

Sequence FAQ's

Sequence: Placing an order

What sequence services does the facility provide?

The facility has three levels of service for automated DNA sequencing:

Cycle Sequence and Sequence Run- The user provides DNA template and primers (see below for a list of primers the facility has available). The facility performs and cleans the cycle sequence reaction and runs the samples on the 3130xl Genetic Analyzer.

Sequence Run Only- The user must set up and clean the Cycle sequence reaction. The facility will run the cleaned cycle sequence reaction on the 3130xl Genetic Analyzer.

Bulk Sequence Orders- This is limited to orders of at least 48 samples. The user will combine the appropriate amount of template, primer and H2O in a 96 well plate. The facility will add the BigDye mix, complete and clean the cycle sequence reactions and then run the samples on the 3130xl Genetic Analyzer.

How do I place an order?

1. Login to iLab.
2. Find the 'Request for Services' tab, and then locate 'Sample Submission and Laboratory Services' heading, open the VCC DNA Analysis Facility folder.

3. Select the correct request for your order:
[Sequencing] – Cycle Sequence and Sequence Run
[Sequencing] – Sequence Run Only
[Sequencing] - Bulk Sequence

Fill out necessary information on the order form. ****Note: Please adjust the quantity field in both locations on the order form.**

The screenshot shows the 'Forms and Request Details' page for a sequencing request. At the top, there is a header with the date 'May 20 10:50 AM' and the facility name 'VCC DNA Analysis Facility'. The request title is '[Sequencing] - Cycle Sequence Rxn and Sequence Run'. The quantity is set to '1.0', the price is '\$9.74', the billing status is 'Not Ready To', and the work status is 'Proposed'. There are buttons for 'View Form', 'Visible', and 'Not Started'. A pink 'page guide' button is also visible.

ATTENTION: Please adjust the Quantity above to reflect the number of sequences you are requesting. Download the Sample Name excel Template, fill out all information on the template and attach to your order.

Save the completed form and submit your request to the core.

To Calculate Number of Sequences Each template and primer combination is counted as 1 sequence. If you have 3 templates and want them each with 2 primers this is 6 sequences.

★ Number of sequences Requested (Change the Quantity field above to this number also!)

Download this document for the required concentrations [DNA_Template_and_Primer_Concentration_Requirements.docx](#)

Template and Primer Names All request forms now require you to attach an excel sheet with the list of template and primers that you want sequenced. Please remember there should one line for each template-primer combination.

New Excel sheet Template added 3-15-13 Attention! We made minor changes to the excel sheet template. Please be sure to download the new excel Template!

Download the Sample Name excel Sheet here [CSRxn_and_Seq_Run_Sample_Name_Template_03-13.xls](#)

★ Did you download and use the new excel sheet added on 3-15-13? yes no

★ Attach the completed Sample Sheet here

Please save your form!

4. All Sequence order forms need to have an excel file attached listing:

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:

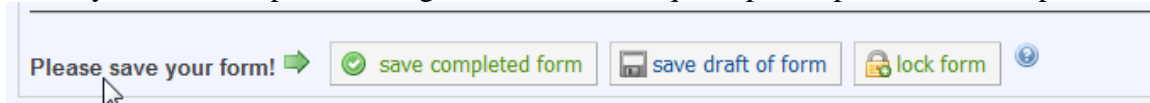
Template Type	Required template concentration	Total amount used in each 15uL Cycle Sequence Reaction*	
Double strand plasmid	50 ng/uL	375 ng	7.5 uL
Large plasmid	50 ng/uL	525 ng	10.5 uL
Single strand plasmid	25 ng/uL	75 ng	3 uL
PCR 0-200 bp	5 ng/uL	10 ng	2 uL
200-500 bp	5 ng/uL	20 ng	4 uL
500-1000 bp	5 ng/uL	40 ng	8 uL
>1000 bp	5 ng/uL	45 ng	9 uL
PCR (Exo-Sap)	no change		1.5 uL
Bac and gDNA	1ug/uL	1-2 ug	1-2 uL

***This amount of template for each primer requested.**

(The first time you place an order you will need to download an excel file template. You can keep a copy of this template on your computer. When you want to submit an order use this template to fill in the information, save it with your name and the date and then this file can be attached to your online order form.

