

Fragment Analysis Protocol

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PCR

1. Estimate genomic DNA from extract by running all samples on an agarose gel with a size marker of known concentration
2. Set up PCR reaction with Qiagen HotStarTaq Master Mix

HotStarTaq PCR Protocol (7.5ul rxn)

3.75uL HotStarTaq Master Mix
0.75uL DNA (I usually dilute the DNA
so 1uL has 10-20ng)
0.75uL primer mix
2.25uL ddH₂O

Primer Mix (400uL)

10uL Forward primer (100uM)
1uL Reverse primer (labeled, 100uM)
9uL Reverse primer (unlabeled, 100uM)
380uL ddH₂O

3. Cap PCR tubes and place in thermal cycler (Note: if PCRing a full 96-well plate, be sure you tightly seal the rubber mats on top of the plate, round sides down!)

Cycling regime

1. 95°C 15min
2. 95°C 30sec
3. 50-60°C 1min
4. 72°C 1min
5. goto 2, 34X
6. 4°C hold

Prepare samples for analysis on ABI 310

1. Make sure ABI310 is running with POP4
2. Thaw an aliquot of HiDi Formamide (DMF)
 - a. DMF degrades to formic acid with multiple freeze-thaw cycles, so don't re-freeze. Check to see if you smell any hint of ammonia – if you do, don't use it. It can be good for a couple days at room temperature.
3. Bring ABI 310 temperature to 60°C
 - a. In the 'Manual Control' window, choose the 'Set Gel Temperature' function
 - b. Set temp to 60°C, hit 'execute'
 - c. Double-check in 'Status' window to be sure temp is set to 60°C
4. In new PCR strip tubes, add 12uL DMF to each tube and 1uL 1:1 ROX Size Standard (mixed 1:1 with dH₂O)
5. Add 1uL (or appropriate amount) PCR product
 - a. Adding more than 2uL PCR product may dilute the DMF too much, so if you want to analyze a really dim band, dry the PCR product down, then add DMF and size standard to the dried product
6. Denature on thermal cycler ("GSDenat") = 95°C 2min
7. Set up GeneScan Sample sheet
 - a. Open ABI Collection Software
 - b. Under 'file', choose 'new' gene scan 96well sample sheet
 - c. Fill in your sample names, making sure the standard is red and the dye color you are using is marked

- d. Close and save sample sheet with the default name, in 'GS sample sheets' folder
8. Set up GeneScan injection list
 - a. Under 'file', choose 'new' 'injection list'
 - b. Choose your sample sheet in the pull down list
 - c. Autoanalysis defaults should be fine
9. Load your samples onto the ABI310, making sure they are in the same positions as listed in the sample sheet you filled out
 - a. Position A1 is in the right rear of the autosampler, as indicated in the diagram on the side of the machine
10. Hit the 'run' button on the injection list and you're set to go!