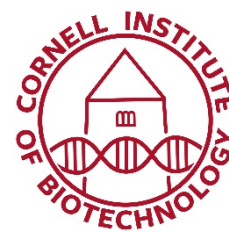


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Sanger Sequencing Handbook

FRAGMENT ANALYSIS

Revised January 2020

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This document (“Sanger Sequencing Handbook FRAGMENT ANALYSIS”) explains all the steps you need to take to prepare and submit material such as microsatellites (STR), fingerprinting (AFLP), etc.

If you are looking for the **Fragment Analyzer** to QC your Next generation sequencing library preparation, look for the document call “Fragment Analyzer”.

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Step 1. Choose your dye set and size standard

Dye sets

By default, we run the Applied Biosystems DS-33 (aka G5) dye set (6-FAM, VIC, NED, PET, LIZ).

If you want to run the DS-30 dye set (6-FAM, HEX, NED and ROX), you **must supply** your sample plates with the fluorescent size standard already added.

Size standards

Most customers supply their own size standard. If you prefer to let the facility add the size standard, we will add LIZ500 and HiDi as a service for a fee.

Step 2. Choose the right plates

All samples must be submitted in plates, no matter how many samples you have.

We require that you use any of the following:

- Applied Biosystems® MicroAmp® Optical 96-Well Reaction Plate (catalog number N8010560)
- Fisher Scientific (catalog number 14230244)
- USA Scientific (catalog number 1402-9200 and catalog number 1402-9300).
- Other plates **that are compatible with the ABI 3730 instruments** are acceptable. If you have a plate that you think is compatible, please email us a picture. We will get back to you if they will work with the sequencers or not.
- Note that “fast plates” or “short plates” are not compatible.

Step 3. Prepare your reaction mix

Typical mix (in each well of the plate):

- 1 uL of PCR product (This is typical, but you may have to optimize for your reaction products. Generally, less, rather than more, PCR product produces better results.)

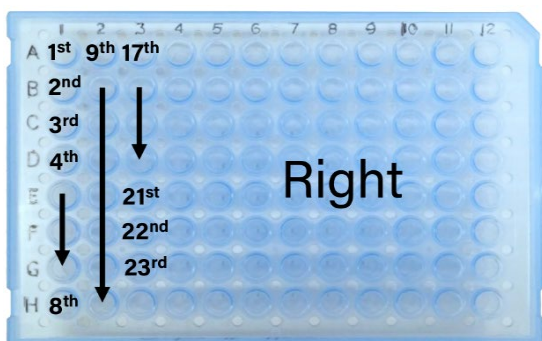
- 0.2 uL LIZ 500 size standard (this is the expected size standard).
- Bring volume up to 10-20 uL with HiDi formamide (available from ABI/LifeTech). **IF YOU DON'T ADD the size standard and require the facility to add it, DO NOT bring the volume to 10-20 uL, and only submit your 1 uL of PCR product**

Step 4. Fill up your plates

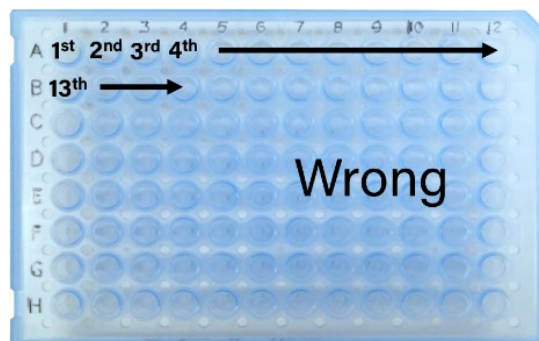
- Fill 10-20uL of your reaction mix *down the columns*.
- All wells in the plate must contain liquid, so wells with no samples should contain 20 uL ddH₂O.
- If you accidentally skip a well, add it to your order so that your samples are not shifted.
- Seal your plate to prevent evaporation.

Example:

Your first sample goes in A1, your second sample in B1, your third samples in C1 and so one. When you are done with the first column, you will fill the next column. Sample 9 will be in A2, sample 10 in B2, sample 11 in C2 etc. If you have 23 samples, your 23rd sample is in G3.



Example with 23 samples



Wrong

Step 5. Place your order online

All requests for sequencing must be submitted online. You can access our online ordering system by clicking "Submit samples" on [our website](#).

We cannot process your samples if your order is not in our online ordering system.

To upload your sample names in the online ordering system, you can either upload a file or type all the sample names manually. If you choose to type in the sample names, go directly to step 2.

1. Create a text file with all the sample names to upload

- Using Microsoft Excel:

Write your sample names with one name per line starting with well A1 and proceeding down the columns (A1, B1, C1, D1...) of the plate.

Name1
Name2
Name3
...
Name23

If you have empty wells, leave the cell blank. You do not need to enter any name for empty wells. Save as a Text (Tab-delimited) file.

- Using a text editor:

Write your sample names with one name per line starting with well A1 and proceeding down the columns (A1, B1, C1, D1...). If you have empty wells, leave the line blank. You do not need to enter any name for empty wells. Save as a plain text file.

2. Log in to your account

3. Scroll down to the bottom of the page, and select the plate you are submitting, 96 or 384 well, under “**Fragment Analysis (e.g. fluorescent microsatellites).**”

4. Enter billing information.

5. **Select size standard used.** If you are not using LIZ500, type in the dye set or size standard you added to your plate.

6. If you are using LIZ500, select **who** is adding the size standard (the facility, or the user).

7. Upload the text file you prepared with your sample names. Don't forget to click “Upload File”. You should see the sample names auto-populate the online form.

8. **Enter a plate name** at the bottom of the form (letters, numbers, hyphens, and underscores only).

9. Click “Proceed to verification” and submit

10. **Write the order number you will receive on the long side of the plate.**

External customers will need a Purchase Order for payment. Please send us a copy of the PO at brc_payment@cornell.edu. We cannot start processing your samples until we receive a copy of the PO. Email is preferred over a paper copy; it will expedite the process.

Step 6. Send your samples or bring them to our facility

There are different methods to send you samples to sequencing.

- **In person.** You can come and drop your samples off in room 147 in Biotechnology Building. Our work hours are written on the first page of this document.
- **By FedEx or UPS.** Avoid USPS as packages are not delivered directly to the lab. Our mailing address is written on the first page of this document. Please note that there is no delivery on weekends or holidays. Also, note that we have received numerous broken plates, so please cushion and seal your plates before mailing.
- **Fed Ex drop box.** This option is available for customers from the Weill Medical College and Memorial Sloan Kettering Cancer Center in NYC. The Office of Sponsored Programs manages the drop box, so please direct all questions regarding the drop box to them.

Whether you send or bring your samples, don't forget to write your name, contact number, lab location and **order number (that you obtained through the online system)** on the outside of the plate and package.

If you have any question, our contact information is on the first page of this document.

Step 7. Receive and analyze your results

Our usual turnaround time is 1-2 business days.

You can track the progress of your samples from our [ordering online system](#)

When your results are ready, we will send you an email pointing you to our secure website where your electropherograms and text files can be downloaded.

The [Bioinformatics Facility](#) can provide software and support for primary sequence analysis.

Step 8. Store your data

Data will be available for 30 days. After this, your results will no longer be available on your account. If you want to access your files, contact us with your order number.

A \$5 internal and an \$8 external fee per order is associated with data retrieval, so please remember to download and save your results as soon as they are ready.

NOTE ON DNA STORAGE:

All samples will be discarded two weeks after they are processed.