

Vollum Automated Sequencing Core User's Guide

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Overview of Automated DNA Sequencing

During sample preparation, the DNA fragments in a sample are chemically labeled with fluorescent dyes. Using PCR, unlabeled deoxynucleotides (dNTPs) and dye-labeled dideoxynucleotides (ddATP, ddGTP, ddCTP, and ddTTP) are incorporated by Taq DNA polymerase into the growing DNA strands. The dye-labeled DNA fragments are separated by electrophoresis within the capillary array of the ABI Prism 3130xl Genetic Analyzer. Once the fragments enter the detection cell, they pass through a laser beam. The light excites the attached dye labels causing them to fluoresce. A computer analyzes the fluorescence to determine the order of DNA bases in the strand. Two files are created for each sequence: a text file with the extension *.seq and an electropherogram (a graph of relative dye concentration against time, plotted for each dye) with the extension *.ab1.

Using the Sequencing Core

The Vollum Institute Sequencing Core is capable of running 174 samples in 24 hours. There is not a sign up sheet. Drop off your samples at any time but pickup is at 10:00 AM. General turn around time is 1–2 days.

Culture

ABI recommends HB101 or DH5a. Mv1190 and XL1Blue give variable results while JM101 usually doesn't work.

Purification

USE: Qiagen kit or standard alkaline lysis miniprep purification followed by 13% PEG8000 precipitation (see below). Resuspend DNA in dH₂O.

DO NOT USE: Promega Magic preps. Avoid purifying DNA with phenol since residual phenol interferes with the sequencing reaction. Do not resuspend DNA in TE.

For organic extractions: Use straight chloroform or chloroform:isoamyl alcohol (24:1) followed by a 13% PEG precipitation. ABI also recommends using ultra filtration such as Centricon-100 Micro-Concentrator columns.

13% PEG8000 precipitation:

- Add 1/4 volume 4M NaCl or 0.16 volume 5M NaCl to plasmid DNA
- Add 1 total volume autoclaved 13% PEG8000
- Mix and incubate on ice for 20 minutes
- Pellet DNA for 15 Minutes in a 4°C centrifuge
- Rinse the pellet with 500 µl 70% ethanol
- Resuspend the pellet in dH₂O

In general, whatever is easiest to prepare should be fine. **But...the cleaner the template provided, the cleaner and better sequence read and read length returned. I will be very upset if your samples contaminate my capillaries!**

Primers

Longer primers work better. We recommend T7, T3, and M13 forward and reverse primers; SP6 is less reliable. Custom primers should be 21- or 22-mers.

Prepare Samples for PCR

Recipe for the CLEAR pre-mix (or Dye Terminator Mix) reaction

Reagent		Quantity
Template	ss DNA ds DNA	0.05–0.10 µg ~0.50 µg
PCR product	100–200 bp 200–500 bp 500–1000 bp 1000–2000 bp >2000 bp	1–3 ng 3–10 ng 5–20 ng 10–40 ng 40–100 ng
Primer		3.2–6.4 pmol
dH ₂ O		quantity sufficient
Final Volume		9 µl

Mix reagents in a small, thin-walled tube labeled with your initials and a unique number (e.g., PIB1). Label each sample horizontally above the line of the tube—**do not** include your primer or template on the label. Writing anywhere else, including the lid, will be rubbed off in the PCR machine, forcing you to repeat your sample. The Core will add Dye Terminator Mix before running your samples.

NOTE: For Double-Stranded Plasmid DNA, increasing the amount of primer more than 6.4 pmol generally doesn't cause problems. Just don't go overboard with the amount. For PCR products, however, it is generally better to stay around 3.2 pmol or only slightly higher if the PCR product is very large.

DNA Order Request Form

A PDF of the DNA Order Request Form is available for download on the Core Services page of the Vollum website (www.ohsu.edu/vollum). The form requests the following information: your name, email address, sample names, concentration, and primer name. The PDF can be filled out electronically using Acrobat or Acrobat Reader, or it may be printed and filled out by hand. Submit a printed copy of the completed form when dropping off your samples.

Submit Samples

Submit samples by 10:00 AM. Place samples in the small refrigerator across from room 4404 on the 4th floor of the Vollum. Results will usually be emailed the next morning. However results may be delayed due to unforeseen problems (e.g., very bad things happening overnight while I'm not here). You will be contacted in that event.

Analyzing Data

View Sequencing results as a text file: Open the appropriate *.seq file using TextEdit on a Mac (OSX) or Notepad on a PC. A dialog box will open and the sequence can be copied & pasted

into Intelligenetics, GCG Strider or other software. NOTE: if there is no *.seq file and the electropherogram (*.ab1 file) is 72K, your sample failed to give a readable sequence.

Analyzing Electropherograms: Several free programs are available through the Internet to view the *.ab1 file:

- **Finch Trace Viewer** is available for either PC or Macintosh OSX users.
- **4Peaks** is available for Macintosh OSX users.
- **Chromas** is available for PC users.
- **Sequence Scanner** is available from Applied Biosystems for PC users.
- **EditView** is available for Macintosh users OS9 or lower.

Cost

Prices vary depending on overall use of the core. Please contact the core (dnaseq@ohsu.edu) for more details. First time users will not be charged until they obtain a usable sequence. Also, the core will notify you of failed runs and you will not be charged.