IMPORTANT: Talk to us and Applied Biosystems (ABI is owned by Thermo Fisher Scientific) technical support (1-800-831-6844) before you order your **primers**. Currently we are set up to run ABI dye set: (DS-30: 6-FAM, HEX, NED, ROX), (DS-32: 6-FAM, JOE, NED, ROX) and (DS-33: 6-FAM, VIC, NED, PET, LIZ). We can also run other Matrix Standards as needed. Our preferred dye set is DS-33. If you are not going to buy your primers from ABI then you must double check with us that your chosen fluorescent tags are compatible with each other and with our equipment. We are not currently set up to run TAMRA or TET.

Your primer sets should have one labeled primer and one unlabeled primer.

No clean up necessary: If you are doing a standard primer labeled PCR reaction to generate your fragments then no clean up is necessary.

If you are here at UC Berkeley, we will supply the **plates**, and the formamide. Pick them up in room 310 Barker Hall.

The plate you use must be compatible with our ABI 3730xl.

We prefer:

4titude
FrameStar 96, semi skirted
Catalogue no. 4ti-0770/C
or
MPX-96M2 (no barcode) or MPX-96M2-BC (with barcode, may need to special order) from Phenix 1-800-767-0665

Each well you are using should have at least 50% **formamide**. It is best to use Hi-Di Formamide from Applied Biosystems Part No. 4311320. Each well must have at least 10 uL total volume. Please put DI water in the wells you are not using.

You will need to add a **size standard**. Most people choose one from Applied Biosystems. Do not buy 1000ROX from ABI it will not work on our equipment. We can add the size standard for you **if** you have just a handful of samples. Call us to ask what we have on hand. 500 LIZ (Part # 4322682), 600 LIZ (Part # 4366589) and 500 ROX (Part # 401734) from Applied Biosystems are the most common.

Amount of sample: .5 uL to 1 uL of your PCR reaction is usually all you need to use, but you should test a few samples to optimize. Loading too much of your PCR reaction can suppress the signal intensity of your size standard, and create problems with the analysis of the data. Overloaded samples can cause interference with the data collected from nearby capillaries.

Be sure to **label the top of the plate** as a "Fragment Analysis" plate.

IMPORTANT: If your plates will be sent through the mail you must be absolutely certain that you have sealed them well. Strip caps probably work best, but test them. If you want to seal the plates with aluminum foil tape, you must rub down the entire surface like your life depends on it. No wrinkles in the surface of the foil. It helps to use a Kimwipe between your glove and the aluminum. The Kimwipe will slide on the surface. Please see attached image of a well sealed plate. You should **test your seal** by adding water to a plate and putting it on a vortex.

Plastic seals from BIO-RAD "MSB-1001" seem to be easiest to use. ("Microseal B Adhesive Sealer") They cost more, but seem to fail less often. Whatever method you want to use you should test it by sealing up some water and putting it on the vortex upside down to see if it leaks. Be sure the water does not get up and mix on the underside of the foil.

If you are sending more than one plate, be sure to put in a separator. The bottom of the wells of one plate can puncture the seals of the wells below. Also, be sure to send your plates in a box or some other stiff container to prevent crushing. No ice is necessary.

You will need to fill out the Fragment Analysis Order Form. Print a copy to include with the plate and e-mail a copy as an attachment. There is space on the order form for you to let us know the expected length of your products and the dyes used.

We will heat denature your plate of samples unless you let us know that you have already done so. We heat to 95 C for 3 min then flash cool on wet ice for 2 min.

We charge UC labs $2.50 per well with a $20 minimum order, LBNL and CHORI affiliated labs add 10%, Outside labs add 61%.

There is **free software** called Peak Scanner from Applied Biosystems to analyze and view your results (older Windows computer compatibility):
https://www.thermofisher.com/order/catalog/product/4381867?SID=fr-cesoftware-3

Use the following settings: Analysis Method: "Sizing Default – PP" Size Standard: "GS500LIZ_3730" or if you are using ROX: "GS500(-250)"

Samples sometimes fail analysis particularly if they have **off scale data** that interferes with the size standard. If you want to get a look before you discard them you will need to lower the quality flags. Go in to "Manage Analysis Methods", create a "New" analysis method, in the "Quality Flags" tab, in "Sizing quality" increase the "Pass Range". This might help you find the source of the problem to improve the re-run of these samples.

Peaks marked with pink have overloaded the camera (off scale). You might want to consider loading less sample on to your plates or putting less labeled primer into your PCR reaction.

Last edited: 02 Oct 2024, Scott Geller