Fragment analysis on the CEQ8800

Instrument description and dyes

Fragment analysis is run on the Beckman CEQ8800 instrument. This is an 8 capillary instrument with non proprietary software for DNA sequencing, STR (Short Tandem Repeat), SNP (Single Nucleotide Polymorfisms) analysis and AFLP (Amplified restriction Fragment Length Polymorphic) fingerprinting. The CEQ8800 has diode lasers that excite infrared dyes, and has four detection channels. For fragment analysis, alleles in each sample can be labeled with up to three separate dyes, the fourth dye is used for the size standard. WellRED dye labeled oligonucleotide can be purchased at ITS or Sigma. Three different WellRED dyes are available: D4-PA (650/670), D3-PA (685/706) and D2-PA (750/770). WellRED dyes have different signal strengths, therefore the signal strengths of amplification products will also depend on the dye employed for labeling. The Molar Extinction Coefficient of these dyes and subsequently their signal strengths are in order D4-PA>D3-PA>D2-PA. Therefore, when pooling reactions, roughly equal signal strengths are obtained by adding the following relative volume of PCR reaction products (assuming the same amplification efficiency for all the alleles):

0.1 μl D4-PA PCR reaction;
0.2 μl D3-PA PCR reaction;
0.4 μl D2-PA PCR reaction.

Size standards

Two different size standards are available at the MCIC:

CEQ DNA Size Standard Kit - 400: Includes DNA fragments of the following sizes labeled with D1-PA fluorescent dye: 60,70, 80, 90, 100, 120, 140, 160, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, and 420 nucleotides, and is used for analysis of fragments up to 400 nucleotides

CEQ DNA Size Standard Kit - 600: Includes DNA fragments of the following sizes labeled with CEQ WellRED fluorescent dye: 60,70, 80, 90, 100, 120, 140, 160, 180, 190, 200, 220, 240, 260, 280, 300, 320, 340, 360, 380, 400, 420, 440, 460, 480, 500, 520, 540, 560, 580, 600, 620, and 640 nucleotides, and is used for analysis of fragments up to 600 nucleotides.

Fragment analysis software

Genotyping data are analyzed with the CEQ800 Series Fragment Analysis Software that estimates DNA fragment sizes and amounts and identifies alleles represented by specific DNA fragments. This is older software and runs on Windows 2007. It requires SQL server capabilities, and administrator privileges to be installed and run. If you do not have a computer to run this software, we have it available at the core. The CEQ 8000 Series Fragment Analysis Training Guide can be downloaded from *http://photos.labwrench.com/equipmentManuals/1730-5285.pdf*.

Instructions for sample submission for fragment analysis on the CEQ8800

Before starting a genotyping project please contact the MCIC staff, as conditions for running each project need to be optimized. Similarly, when you change established genotyping conditions, or use new primers you need to let us know. To submit samples, you need to:

- Download the "**Fragment Analysis Order Form**" (Excel format), fill it in completely, and e-mail it as an attachment to: mcic_seq@osu.edu.
- Submit 6 μl of samples in a microtiter plate .
- Place microtiter plate(s), labeled with your name (Samples need to be labeled with your name, PI name and date) in the freezer (there is a sign) in room #009.
- If you have more then one plate, don't forget to label them so that they can be distinguished.
- You will be invoiced at the end of the project.

Samples are submitted in any microtiter plate. Facility staff will combine them with formamide and size standards just prior loading on the instrument. The amount of PCR product used will depend upon the efficiency of the PCR reaction.

If samples contain unincorporated primers and excess of salts, they need to be desalted as follows:

- add 0.25 μl of 20μg/μl Glycogen (Boeringer Mannhein, Cat.#901393) to the PCR reaction
- add 1/10 volume 3M NaAc pH 5.2
- add 2.5 volume 95% EtOH (from -20^{O}C)
- centrifuge at max speed at 4 degree C for 15 minutes. Flip plate quickly to eliminate supernatant.
- rinse the pellet twice with 70%EtOH (from -20^{O}C); after each rinse, spin at max speed at 4^{O}C for 2 minutes, invert the plate onto a paper towel to completely remove the EtOH
- spin at 180g for a minute, then remove the plate from the centrifuge and let the pellet dry.
- Re suspend in the appropriate amount of dd water.

How to retrieve your sequence data

Once the run is completed you will be alerted via e-mail and receive instructions of how to access your data.