5. In the iLab request form, browse to locate the excel file with your sample name, sample type and primer, then click ‘please upload’ at the bottom of the page.

When you have completed filling out the service request, please press ‘save completed form’



6. Remember to press ‘submit request to core’



How do I print a copy of my service request?

After your service request has been submitted, locate the pencil icon on the right hand side of the screen. We suggest that you print to the web format.



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 request.**

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.

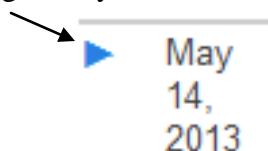
When will my samples be run and how will I be notified?

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. You can track the progress of your request in iLab. When you see “Processing” you will know that we are working on your samples.

You will receive an email from iLab 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 view this document, “[Sequence email messages with explanations](#)” located at the VCC website, DNA Analysis Facility, FAQs to see explanations of these messages that address the most common sequencing problems that we encounter.

How do I download my data from iLab?

You will receive an email from iLab letting you know you have data available. Please login to iLab and navigate to your service request. To find your data, open your service request by clicking the blue arrow.



Your data will be attached to your service request under the heading “Attachments and URL’s in a zipped file.



Service requests are only stored on the iLab system for 18 months. We strongly urge you to download your data to a safe place.

Sequence: Format of data

What format will my sequence data be in?

The sequencer generates .ab1 sequence files.

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.

PC: Chromas: <http://www.technelysium.com.au/chromas.html>

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

Mac: Editview: <http://www.appliedbiosystems.com/support/software/dnaseq/installs.cfm>

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

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

The facility has a copy of Sequencher 4.8 for your use. Please let the staff know if you would like to arrange for a tutorial.

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>).

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 Mg²⁺ 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?

Template Type	Required template concentration	Total amount used in each 15uL Cycle Sequence Reaction*	
Double strand plasmid	50 ng/uL	375 ng	7.5 uL
Large plasmid	50 ng/uL	525 ng	10.5 uL
Single strand plasmid	25 ng/uL	75 ng	3 uL
PCR 0-200 bp	5 ng/uL	10 ng	2 uL
200-500 bp	5 ng/uL	20 ng	4 uL
500-1000 bp	5 ng/uL	40 ng	8 uL
>1000 bp	5 ng/uL	45 ng	9 uL
PCR (Exo-Sap)	no change		1.5 uL
Bac and gDNA	1ug/uL	1-2 ug	1-2 uL

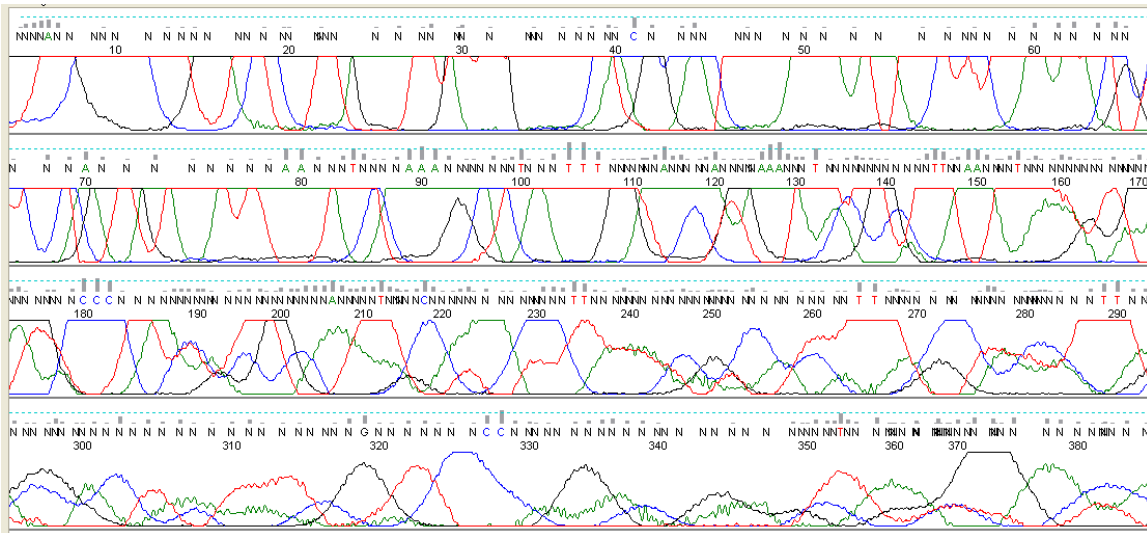
What if my template concentrations are lower than the required amount?

