Capillary Electrophoresis Instrumentation and Available STR Kits

John M. Butler, Ph.D.
Canadian Forensic DNA Technology Workshop
Toronto, Ontario
June 8, 2005

Steps in DNA Analysis

Collection
Specimen Storage
Extraction
DNA Extraction
Quantitation
DNA Quantitation
Genotyping
STR Typing (DNA separation)
Interpretation
of Results
DNA Database

STR Typing Technologies

http://www.cstl.nist.gov/biotech/strbase/tech.htm

Typical Instruments Used for STR Typing

GeneAmp 9700
Thermal Cycler for PCR Amplification

Capillary electrophoresis instruments for separating and sizing PCR products

single capillary
ABI 310

16-capillary array
ABI 3100

Review Article on STRs and CE


NEAFS Workshop Slide Handouts

Handouts available as downloadable pdf files from
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm#NEAFSworkshop

2 day workshop with >500 slides describing STRs and CE
(ABI 310 and ABI 3100)

NEAFS CE-DNA Workshop (Butler and McCord)
Sept 29-30, 2004

Capillary Electrophoresis in DNA Analysis

Outline for Workshop

1. Introduction
2. Sample preparation and injection
3. Sample separation
4. Sample interpretation
5. Software usage
6. Assessing resolution of DNA separations
7. Applications of forensic DNA testing
8. Time honored
9. Gas and Montana
10. Increasing sample throughput
11. Capillary array electrophoresis systems
12. Microchip CE systems
13. Future methods for capillary typing
14. Conclusions

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Why Use CE for DNA Analysis?

1. Injection, separation, and detection are automated.
2. Rapid separations are possible
3. Excellent sensitivity and resolution
4. The time at which any band elutes is precisely determined
5. Peak information is automatically stored for easy retrieval

In the early 1990s the real question was how to transition from a gel to a capillary

- Cross-linked acrylamide gel filled capillaries were tried first
  - Reusable?
  - Bubble formation
  - Thermal degradation
- Alternative was to not use a gel at all
  - Refillable sieving polymers
  - However, resolution was poor early on

Early Work with CE and STRs

- Barry Karger’s group (1988-1990)
  - Utilized gel-filled capillaries to separate ssDNA
  - Introduced sieving polymers in the form of linear polyacrylamide to separate restriction digests
- Beckman P/ACE 2050 is introduced in 1992 as the first commercially available CE coupled to a laser to enable fluorescence detection
- John Butler and Bruce McCord (1993-1995)
  - First STR typing with single color CE using intercalating dyes and dual bracketing internal size standards
  - First STR typing with multi-color CE (and multi-capillary) using dye-labeled primers
- ABI 310 is introduced in July 1995 as the first commercially available multi-color CE

First Rapid STR Typing with Capillary Electrophoresis

Butler et al. (1994) BioTechniques 17: 1062-1070

Research performed at FBI Academy in the Forensic Science Research Unit

Requirements for Reliable STR Typing


- Reliable sizing over a 75-500 bp size region
- High run-to-run precision between processed samples to permit comparison of allelic ladders to sequentially processed STR samples
- Effective color separations of different dye sets used to avoid bleed through between 4 or 5 different colors
- Resolution of at least 1 bp to >350 bp to permit reliable detection of microvariant alleles

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Close-up of ABI Prism 310 Sample Loading Area

**Process Involved in 310 Analysis**

- **Injection**
  - electrokinetic injection process (formamide, water)
  - importance of sample stacking

- **Separation**
  - Capillary – 50μm fused silica, 43 cm
  - POP-4 polymer – Polymethyl acrylamide
  - Buffer - TAPS pH 8.0
  - Denaturants – urea, pyrolidinone

- **Detection**
  - fluorescent dyes with excitation and emission traits
  - CCD with defined virtual filters produced by assigning certain pixels

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**Injection**

**Steps in STR Typing with ABI 310**

- **Size Separation**
  - ABI Prism spectrograph

- **Sample Separation**
  - Argon ion LASER (488 nm)
  - Fluorescence

- **Sample Injection**
  - Capillary (filled with polymer solution)
  - Outlet buffer
  - Inlet buffer

