Sequencing Flow Charts

(III) Reaction Product Purification, and Preparation for Automated Sequencing

The extension products (ssDNA frgments of varied sizes) from your cycle sequencing reactions must be (a) separated from any unincorporated dye-terminators; this removal process is the same for plasmid and PCR fragment extension products, (b) dried thoroughly in a Vacufuge, and (c) resuspended in Sample Loading Solution (SLS)

We use Edge Performa columns (and see Edge DTR notes) for plasmid templates (we slightly modified Edge's procedure); SigmaSpin columns are a recent addition and work as well as the Edge ones (and are a bit cheaper).

Add 5 ul of sterile water to each 15 ul cycle-seq reaction to bring the final volume to 20 ul, as recommended in the Edge Performa purification protocol

Purify all your cycle sequencing extension products (now in 20 ul volumes) with the Edge Performa columns – see the notes on our procedure for Edge (or this one for SigmaSpin).

You will have slightly > 20 ul in the final collection tube from the Edge Performa columns for each reaction; place all these tubes in the Vacufuge according to our procedure

Add 40 ul SLS (Sample Loading Solution) to each dried reaction tube to solubilize the extension products, according to our procedure

Proceed to (IV) Sequencing in the CEQ 8000