Framingham Heart Study

Manual of Procedures

MOP-version 1.0

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DNA Quality Control

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

Table of Contents

1.0 Agarose Gels

Agarose gels are run as a complement to spectrophotometry to quality control the DNA produced by the lab. Visualizing the DNA after an agarose gel run provides confirmation of DNA quality and quantity.

Large gels for running two 96 well plates at once: NOTE: all electrophoresis equipment should stay on the designated bench. The only exception is when preparing the DNA and loading buffer mixture. Make sure there is enough 1X TBE in the gel box to run your gel.

1.1. Preparing to run a gel

1. Set up the larger gel tray. Wet down the tray walls with deionized water and slide it into the tray grooves at the top and bottom of the gel box to keep the gel in place. Place combs into designated slots being sure to leave plenty of room for the samples to run.

2. In a flask, mix 400 mL of 1X TBE and 4 grams of agarose. Swirl to mix, then microwave for ~ 3 minutes. If the agarose isn't dissolved, microwave again in increments of 30 seconds until it is. Once dissolved, wrap paper towels around neck as the flask will be VERY HOT. Swirl under cold running water until the agarose mixture is warm to the touch. Keep swirling the gel otherwise it will solidify in the flask.

If the agarose is poured into the tray before it has cooled, it can crack the tray. Do not rush this step.

3. Once warm to the touch for 10 seconds, add 30 uL of Ethidium Bromide (EthBr) and swirl to mix. Use specified EthBr pipettes and holders and ONLY use on the one corner of bench designated for this activity. Remove and disposed of any gloves worn while handling Ethidium Bromide.

4. Pour liquid into gel box and remove any bubbles or, using a pipette tip, swish them to the bottom of the box, out of the way. Put combs in the appropriate slots. Gel should solidify in about 15-20 minutes.

5. Once the gel is solidified, pull out the combs carefully and remove the top and bottom walls of the gel box. If you forget, the current will be disrupted and the gel won't work. Place entire tray in the electrophoresis box, remove any bubbles trapped under the gel or gel tray then pour 1X TBE over the entire gel to the fill line in the gel box, if need be.

6. DNA must be normalized to a concentration of 100ng/uL. The protocol uses 200 ng of DNA per run.

7. In a buffer tray, pour some loading buffer (50 ml tube with blue liquid on door of mini fridge) and using a 0.5-10 uL multi-channel pipette, aliquot in 3 uL into each well of 96-well Med-supply plate. You can use the same tips until you see air bubbles start to form or until the liquid being sucked up isn't uniform in the tips. **Make sure you pipette the remaining loading buffer from the tray back into its 50 mL tube.**

8. Next, add 2 uL of Normalized DNA using multichannel pipette and a new box of tips, CHANGING TIPS EVERY TIME. You don't have to mix. COVER & SPIN PLATE DOWN on program 6 of the centrifuge.

9. Once plates are spun down, pipette DNA-Buffer mixture with multichannel set at 5.1 uL to first well and every other corresponding well of the gel. Place tips just to where it touches the bottom of the well, lift up slightly and slowly expel DNA while moving pipette gently from side to side. This greatly reduces the "tail effect" of dragging the DNA back out of the well when the tips are removed. Dispose of tips every time.

10. Take up your next row and place the first tip in the next set of empty well directly next to the last one (see diagram for example).

11. Skip 2 wells and repeat steps 8 and 9.

12. Once all of the DNA is loaded, take the controls out of fridge and put 2 uL of the 50 ng size standard in 1 of the empty wells between your rows of DNA on the top and 4 uL in the other empty well. Do this again for the second plate in your gel. A set of standards needs to be run per plate.

13. Once everything is set, slide top of gel box on top making sure the electrodes are on snug and turn on voltage, setting it at 150 Volts. Turn off the voltage source when the DNA has run ¾ of the way down the gel (~1.5 hours).

For smaller gels, use 175 ml of TBE, 1.75 grams of agarose and 10 uL of Ethidium Bromide.

1.2. Printing Gel Image

1. Bring the gel over to gel documentation instrument and separate the 2 plate run with a razor blade. Making sure the UV is OFF, open the door and CAREFULLY slide gel off platform into the instrument. The gel can break very easily so use caution when making the transfer.

2. Shut the door and turn on UV light. Press the live button. Make sure the gel is lined up on the LCD screen. Focus the image using the focus rings on top of the instrument. Check the contrast to ensure a good picture. Put the jump drive into the port on the machine.

3. Once it's positioned, press the **capture** button. If the image is satisfactory, press **print** on the printer and on the instrument to save the image electronically on the jump drive. Repeat for second plate gel.

4. Once done, discard the gel into the gel bucket located under the EthBr section of the bench. Rinse the gel plate with deionized water and place on the gel bench to dry. Wipe down the gel doc with a damp paper towel.

5. Load the image into the appropriate file and labels all controls, empty wells, lanes & bad samples for further QC and save.

