Nemours | Alfred I. duPont Hospital for Children
Nemours Children's Clinic

BCL

Biomolecular Core Facility
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3130xl Full DNA Sequencing Request Protocol

Full Sequencing is available for investigators who occasionally have sequencing needs but do not wish to set up the cycle sequencing and the subsequent cleanup in their own lab. Researchers are asked to bring a tube with DNA and primer to the core, along with a submission sheet, disk and gel picture of their DNA. For high volume sequencing please inquire with the Biomolecular Core Director.

Sequencing sample Setup

Label the side of a 0.2ml PCR tube (strip tubes with **individual caps** may be used) with the principal investigator or submitter's initials and a number (Ex. KS1, KS2 etc.). More detailed names can be linked to these numbers on the sample submission sheet, which can be requested by email or picked up at the core.

The tube should contain: DNA (amount as recommended below)
Sequencing Primer (3.2pMoles)
H20
12ul Total

Important note: Automated DNA sequencing requires **high-quality template DNA**. Either plasmid DNA or PCR product can be used as template for cycle sequencing. The quantity of DNA needed for sequencing varies with the size of the template (plasmid or PCR). For best results it is important to follow the instructions below.

Sequencing plasmid DNA

DNA Prep

For plasmids, cosmids, PACs and BAC's columns from Qiagen, Promega or Edge Biosystems are recommended. Depending on how much DNA you need either small spin columns or large Maxiprep columns will work. DNA concentration should be estimated after purification by comparison to standards on an agarose gel or by spectrophotometry.

DNA amount per sequencing request

For standard plasmid preps, 100ng per kb of the total plasmid is a good place to start. In general, 500-750ng of DNA is sufficient for a single sequencing reaction. Cosmids, YACs, PACs and BACs will require more DNA (please inquire).

Plasmid size in bp	Amount of DNA in ng
1000	100
2000	200
3000	300
4000	400
5000	500
6000	600
etc.	

Sequencing PCR Products:
It is possible to sequence fragments ranging from 150-12,000bp, although very small (<150bp) and very large (>5kb) products can be challenging.

PCR products should be purified with PCR clean up columns such as those from Qiagen or Promega. The Core routinely uses Qiagen QIAquick PCR Purification Kit (250), Cat. # 28106.

PCR size in bp	Amount of DNA in ng
150-250	25-40
250-500	40-70
500-700	70-90
700-900	90-110
900-1200	110-140
1200-2000	140-180
2000-12,000	180-250

Primer Design
The length and sequence of a primer determines its melting temperature and specificity. Most recommendations are for primer lengths of 18-25bp and CG content between 40-60%. There should also be no runs of a single nucleotide greater than 3 within the primer. The primer should also be checked for potential self-annealing or hairpin formation, especially at its 3' end. In addition, possible secondary priming should also be identified, again stressing matches involving the 3' end of the primer.

Submission and Turnaround Time
This service is by advance notification only. Contact the core (x6712) to arrange for your samples to be processed. Samples may be placed into the Core –20 freezer in G25 A/R building or in the BCL lab room 214, Rockland Center One. We pickup samples at 1:30 PM from ARB G25. We typically begin processing the full sequencing samples in the morning for inclusion on the afternoon 3130 run (sample load permitting). Samples will be run on a first-come first-serve basis and turn around time is typically 24-48 hours.

The sequencing submission sheet along with a PC formatted disk, CD or flash drive, should be placed into the sequencing submission slot on top of the Core freezer in G25 A/R building or in the submission box in room 214, Rockland Center One. In addition, we also require a gel picture of your DNA sample after its final purification. Please indicate the quantity or ul amount of DNA run on the gel and how it relates to the amount you submitted. The gel picture along with the info on the submission sheet is very important for troubleshooting if any problems should occur.

Data
Samples are usually processed overnight and results can be picked up from the mail slots outside the core after 10:00 AM the following morning. There are two files generated per sample:

- The first is a chromatogram file. These are available upon request. You can use any of the available free downloads available to print and view your chromatograms.
- The second is a text file of the sequence, which will be saved to your shared folder, disk, flash or CD. Text files can be imported into programs such as Macvector as well as PC software.

In addition, a hard copy printed color chromatogram containing the first 600-625 bps of sequence (the second page can also be printed on request) will be printed by the Core and made available for pickup after 10:00 AM or delivered to your customer mail slot in G25 A/R building by 1:30 PM.