EXTRACTION OF THYMUS DNA

David B. Fankhauser, PhD
Modified from protocol by Lana Hays
Modified 18 April 2016

Students will make their own glass hook from Pasteur pipets ACCORDING TO THE DEMONSTRATION before isolation is begun. CAUTION, MELTED GLASS IS HOT...

MATERIALS
- fresh thymus
- knife and cutting board
- blender: NOTE: must have narrow neck, fits Mason Jars
- sucrose
- Bufferin tablets (325mg)
- Epsom salts (MgSO₄)
- Palmolive detergent (SDS)
- non-iodized salt
- 15 mL centrifuge tubes with caps
- 1 mL & 5 mL displacement pipets
- graduated cylinders (10ml,100ml)
- distilled water
- clinical centrifuge
- -20° C 100% ethanol (store in freezer)
- 10 mL beakers, one per student
- glass hook (or Pasteur pipet and bunsen burner to make it)

PREPARATION OF SOLUTIONS
(All ice cold!)

- prep buffer solution (turbid):
  - 57 g granulated sugar
  - 1 buffered aspirin
  - 3 g epsom salts
  - Q.s. with dH₂O to 500 mL

- 10% detergent solution:
  - 90 mL distilled water
  - 10 mL Palmolive detergent (SDS?)

- salt solution:
  - 29.2 g non-iodized salt
  - Q.s. with dH₂O to 250 mL

PROTOCOL (Latex gloves should be worn to protect DNA from endonucleases, and all materials kept as cold as possible.)

1. Mince about 15 g of fresh thymus (~2 cm square) into small pieces, record weight.
2. Load the blender: 100 mL prep buffer, 10 mL detergent solution, 15 g minced thymus.
3. Blend at high speed for 1 minute, or until the mixture is smooth. (Inadequate blending = failure!)
4. Take aliquot of homogenate: Transfer 1 mL of the homogenate to a labeled capped 15 mL centrifuge tube. (Trim 2 mm off the end of the pipet tip (= mochos) if fluid is too thick to pipet.)
5. Make hypertonic, shake: Add 2 mL of salt solution, cap, and shake vigorously for 2 minutes.
6. Centrifuge: Place shaken centrifuge tube in a clinical centrifuge so that label is to the outside, spin in a balanced configuration at top speed for 7 minutes.
7. Decant the supernatant: Carefully remove the tube from the centrifuge and note the two phases: upper layer = supernatant DNA is dissolved here. lower layer = pellet cell debris and precipitated protein. Hold the tube so the centrifuge label (and pellet) is down, carefully, in one smooth motion, decant most of the supernatant into a clean 10 mL beaker. Leave some supernatant to avoid any of the pellet.
8. Add a layer of ice-cold ethanol on top of supernatant: Carefully pour 5 mL ice-cold EtOH down the side of the beaker. Do not disturb or mix. Should get two phases: EtOH is on the top.
9. Let sit undisturbed for a minute or two. Note appearance of filmy white ppt at the interface.
10. The DNA will float in the alcohol just above the aqueous phase in the alcoholic phase. The white lacy interface is precipitated thymus DNA. It should form long threads that easily spool with the glass hook.

Modified from:
"Generic, All Purpose DNA Extraction from Meat Protocol" Judy Brown
"Mammalian DNA Extraction" Theresa Knapp