Fragment Analysis

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SUMMARY

This protocol outlines the procedure of setting up fragment analysis samples.

MATERIALS & REAGENTS

Materials/Reagents/Equipment ***Vendor*** ***Catalog Number***

DISPOSABLES

Half Skirted 96 well Plates	ISC BioExpress	T-3060-1
Stack Rack 1uL-200 uL Natural Tip	USA Scientific	1111-0200
ThermalSeal Tape	ISC BioExpress	T-2417-5

REAGENTS

Hi-Di Formamide (25mL)	Applied Biosystems	4311320
GeneScan 500 LIZ Size Standard	Applied Biosystems	4322682

EQUIPMENT

Centrifuge	Beckman Coulter	
SpectraFlour Plus Plate Reader	Tecan	

PROCEDURE

1. Take out the aliquoted Hi-Di Formamide from the –20 C freezer C to let it thaw.
2. Take out the required amount of GeneScan 500 LIZ size standard labeled with the green sticker and place it on ice.
3. Check the original genotyping plate with the plate reader to figure out the proper dilution before running fragment analysis.
4. In the black 96 well plate, add 200 uL of water and 1 uL of the original genotyping sample.
5. On the desktop click on Megallan2.
6. In the WIZARDS LIST window select Run a Method. Press OK.
7. In the RUN A METHOD window select Genotyping DNA.mth. Press Next.
8. In the WORKSPACE box type in the desired file name, the name you would like to save it under.
9. Then press the START button.
10. After the run, in the EVALUATE RESULTS window click the View Button.
11. View the results and the samples should be in the range of 300-1000.
12. If the results are within this range then 0.75 uL of genotyping sample should be added. If the results are less than the range then more genotyping sample should be added about 1-2 uL. If the results are more than the range then less genotyping or more diluted sample should be added.
13. After viewing the results click the Next button.
14. Press the Save button and then press the Finish Button.

15. The master mix requires per sample: 10 uL of Hi-Di Formamide
    0.25 uL of GeneScan 500 LIZ size standard
    0.75 uL of the original sample

	1 Plate (100 Rxns)	2 Plates (196 Rxns)	4 Plates (388 Rxns)	6 Plates (580 Rxns)	8 Plates (772 Rxns)
Formamide	1000 uL	1960 uL	3880 uL	5800 uL	7720 uL
Standard	25 uL	49 uL	97 uL	145 uL	180 uL

Note: Each mixture has added 4 more reactions for room for error. For example for 2 plates: 96 wells x 2= 192 wells/reactions. 192 reactions + 4 more reactions for error= 196 reaction total.

16. Mix the master well with the mini-vortexer for 15 seconds or by hand.
17. Label the semi-skirted 96 well plate with the appropriate name.
18. Add 10.2 uL of master to well.
19. Then add 0.75 uL (the proper amount depending on the range from the plate reader) of the original sample to the appropriate wells.
20. Vortex it for a couple of seconds to ensure mixing.
21. Place the plates in the centrifuge and spin up to 2000 RPM.
22. Place the plates on the heat block for 2 minutes to denature the samples.
23. Then place the plates on ice for 2 minutes.
24. Assemble the plate for 3730 run. Note: The black plate base, sample plate, plate septa, and plate retainer are assembled in this order with the black plate base at the bottom.
25. Centrifuge for a couple of seconds the assembled plates to ensure that all the products are in its proper place.
26. Place the assembled plate in the plate stacker.
27. Please refer to the ABI 3730 protocol for running these samples.