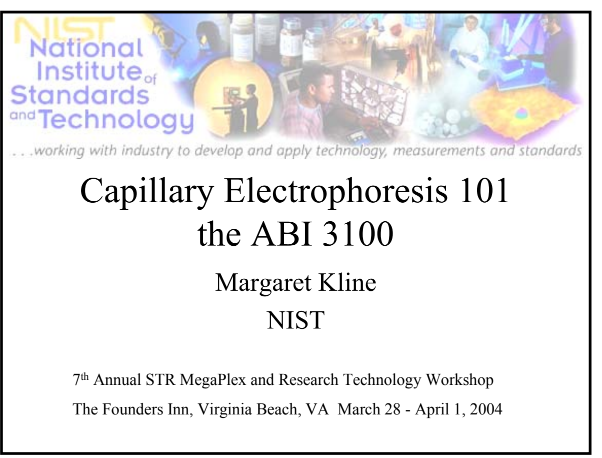
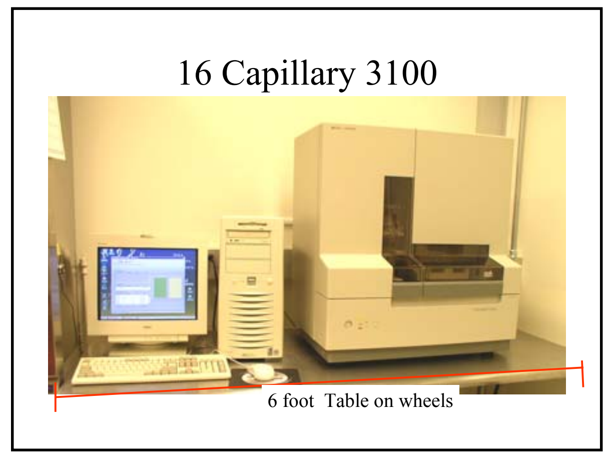
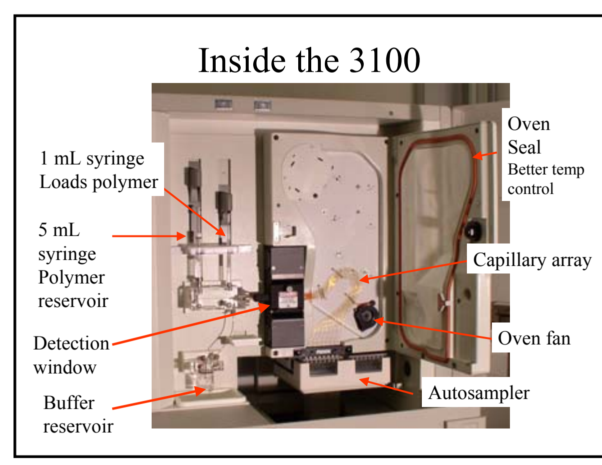
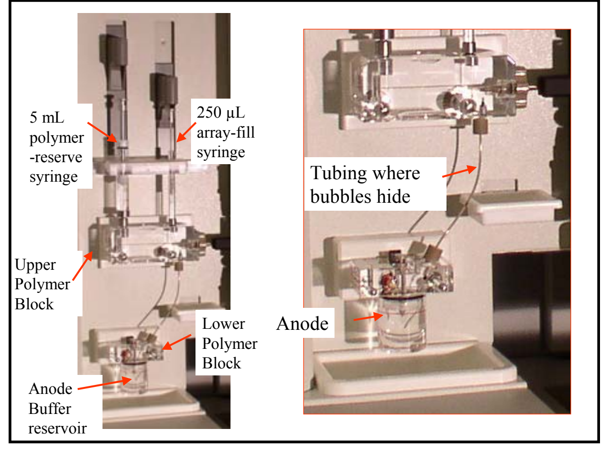
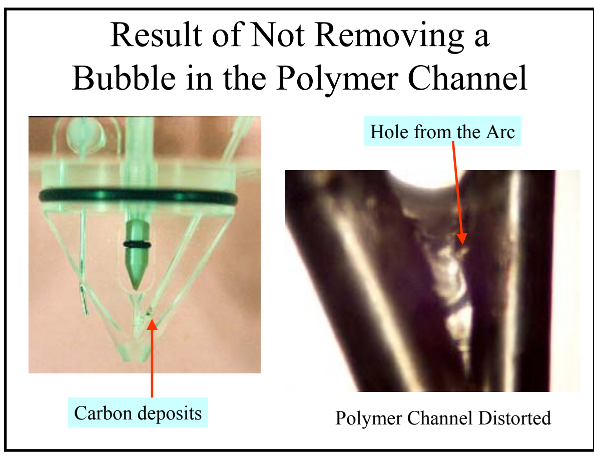

