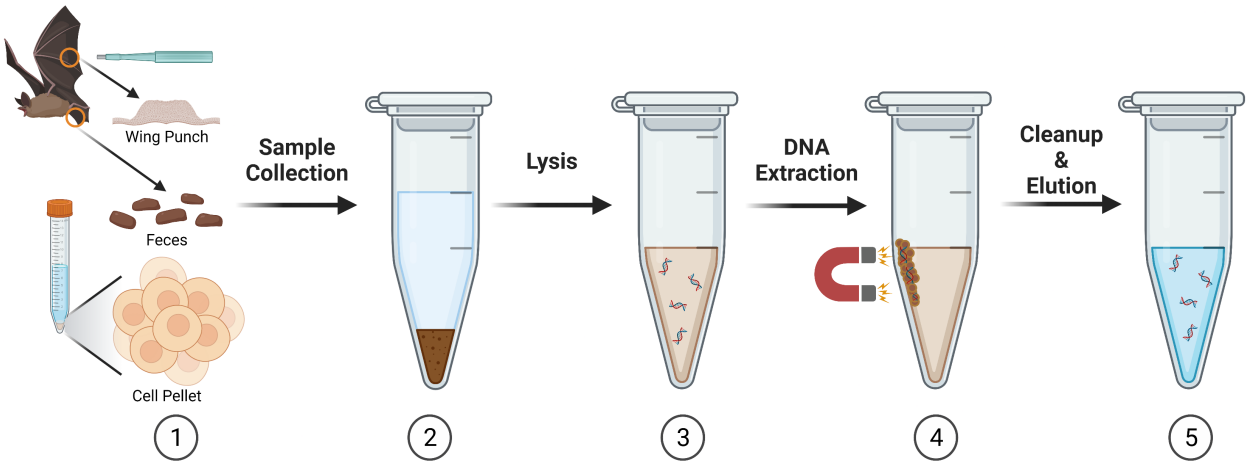
Day 1: DNA Extraction and Quantification



Materials

- Zymo tissue buffer ("Biofluid & Solid Tissue Buffer")
- Proteinase K (20mg/uL)
- Dithiothreitol (DTT) (1M)
- RNAse A
- 80% ethanol, ice-cold
- Elution Buffer (10mM Tris-HCL pH 8.0)

Equipment

- Magnetic rack, 1.5mL
- Microcentrifuge tube rack
- Vortexer
- Microfuge
- Thermomixer
- Rotator
- Pipettes:
  - P1000

Consumables

- Microcentrifuge Tubes, 1.5mL
- Pipette Tips
  - P1000
  - P200
  - P20
  - P2

- SPRI beads
- Autoclaved toothpicks
- Quantus DNA ONE dye
    - P200
    - P20
    - P2
- Quantus fluorometer

▼ Receiving and Tracking Your Samples

1. We will give each person two samples: either a tissue sample, or a cell culture sample.

   Make sure to keep the sample comfortable and safe! If its on ice, keep it on ice; otherwise, just keep it in the tube.

   If you receive the purified fecal matter, you will be following the “DNA Power Soil Extraction” Kit along with a different group.

2. Once you receive your sample, you will note in your notebook the identifying data: sample number, date, coloration, amount of the sample, etc.

▼ Lyse the Samples

In order to free the DNA, the first step is to *lyse* your samples: essentially using a combination of detergents and enzymes to rip apart cells and connective material, and release everything into solution.

1. In a fresh tube, prepare a master mix of the DNA lysis solution by combining the following:

Chemical	Amount for 1 Reaction	2.1x Reactions
Zymo Tissue Buffer	100 uL	
Proteinase K	20 uL	
DTT	1 uL	
TOTAL	121 uL	

2. Add **120uL** of lysis solution to each sample and mix well

Mixing well in this step is key to making sure everything dissolves and lyses properly

For cells, mix by pipetting up-and-down several times until the pellet of cells disappears fully; then vortex for ~10 seconds in 1-second pulses.

For tissues, just vortex for ~10 seconds in 1-second pulses.

3. Place the samples in the thermomixer at **55*C for 30 minutes.**

   Proteinase K works best at this temperature. Setting an appropriate shaking speed (~800RPM) helps keep the mixture homogenous and prevents undigested material from accumulating at the bottom.

