

Sequencing Protocol

| | |
|---------------|--------------------|
| Big Dye V3.1 | 2.6ul |
| Half-Dye | 5.4ul |
| Primer | 2.0ul (1.6pmol/ul) |
| DNA template | ul (50-100ng/kb) |
| Water to 20ul | ul |
| Total | 20ul |

Run sequencing program in a PCR machine (Thermal Cycler) with a heated lid so that you do not have to use oil.

Notes:

- Reactions are light sensitive.
- Template amount is critical. Too much can clog the system so be sure to quantitate your samples

Sequencing reaction in Thermal Cycler:

2 min 98C

15sec 96C }
15sec 55C* } x 25
4 min 60C }

4C soak

Ramp speed is critical and should not exceed 1C/sec.

*The extension time may need to change depending on the T_m of the primer.

Transfer reaction to a 1.5ml tube. Label tubes clearly. Fill out sequencing form and take reactions to PNACL to be cleaned and run on gel. There is a PNACL drop off point on the 5th floor of MPRB.

If your Chromatogram stops abruptly, you may have a hairpin loop. Follow protocol below in this case.

Sequencing through Hairpin Loops:

| | |
|-------------------|----------------|
| Big Dye V3.1 | 6ul |
| dGTP Big-Dye v3.0 | 2.0ul |
| Primer | 2.0ul (10pmol) |
| 5% DMSO | 1.0ul |
| DNA template | ul (800ng) |
| Water to 20ul | ul |
| Total | 20ul |

Cycle conditions for the PE 9700, 9600 emulation mode

10 min 98C
20sec 96C }
15sec 55C* } x 35
4 min 60C }
4C soak

Sequencing Genomic DNA templates:

| | | |
|------------------------|-------------|---|
| Big Dye Terminator Mix | 16ul | <u>Cycle Conditions:</u> |
| Primer (18-24mer) | 15-30pmoles | 5 min 95C |
| Genomic DNA Template | 3-6ug | 30sec 96C } 20sec 55C } x45 4 min 60C } |
| DMSO | 2ul | |
| Final Reaction Volume | 40ul | 4C soak |

To access your sequence data, go to the PNAACL website and download and install the SSH Client.

<http://molecool.wustl.edu/compsystutor.html>

After installation, open the SSH Secure File Transfer Program. Click on Quick connect. It will ask for the following information:

Host: pnacl.wustl.edu

User: kellyd

Port Number: 22

Once you hit OK, it will ask for your authentication response: th3;leak

Once you have the file open, Go to New File Transfer under the Window tab. All the Kelly Lab sequences will be shown. You can simply drag your folder to the desktop.

To transfer into Vector NTI: Open VNTI, Open the Local Database, Under Table, Go to Import, Sequence from text file. Browse and choose your sequence with the .seq suffix.

To transfer into Contig express: Open Contig Express and open the folder with your sequences in it. DRAG and DROP your sequence file into Contig Express.