- **Sample Preparation**
  - Mixture of dye-labeled PCR products from multiplex PCR reaction

- **Sample Detection**
  - CCD Panel (with virtual filters) produced by assigning certain pixels

- **Sample Interpretation**
  - Processing with GeneScan/Genotyper software

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**CE Injection Methods**

- **Hydrodynamic** (pressure)
- **Electrokinetic** (voltage)
Electrokinetic Injection Process

- Amount of DNA injected is inversely proportional to the ionic strength of the solution
- Salty samples result in poor injections

Two Major Effects of Sample Stacking
1. Sample is preconcentrated. Effect is inversely proportional to ionic strength
2. Sample is focused. Ions stop moving in low electric field
3. Mobility of sample = \( \mu = \frac{velocity}{electric\ field} \)

Typical Sample Preparation for ssDNA
1. Perform PCR with dye-labeled primers
2. Dilute 1 µL PCR product with 24 µL deionized formamide; add 1 µL ROX-labeled internal sizing standard
3. Denature 2 minutes at 95 °C with thermocycler
4. Cool to 4 °C in thermocycler or ice bath
5. Sample will remain denatured for at least 3 days

Comments on Sample Preparation
- Use high quality formamide (<100 \( \mu \)S/cm)!
  - ABI sells Hi-Di formamide
  - Regular formamide can be made more pure with ion exchange resin
- Deionized water vs. formamide
    - Water works fine but samples are not stable as long as with formamide; water also evaporates over time...
- Denaturation with heating and snap cooling
  - Use a thermal cycler for heating and cold aluminum block for snap cooling
  - Heat/cool denaturation step is necessary only if water is substituted for formamide...

Sample Conductivity Impacts Amount Injected

\[
[\text{DNA}_{\text{inj}}] = \frac{Et(\pi r^2) (\mu_{\text{ep}} + \mu_{\text{eof}})[\text{DNA}_{\text{sample}}]}{\lambda_{\text{buffer}}}
\]

- \([\text{DNA}_{\text{inj}}]\) is the amount of sample injected
- \(E\) is the electric field applied
- \(t\) is the injection time
- \(r\) is the radius of the capillary
- \(\mu_{\text{ep}}\) is the mobility of the sample molecules
- \(\mu_{\text{eof}}\) is the electroosmotic mobility

Cl- ions and other buffer ions present in PCR reaction contribute to the sample conductivity and thus will compete with DNA for injection onto the capillary.
DNA and Electrophoresis

“From a practical point of view it is disappointing that electrophoresis cannot be used to fractionate or analyze DNA’s on the basis of size” Olivera, Biopolymers 1964, 2, 245

\[ \mu_{ep} = \frac{q}{6\pi \eta r} \]

small ions with high charge move fastest

As size increases so does charge!

Separation Issues

- Capillary wall coating — dynamic coating with polymer
  - Wall charges are masked by methyl acrylamide
- Electrophoresis buffer —
  - Urea for denaturing and viscosity
  - Buffer for consistent pH
  - Pyrrolidone for denaturing DNA
  - EDTA for stability and chelating metals
- Polymer solution — POP-4 (but others work also)
- Run temperature — 60 °C helps reduce secondary structure on DNA and improves precision. (Temperature control affects DNA sizing)

Capillary Wall Coatings Impact DNA Separations

Electrophoretic flow

DNA Separations in Entangled Polymer Sieving Solutions

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

What is in POP-4 and Genetic Analyzer Buffer?

POP-4 (4% poly-dimethylacrylamide, 8 M urea, 5% 2-pyrrolidinone)

Running buffer contains 100 mM TAPS and 1 mM EDTA (adjusted to pH 8.0 with NaOH) TAPS = N-N-Tris(hydroxymethyl)methyl-3-amino propane-sulfonic acid

See also Wenz et al. (1998) Genome Research 8: 69-80

US Patent 5,552,028 covers POP-4 synthesis
How to Improve Resolution?

