

## DNA SEQUENCING ORDERS

Please use our GeneSifter Lab (formerly Finch) data management server to electronically submit your sample request <http://idaho.genesifter.net/Finch/>. Follow the steps below to submit a sample request. If you are not a current GeneSifter Lab user, please click on the following link [Registration Form](#). If you are a past Finch user, you must create a new account in GeneSifter.

1. Log in to GeneSifter Lab at <http://idaho.genesifter.net/Finch/>. If you are not a current user, please click on the following link [Registration Form](#). If you are a past Finch user, you must create a new account in GeneSifter.
2. In the left column under Orders, select Place Order
3. Choose Sequencing by clicking either the text or icon
4. Complete the **top section (Order Information)**. All areas with an asterisk\* must be filled
  - A. Order Comment – Enter specific information or requests concerning your samples (for example, if you want the MRCF to add primers, enter that request here)
  - B. Plate Label – you may enter any label for your plate (no spaces)
5. Complete the **bottom section (Sample Information)** using one of the following two methods:
  1. Excel GeneSifter Input Grid
  2. Manual entry using GeneSifter Sequencing order form

### INSTRUCTIONS FOR EXCEL GENESIFTER INPUT GRID

The screenshot shows the 'Sample Information' section of the GeneSifter interface. It features a table with columns for 'Position', 'Sample', 'Primer', 'DNA type', and 'PCR product size (bp)'. Above the table are various utility buttons like 'Fill Down', 'Clear Column', and 'Go'. Three callout boxes are present: box '1.' points to the 'DNA type' dropdown menu; box '2.' points to the left Excel icon; and box '5.' points to the right Excel icon.

1. Select DNA type under the DNA Type drop down menu (see #1 above), then select the first radio button (arrow) to fill down *Note: DNA type will fill through H12. This will not affect your ability to submit the order*
2. Locate GeneSifter Input Grid by clicking on the left Excel icon (see # 2 above) in the blue region under Sample Information
3. Enter Sample, Primer, and PCR product size (bp), and delete any extra rows of Size Standard
4. Save the Excel file
5. Click on the right Excel icon (see # 5 above) in the blue region under Sample Information
6. Browse for your saved Excel file and click Upload

7. Click Next at the bottom of the screen
8. Verify that your order is correct
9. Complete your purchasing information by going to the “purchasing information” link. Select your name under Payer, Select Purchase Order under Charge Code Type, enter your PI or account number, then click Next
10. Click on Submit Order
11. After your order is complete, please record the GeneSifter Tracking ID# on the [Job Request Form](#), which must be completed and submitted with your samples

**INSTRUCTIONS FOR MANUAL ENTRY USING GENESIFTER SEQUENCING ORDER FORM**

1. Range – enter the range from A01-H12 for the number of samples you are submitting, then select Go. Examples: If you have 15 samples you would enter A01-G02 under Range, then select Go. If you have 31 samples you would enter A01-G04 under Range, again followed by Go
2. Enter your sample name under \*Sample. If possible, label your samples with a simple name such as test1. If you enter the sample name followed by the number 1 and then choose the second radio button (arrow+), your samples will fill down consecutively (i.e. test1, test2, test3, etc.)
3. Enter your primer name under \*Primer, then select the first radio button (arrow) to fill down
4. Select DNA type under the DNA Type drop down menu, then again select the first radio button (arrow) to fill down
5. Select your PCR product size (bp) and click on arrow to fill down. If your DNA type is Plasmid, enter 0 and fill down
6. Click on Next at the bottom of the screen
7. Verify that your order is correct
8. Complete your purchasing information by going to the “purchasing information” link. Select your name under Payer, Select Purchase Order under Charge Code Type, enter your PI or account number, then click Next
9. Click on Submit Order
10. After your order is complete, please record the GeneSifter Tracking ID# on the [Job Request Form](#), which must be completed and submitted with your samples

**VOLUME**

Submit all DNA templates at the concentrations listed below under **QUANTITY** in 1 to 4  $\mu$ l of molecular grade water (depending on your starting concentration) together with 1  $\mu$ l

of primer (primer concentration should be 3.2 pmoles/ul) for a total of 6 µl. Please do not use TE as this can interfere with the sequencing reaction.

Example 1: If you have a 1000 bp sample at a concentration of 5 ng/µl, add 4 µl of the DNA to 1 µl of water and add 1 µl of primer for a total volume of 6 µl.