2.0 Spectrophotometry

PLACE HOLDER

Sample Preparation for Forensic Genotyping using the ABI Ampflster ProfilerPlus Kit

PCR

PCR protocol

	50ul/each	5ul/each	*20	*60	*110	*440	*500
reaction mix	21.00	1.91	38.18	114.55	210.00	840.00	954.55
primer set	11.00	1.00	20.00	60.00	110.00	440.00	500.00
Taq	1.00	0.09	1.82	5.45	10.00	40.00	45.45
total master mix	***33.00***	***3.00***	***3.00***	***3.00***	***3.00***	***3.00***	***3.00***
DNA 0.1ng/ul		2.00	2.00	2.00	2.00	2.00	2.00

Hidi-formamide and size standard

	*16	*32	*48	*64	*80	*96
HD400 (ul)	8	16	24	32	40	48
Hi-Di formamide (ul)	175	350	525	700	875	1050

1. Place normalized DNA in a 96-well plate into the Thermocycler

2. The Thermocycler is programmed as follows:

- 95^0 for 11 minutes
- 94^0 for 1 minute
- 59^0 for 1 minute
- 72^0 for 1 minute
- Steps 2-4 repeated 27 times
- 60^0 for 45 minutes
- Hold at 4^0 ∞

3. The protocol takes 4 hours to complete. Plates are usually run overnight then loaded into the genetic analyzer the next morning.

3.0 Forensic Genotyping on the ABI 3130XL

ABI AmpFLSTR ProfilerPlus PCR Kit (P/N: 4303326)

PCR

(1) Dilute genomic DNA to 0.1ng/uL with water.
(2) Make master mix according to the number of samples
(3) Add 3ul master mix to each well leaving one well (H05) empty for positive control.
(4) Add 2ul DNA to the master mix
(5) Add 1ul of Positive Control to empty well
(6) Run the plate on thermocycler with program “Forensic”

Prepare the 3130xl

(1) Thaw the hidi-formamide (1050ul).
(2) Add HD400 size standard to hidi-formamide (36 uL)
(3) Add 9ul hidi-formamide with HD400 to each well of a 96 well plate.
(4) Add 1 uL of PCR’d sample to plate.
(5) Add 1 uL of ladder to empty well (H02 & H10)
(6) Denature at 95C for 5 minutes and immediately cool it on ice for ~2 minutes. Centrifuge and cover it with septa and assemble it with a plate base.
(7) Ready for 3130xl.

Run a plate on the 3130xl

(1) Create a plate map on 3130xl.
(2) Check the buffer and pop7. Buffer needs to be changed after a batch of run. Pop7 is good at room temperature for only 7 days. All reservoirs and septa need to be cleaned after a batch of run.
(3) Load the plate on 3130xl.
(4) Link the plate with plate map.
(5) Start the run.
(6) After the run, copy the data to a CD or external hard drive

Data analysis of forensic data output

(1) Open GeneMapper ID 3.2 (user name: gmid Password: 3130User)
(2) Go to “file” → “add samples to project” → locate your sample files→ “Add to list” → “add”.
(3) In “sample type”, choose allelic ladder and PTC. In “analysis method”, choose “Hid_advanced”. In “panel” choose “Profiler_plus_v1”. In “sizes standard” choose “Rox400size standard”. You leave the rest of fields as they are.
(4) Fill down all the samples using “Ctrl+D”.
(5) Click green arrow to start the analysis. You will be asked to give a project name before analysis.
(6) After the analysis, manually inspect the quality of data.
(7) Make sure “Standard Export Table for HID” is chosen for exporting tables.
(8) Go to “file” → “Export combined table” → give a name of table → click “export combined table”.

4.0 ABI 3130xl Genetic Analyzer Maintenance

Purpose: To describe maintenance procedures for the ABI 3130xl Genetic Analyzer.

4.1. Instrument is idle

4.1.1. Replace the Running Buffer weekly

- Running Buffer (10X) is stored at 4°C
- Dilute to 1X with Molecular Grade Water and store at 4°C
- Open the doors to the instrument to gently remove the Buffer Jar
- Discard any buffer still in the jar, rinse with Molecular Grade Water, dry with a Kimwipe then refill with fresh buffer
- Gently place back into position and shut doors. The instrument will reset itself.

4.2. Instrument in use

4.2.1 Replace the Running Buffer at the start of a run

- Prepare and replace the Running Buffer as described above.

4.2.2 Empty the water from the waste container and replace water in the water reservoir.

4.2.3 Replace Pop 7 Polymer. The polymer is only good for a week. Be sure to date the bottle when it is replaced.

4.3. Maintenance Protocol

4.3.1 Press the “tray” button on the machine. This brings your reservoirs forward.

4.3.2 Once it stops, remove each reservoir one at a time, remove the septa, empty the contents in biohazard, rinse with the purified water from the squeeze bottle and wipe out with a kimwipe.

4.3.3 Refill with the appropriate liquid to the fill line, place the septa back (make sure its not on wrong or it could jam the capillaries) and replace into correct slot back on the tray.

4.3.4 Remove buffer jar (the round container hanging next to the polymer bottle) GENTLY empty, rinse and wipe down and refill with fresh buffer. Gently replace back onto port.

4.3.5 Shut doors. Machine will reset itself.

If doing a run, make sure you do the above maintenance as well as the “Replenish Polymer” wizard if the polymer is too low, gone or has expired. The wizard will take you through step by step. Always do a bubble flush when replenishing polymer, even if you don’t see any bubbles.