Framingham Heart Study

Manual of Procedures

MOP-version 1.0

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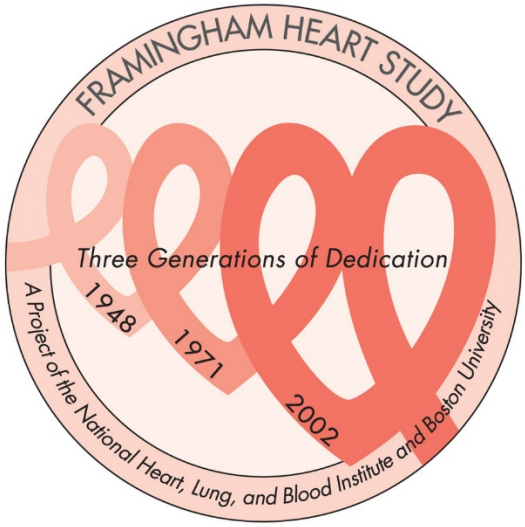
Heather Arruda and Jessica Rumpf

Genetics Lab

DNA Extraction



Tracking of Revisions to this FHS Protocol MOP

Revised Section	Revision Author	Date (s) of Revisions; source	Approved by, Date	Revisions	Previous Pages #s section changed	Distribution Date

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1.0 DNA Extraction from Buffy Coat and Whole Blood

1.1. Equipment and Consumables

- Table-top swing bucket centrifuge with 750 mL capacity.
- Rotor and adapters to accommodate 50 ml and 15 ml conical centrifuge tubes.
- Water bath
- 50 ml polypropylene conical centrifuge tubes
- 15 ml polypropylene conical centrifuge tubes
- 10 ml pipets
- 5 ml pipets
- 1 ml pipetman and tips
- Fluid resistant gown and gloves

1.2. Reagents

- Solution A (RBC lysis)
- Solution B (WBC lysis)
- 5M Sodium Perchlorate
- Phenol/Chloroform

1.3. Recipes

- **Solution A (1L)**

  Tris……..1.21g
  Sucrose……. 109.50g
  $MgCl_2*6H_20$……1.02g
  100% Triton……..10ml

Bring solution to volume with purified water
Adjust pH to 8.0 with NaOH
Autoclave

- **Solution B (1L)**

  Tris…….. 48.50g
  EDTA…...22.30g
  NaCl……...8.77g

Bring solution to volume with purified water
Autoclave
Add 10g SDS

- **5M Sodium Perchlorate (250ml):**

Add 175.57g of Sodium Perchlorate to 100 mL of autoclaved water. Mix well.
This is an endothermic reaction and the solution will expand and be cool to the touch.
SLOWLY bring to volume.

- **Phenol/Chloroform**

  Make this 1:1 mixture up fresh the day of extraction.
  Prepare enough to use 2.5 mL per sample.
  Mix in a **polypropylene** 50 mL tube.

1.4. Cell Lysis

1. Transfer the blood sample using a 5 mL pipette into a 50 mL centrifuge tube containing 45-50 ml of Solution A. Wash the sample container with 1 mL of Solution A to ensure the entire sample is transferred.

2. Invert the tube several times to mix. Centrifuge at room temperature for 30 minutes at 2800 rpm in order to pellet the WBC.

3. **CAREFULLY** decant the supernatant into a waste collection vessel. **The resulting pellet is small and slippery and can be easily dislodged and poured off with the supernatant**. **Pour with caution**. Use a pipette to remove the residual liquid from the pellet.

4. Add 2 mL Solution B. Vortex to mix.

5. Add 0.5 mL 5M Sodium Perchlorate to the 50 ml tube and vortex to mix.

6. Transfer solution to a 15 ml centrifuge tube and incubate in a 65^0 C shaking water bath for 1 hour.

1.5. DNA Extraction

7. Remove from water bath and add 2 mL Phenol/Chloroform. Vortex to mix. *DO THIS IN THE FUME HOOD AND POLYPROPYLENE TUBES are required (phenol melts polystyrene).

8. Centrifuge for 30 minutes at 2800 rpm.

9. VERY CAREFULLY pipet off the top, DNA containing layer, and transfer to a new 15 mL centrifuge tube. Leave the old tube in the fume hood to evaporate off residual Phenol/Chloroform.

10. Add 2x volume (generally 5 mL) 100% ethanol.

11. Gently invert the tube10-20 times to precipitate the DNA.

12. Centrifuge at 1500 rpms for 1 minute and pour off ethanol.

13. Add 5 ml of 70% ethanol to wash the DNA pellet. Invert tube 15 times then centrifuge at 1500 rpms for 2 minutes.

14. Pour off ethanol and allow the pellet to dry until almost clear.

Add appropriate amount of 1X low E TE to re-suspend the DNA and incubate at 65^0 C for an hour. Leave at room temperature overnight. Make sure your caps are on ti

2.0 DNA Extraction from Lymphoblast Cell Lines

2.1. Equipment and Consumables

- Table-top swing bucket centrifuge with 750 mL capacity.
- Rotor and adapters to accommodate 50 ml and 15 ml conical centrifuge tubes.
- Water bath
- 5 mL pipettes
- 10 mL pipettes
- 50 ml polypropylene conical centrifuge tubes
- 15 ml polypropylene conical centrifuge tubes
- 2 ml tubes
- Labels
- 100 and 1000 uL pipetman and tips

2.2. Reagents

- 0.85% NaCl Solution
- TKM #2 Buffer
- 20% SDS Solution
- Proteinase-K (41.7mg/ml)
- RNase A (100mg/ml)

2.3. Reagent Recipes

- **0.85% NaCl (1L)**

  NaCl……..8.5 g
  Bring to volume with autoclaved water

- **1M Tris-HCl (250 mL)**

  Tris-HCl…….. 39.4 g
  Bring to volume with autoclaved water
  Adjust to pH 7.6 with NaOH

- **0.5M $MgCl_2$ (250 mL)**

  $MgCl_2$. …….25.4 g
  Bring to volume with autoclaved water

- **0.5M EDTA (500 mL)**

  EDTA disodium salt……..93.06 g
  Bring to volume with autoclaved water.
  Adjust to pH 8.0 with NaOH and gently heat to get into solution.