National Institute of Standards and Technology
. . .working with industry to develop and apply technology, measurements and standards
Capillary Electrophoresis 101
the ABI 3100
Margaret Kline
NIST
7th Annual STR MegaPlex and Research Technology Workshop
The Founders Inn, Virginia Beach, VA March 28 - April 1, 2004

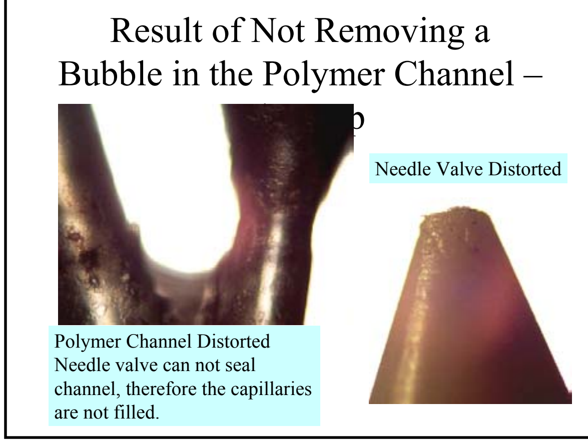

16 Capillary 3100
6 foot Table on wheels

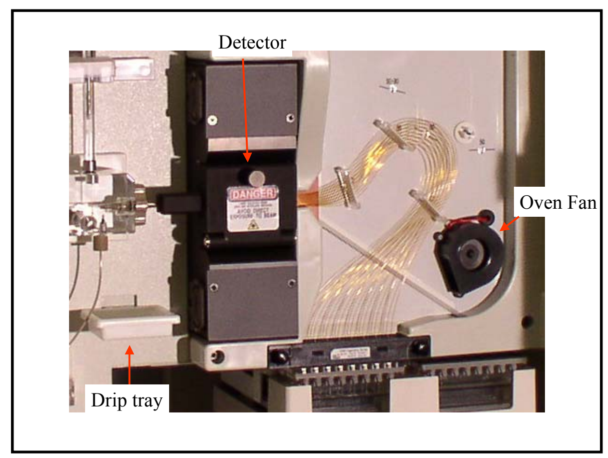
ABI PRISM® 3100 Genetic Analyzer
Applied Biosystems | HITACHI

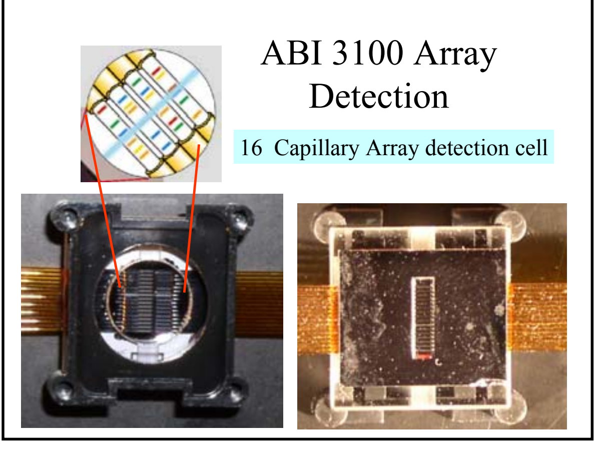

Inside the 3100
1 mL syringe
Loads polymer
5 mL
syringe
Polymer
reservoir
Detection
window
Buffer
reservoir
Oven
Seal
Better temp
control
Capillary array
Oven fan
Autosampler