4. After 30 minutes, take the samples off the heat and add **10 uL** of RNAse A to each sample. Vortex for 10x 1-second pulses and let sit on the rack at room temperature for **10 minutes**.

▼ Extract the DNA

Now, we will begin to extract the DNA. While there are many ways to do this, one of the easiest and cheapest ways to do so is to use magnetic SPRI beads. These negatively-charged beads are the perfect size for DNA to wrap around and bind to, allowing us to pull the DNA out of solution using a magnetic rack and thus allowing us to remove protein and other unwanted junk from the sample. We use 80% ethanol to clean the DNA on the pellets, since it can dissolve proteins and salts but cannot dissolve DNA (DNA does not dissolve in >60% ethanol).

1. Vortex your SPRI bead solution for **30 seconds continuously**.
   It is vital that all the beads be resuspended properly.
   The beads should also be at room temperature, but if they're not, warm them up in your hands for a few minutes.

2. Add an **equal volume** of SPRI bead suspension to the sample.

Volume of your sample	Volume of SPRI bead to add
eg: 200 uL	*200 uL*
_____ uL	_____ uL

3. Vortex your sample for 2 quick 1-second pulses to mix, and then place on the rotator for **20 minutes**.

At this step, the DNA will begin to bind to the beads, and come out of the solution. Keeping the sample tubes on the rotator makes sure the beads stay suspended and in constant contact with the solution.

4. Spin down the tubes on a micro centrifuge for 10 seconds to collect all the liquid at the bottom.
5. Place the tubes on the magnetic rack provided. Wait 10 minutes for the beads to collect in the back
   You'll want to keep the hinge of the tube facing away from you, towards the magnet
6. Using a P200 pipette, remove all the supernatant from the tube, taking care to not disturb the pellet.
   Make sure to discard the supernatant in an appropriately-labeled waste container - or keep it in a new tube as a backup! We do the latter a lot in the lab in case something went wrong with the beads.
7. **Without removing the tube from the magnet**, add 500 uL of ice-cold 80% ethanol, and close the tube. Gently invert and rotate the rack in your hands around for 10 seconds.
8. Using a P200 pipette, remove all the supernatant and discard it in the liquid waste container. Also be sure to remove the liquid off the lid of the tube.
9. Repeat steps 7-8 **two more times** for a total of 3 washes. **Do not let the pellet get dry at any point of this wash.**
   It takes a few minutes for the pellet to dry out, but if it does its game over - the DNA will never come off the pellet!
10. After the final wash, use one of the autoclaved toothpicks to dry off any extra ethanol on the walls of the tube, **without disturbing the pellet**.

▼ Elute the DNA

Your DNA is now clean! Time to dissolve it in our final buffer.

1. Add 50 uL of the Elution Buffer into each of the sample tubes. Remove the tube from the magnetic rack.
2. Using a P200, resuspend the beads in the buffer.

3. Vortex samples continuously for 10 seconds.
4. Place samples in the thermomixer at **55*C for 20 minutes.**
   Placing the samples in heat helps get rid of the remaining ethanol while also ensuring all the DNA dissolves.
5. Spin down the tubes in the microfuge for 10 seconds.
6. Place the samples back on the magnetic rack, and allow the beads to pellet for 5 minutes or until the solution is clear.
7. Label a fresh tube with all the information from the original tube.
8. Transfer the supernatant (which contains your DNA!) into a fresh tube.
   Sometimes, if the DNA is very high molecular weight, some beads come with the DNA into the new tube. This is fine for our next steps.

▼ Quantify the DNA

We now want to know *how much* DNA we got from each sample. To do this, we are going to use a fluorometric assay: adding a known volume of our sample to a dye that only fluoresces when it binds to double-stranded DNA. Our assay is accurate for concentrations between ~100pg/uL to 1ug/uL of DNA.

1. For each sample, prepare a 0.5mL tube by adding 200uL of Quantus DNAONE dye to an empty tube.
2. Add **1uL** of your sample to the dye tube and label it
3. Vortex the DNA-Dye mix for 5 seconds and place it in the dark for 5 minutes
4. Place the tube in the machine and read out the concentration. **Write it down!**
5. Also label your tube with the concentration - redundancy is your friend!

Now, place your tubes in a sample box with your instructors, which will be kept in the fridge overnight.

Day 2: Polymerase Chain Reactions and Cleanup

Materials Equipment Consumables