Sequence FAQ’s

Sequence: Placing an order

What sequence services does the facility provide?
The facility has two levels of service for automated DNA sequencing.
Cycle Sequence Reactions- The user provides DNA template and primers (see below for a list of primers that the facility has available). The facility performs and cleans the cycle sequence reaction. If the user prefers, they can do their own cycle sequence reaction, clean the cycle sequence product, and bring the samples ready to run on the instrument. The cleaned product should be in water and does not need to be dried in a speed-vac.

Sequence Runs- This is the run of the cleaned cycle sequence reaction on the 3130xl Genetic Analyzer. All data will be uploaded to the user’s BioDesktop account.

How do I place an order?
Login to the DNA Depot, you will see the Order Forms under the “Orders” menu on the left hand side of the page. Select the Sequence Request, and fill out all information. Click OK at the bottom of the page. You will receive an email confirmation of your order.

What information will I need to provide when I place an order?
You will need to provide the following information:

Your name, principle investigator of lab, phone, and chart string.

Primer Info: Name: (no more than 6 characters)
Concentration: Primers should be at 2uM. If you are using a facility primer choose “Fac. Primer” in this field.

Template Info: Name: (no more than 10 characters)
Sample type: ds plasmid, ss DNA, PCR product, genomic, BAC, cosmid
PCR product length: PCR products only
Template concentration: Templates must be provided at following concentrations:

<table>
<thead>
<tr>
<th>Template Type</th>
<th>Required concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double strand plasmid</td>
<td>50 ng/uL</td>
</tr>
<tr>
<td>Single strand plasmid</td>
<td>25 ng/uL</td>
</tr>
<tr>
<td>PCR 0-200 bp</td>
<td>5 ng/uL</td>
</tr>
<tr>
<td>200-500 bp</td>
<td>5 ng/uL</td>
</tr>
<tr>
<td>500-1000 bp</td>
<td>5 ng/uL</td>
</tr>
<tr>
<td></td>
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<tr>
<td>----------------</td>
<td>------------------</td>
</tr>
<tr>
<td>&gt;1000 bp</td>
<td>5 ng/uL</td>
</tr>
<tr>
<td>PCR (Exo-Sap)</td>
<td>1uL/Rxn</td>
</tr>
<tr>
<td>Bac and gDNA</td>
<td>1ug/uL</td>
</tr>
</tbody>
</table>

How do I print a copy of my order form?

See the BioDesktop FAQ.

Where do I put my samples?
Your samples should be put in the freezer labeled “Sequence Samples” in Room 305 HSRF. Boxes are in alphabetical order by the lab Investigator’s name. Please group them together and place any of your own primers that will be used with the template. **Make sure your DNA template and primers are labeled on the top of the tube with the same name that was entered on the order.**

If you have done your own cycle sequence reactions, your tubes should go in a rack on the second shelf of the freezer marked “Samples Ready to Run”. If your samples are in a 96 well plate, be sure the plate is well sealed and labeled with your name, Investigator name, and date order was placed.

What do I do if my box is full?
Empty it! Please go through your box occasionally and remove template that have already been sequenced. It is fine to leave primers that you expect to use again. If you are dropping off large numbers of samples to be sequenced it is sometimes easier to have them in their own box, please see a staff member to make sure it is properly marked.

When will my samples be run and how will I be notified?
Turn-around is usually 1-2 days. We occasionally experience periods of high demand and it may take 3-4 days. Cancer-qualifying projects will receive priority during these times. If you need data quickly, please feel free to talk to Tim or Mary Lou.

You will receive an email from the BioDesktop when your data is ready. We sometimes edit this message to have specific remarks about how your samples ran, so please look at this message! Please read our “Sequence email Message Explanations” FAQ to learn more about these messages.

Sequence: Sample information

Template

What host strains of bacteria are recommended for sequencing?
DH5a and HB101 work well.
MV1190 and XL1 Blue yield variable results.
JM101 usually yields poor quality DNA for sequencing.
Can my template be in TE?
No, template should be suspended in water and not TE buffer for submission for sequencing. Excess salts may cause a no signal. EDTA chelates Mg$^{2+}$ which is necessary for Taq FS polymerase used in the cycle sequencing reaction.

Do I need to quantitate my DNA?
Yes, we need an accurate measure of the concentration of your DNA in order to set up a good cycle sequence reaction. Your 260/280 ratio should be 1.8-2.1. A lower number indicates possible protein contamination and a higher number indicates possible RNA contamination. Our NanoDrop is available during regular hours in room 305 HSRF and the NanoDrop in room 307 HSRF is available 24 hours/day once you have obtained swipe access to the room. See the Imager FAQ page to get details on how to obtain 24 hour access to room 307).

What are the required template concentrations?
- DS DNA: 50 ng/uL
- SS DNA: 25 ng/uL
- PCR: 5 ng/ul
- Genomic: 1 ug/uL
- Cosmid or BAC: 1 ug/uL

What if my template concentrations are lower than the required amount?
You can submit samples with lower than the recommended amount. Please note this in the “comments” section of the order form. We will use the maximum volume available to us in the reaction. This is not ideal, however, and may result in poorer quality sequence results.

What if my template concentrations are higher than the required amount?
It is important to make dilutions to the required concentration before submitting your template so that the cycle sequence reaction is set up with the optimal amount of template. Submitting template at too high a concentration can result in poor sequence data and can lessen the life of the capillary array in our sequencing instrument.