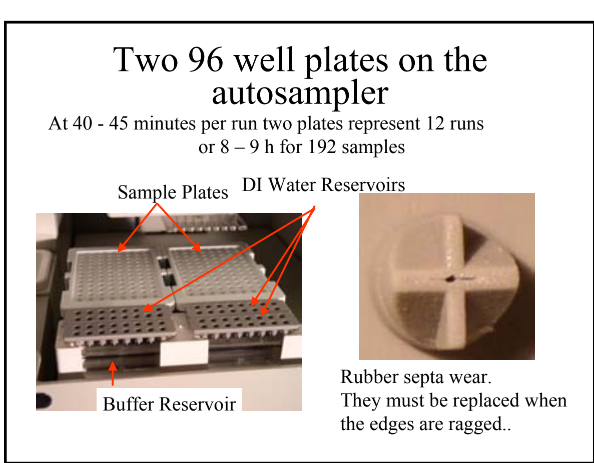

5 mL
polymer
-reserve
syringe
250 µL
array-fill
syringe
Upper
Polymer
Block
Lower
Polymer
Block
Anode
Buffer
reservoir
Tubing where
bubbles hide
Anode

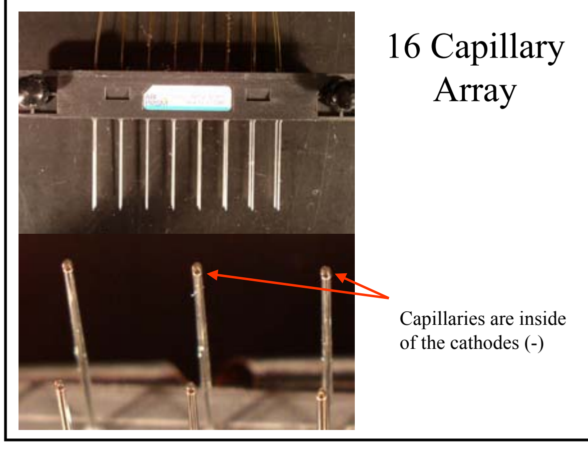

Result of Not Removing a
Bubble in the Polymer Channel
Hole from the Arc
Carbon deposits
Polymer Channel Distorted

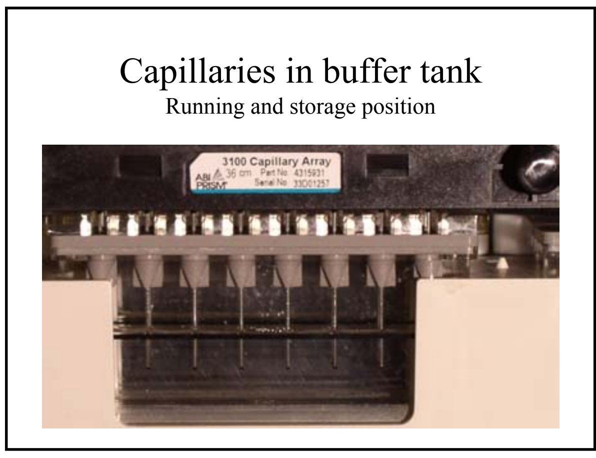

Result of Not Removing a
Bubble in the Polymer Channel –
Needle Valve Distorted
Polymer Channel Distorted
Needle valve can not seal
channel, therefore the capillaries
are not filled.


Detector
DANGER
Oven Fan
Drip tray


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
Sample Plates
DI Water Reservoirs
Buffer Reservoir
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
3100 Capillary Array

Spatial Calibration

Performed after:

