DNA Sequencing

(using ABI PRIMS™ BIG Dye terminator cycle sequencing)

PCR reaction

1. Assemble on ice in 0.2 ml PCR reaction tube:

DNA:	ssDNA	50-100 ng
	dsDNA	200-500 ng
	PCR product	30-90 ng
Big Dye reaction mix		4 µl
Diluent buffer*		4 µl
Primer		3.2 pmol
dH_2O		to a final volume of 20 µl

2. Place tube in thermalcycler programmed for the following:

   96˚C for 30 sec
   50˚C for 15 sec
   60˚C for 2 min

3. Repeat for 25 cycles then soak at 4˚C

-Note: The purity and quantity of the DNA template and primer are critical to the success of this reaction
Minimize the exposure of the Big Dye reaction mix to fluorescent light as the dye terminators are photobleached easily。

Purification of sample for sequencing

1. Remove the bottom of Spin Autoseq™ G-50 column (BioRad) and place in the microcentrifugation tube.
2. Spin column at 3,500 rpm for 5 min and remove liquid from the bottom of column.
3. Apply PCR reaction mixture on the column and centrifuge at 3,500 rpm for 5 min.
4. Dry up purified sample by Speed vac for at least 30 min at high temperature.
5. Resuspend in 4 µl sequencing dye and subject to autosequencer (5th floor).