1. Lower Field Strength
2. Increase Capillary Length
3. Increase Polymer Concentration
4. Increase Polymer Length

All of these come at a cost of longer separation run times

Detection

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)

Filters determine which wavelengths of light are collected onto the CCD camera

Laser Used in ABI 310

- Argon Ion Laser
- 488 nm and 514.5 nm for excitation of dyes
- 10 mW power
- Lifetime ~5,000 hours (1 year of full-time use)
- Cost to replace ~$5,500
- Leads to highest degree of variability between instruments and is most replaced part
- Color separation matrix is specific to laser used on the instrument

Methods for Fluorescently Labeling DNA

- Intercalating Dyes (post-PCR)
- Dye-labeled nucleotide insertion during PCR
- Dye-labeled primer insertion during PCR

Fluorescent Labeling of PCR Products

- Dyes are attached to one primer in a pair used to amplify a STR marker
- Dyes are coupled to oligonucleotides (primers) through NHS-esters and amine linkages on the 5’end of the primer: Dye-(CH₂)₆-primer
- Dye-labeled oligonucleotides are incorporated during multiplex PCR amplification giving a specific color “tag” to each PCR product
- PCR products are distinguished using CCD imaging on the 310
Fluorescent Dyes Used in 4-Color Detection

- FAM (Blue)
- JOE (Green)
- TAMRA (Yellow)
- ROX (Red)

Virtual Filters Used in ABI 310

Visible spectrum range seen in CCD camera

Commonly used fluorescent dyes

- FL (Blue)
- JOE (Green)
- TAMRA (Yellow)
- ROX (Red)

Filter sets determine what regions of the CCD camera are activated and therefore what portion of the visible light spectrum is collected.

Filter A
- FL
- JOE
- TAMRA
- ROX

Filter C
- 6FAM
- TET
- HEX
- ROX

In-house assays

Filter F
- 5FAM
- JOE
- NED
- ROX

Profiler Plus

Filter G5
- 6FAM
- VIC
- NED
- PET
- LIZ

Identifiler

Fluorescent Emission Spectra for ABI Dyes

5-FAM
- JOE
- NED
- ROX

NED is a brighter dye than TAMRA

Please Note!

- There are no filters in a 310
- It's just the choice of pixels in the CCD detector
- All the light from the grating is collected
- You just turn some pixels on and some off

Comments on Matrices (Multi-Component Analysis)

- Make sure that the right filter set and matrix are applied when collecting data.
- You can always apply another matrix to a sample collected on the ABI 310 but it must be run with the right filter set (matrix must be run first with ABI 3100).
- It is important to update matrices on a regular basis (depending on use) due to differences in laser power over time.
- A good indication of when to run a new matrix is the observation of pull-up between dye colors when peaks are smaller than ~4,000 RFUs.

Same Dye Set and Filter F with Different ABI 310s

Instrument lasers make a big difference.

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Injection List in Data Collection Software

- Lists samples to be analyzed (repeats can be easily performed)
- Sets virtual filter on CCD camera
- Sets electrophoresis time and voltage
- Sets injection time and voltage
- Sets run temperature
- If desired, sample analysis can be set up for automatic matrix color separation and sizing with internal standards using defined analysis parameters

Steps Performed in Standard Module

See J.M. Butler (2005) Forensic DNA Typing, 2nd Edition; Chapter 14

- Capillary fill – polymer solution is forced into the capillary by applying a force to the syringe
- Pre-electrophoresis – the separation voltage is raised to 10,000 volts and run for 5 minutes
- Water wash of capillary – capillary is dipped several times in deionized water to remove buffer salts that would interfere with the injection process
- Sample injection – the autosampler moves to position A1 (or the next sample in the sample set) and is moved up onto the capillary to perform the injection; a voltage is applied to the sample and a few nanoliters of sample are pulled onto the end of the capillary; the default injection is 15 kV (kilovolts) for 5 seconds
- Water wash of capillary – capillary is dipped several times in waste water to remove any contaminating solution adhering to the outside of the capillary
- Water dip – capillary is dipped in clean water (position 2) several times
- Electrophoresis – autosampler moves to inlet buffer vial (position 1) and separation voltage is applied across the capillary; the injected DNA molecules begin separating through the POP-4 polymer solution
- Detection – data collection begins; raw data is collected with no spectral deconvolution of the different dye colors; the matrix is applied during Genescan analysis

