SAMPLE SUBMISSION GUIDELINES Fragment Analysis – Ready2Load Service

Eurofins Genomics (US) offers the 'Fragment Analysis – Ready2Load' service using ABI 3730xl DNA analyzers. Our workflow is optimized to offer the service for the PCR products generated by microsatellite, SNP, LOH, AFLP, RFLP, TRFLP, ARISA or other protocols.

Dye Set

Only the following dye combinations can be used as matrix and for labelling the PCR products: ABI Standard Dye Set DS-33—6-FAM, VIC, NED, PET and LIZ* *the dye LIZ is typically reserved for the size standard and should not be used for PCR product labelling.

Size Standard

The protocol for the Ready2Load fragment analysis at Eurofins Genomics US is optimized for analysis of DNA fragments 50–600 bp. The LIZ 600 size standard must be included in your samples for fragment analysis.

Sample preparation for 'Fragment Analysis – Ready2Load' Service

Please adhere to the following guidelines, to assemble samples for Fragment Analysis – Ready2Load service.

- Run PCR using dye labeled primers that can be analysed with ABI Standard DS-33 filter set
- Prepare solutions of PCR products at 50–100 ng/µL
- Assemble the samples for 'Fragment Analysis Ready2Load' Service, by mixing the following:
 - 1) Hi-Di Formamide loading buffer, 10 µL (Applied Biosystems)
 - 2) Dye-Labeled internal size standard, 0.5 µL (use only LIZ-600).
 - 3) PCR product, 2 µL
- The total sample volume should be 10–15 μL.

Sample Submission formats

Please note that you can submit your samples in one of the following two formats:

- 1.75 mL centrifuge tubes
- 96-well plates (96-well plates (MicroAmp® Optical 96-Well Reaction Plate, ThermoFisher, catalogue no. 4316813, or equivalent)

Shipping

• Ship the liquid samples at ambient temperature to:

Eurofins Genomics 12701 Plantside Drive, Louisville, KY 40299

- Use 8-cap strips for sealing 96-well plates (recommended)
- Alternatively, adhesive PCR foil can also be used for sealing 96-well plates
 - ensure that the plate has reached room temperature and the plate surface is absolutely dry before you stick the foil on the plate
 - Seal the plate tightly and avoid folding; don't freeze and thaw the plate after sealing.

General Instructions

To place an order for 'Fragment Analysis – Ready2Load' service, you can submit the details of your assay samples and payment information in an excel file via email to GenomicsSupport@eurofins.com. Additional details are included below

- Download the excel order form from :
- Send the filled order form electronically to genomicsSupport@eurofins.com and as a hard copy together with the samples
- Label your samples according to the sample names indicated in your sample list:
 - Single tubes on the lid
 - 96-well plates on the plate frame
- The data will be delivered in .fsa format in 1-3 days after receiving the samples
- Results can be viewed using any of the following free/paid softwares (Dustin/Danielle/Daria can you please verfiy this list)
 - GeneMapper (Applied Biosystems) Software
 - Peak Scanner ((Applied Biosystems), <u>https://www.thermofisher.com/order/catalog/product/4381867</u>)
 - GeneMarker (SoftGenetics https://softgenetics.com/GeneMarker.php)
 - Peak Studio (http://fodorlab.uncc.edu/software/peakstudio)
 - Xplorer (http://www.dnatools.com/download.html)
 - STRand (http://www.vgl.ucdavis.edu/informatics/strand.php)
 - DAx (http://www.dax.nl/dax.htm)

A note about sample concentration

- Concentration across all samples submitted in an order must be normalised
- For multiplex analysis, the concentrations of the PCR products in a sample must be normalised in order to get a balanced profile (recommended variations in concentration <50%)
- The concentration of each PCR product resp. marker should be 50–100 ng/µL

Contact

Please do not hesitate to contact us in case of any questions! Email: GenomicsSupport@eurofins.com Phone: 1-800-688-2248 Registered trademarks are listed at eurofinsgenomics.com