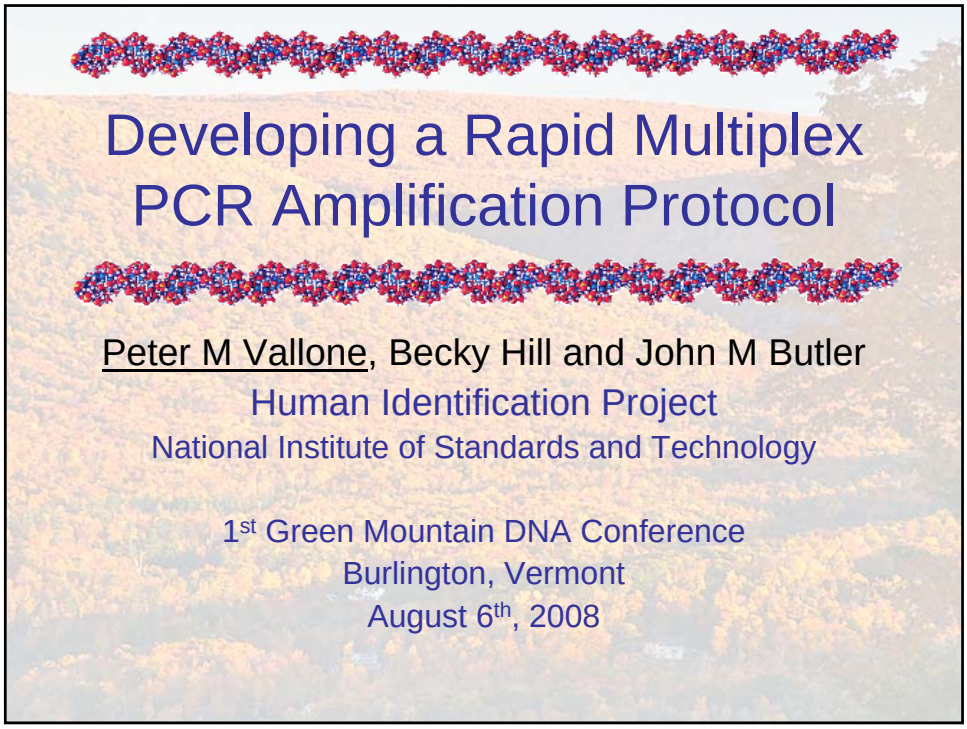
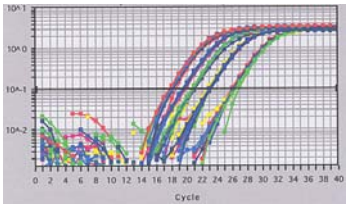


Outline

- Rapid PCR
- Conditions and Parameters
- miniSTR 3plex
- Commercial Kits
- Larger Custom Multiplexes
- Current Work
- Conclusions

Things That Are Rapid

PCR?



Typical STR Workflow

Sample Extraction ~2 h	Quantitation ~1.5 h	**PCR ~3 h**	CE Run ~1.5 h	Data Review ?

How can the time needed for cycling be reduced?

What happens when we alter cycling parameters?
How will existing commercial kits work?
How will different polymerases perform?
How robust will the results be?
Can we develop novel assays and further the understanding/limits of rapid multiplex PCR?

Applications for Rapid PCR

- Integrated devices ('Lab on a Chip')
- Screening at a point of interest (airport, border, crime scene, intelligence community)
- Rapid STR typing 'in the field'
  - Potential for situations/cases when a quick result is needed
  - Provide initial screening information
- Decrease overall time required for STR typing
- Do not necessarily have to use CODIS 13+ loci (fewer loci or alternative loci?)

Growing Interest in DNA for Biometrics

Efforts towards Portable/Mobile DNA Devices

- NEC (Japan)
  - Poster at Promega meeting in Hollywood, CA (Oct 1-4, 2007)
  - Press release on October 15, 2007 (http://www.nec.co.jp/press/en/0710/1501.html)
- Network Biosystems (based on Dan Ehrlich's work at Whitehead)
  - http://www.netbio.com
- Mathies group at UC-Berkeley and Microchip Biotech
  - Publications… in *Analytical Chemistry*, *FSI Genetics*, etc.
  - http://www.microchipbiotech.com
- Landers group at UVA and MicroLab Diagnostics
  - Publications… *Proc Natl Acad Sci USA* 2006; 103:19272-19277
  - http://www.microlabdiagnostics.com

