

Biomolecular Core Facility

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1/09

3130- LONG DNA Fragment Analysis Submission Protocol

Researchers are asked to provide a fluorescently labeled diluted PCR reaction along with a submission sheet to the core for processing. Long FA is for products ranging in size from 50 to 1200 base pairs.

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 12/9/08 the following dye combinations can be used:

- 6-FAM, VIC, NED, PET with LIZ 1200standard (from the G5 set)

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, which can be requested by email or picked up at the core. For PCR products that are normally visible on a gel as a strong band, a good starting range is a 1 in 10 or 1 in 20 dilution.

Sample Submission and Turnaround Time

Cover your sample in a labeled box or aluminum foil and place it in the -20° Core freezer in G25 A/R building by 1:30 PM or room 214 Rockland Center One by 2:30 PM. Place your submission sheet in the FA inbox. 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 2 μ L of that dilution and adding it to a microplate well containing the LIZ 1200 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 a printout of the electropherogram and the run files (.FSA files) for each of your samples. 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.

Fee

The cost per injection is \$4.00 and will be billed to your Lawson activity number on a quarterly basis. For high volume discount, please contact the Director, Dr. Katia Sol-Church. All non-AIDHC affiliated customers will be charged a 10% surcharge.