This is data generated from template that was submitted at 350ng/uL instead of 50ng/uL:
What is the recommended method for cleaning PCR products prior to sequencing?

If your PCR generates a single product, you can do an enzyme treatment known as Exo-Sap to remove the excess primer and nucleotides from the PCR reaction.

If your PCR generates multiple bands, you will need to run the product out on a gel, and gel purify the bands.

Contact the facility for more information about these procedures.
What special template conditions should I note on my order form?
The following conditions are important to note on your order form: GC rich templates, sequence with homopolymeric runs or possible secondary structure, plasmids with large vectors (>10 kb) or plasmids in low copy vectors.

**Primers**

What primers does the facility have available for use?
- M13For (-21): TGT AAA ACG ACG GCC AGT
- M13Rev: CAG GAA ACA GCT ATG ACC
- T3: ACC CTC ACT AAA GGG AAC AA
- T7 Promoter: TAA TAC GAC TCA CTA TAG GG
- T7 Terminator: GCT AGT TAT TGC TCA GCG G
- Sp6: ATT TAG GTG ACA CTA TAG
- 5’pGex: CCA GCA AGT ATA TAG CAT GG
- 3’pGex: CCG GGA GCT GCA TGT GTC AGA GG
- cmvFor: CGC AAA TGG GCG GTA GCG GTG
- BGHRev: TAG AAG GCA CAG TCG AGG

What is the required primer concentration?
Primers should be brought to the facility at 2uM.

What are the recommended characteristics of a sequencing primer?
Primers should have a 100% match with the target sequence. The primer should be at least 18 bases long with a Tm between 55 and 65, and a GC content of 50%. The primer should not have hairpins or long repeats (more than 3 of the same base in a row).

**Cycle sequence reactions**

What chemistries does the facility have available for cycle sequence reactions?
The facility regularly uses Applied Biosystems BigDye Terminator v3.1. We have an AB dGTP chemistry which is helpful when sequencing through runs of G’s. We will use this chemistry when the user has indicated a need for it in the comment section of their order. We also use modified protocols for sequencing difficult templates.

How much template and primer do you use in a cycle sequence reaction?

<table>
<thead>
<tr>
<th>Template Type</th>
<th>Required template concentration</th>
<th>Total amount used in each 15uL Cycle Sequence Reaction*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Double strand plasmid</td>
<td>50 ng/uL</td>
<td>375 ng</td>
</tr>
<tr>
<td></td>
<td>Amount</td>
<td>Volume</td>
</tr>
<tr>
<td>----------------------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Large plasmid</td>
<td>50 ng/uL</td>
<td>525 ng</td>
</tr>
<tr>
<td>Single strand plasmid</td>
<td>25 ng/uL</td>
<td>75 ng</td>
</tr>
<tr>
<td>PCR 0-200 bp</td>
<td>5 ng/uL</td>
<td>10 ng</td>
</tr>
<tr>
<td>200-500 bp</td>
<td>5 ng/uL</td>
<td>20 ng</td>
</tr>
<tr>
<td>500-1000 bp</td>
<td>5 ng/uL</td>
<td>40 ng</td>
</tr>
<tr>
<td>&gt;1000 bp</td>
<td>5 ng/uL</td>
<td>45 ng</td>
</tr>
<tr>
<td>PCR (Exo-Sap)</td>
<td>no change</td>
<td></td>
</tr>
<tr>
<td>Bac and gDNA</td>
<td>1 ug/uL</td>
<td>1-2 ug</td>
</tr>
</tbody>
</table>

*This amount of template for each primer requested.

**What is your standard cycle sequence profile?**
We run 25 cycles of the following profile:
- 96° 10sec
- 50° 5 sec
- 60° 4 min

**How do I clean my cycle sequence products?**
We recommend cleaning the cycle sequence reactions with a sephadex spin column.

Contact the facility for more information about this procedure.

**Sequence: Format of data**

**What format will my sequence data be in?**
The sequencer generates .ab1 sequence files.
The BioDesktop also can generate phred files.

**What are phred files?**
For an explanation of phred please go to:
[http://www.genome.washington.edu/UWGC/analysistools/Phred.cfm](http://www.genome.washington.edu/UWGC/analysistools/Phred.cfm)

Please read our phred FAQ before downloading phred data:

**How do I download my data from the BioDesktop?**
You will receive an email from the BioDesktop letting you know you have data available. The email will have a link to your DNA Depot page. You are prompted to type in your user name and password. You will see your new data listed. You can selectively choose the ones to download or click select all. Click on the “Data Files (in Zip Archive)” button under the Download Menu on the left-hand side of the DNA Depot page. This will download a zipped file which will need to be expanded.
Sequence: How to view data

How do I view my chromatograph?

It is important to view your sequence data to assess the quality of the data before using the text data for alignments, contigs, Blast searches, etc. The following are a few of the freeware trace viewers available to view your data.


FinchTV: http://www.geospiza.com/finchtv/


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What software does the facility have available for doing sequence alignments?

The facility has a copy of Sequencher 4.8 is available on the Dell Optiplex GX270 in the workstation.

A demo version of Sequencher can be found at: (http://www.genecodes.com/demos/) and a freeware called BioEdit can be found at: (http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

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