01-17-2008

NYC Prize

- In the months ahead, we will also challenge the private sector to speed up DNA fingerprinting so that when DNA is left behind, officers can identify suspects more quickly and avoid wrongful arrests. And to do this, we will establish **a six-figure prize for anyone who can invent a device tailored to the NYPD that analyzes DNA right at the crime scene**. It's just one more way we are trying to bring private sector innovation into the public sector

http://nyc.gov/portal/site/nycgov/?front_door=true

Rapid PCR

- What do we mean by rapid PCR?
  - Rapid hot start polymerases (save ~10min)
  - Shortening cycling hold times (5 sec vs 1 min)
  - Utilizing existing thermal cycling technology (AB 9700)
    - Eliminating 1 ^{o}C/sec ramp rate (9600 emulation)
    - Utilize the 9700 4 ^{o}C/sec ramp rate
  - Using commercial polymerases that are 'faster'

Goal: cycling in less than 45 minutes
Trying simple things first…

Thermal Cyclers

AB 9800

http://www.appliedbiosystems.com

RapidCycler 2 Instrument
http://www.idahotech.com
Ramp rate = **10°C/sec**

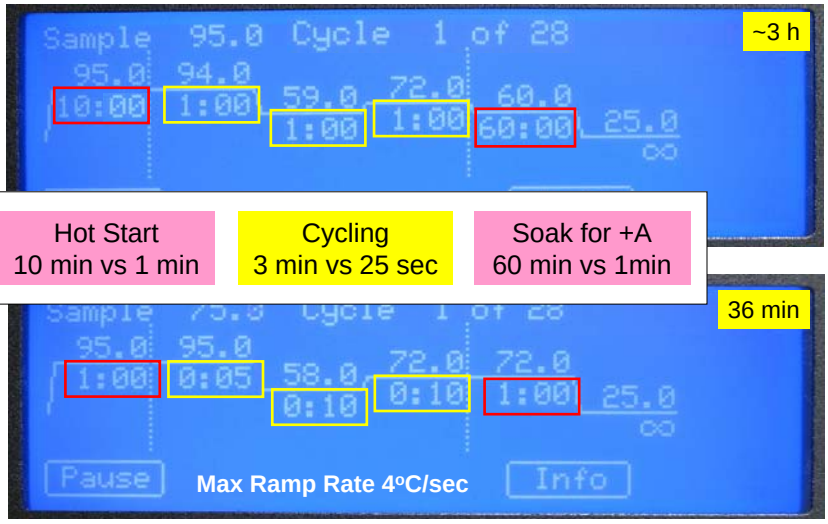
Eppendorf
Mastercycler ep
6°C/sec
http://www.eppendorfna.com

AB 9700

Ramp rate = **4°C/sec**

http://www.appliedbiosystems.com

Thermal Cycling



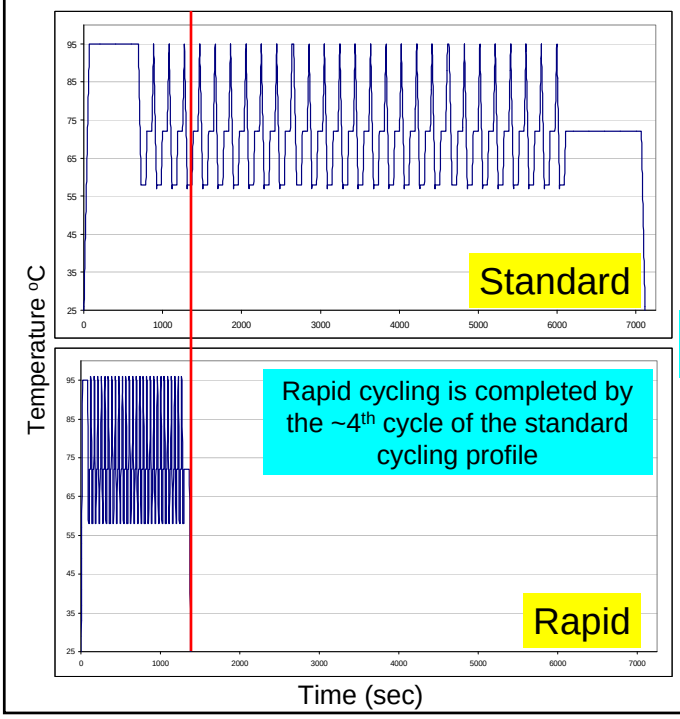
Rapid Thermal Cycling Conditions used

Thermal Cycling

Parameter	Unit	Trad	Rapid	Difference (min)	%
Hot Start	Min	10	1	9.0	6.3
Hold	Sec	60	5/10	72.3	50.6
Soak	Min	60	1	59.0	41.2
Ramp rate	(deg/sec)	1	4	22.4	15.7
Cycles		28	28		
Time		2:58:41	0:35:38	2:23:03	

