

Running Sequences on the ABI 310

By Alisa Ramakrishnan, Tuesday, July 06, 2004, updated May 2005

Make sure you have enough polymer for the samples you're going to run – it takes about 3-5uL of polymer for each sample on the short capillary, 7-10uL on the long capillary.

Prepare Sequences

1. Sequences should be cleaned (Sephadex and/or EtOH-ppt) and lyophilized (dried)
2. Add 15uL of DMF ('Hi-Di Formamide', dimethyl formamide)
 - a. If you smell any ammonia in the DMF, don't use it
3. Vortex for 20 seconds
4. Transfer samples to 12-strip tubes (or fewer, depending on the number of your rxns)

In Manual Control, set gel temperature (function) to 50°C, hit 'execute'

Prepare Sample Sheet

1. In the ABI Collection software, pick 'file', 'new', 'sequencing sample sheet 96'
2. Fill out sheet (I don't remember which of following are required:)
 - a. Dye Set/Primer Files = DT310POP4(BDv3)v1.mob
 - b. Module
 - i. SeqPOP6(1mL)E.md4 for long capillary
 - ii. SeqPOP6Rapid(1mL)E.md4 for short capillary
 - c. Matrix = BDT matrix (any one of several)
3. Close sample sheet (save in Runs folder as default name)

Load Samples

1. Place strip tubes in a tan 96-well plastic ABI holder thing, with your first sample in the A1 position (following the sample sheet)
2. Load onto autosampler with A1 towards the right-hand rear of the autosampler (see diagram on right-hand side of machine)
3. Make sure the tan 96-well holder is firmly on the autosampler and that the white retainers are snapped onto the 96-well holder

Prepare Injection List

1. Select 'file', 'new', 96-well injection list
2. Under the 'sample file' pulldown menu, find your sample file and select it
3. Fill in the module (see 'prepare sample sheet' above if necessary)
4. Hit 'run'

I'd watch it for awhile to make sure it's running okay – on a short capillary, your samples should show up after maybe 10min, but on a long capillary, it takes longer. ☺