

Vollum Institute DNA Sequencing Core User's Guide

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Overview of Sanger sequencing by capillary electrophoresis

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 96-capillary array of the ABI (Applied Biosystems) 3730x1 DNA 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.

Three files are created for each sequence:

- .seq** — a simple sequence text file
- .ab1** — an electropherogram (sequence data plotted as a graph of relative dye concentration against time, plotted for each dye)
- .phd.1**— (Phred file) simple text file showing bases with quality values for each base

Using the Vollum Sequencing Core

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

1. Fill out a Vollum Sequencing Core service request — either online or using the downloaded PDF. If you are using the PDF form, please print a copy and include it with your samples.
2. Drop off samples any time. There are three sample delivery options:
 - a. Small refrigerator by room 4404, 4th floor of the Vollum Institute
 - b. Small refrigerator in the loading dock of OHSU's Robertson Life Sciences Building
 - c. Send via campus mail to Vollum DNA, Mail code L474
3. **Samples are picked up promptly at 11:00 AM every day.**
4. Standard turnaround 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.

Two options for submitting a service request

- **Submit online** using the Vollum Sequencing Core's online form and drop off your samples for processing.
- **Download the service request PDF** (598 KB) and fill out by hand or electronically using Acrobat. Then submit a printed copy of the completed form with your samples.

Cost of services

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

Sample Preparation

Culture

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

Purification

Recommended by the Sequencing Core: Qiagen kit or standard alkaline lysis miniprep purification followed by 13% PEG8000 precipitation. Resuspend DNA in dH₂O.

What to avoid:

- 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

Mix reagents in a small, thin-walled, 0.2 µl PCR tube.

Use the following 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 |

Labeling the PCR tube

- Label each sample in the service request with your initials and a unique number (e.g., PIB1, PIB2, PIB3).
- Write above the line of the tube AND only write horizontally.
- Writing anywhere else, including the lid, will be rubbed off in the PCR machine, forcing you to repeat your sample.
- Do not include your primer or template on the label.

The Core will add Dye Terminator Mix before running your samples.

SPECIAL NOTE about primer volumes

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

Any simple text editor, such as TextEdit (Mac) or Notepad (PC), can open the **.seq** file. SnapGene Viewer and ApE are also options.

NOTE: If there is no **.seq** file and the **.ab1** electropherogram file is 72 KB, your sample failed to give a readable sequence.

Analyzing the electropherograms

Several free programs are available to view the **.ab1** files and analyze your data.

- 4Peaks
- SnapGene Viewer
- Benchling
- FinchTV