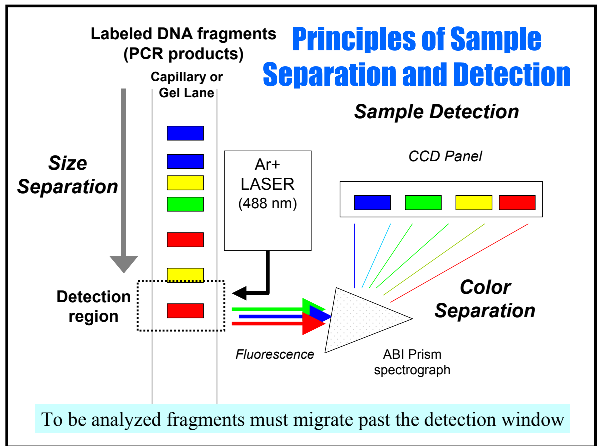
Installing or replacing a capillary array
Removal of the array from the detection block,
(Due to the design, to remove the upper polymer block for cleaning you must remove the Array from the detection window)

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

Spatial Results

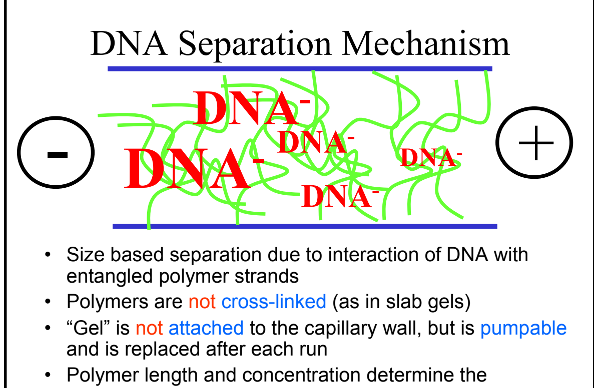
Good Results

Bad results
Try again



To be analyzed fragments must migrate past the detection window

DNA Separation Mechanism



- 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

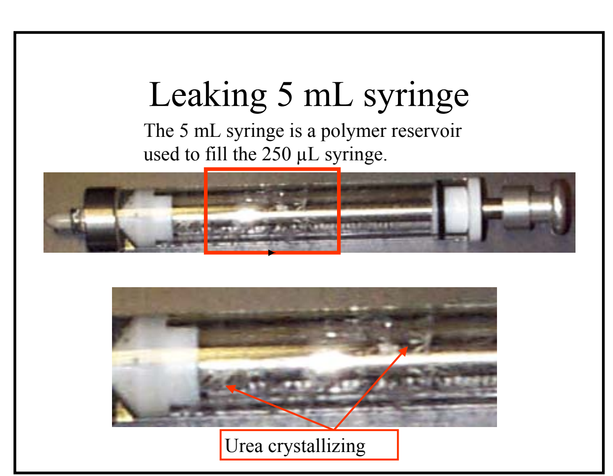
Maintenance of ABI 3100

- 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

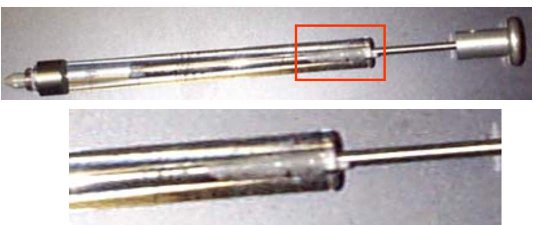
YOU MUST BE CLEAN AROUND A CE!

Leaking 5 mL syringe

The 5 mL syringe is a polymer reservoir used to fill the 250 µL syringe.



250 µL syringe leaking



Urea crystallizing after polymer leaked around the worn plunger. Since this is the high pressure syringe which fills the capillaries this is a problem.

3100 Laser

- Argon-ion 25mW
- Primary emissions
  - 488 nm
  - 514.5 nm

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

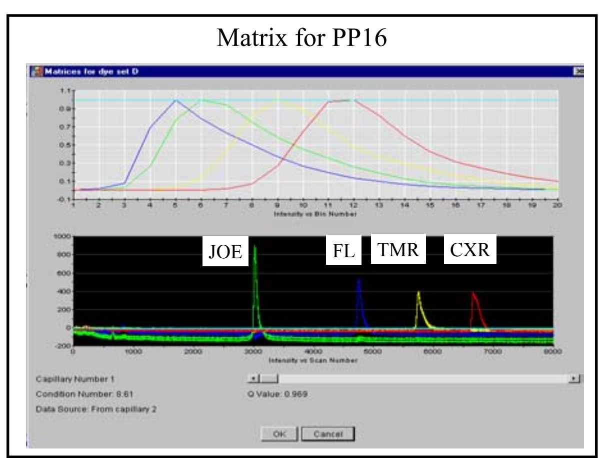
There are 7dye set place holders available on the 3100

