Biomolecular Core Facility

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3130- DNA Fragment Analysis Submission Protocol

Currently used for quantative analysis, mutation detection and genotyping for linkage analysis. Researchers are asked to provide a fluorescently labeled diluted PCR reaction along with a submission sheet to the core for processing.

Sample sign-up

Contact the Core via phone (302-651-6712) or email (mbcore@nemours.org) by 11:00AM the day you would like your samples to be run.

DNA Prep

Primers can be labeled with a number of different dyes. See Jennifer Holbrook for an updated list of what dyes can be visualized on the 3130XL. As of 4/01/2005 the following dye combinations can be used:

- 6-FAM, VIC, NED, PET with LIZ standard (from the G5 set)
- 5-FAM, JOE, NED with ROX standard (from Filter set F)
- 6-FAM, HEX, NED, ROX (from Filter set D)

Dyes from one filter set cannot be analyzed at the same time as dyes from the other set, so don't "mix 'n' match" dyes.

Post PCR prep

Dilute your labeled PCR products in a 0.2ml tubes (strip tubes with individual caps may be used). Tubes must be labeled **on the side** with the **principal investigator or submitter's initials and a number** (Ex. KS1, KS2 etc.). More detailed names can be linked to these numbers on the sample submission sheet. For microsatellites where the product is normally visible on a gel as a strong band, a good starting point is a 1 in 50 dilution of the PCR product.

Sample Submission and Turnaround Time

Cover your sample in a labeled box or aluminum foil and place it in the -20^{o} Core freezer in G25 A/R building by 1:30 PM or room 214 Rockland Center One by 2:30 PM. A sample submission sheet must be completed. Samples must be submitted in 0.2ml tubes (strip tubes with individual caps may also be used), labeled on the side with the principal investigator or submitter's initials and a number (Ex. KS1, KS2 etc.), along with a sample submission sheet.

Core sample processing
The processing of your sample by the Core staff includes taking 2µL of that dilution and adding it to a microplate well containing the LIZ 500 Size Standard in Hi-Di Formamide. Quantities of LIZ and Hi-Di Formamide may need to be optimized for individual PCR's or problematic samples.

Data
You will receive the run files (.FSA files) for each of your samples (a printed chromatogram can be requested). PeakScanner (an ABI free software) or GeneMapper may be used to visualize your run files. Please contact the Core for information.

GeneMapper® Software v3.5 is a software package that provides quality allele calls on samples electrophoreses using Applied Biosystems' genotyping systems such as the ABI PRISM® 3130XL DNA Analyzer. This software package replaces the 2 analysis systems previously known as GeneScan and Genotyper. Please inquire for literature and training for the new software. This software is currently only available on the Core PC that runs the 3130 instrument and is only available at specific times.

The DNA Fragment Analysis Core offers several levels of services depending on the needs of the researchers. Please consult with the core director, Dr. Katia Sol-Church for the best fit for your experimental design. High throughput genotyping may require the need for robotics set-up. We also offer genotyping software training to anyone interested.