Sequencing Flow Charts
(II) Cycle Sequencing Reactions

Cycle sequencing reactions are performed in a typical PCR machine, and contain the following components in a 0.2 ml thin-walled PCR tube:

1. your purified template DNA of known concentration
2. one primer suitable for your template (plasmid or PCR fragment
3. a sequencing buffer if half-reactions are used instead of full rxns
4. Beckman-Coulter's DTCS (Dye-Terminator-Cycle-Sequencing) reagents
(a master mix of flourescent dye-terminators, DNA polymerases, dNTPs and buffers)
5. highly purified water (17 megohm) to make a final volume of 15 ul

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(a) Get a standard Cycle-Sequencing data sheet from our web site or from the sequencing lab in the Olin Biology building, room 219
(b) Fill in all the necessary information for the data sheet; use a separate sheet for each row of the final microtiter plate, so you may set up as many as 8 reactions per sheet

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Set up your cycle sequencing reactions with the listed reagents according to the protocols described in the BIO 315 Lab; all reactions will have exactly the same reagents EXCEPT for your template, and possibly the primer (hence the use of 'master mixes' for distributing your reagents to the 0.2 ml tubes).

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(a) Place your completed reactions in the PCR machine's temperature block (be sure to use only the smaller wells for the 0.2 ml tubes!)
(b) Select the BC-Seq program and start the machine
(c) Return **promptly** to the lab when your run is finished (others may want to use the PCR machine) and remove your tubes to the refrigerator

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Proceed to (III) Product Purification & preparation for the CEQ 8000