2019v2

Sanger User Guide (FY21)
CCR Genomics Core
Location: Building 37, Room 2135
Core e-mail: ncilecdnacore@mail.nih.gov
Core website: https://genomics.ccr.cancer.gov/

I. Requests for Sanger should be made on the iLab website.

A. Go To URL: https://nci.corefacilities.org/account/login

B. Log-in using your NIH credentials (Username and password, not your PIV card) as an internal NCI user (even if you are from a different institute).

a. If you are a new member, register under your PI from the drop-down list. Please note that you will not be able to register unless your PI is already in system with a CAN number. Contact the Core manager (liz_conner@nih.gov) if your PI is not listed.

C. Under the Request Services tab, select your service of interest and initiate a request.

II. Sanger Sample Submission

A. Complete the service request for Sanger on the iLab website

a. Choose your *Area of Research* from the dropdown menu.

b. Select your service, i.e. Sequencing Reaction & Electrophoresis, Electrophoresis, Bulk Capillary As-is Run, etc…

c. Enter sample names into the "Sample Grid." Copy and paste sample names into the sample grid rather than uploading an excel file or typing in sample names. Make sure to click the *Confirm.*

a. Sample names can consist of any letter or number. Preferably starting with your initials, i.e. LC_1. Avoid spaces, which may be replaced by an underscore ("_") but not a dash and special characters. Names should not be longer than 15 characters.

b. Remember to include the name of your folder on the DNACore drive under "destination" on the sample grid, i.e. ConnerL

B. Deliver samples to bldg. 37/2135 in a light protected box between 8:00 AM to 4:30 PM Monday through Friday. The box should be clearly labeled with your name, building/room number and phone number.

a. Samples should be in PCR strip tubes and left in the freezer section (left side of refrigerator) in 37/2135.

b. Samples should be labeled on the side of the tubes, with simple sequential numbers (i.e. 1, 2, 3,)

c. Samples are kept for 5 days after electrophoresis.

C. There is a charge for this service:

a. ELECTROPHORESIS ONLY $2.00/sample

b. SEQUENCING RX And Electrophoresis $7.00/sample

c. SEQ REACTIONS, CLN-UP & ELECTOPHORESIS (96 SBP) $440

d. Bulk capillary As-Is Run (SEQ RX & CLN-UP performed by customer):

(0-16 samples) $22.00
(16-32 samples) $44.00
(33-48 samples) $66.00
(49-64 samples) $88.00
(65-80 samples) $110.00
(81-96 samples) $132.00

These samples are submitted in 96 well MicroAmp plates and are completely ready to be placed on our capillary sequencer.

D. Data Processing

a. Sanger sequence data is transferred to the group drive, NCI GP DNACore Access (**//nciis-p001.nci.nih.gov/Group05/DNACore**), in a subfolder that is named the same as your network login name (e.g. Connerl for Liz Conner) or group name. Each set of data is stored in a subdirectory that indicates the date the electrophoresis was run (e.g. 20180310 for March 10, 2018). The data is read-only and is deleted after four weeks; you are responsible for copying the data to storage of your OWN before it is deleted.

b. An email message from iLab is sent to you after we upload your data. If you have not received a message from us check your DNACore folder. If your samples are not there, give us a call (240-760-7373) or send us an e-mail (ncilecdnacore@mail.nih.gov).

E. Sequencing Reactions Done by the User:

THE FASTEST AND CHEAPEST WAY TO GET YOUR DATA IS TO DO THE SEQUENCING REACTIONS YOURSELF!

a. The CCR Genomics Core sells the ABI Big Dye Terminator sequencing reaction kits at cost. These are sold, at cost, in 50 (400ul) or 100 (800ul) reaction lots at $414 and $828 respectively. We will also give you the buffer to dilute the Big Dye at no cost when you buy the Big Dye. The cost is about 60% of what ABI charges if ordered directly from them.

b. After the sequencing reaction is complete, the unincorporated, dideoxy-nucleotides left in the reaction must be removed before electrophoresis. Use columns/plates to remove them, not ethanol precipitation!

c. We use the Edge BioSystems Gel Filtration columns in the Core [(800) 326-2685, Cat. No. #42453 or V3 short plate #47938]. The company is located in Gaithersburg and the columns are usually delivered within a day of ordering.

