

EXTRACTION OF THYMUS DNA

page 17

David B. Fankhauser, PhD

Modified from protocol by Lana Hays

30 January 2001, 2 Feb 01, 16 Feb 01, 2 Jan 02, 2 Jan 04, 3Jan06, 30Dec08, 28Dec10, 5Jan11

http://biology.clc.uc.edu/fankhauser/Labs/Genetics/Genetics_web/DNA_extraction/DNA_extraction.htm

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

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 if you wish to protect DNA from endonucleases.)

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 decant supernatant into a clean 10 mL beaker. 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 between the aqueous and alcoholic phases. 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

Did single homogenization, distributed aliquots to students, and each then spooled their own DNA. Worked very well except maybe too MUCH DNA...

Tried mushrooms—they formed a white band at the bottom of the EtOH, just above the aqueous phase. In both cases, forgot to add the detergent... The thymus formed so much DNA that it globbed up into “snot balls.” 30 gm/100 mL of buffer was probably too much thymus. Should try liver since thymus was hard to find. (Amy got it frozen from Suhshine Foods in Hyde Park for \$10/pound.)