Example 2: If you have a 500 bp sample at a concentration of 10 ng/µl, add 1 µl of the DNA to 4 µl of water and add 1 µl of primer for a total volume of 6 µl.

## **SAMPLE FORMAT**

Samples may be submitted in 96-well plates, 200 µl strip tubes, .5 ml. tubes, or 1.5 ml. tubes. Please do not submit samples in individual 200 µl PCR tubes. For a full plate, leave one well empty for a control (95 samples per plate + 1 control).

You may also submit fewer than 96 samples in a plate, but you must arrange your samples by COLUMNS (A1-H1, A2-H2, etc.), not rows (A1-A12, etc.).

If you are shipping samples in 96-well plates, please use an adequate cover to prevent sample evaporation. We recommend foil covers, but you may use anything that effectively seals your samples. Strip tubes with caps should be sealed with Parafilm.

## **QUANTITY**

The correct DNA concentration (within the range listed below) and proper sample purification are critical for successful sequencing. Examples of sample purification methods are:

Spin column (Qiagen)

Enzymatic (Exo-Sap)

Ultra-filtration (Millipore Montage)

## **Template**

We have provided a broad range for DNA quantity based on Applied Biosystems guidelines. Pure DNA is critical to success and will allow us to generate optimal sequence data. If your sample has been purified adequately (see suggestions for sample purification methods above), we recommend that you start at the lower end of the range.

If low signal strength is observed, increase the quantity.

If off scale signal is observed, decrease the quantity.

PCR Product:

100-200 bp	0.5-3 ng
200-500 bp	1-10 ng
500-1000 bp	2-20 ng
1000-2000 bp	5-40 ng
>2000 bp	10-50 ng

Single-Stranded	10-50 ng
Double-Stranded	50-300 ng
Cosmid, BAC	200-1000 ng
Bacterial Genomic DNA	2-3 µg

Please quantitate the DNA. The MRCF's NanoDrop spectrophotometer is available for your use. If you're an off-campus researcher we would be happy to quantitate your samples for an additional \$.25/sample. If you wish to do this you can simply send us your DNA template and primer in separate tubes. We'll NanoDrop the samples, combine the template and primer, and process the samples as usual.

**Primer** 3.2pmol/µl

We maintain stocks of the following primers. Please notify us if you would like to use these primers in your sequencing reaction.

Primer name	Primer sequence
M13F (-40)	GTTTTCCCAGTCACGAC
M13R	CAGGAAACAGCTATGAC
T7	TAATACGACTCACTATAGGG
T3	ATTAACCCTCACTAAAGGG
SP6	TATTTAGGTGACACTATAG
SP6R	TATAGTGTACCTAAAT
T7R	TATAGTGAGTCGTATTA
T7terminal	GCTAGTTATTGCTCAGCGG
8F	AGAGTTTGATCCTGGCTCAG
907R	CCGTC AATTCCTTTRAGTTT (R=A or G)
704F	GTAGCGGTGAAATGCGTAGA
1492R	GGTTACCTTGTTACGACTT
M13F (-20)	GTA A A A C G A C G G C C A G T
M13R (-48)	A G C G G A T A A C A A T T T C A C A
PUCuniversal	GGCTGGCTTAACTAT

## **DATA VIEWING, DOWNLOADING, AND ANALYSIS**

Samples are analyzed on an Applied Biosystems 3130XL DNA analyzer. When your sequencing samples have finished processing, you will receive an e-mail message with instructions for downloading your data from GeneSifter.

The MRCF uses the Geospiza GeneSifter Lab (formerly Finch) server for data management as well as sample submission. Data can be accessed or downloaded from the server, which calculates Phred Q>20 values for all sequences and reports these in

graphical form. The vector sequences are identified and can be trimmed from the downloaded sequences. E. coli sequence is identified. Researchers can download the sequence information or the original chromatograms individually or by folder, and the chromatograms can also be viewed or printed.

Download the [free sequence trace viewer](#) and editor from Applied Biosystems. You may also download the [freeware version of FinchTV](#) from the Geospiza website. In addition, Sequencher software from Gene Codes Corporation is installed on a common use Macintosh G4 computer in the MRCF for sequence data analysis.

[Sequencing Data Quality Questions or Problems?](#)