Parameter	Purpose
Hot Start	Primer Dimer, non-specific amplification
Hold	Denature, annealing, elongation, Inter and intra locus balance
Soak	Full adenylation of PCR products

Evaluate robustness and reproducibility



Comparison of Thermal Cycling Profiles

Initial Work/Assumptions

- Using common materials/conditions
  - AB 9700 (10 μL volume)
  - Standard plastics
  - Commercial Polymerases
  - Final primer concentration ~0.2 μM
  - ~250 μM dNTPs, 2 mM Mg^{++}
  - 5 color dye chemistry for labeling primers
  - Separation on AB 3130
  - Not sample limited (>500 pg of DNA)

Loci for Testing

- 26 autosomal loci characterized in our laboratory
  - Small 3plex panels
  - Larger 26plex *Hill, C.R., Butler, J.M., Vallone, P.M. A New 26plex Autosomal STR Assay to Aid Human Identity Testing (submitted to JFS)*
- Available in kits - 13 CODIS +
- Existing commercial STR typing kits are not optimized for rapid PCR
- Challenge for miniaturized/integrated STR typing platforms – since they have to use a commercial kits or develop their own…

Hill, C.R., Coble, M.D., Butler, J.M. (2008) Characterization of 26 miniSTR loci for improved analysis of degraded DNA samples. J. Forensic Sci. 53(1):73-80.
Coble, M.D. and Butler, J.M. (2005) Characterization of new miniSTR loci to aid analysis of degraded DNA. J. Forensic Sci. 50: 43-53.

Polymerases

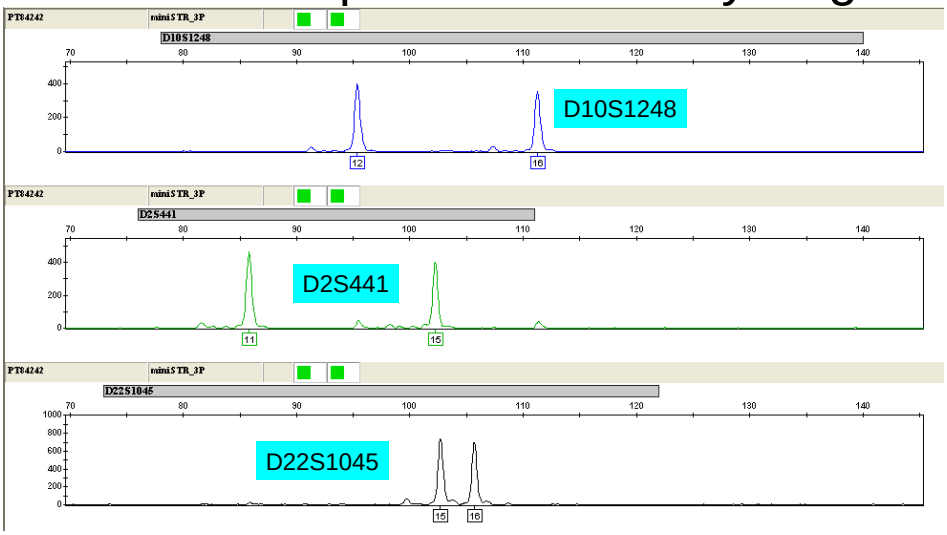
Polymerase	Vendor	MasterMix	Hot Start
TaqGold	Applied Biosystems	no	10 min
GeneAmp	Applied Biosystems	yes (2x)	1 min
SpeedSTAR	Takara	no	1 min
PyroStart	Fermentas	yes (2x)	1 min
Qiagen Fast Cycling PCR Kit	Qiagen	yes (2x)	5 min