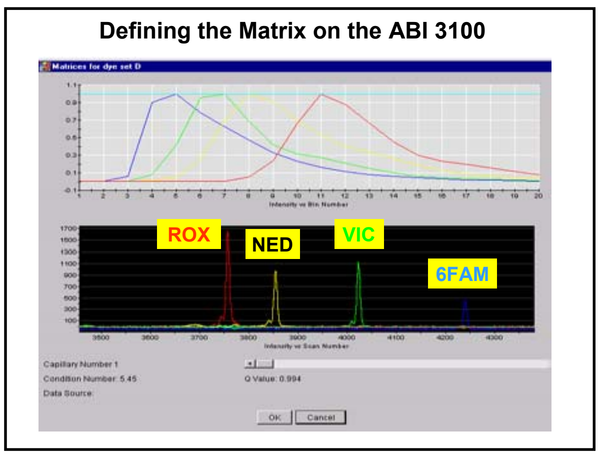
C D E E5 F G5 Z

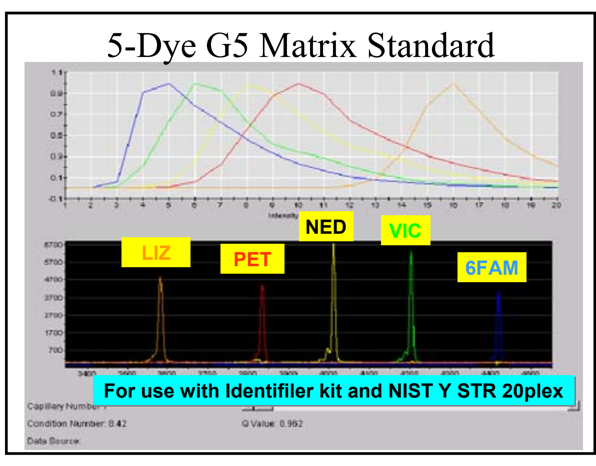
E5 and G5 are for 5 dye systems

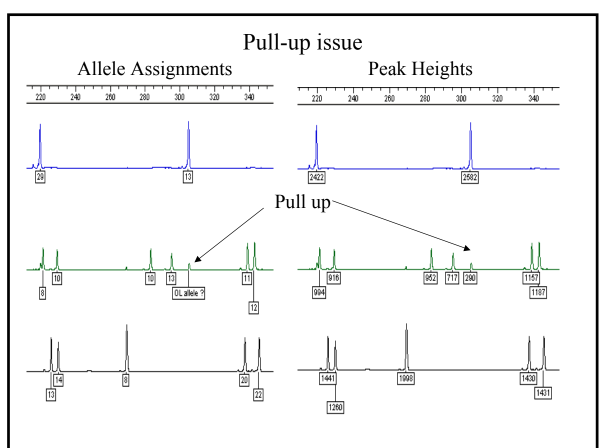
While there are parameters associated with each dye set You can use the parameters of one dye set and then name it a different Dye Set so keep track of things.

