

Microsatellite Protocol

Prepare gel:

1. Pour Acrylamide gel (mix up 25 mL Acrylamide solution, 125 uL APS, 17 uL TEMED)
2. Wait 30 minutes, add damp paper towel to open bottom of plates to keep from drying out.
3. Wait 1:30 hours longer (2 hours total) for gel to finish setting up. Wash plates with Alcanox, rinse with tap water, then rinse with DI water. Remove well comb and rinse out well with DI water to remove excess acrylamide. Wick out water from well and place upside down in drying rack to dry plates.

While plates dry, setup templates:

4. Make master mix of 2.75 uL dye:formamide solution and 0.25 uL ROX size standard for each sample (allowing extra for pipetting error). Add 3 uL of master mix for each sample (either in strip tubes or 96 well plate).
5. Add 0.5 – 1.0 uL of each template to formamide/ROX solution. You can add multiple templates if they use different dyes and are separated in length.
6. Spin plate (or strip tubes) to remove any bubbles (700 rpm for 15 seconds)
7. Heat samples at 95 C for 3 minutes then chill at 4 C. This can be accomplished on the thermocycler or with heating block and frozen block.

When the plates are dry:

8. Turn on ABI 377
9. Load lower buffer chamber into machine
10. Small amounts of water can be removed with the canned air.
11. Wick out any remaining water in well
12. Load plates into cassette taking care to hang the reading frame over the edge of the counter top.
13. Load cassette into machine and close door.
14. Start collection software (icon labeled 377)
15. Start new Genescan Run
16. Change Plate Check parameters to: Platecheck_D and run plate check
17. While plate check is running, make up 1500 mL of 1X TBE (300 mL of 5X TBE in a total volume of 1500 mL).
18. If plate check is good, then cancel run. Open machine and add lower buffer to MAX fill line. Attach heating plate and close door.
19. Start Pre-run and wait for pump to turn on. The program will send out an error about no current due to the upper buffer chamber not being attached. This is ok to ignore.

Loading the membrane comb with template:

20. Remove the samples from the thermocycler and place in frozen block.
21. Select appropriate size membrane comb and loading tray.
22. Load 1 uL of sample into tray.
23. Dip comb into tray and absorb sample until the liquid has just reached the top of each tooth but not gone up into the main part of the comb.
24. Rest comb with teeth hanging off on tray.

Starting the run:

25. Cancel the prerun.
26. Set the number of lanes (if you used a 96 well comb, tell the computer you used 64, or else GeneScan will not be able to read your samples), sample sheet and collection time (usually 1.5 - 3 hours).
27. Start the Run. You'll need to enter in a name for the gel file.
28. Open the door and load the well with 20% ficoll solution. It takes about 500 uL.
29. Load membrane comb into gel
30. Attach upper buffer chamber and fill to MAX line with buffer.
31. Close door and immediately resume run
32. After 1 min, open door (pauses run) and remove comb to safe place.
33. Rinse out ficoll solution with syringe (run across well approximately 10 times).
34. Add upper buffer chamber lid, close door and resume run.
35. Check the Status window and the gel image to ensure that the run has started.
36. Gel takedown is just like regular sequencing procedures.