Ways to Increase Sample Throughput

- Run more gels (FMBIO approach)
- Increase speed of single sample analysis (microchip CE systems)
- Multiplex fluorescent dyes of different colors (higher level PCR multiplexes)
- Parallel separations using capillary arrays (e.g., ABI 3100 or 3130)
- New detection technologies (MALDI-TOF mass spectrometry)

Methods for Parallel Sample Processing

- Multiplex by Size
- Multiplex by Dye Color
- Multiplex by Number of Capillaries

Increasing Sample Throughput with Parallel Processing

ABI 3100: 16 capillaries
ABI 3730: 96 capillaries
ABI 3100 Avant: 4 capillaries

256 data points in 45 minutes with STR 16plex and 16 capillaries
Inside the 3100

- 1 mL syringe loads polymer
- 5 mL syringe loads polymer reservoir
- Detection window
- Oven fan
- Autosampler
- Oven seal
- Better temp control

ABI 3100 Array Detection

- 16 Capillary Array detection cell
- ABI 3130xL uses pump rather than syringe
- Tubing where bubbles hide

Two 96 well plates on the autosampler

- At 45-60 minutes per run two plates represent 12 runs or ~10-12 hours 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

Process Overview for Using the ABI 3100 for STR Typing

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

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!

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.
Allele Assignments

Peak Heights

Pull-up issue

Pull-up

<table>
<thead>
<tr>
<th>Peak Heights</th>
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<tbody>
<tr>
<td>1000 rfu</td>
</tr>
<tr>
<td>700 - 800 rfu</td>
</tr>
<tr>
<td>500 - 700 rfu</td>
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<tr>
<td>500 rfu</td>
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</table>

Pull-up issue

Powerplex 16 data

Time for a new matrix

<table>
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<th>Peak Heights</th>
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</thead>
<tbody>
<tr>
<td>1000 rfu</td>
</tr>
<tr>
<td>700 - 800 rfu</td>
</tr>
<tr>
<td>500 - 700 rfu</td>
</tr>
<tr>
<td>500 rfu</td>
</tr>
</tbody>
</table>

Defining the Matrix on the ABI 3100

Matrices Created on NIST ABI 3100 with Various Dye Combinations

<table>
<thead>
<tr>
<th>Color</th>
<th>FL</th>
<th>E</th>
<th>ES</th>
<th>E</th>
<th>GS</th>
<th>Z</th>
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<tbody>
<tr>
<td>Blue</td>
<td>dR110</td>
<td>dR110</td>
<td>SFAM</td>
<td>SFAM</td>
<td>SFAM</td>
<td></td>
</tr>
<tr>
<td>Green</td>
<td>dR66</td>
<td>dR66</td>
<td>JOE</td>
<td>VIC</td>
<td>VIC</td>
<td></td>
</tr>
<tr>
<td>Yellow</td>
<td>dTAMRA</td>
<td>dTAMRA</td>
<td>NED</td>
<td>NED</td>
<td>NED</td>
<td></td>
</tr>
<tr>
<td>Red</td>
<td>dROX</td>
<td>dROX</td>
<td>ROX</td>
<td>PET</td>
<td>ROX</td>
<td></td>
</tr>
<tr>
<td>Orange</td>
<td>LIZ</td>
<td>LIZ</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CXR TMR JOE FL

Different ABI 3100 matrix sets used at NIST in order to address a variety of applications and dye combinations.
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...

Parameters in Run Modules

Default injection changes between 3100 data collection versions:
Version 1.0.1 = 10s @ 3kV
Version 1.1 = 22s @ 1kV

Consumables for ABI 310/3100

What we use at NIST

- A.C.E.™ Sequencing Buffer 10X (Amresco)
  - $155/L = $0.0155/mL 1X buffer (costs 20 times less!)
  - http://www.amresco-inc.com
- 3700 POP-6 Polymer (Applied Biosystems)
  - $530 / 200 mL = $2.65/mL (costs 20 times less!)