F. Sequencing Reactions Done by the Core:

a. We will run sequencing reactions for you. They start daily at noon. If you miss that time we will do an afternoon set.

b. We will only do dye-terminator reactions using the ABI Big Dye V1.1 sequencing kits.

c. There is a $7/sample charge.

d. Submit samples to us in a form ready for the sequencing reaction to be performed. Samples should be a mixture of template and primer.

In 15 ul of solution there should be:

50-100 ng/ul double stranded DNA or

10-50 ng/ul PCR product DNA or

2-3 ug/ul Bacterial genomic DNA

1 ul of 10 uM primer DNA (one primer per reaction)

e. There should be a minimum of 15 micro-liters, preferably more, of this solution in each tube. Scale your template and primer accordingly.

f. Number the top of each tube in the order you have set in your sample submission form. Sort them in the order in the sample submission form. We often run over 100 samples a day. It's easy to make a mistake if the samples are submitted in a random order.

g. A pGEM control will be run with each sample. If it works and the samples don't, we will assume the problem lies with DNA/primer mix.

h. General Rules for Primer Design:

a. Primers should be at least 18-20 nucleotides in length to minimize the chances of encountering problems with a secondary hybridization site on the vector or insert.

b. Primers with long runs of a single base should be avoided. It is especially important to avoid 3 or more G's or C's in a row.

c. For cycle sequencing, primers with melting temperatures above 50°C, as determined by a primer design program, such as Oligo 4.0, generally produce better results than primers with lower melting temperatures.

d. Primers should have a G/C content between 40 and 60 percent. For primers with a G/C content of less than 50%, it may be necessary to extend the primer sequence beyond 18 bases to keep the melting temperature above the recommended lower limit of 50°C.

e. Primers should be "stickier" on their 5' ends than on their 3' ends. A "sticky" 3' end, as indicated by a high G/C content, could potentially anneal at multiple sites on the template DNA. A "G" or "C" is desirable at the 3' end but the first part of this rule should apply.

f. Primers should not contain complementary sequence (palindromes) within themselves; that is, they should not form hairpins. If this state exists, a primer will fold back on itself and result in an unproductive priming event which decreases the overall signal obtained.

g. Primers should not contain sequences of nucleotides that would allow one primer to anneal to itself or to the other primer used in a PCR reaction (primer dimer formation).

h. If possible, run a computer search against the vector and insert DNA sequences to verify that the primer and, especially, the 8-10 bases of its 3' end are unique.

G. How to map the group drive NCI GP-DNACore-Access (**//nciis-p001.nci.nih.gov/Group05/DNACore)** to your computer. Please restart computer for the permissions to take effect.

On Windows 7

1. On the **Start** menu, right click **Computer** and select **Map Network Drive**.
2. Select an unused drive letter
3. In the folder box, type the network path \\nciis-p001.nci.nih.gov\Group05\DNACore
4. Click **Finish**

On Windows 10

1. Open File Explorer and select This PC.
2. Select the **Computer tab** and select **Map network drive button** in the ribbon menu at the top, then select **Map network drive.**
3. Select an unused drive letter
4. In the folder box, type the network path \\nciis-p001.nci.nih.gov\Group05\DNACore
5. Click **Finish**

On Mac

1. From the **Finder**, hit **Command+K**
2. Enter the network path you want to map – smb://nciis-p001.nci.nih.gov/Group05/DNACore
3. Click **Connect**

H. Software supported

a. NCI Supported Software: MacVector and Sequencher

b. Contact ncisupportedsoftware@mail.nih,gov

The CCR Genomics Core would like to remind our customers that it is important to acknowledge the core in scientific publications, posters, and presentations that include data derived from the facility. Proper acknowledgment provides a visible measure of the impact of the core and is thus essential for our existence both for our continued funding and leadership support. It also helps tremendously in our future effort to secure additional instruments and services. Acknowledgment at the authorship-level would be strongly appreciated when extensive collaborative efforts are involved. Please send us a reprint of the paper, or an e-mail including the reference information for any publication in which the CCR Genomics Core is acknowledged.

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