- **TKM #2 Buffer (1L)**

  KCl……0.75 g
  NaCl…… 23.38 g
  Dissolve in 600 ml water.
  Add 10 ml of 1 M Tris-HCl (pH 7.6)
  Add 20 ml of 0.5 M $MgCl_2$
  Add 4 ml of 0.5 M EDTA (pH 8.0)

  Bring to volume with purified water
  Adjust to pH 7.2 with 1M HCl or NaOH
  Autoclave

- **20% SDS (100 mL)**

  Wear a mask!!!
  SDS……20 g
  Slowly bring to volume with autoclaved water

- **Proteinase K (12 mL at 41.7 mg/mL)**

  Add 12 mL autoclaved water to 500 mg Proteinase K, mix gently
  Make 1 ml aliquots and store at –20°C

- **6M NaCl (1L) Supersaturated**

  NaCl……..351 g
  Bring to volume with autoclaved water.

- **1X Low E TE (500 mL)**

  Dilute 100x commercially prepared solution 1:100 with autoclaved water

Always remember to wipe down work surface with 70% Ethanol before AND after working in the BioSafety Cabinets (BSC). If you use a solution up or are running low, PLEASE make more.

2.4. DNA Extraction

Stage 1

1. Harvest 50 mL of a 100 mL saturated cell suspension from a T-75 cell culture flask into a 50 mL centrifuge tube and centrifuge at 1500 rpm for 10 minutes. Decant supernatant, then harvest the second 50 mL of cell suspension into the same 50 mL

conical centrifuge tube and repeat spin to form a single cell pellet. Decant supernatant.

2. Wash the resulting cell pellet in **9 mL** of **.85% NaCl** by pipetting up and down the transfer into a new 50mL tube and centrifuge at 1500 rpm for 10 minutes. Decant supernatant.

3. Wash the cell pellet a second time in **4 mL** of **.85% NaCl** solution. Centrifuge at 1500 rpm for 10 minutes. Decant supernatant.

4. Re-suspend the cell pellet in **4.3 mL** of **TKM #2** cell lysis buffer solution.

5. Add **135 uL** of **20% SDS**.

6. Add **10 uL** of **RNase** and invert to mix.

7. Incubate in a **37**°C water bath for 1 hour. Take Proteinase K out of fridge to reconstitute.

8. After an hour, add **35 uL** of reconstituted **Proteinase K** to each sample.

9. Incubate at 56°C in an orbital shaking water bath for 2 hours.

10. Remove from incubation and allow the samples cool to room temp.

Stage 2

1. Add **1.6 mL** of **6M NaCl** and vortex for 15 seconds.

2. Let sit at room temperature for 5 minutes.

3. Centrifuge at 3350 rpm for 15 minutes.

4. Decant supernatant into a new 50 mL centrifuge tube.

5. Add an equal volume of **isopropanol** (usually ~6 mL) and invert GENTLY to precipitate DNA.

6. Centrifuge the tubes at 2500 rpms for 3 minutes to pellet the DNA. The DNA will be visible as a small white pellet.

7. Pour off supernatant into an alcohol waste container, briefly drying the tube onto a clean paper towel. Add 15 mL of **70 % Ethanol** and invert tube gently ~ 12 times to wash the DNA pellet.

8. Centrifuge at 2500 rpms for 15 seconds and then **CAREFULLY** pour off supernatant into the alcohol waster container. *Pellet may be loose so pour slowly and keep your eye on your pellet.* (If you lose your cell pellet, the culture will need to be regrown and the entire process redone.)

9. Invert tubes, caps off, on the blue rack in the fume hood to allow the ethanol to evaporate leaving a clean, dry DNA pellet. This will take at least 20 minutes. If this step is rushed and do all the ethanol has not evaporated, the QC results from the spectrophotometer will give false readings. (The culture will need to be regrown and the entire process redone.)

10. Once the ethanol has evaporated and the pellet looks opaque, bring the samples back into the hood and add an appropriate volume of **DNA Hydration Solution (Low E-TE).** It takes a bit of experience to learn how to make this judgement, so new technicians should ask a senior person for assistance until competent.

11. Cap your tubes **TIGHTLY** and incubate in a 65°C orbital shaking water bath for 1 hour.

12. Remove samples and place on bench top in main lab to incubate at room temperature overnight. Be sure to label the rack with your initials and the date of extraction. Put a label per cellid extraction in the extraction book. Be sure to initial and date as well. Make sure the caps are tight.

13. Add bleach to waste bottles and let sit overnight. Flush down the drain with copious amount of water.

14. The next day, vortex tube slightly, on pulse, and spin down at 3350 rpms for 40 seconds then place in 4° to await transfer and quantification.