Brief survey of 'fast' commercial polymerases

Initial Testing with miniSTR 3plex

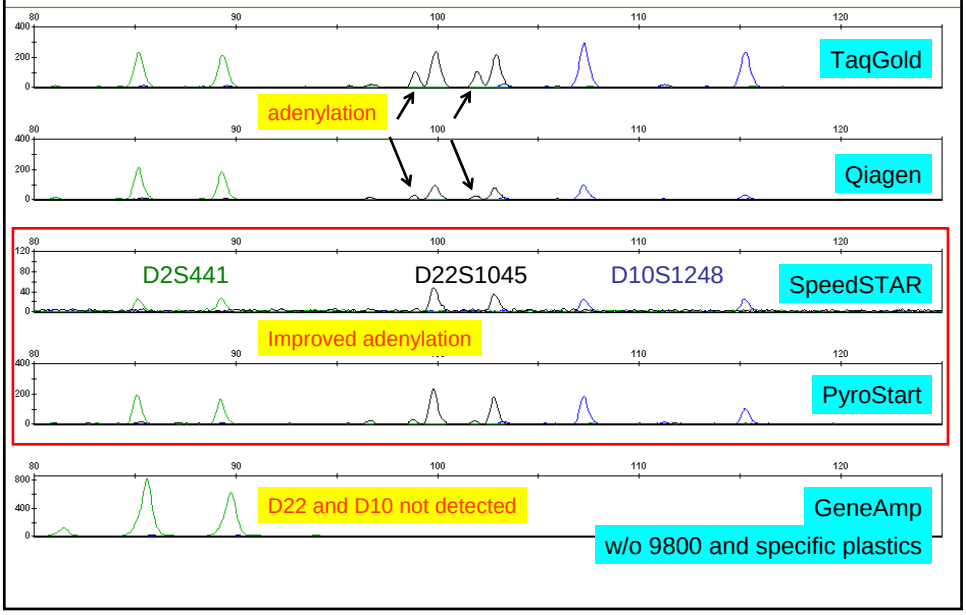
- A simple 3plex in 3 dye colors (FAM, VIC, NED)
- MiniSTR loci
  - D2S441, D10S1248 and D22S1045
  - 'European loci'
  - Amplicon size range 65-140 bp
- These loci were previously tested under standard cycling conditions in a miniSTR multiplex in our lab

miniSTR 3plex Standard Cycling



750 pg template, 28 cycles, TaqGold, ~3 hour cycling time

Initial Rapid Testing Results



w/o 9800 and specific plastics

Rapid PCR 28 cycles in 36 minutes

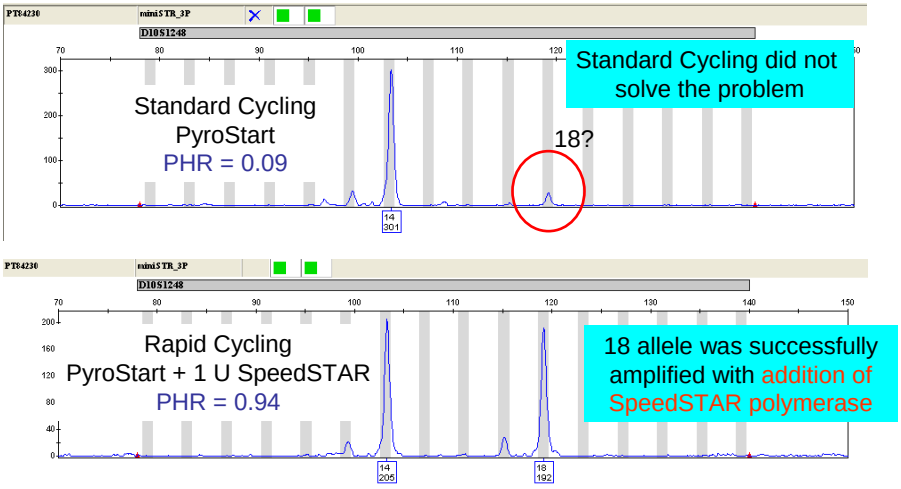
750 pg template, 28 cycles, PyroStart, 36 minute cycling time

miniSTR 3plex Concordance

- 3plex run on a plate of samples (n = 95)
- Concordance was checked with genotypes obtained with Standard Cycling and TaqGold
- 2/285 (0.7%) of the genotype calls were discordant
- Both cases due to D10S1248 heterozygote imbalance