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.

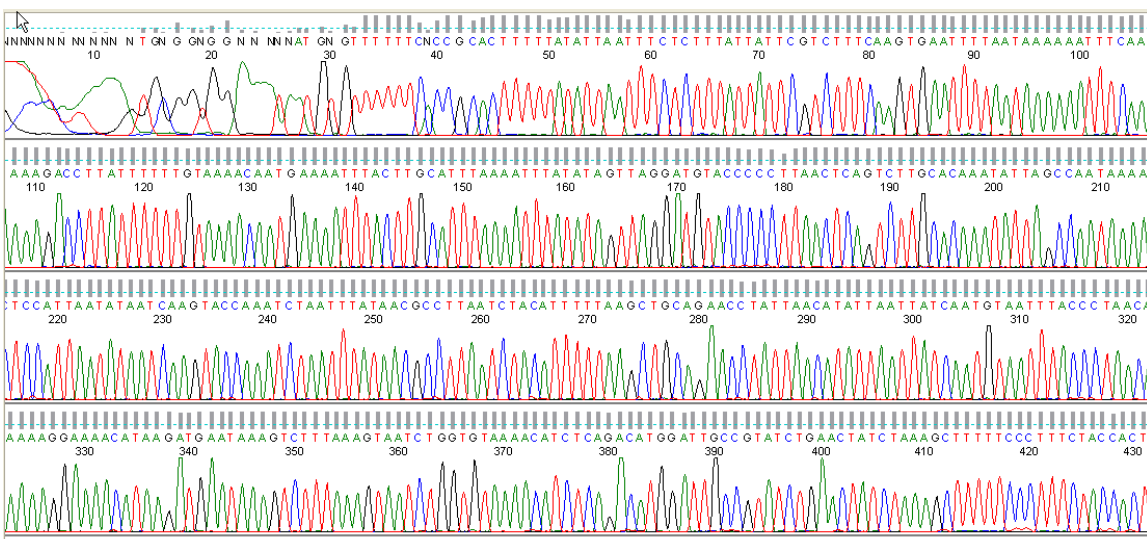
What if my template concentrations are higher than the required amount?

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:



This is data from the same template after it was diluted to the proper 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 ExoSap-IT 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 GGC 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 T_m 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?

Template Type	Required template concentration	Total amount used in each 15uL Cycle Sequence Reaction*	
Double strand plasmid	50 ng/uL	375 ng	7.5 uL
Large plasmid	50 ng/uL	525 ng	10.5 uL
Single strand plasmid	25 ng/uL	75 ng	3 uL
PCR 0-200 bp	5 ng/uL	10 ng	2 uL
200-500 bp	5 ng/uL	20 ng	4 uL
500-1000 bp	5 ng/uL	40 ng	8 uL
>1000 bp	5 ng/uL	45 ng	9 uL
PCR (Exo-Sap)	no change		1.5 uL
Bac and gDNA	1ug/uL	1-2 ug	1-2 uL

*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.