Capillary Electrophoresis 101
the ABI 3100
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16 Capillary 3100
6 foot Table on wheels

Inside the 3100
Oven fan
Capillary array
Autosampler
Buffer reservoir
Detection window
5 mL syringe
Polymer reservoir
1 mL syringe
Loads polymer

Result of Not Removing a Bubble in the Polymer Channel
Tubing where bubbles hide
Anode
Lower Polymer Block
Upper Polymer Block
250 µL array-fill syringe
5 mL polymer reserve syringe

Carbon deposits
Hole from the Arc
Polymer Channel Distorted
Result of Not Removing a Bubble in the Polymer Channel – Close-up

Polymer Channel Distorted
Needle valve can not seal channel, therefore the capillaries are not filled.

ABI 3100 Array Detection

16 Capillary Array detection cell

Two 96 well plates on the autosampler
At 40 - 45 minutes per run two plates represent 12 runs or 8 – 9 h for 192 samples

Rubber septa wear. They must be replaced when the edges are ragged.

16 Capillary Array

Capillaries are inside of the cathodes (-)

Capillaries in buffer tank
Running and storage position
Spatial Calibration

Performed after:
- Installing or replacing a capillary array
- Removal of the array from the detection block,
  (Due to the design, to remove the upper polymer block for cleaning you must remove the Array from the detection window)

Information Provided:
- Position of the fluorescence from each capillary on the CCD

Spatial Results

- Good Results
- Bad results

Sample Detection

CCD Panel

Color

Ar+ LASER (488 nm)

Fluorescence

ABI Prism spectrograph

Principles of Sample Separation and Detection

- Size based separation due to interaction of DNA with entangled polymer strands
- Polymers are not cross-linked (as in slab gels)
- "Gel" is not attached to the capillary wall, but is pumpable and is replaced after each run
- Polymer length and concentration determine the separation characteristics

DNA Separation Mechanism

- Size Separation
- Detection region

DNA Separation

Maintenance of ABI 3100

- Syringe – leaks cause capillary to not fill properly
- Capillary storage & wash – it dries, it dies
- Pump block – cleaning helps insure good fill
- Change the Running buffer regularly

YOU MUST BE CLEAN AROUND A CE!

Leaking 5 mL syringe

The 5 mL syringe is a polymer reservoir used to fill the 250 µL syringe.
Urea crystallizing after polymer leaked around the worn plunger. Since this is the high pressure syringe which fills the capillaries this is a problem.

Detection Issues

- Fluorescent dyes
  - spectral emission overlap
  - relative levels on primers used to label PCR products
  - dye “blobs” (free dye)
- Virtual filters
  - hardware (CCD camera)
  - software (color matrix)

Dye set determines which wavelengths of light are collected onto the CCD camera

Spectral Calibration

- Performed:
  - New dye set on the instrument
  - After Laser or CCD camera has been realigned
  - You begin to see a decrease in the spectral separation (pull-up, pull-down).
- You must have a valid separation matrix on the instrument prior to running samples.

3100 Dye Sets

There are 7 dye set place holders available on the 3100

C  D  E  E5  F  G5  Z

E5 and G5 are for 5 dye systems

While there are parameters associated with each dye set you can use the parameters of one dye set and then name it a different Dye Set so keep track of things.
Defining the Matrix on the ABI 3100

Matrix is applied during the data collection so if there is a problem, the sample must be REINJECTED after a new matrix is applied rather than applying a new matrix to any raw data as can be done on the ABI 310...
Consumables

- ABI Optical Reaction Plates
  - $2,200 / 500 plates = $4.40 / plate
  - Phenix (mps-3590)
    - Plates $291/100 plates = $2.91 / plate
- Hi – Di Formamide
  - $28 / 25 mL
- 36 cm 3100 Capillary Array (100 runs) $695
  - 281 runs and still going (replace by resolution not # of injections)
- 36 cm 3100 Avant Capillary Array (150 runs) $560

Consumables

- 10X Genetic Analyzer Buffer with EDTA
  - $75/25 mL = $0.30/mL 1X buffer (ABI)
  - Or A.C.E.™ Sequencing Buffer 10X
    - $155/L = $0.016/mL 1X buffer (Amresco)
- 3100 POP-4 Polymer $365 / 7 mL
- 3100 POP-6 Polymer $365 / 7 mL
- 3700 POP-6 Polymer $465 / 230 mL
  - What we have been using, runs take longer but you also get better resolution.

Powerplex 16 Ladder with POP 6

Other Applications with POP6

Identifiler 5 uL PCR Protocol

Identifiler PCR amplification was carried out on a GeneAmp® 9700 using 1 ng of DNA according to kit protocols with the exception of reduced volume reactions (5 µL instead of 25 µL) and reduced cycles (26 instead of 28).

Amplification products were diluted 1:15 in Hi-Di™ formamide and GS500-LIZ internal size standard (0.3 µL) and analyzed on the 16-capillary ABI Prism® 3100 Genetic Analyzer without prior denaturation of samples.

POP®-6 (3700 POP6) rather than POP®-4 was utilized for higher resolution separations.

Allele calls were made in Genotyper® 3.7 by comparison with kit allelic ladders using the Kazaam macro (20% filter).

Some Example Data
Identifiler 5 uL PCR
(lower 3100 injection; 5s@2kV instead of 10s@3kV)

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