Peak Height Ratio Imbalance for D10S1248

Peak Height Ratio Imbalance for D10S1248



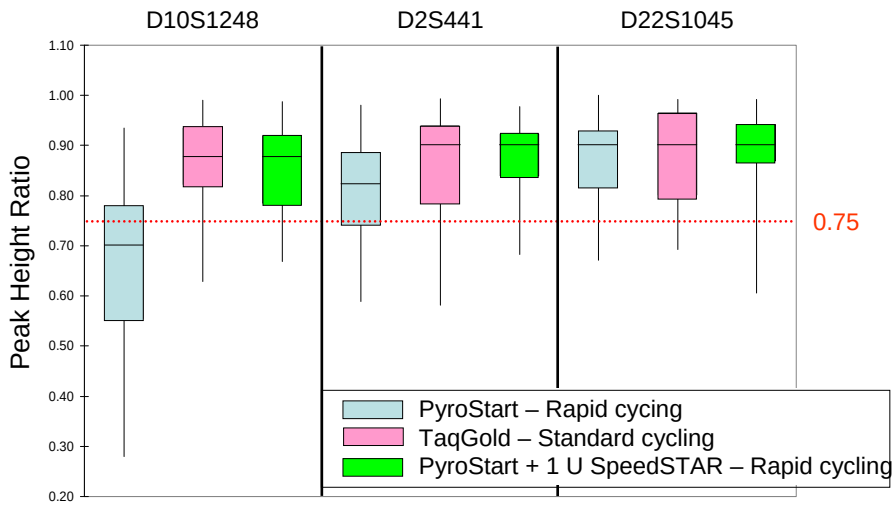
Peak Height Ratio for 16 Samples

Sample Name	Cycling TaqGold	Normal Pyro	Rapid Pyro+SS	Genotype
MT94859	0.70		0.67	**14,19**
PT84230	0.68		0.94	**14,18**
PT84243	0.63	0.28	0.73	14,17
OT05890	0.66	0.30	0.69	14,17
WT51354	0.67	0.33	0.97	14,17
UT57303	0.70	0.37	0.79	13,16
MT97172	0.70	0.37	0.87	13,16
WT51342	0.71	0.40	0.99	13,16
WT51355	0.73	0.41	0.88	13,16
ZT80865	0.74	0.41	0.91	13,16
UT57310	0.75	0.42	0.88	14,16
PT84242	0.78	0.42	0.95	12,16
PT84241	0.78	0.46	0.77	13,16
GT37862	0.78	0.47	0.87	13,16
WT51362	0.78	0.50	0.85	14,16
ZT80863	0.81	0.51	0.90	12,15
avg	0.72	0.40	0.85	
std	0.05	0.07	0.10	

- 2 samples were typed as ‘homozygous”
- 16 samples with lowest PHR values were amplified with extra polymerase
- Balance was improved with extra SpeedStar polymerase

Samples with larger allele spreads for D10 exhibited greater imbalance
e.g. 14,16 better balance than 14,19

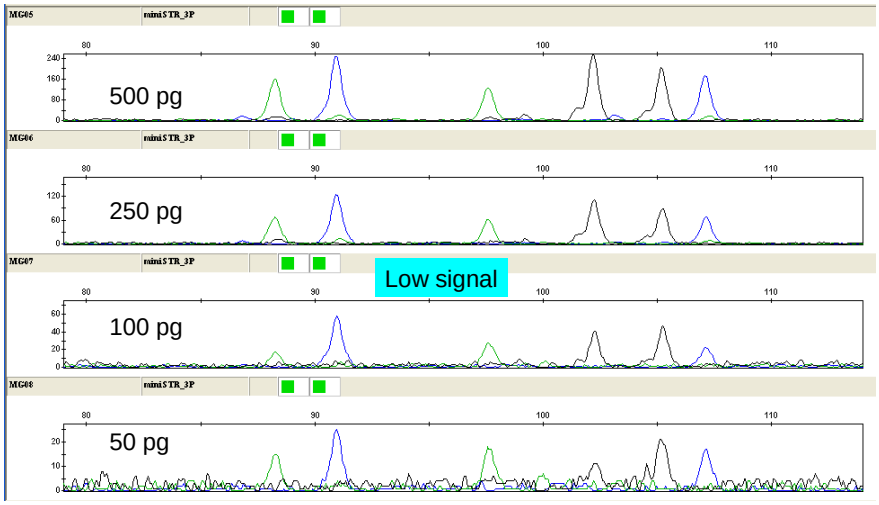
Peak Height Ratios for 3plex Loci



D10S1248 PHR Imbalance

- Imbalance is not solely related to amplicon size
- Improved with additional SpeedStar polymerase
- Not an artifact of rapid cycling conditions
- Other reasons
  - Repeat motif?
  - Primer T_m ?
  - Sequence region for SNPs?