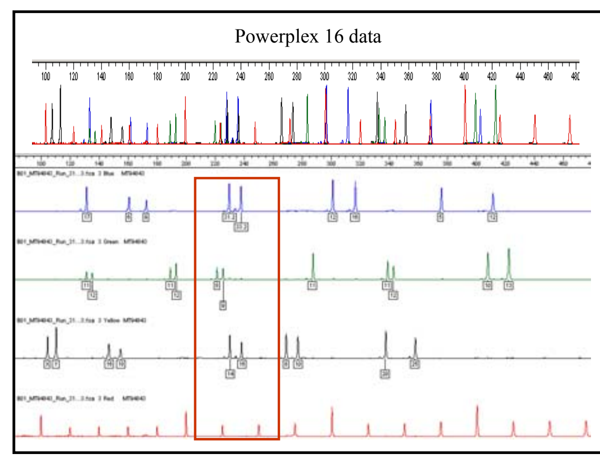
Matrix for PP16

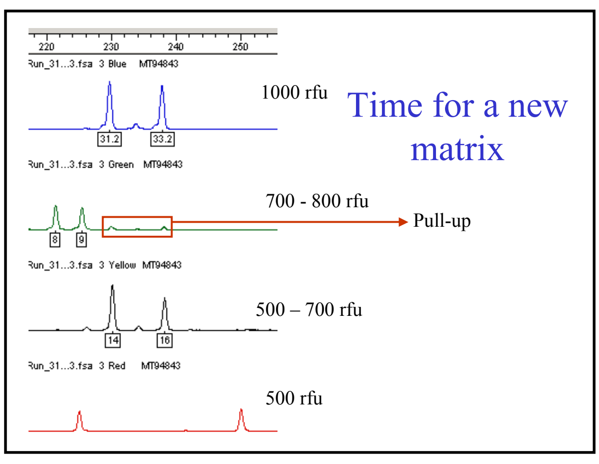


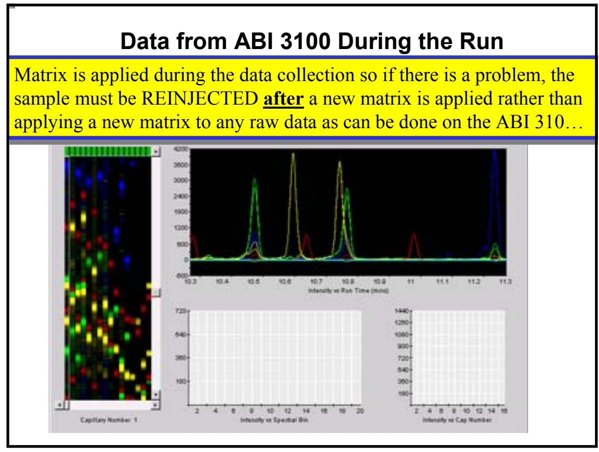

Defining the Matrix on the ABI 3100
ROX
NED
VIC
6FAM


5-Dye G5 Matrix Standard
LIZ
PET
NED
VIC
6FAM
For use with Identifiler kit and NIST Y STR 20plex


Pull-up issue
Allele Assignments
Peak Heights
Pull up


Powerplex 16 data


1000 rfu
Time for a new matrix
700 - 800 rfu
Pull-up
500 – 700 rfu
500 rfu


Data from ABI 3100 During the Run
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…