What ABI protocols suggest

- 10X Genetic Analyzer Buffer with EDTA
  - $78/25 mL = $0.312/mL 1X buffer (ABI)
- 3100 POP-4 Polymer
  - $365 / 7 mL = $52/mL

SNaPshot SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)

mtDNA Sequencing (HV1)

NIST ABI 3100 Analysis Using POP6 Polymer

High Resolution STR Typing

SNAPshot SNP Typing
(Coding Region mtSNP 11plex minisequencing assay)

mtDNA Sequencing (HV1)

ABI 310 Reagents and Operating Costs

<table>
<thead>
<tr>
<th>ABI 310 Reagent Costs</th>
<th>Part Number</th>
<th>Quantity Provided</th>
<th>Cost for 500</th>
<th>Actual Cost for 1000 runs</th>
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<tbody>
<tr>
<td>Capillaries</td>
<td>402839</td>
<td>5/pk (47cm x 50 um uncoated)</td>
<td>$294</td>
<td>$588 (with P+C)</td>
</tr>
<tr>
<td>POP-4 polymer</td>
<td>402838</td>
<td>5 mL</td>
<td>$196</td>
<td>$392 (with P+C)</td>
</tr>
<tr>
<td>Buffer, Genetic Analyzer 10X</td>
<td>402824</td>
<td>25 mL</td>
<td>$78</td>
<td>$156 (with P+C)</td>
</tr>
<tr>
<td>Sample tubes (0.5 mL)</td>
<td>401957</td>
<td>500/pk</td>
<td>$52</td>
<td>$104 (with P+C)</td>
</tr>
<tr>
<td>Septa for tubes</td>
<td>401956</td>
<td>500/pk</td>
<td>$163</td>
<td>$326 (with P+C)</td>
</tr>
<tr>
<td>Formamide, Hi-Di</td>
<td>4311320</td>
<td>25 mL (for ~1000-1500 samples)</td>
<td>$29</td>
<td>$58 (with P+C)</td>
</tr>
<tr>
<td>GS500-ROX size standard</td>
<td>401734</td>
<td>800 tests/pk</td>
<td>$260</td>
<td>$520 (with P+C)</td>
</tr>
<tr>
<td>Matrix standards</td>
<td>4312131</td>
<td>5FAM, JOE, NED, ROX</td>
<td>$70</td>
<td>$140 (with P+C)</td>
</tr>
<tr>
<td>PCR tubes, strips</td>
<td>N801-0580</td>
<td>1000/pk</td>
<td>$76</td>
<td>$152 (with P+C)</td>
</tr>
<tr>
<td>PCR tube caps</td>
<td>N801-0535</td>
<td>1000/pk</td>
<td>$60</td>
<td>$120 (with P+C)</td>
</tr>
<tr>
<td>Pipet tips</td>
<td>~$0.10/ tip</td>
<td>x 550</td>
<td>$55</td>
<td>$110 (with P+C)</td>
</tr>
<tr>
<td>Profiler Plus STR kit</td>
<td>4303326</td>
<td>100 tests/kit</td>
<td>$2,018.94</td>
<td>$4037.88 (with P+C)</td>
</tr>
<tr>
<td>COfiler STR kit</td>
<td>4305246</td>
<td>100 tests/kit</td>
<td>$1,816.54</td>
<td>$3633.08 (with P+C)</td>
</tr>
<tr>
<td>Syringe, Kloehn 1.0 mL</td>
<td>4304471</td>
<td>each</td>
<td>$82</td>
<td>$164 (with P+C)</td>
</tr>
<tr>
<td>Genetic Analyzer vials, 4 mL</td>
<td>401955</td>
<td>50/pk</td>
<td>$62</td>
<td>$124 (with P+C)</td>
</tr>
<tr>
<td>48-tube sample tray kit</td>
<td>402867</td>
<td>each</td>
<td>$230</td>
<td>$460 (with P+C)</td>
</tr>
</tbody>
</table>

*following manufacturer’s protocols (based on 500 samples total)

Total per Sample Cost to Obtain Result on 13 CODIS core loci (with Profiler Plus and COfiler STR kits): $43.42
(materials other than STR kits = $5.06)
10 µL PCR (1/5 vol) = $12.73

http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Identifiler 5 µL 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 G3500-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).


