

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 no sign up sheet. Here's how it works:

1. Prepare a Vollum Sequencing Core service request—using either the online form or downloaded PDF form.
2. Drop off your samples any time before 11:00 AM by placing in the small refrigerator across from room 4404 on the 4th floor of the Vollum or sending via campus mail.
3. **Samples are picked up promptly at 11:00 AM every day.**
4. Standard turn around time is 1–2 days, but results are usually emailed the morning following submission.
5. If there is a problem with your run, you will be contacted via email and will not be charged.

DNA Service Request Form

There are two options for submitting a DNA sequencing service request:

1. Submit a service request online using the Vollum Sequencing Core's web form (<http://www.ohsu.edu/xd/research/centers-institutes/vollum/core-services/dna/service-request.cfm>).
2. Download the service request form PDF from the Vollum Sequencing Core web page (<http://www.ohsu.edu/xd/research/centers-institutes/vollum/core-services/dna/index.cfm>). The PDF can be filled out electronically using Acrobat, or it may be printed and filled out by hand. Submit a printed copy of the completed form with your samples.

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. You will not be charged for any failed runs, but the core will notify you of such events.

SAMPLE PREPARATION

Culture

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

Purification

Recommended for use: Qiagen kit or standard alkaline lysis miniprep purification followed by 13% PEG8000 precipitation. 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.

In general, whatever purification process is easiest to prepare should be fine. However, the cleaner the template provided, the cleaner and better the sequence read and read length returned. You are responsible for the purity of your samples...do NOT send contaminated samples!

Primers

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

Preparing 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 μ g ~0.50 μ g
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 μ l

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. But don't add too much! 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.

ANALYZING DATA

Viewing Sequencing Text File

Open the appropriate *.seq file using **TextEdit** on a Mac (OSX) or **Notepad** on a PC.

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 (FinchTV)** 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.