Sequencing Protocol for BIC Genomics Core Facility

I. **Reagents**

BigDye Terminator v.3.1 or v.1.1 Cycle Sequencing Kit

II. **Storage**

1. Store kit at -15 to -25°C.
2. Before each use, allow to thaw at room temp (do not heat).
3. Materials should be kept on ice during use.
4. Avoid repeated freeze/thaw; instead, aliquot into multiple tubes.

III. **Template Preparation Methods**

1. Starting concentration of templates:
    a. double-stranded plasmid less than 7K: 0.300µg-0.800µg
    b. double-stranded plasmid 7K-12K: 0.800µg-1.500µg*

*Certain expression vectors sequence very poorly regardless of the concentration.

2. Genomic questions: see Zhao Li, 295-3657
3. Expected amount of PCR product:
    a. 50bp-200bp: 10ng-30ng
    b. 200bp-700bp: 30ng-60ng
    c. 700bp-1000bp:60ng-100ng

IV. **Reaction Conditions**

1. X µl of template
2. Y µl of H_2O
3. 10 pmol primer (If concentration is unknown, more is better than less.)
4. 4.0µl of BigDye v.3.1 or v.1.1
5. Total volume=20µl
6. Mix well and spin briefly.

V. **Thermal Cycler Profile**

Hot start; 25 cycles; 3-5min final extension at 60°C

Step	Action
1	Place tubes in thermal cycler and set the volume to 20µl.
2	Repeat the following for 25 cycles: a. Rapid thermal ramp to 96°C b. 96°C for 10sec c. Rapid thermal ramp to 50°C d. 50°C for 5sec* e. Rapid thermal ramp to 60°C f. 60°C for 4min
3	Rapid thermal ramp to 4°C and hold until ready to purify.
4	Spin down the contents of the tubes in microcentrifuge.

*Set temperature 1-2°C below Tm of primer used.

VI. **Purification**

Purify PCR product using a DTR gel filtration column.