Overall Thoughts on the ABI 310/3100

• Setting on a common instrument platform has been good for the forensic DNA community in terms of data consistency (this is also true with the use of common STR kits)

• I am concerned that the community is very dependent primarily on one company…

• I really like using the instrument and can usually get nice data from it

• Like any instrument, it has its quirks…

Available STR Kits

For positions of the core CODIS loci, please see page 96 of J.M. Butler’s Forensic DNA Typing, 2nd Edition. Review article on core CODIS loci genetics and genomics to be published this fall.

Conclusions

STR typing is here to stay for a few years because of DNA databases that have grown to contain millions of profiles.

SOURCES

http://www.ojp.usdoj.gov/nij/pubs-sum/183697.htm

• Report published in Nov 2000

• Asked to estimate where DNA testing would be 2, 5, and 10 years into the future

From Table 5.2, Forensic DNA Typing, 2nd Edition, p. 96 (J.M. Butler, 2005)
Canadian Forensic DNA Technology Workshop
J.M. Butler, “CE Instrumentation and STR Kits”

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http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm
Value of STR Kits

Advantages
- Quality control of materials is in the hands of the manufacturer
- Improves consistency in results across laboratories – same allelic ladders used
- Common loci and PCR conditions used – aids DNA databasing efforts
- Simpler for the user to obtain results

Disadvantages
- Contents may not be completely known to the user (e.g., primer sequences)
- Higher cost to obtain results

FSS: 5X higher cost with SGM Plus kit

Importance of DNA Quantitation (prior to multiplex PCR)

- Too much DNA
  - Off-scale peaks
  - Split peaks (+/- A)
  - Locus-to-locus imbalance

- Too little DNA
  - Heterozygote peak imbalance
  - Allele drop-out
  - Locus-to-locus imbalance

Stochastic effect when amplifying low levels of DNA produces allele dropout

miniSTRs: new tool for degraded DNA

- Smaller PCR products work better with low copy number or fragmented DNA templates
- No commercial miniSTR kits available yet

Recent Publications on miniSTRs

http://www.cstl.nist.gov/biotech/strbase/miniSTR.htm
http://www.cstl.nist.gov/biotech/strbase/miniSTR/timeline.htm
New miniSGM miniplex assay
Provided to EDNAP/ENFSI group for degraded DNA study (Fall 2004)

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<th>Locus</th>
<th>Size (bp)</th>
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<tr>
<td>TH01</td>
<td>-71</td>
</tr>
<tr>
<td>Amelogenin</td>
<td>-41</td>
</tr>
<tr>
<td>FGA</td>
<td>+20</td>
</tr>
<tr>
<td>D18S51</td>
<td>-151</td>
</tr>
<tr>
<td>D16S539</td>
<td>-151</td>
</tr>
<tr>
<td>D2S1338</td>
<td>-151</td>
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<tr>
<td>D5S818</td>
<td>-151</td>
</tr>
<tr>
<td>D3S1358</td>
<td>-151</td>
</tr>
<tr>
<td>D19S433</td>
<td>-151</td>
</tr>
</tbody>
</table>

Size relative to ABI kits

-71 bp
-71 bp
+20 bp
+20 bp
-151 bp
-151 bp
-151 bp
-151 bp
-151 bp
-151 bp

New combination of miniSTR loci
- Core with highest PD: D18S51, FGA, D2S1338
- Extra European concordance: TH01, D16S539
- Sex-typing added: Amelogenin


Content of STRBase Website
http://www.cstl.nist.gov/biotech/strbase

- ../../str_fact.htm STR Fact Sheets on Core Loci
- ../../multiplex.htm Multiplex STR Kit Information
- ../../y_strs.htm Y-Chromosome Information
- ../../var_tab.htm Variant Alleles Reported
- ../../mutation.htm Mutation Rates for Common STRs
- ../../ref.htm Reference List with ~2,300 Papers
- ../../training.htm Downloadable PowerPoints for Training
- ../../validation.htm Validation Information
- ../../miniSTR.htm miniSTR Information
- ../../address.htm Addresses for Scientists
- ../../NISTpub.htm Publications & Presentations from NIST

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Thank you for your attention!

Questions!

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Our publications and presentations are available at:
http://www.cstl.nist.gov/biotech/strbase/NISTpub.htm

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