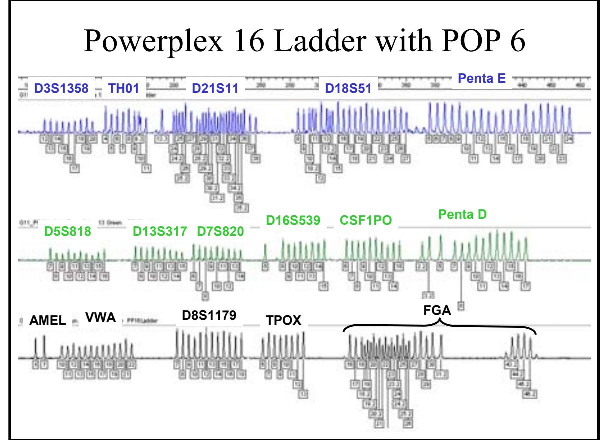
Consumables

- 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

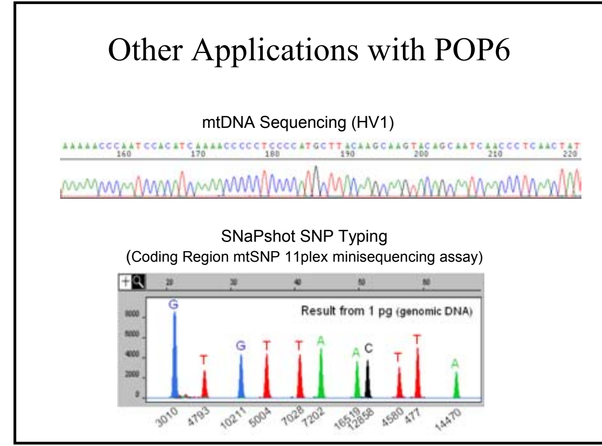
Consumables

- 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

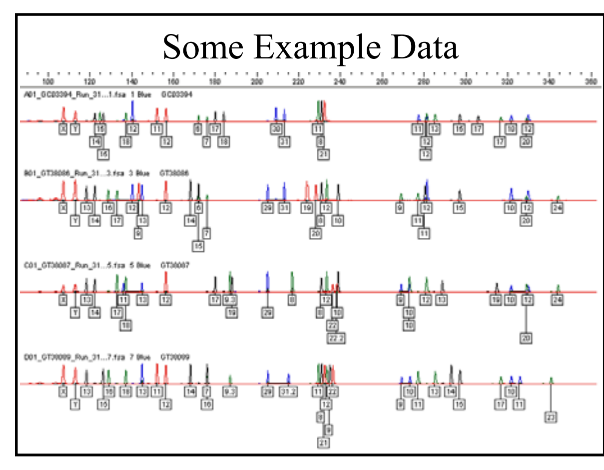
Identifiler PCR amplification was carried out on a GeneAmp® 9700 using 1 ng of DNA according to kit protocols with the exception of reduced volume reactions (5 µL instead of 25 µL) and reduced cycles (26 instead of 28).

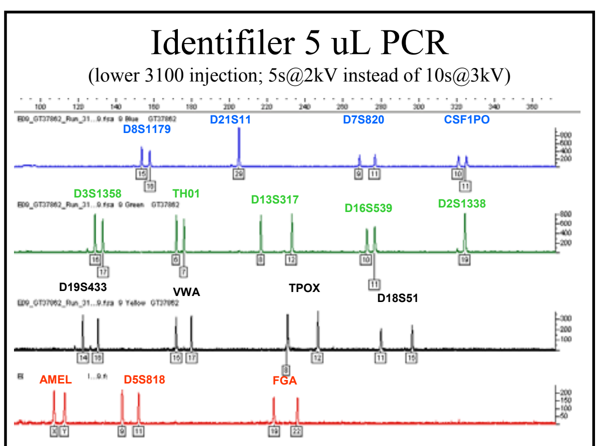
Amplification products were diluted 1:15 in Hi-Di™ formamide and GS500-LIZ internal size standard (0.3 uL) and analyzed on the 16-capillary ABI Prism® 3100 Genetic Analyzer without prior denaturation of samples.

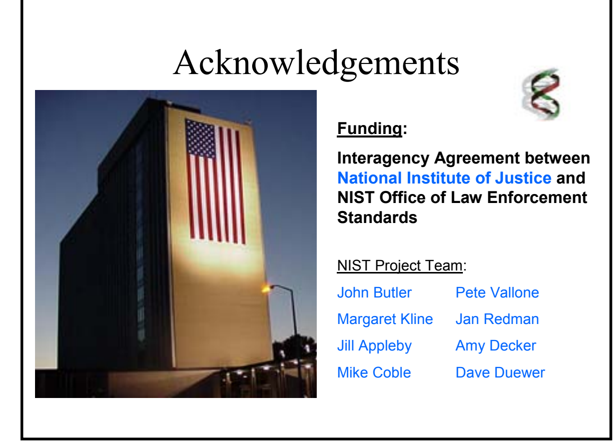
POP™-6 (3700 POP6) rather than POP™-4 was utilized for higher resolution separations.

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)
D8S1179
D21S11
D7S820
CSF1PO
D3S1358
TH01
D13S317
D16S539
D2S1338
D19S433
VWA
TPOX
D18S51
AMEL
D5S818
FGA


Acknowledgements
Funding:
Interagency Agreement between National Institute of Justice and NIST Office of Law Enforcement Standards
NIST Project Team:
John Butler
Pete Vallone
Margaret Kline
Jan Redman
Jill Appleby
Amy Decker
Mike Coble
Dave Duewer