Sensitivity of miniSTR 3plex

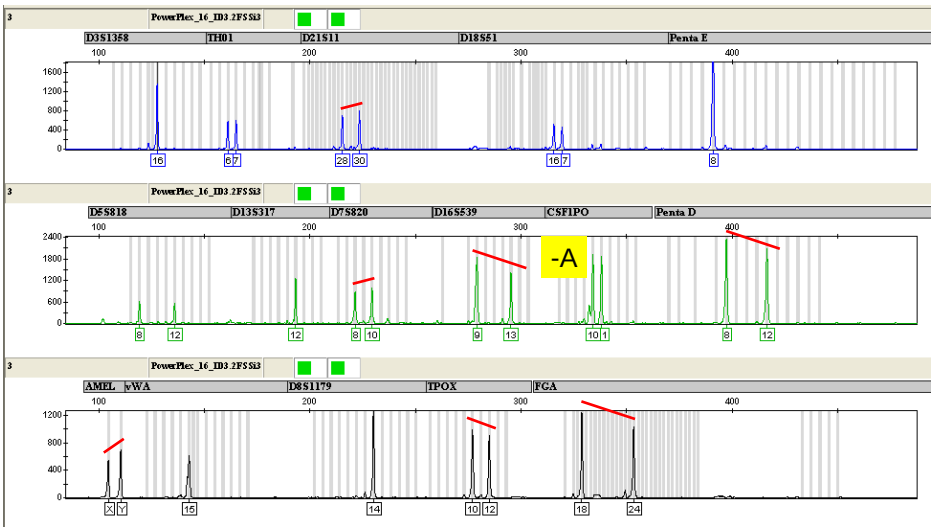


28 cycles, PyroStart polymerase, 36min

Testing Commercials Kits

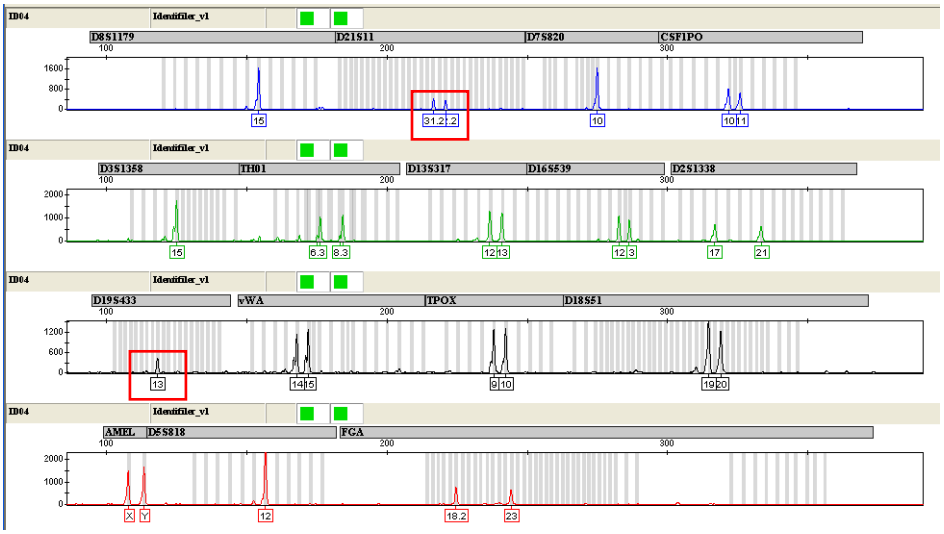
- Tested PP16, COfiler, Profiler Plus ID, Identifiler, and Minifiler primer mixes
  - 10 μL volume
  - 2 μL primer mix
  - PyroStart +1 U SpeedSTAR polymerase
  - 1 ng of template DNA
  - 28 cycles (rapid cycling parameters)
  - 36 min

PowerPlex 16 Rapid Cycling

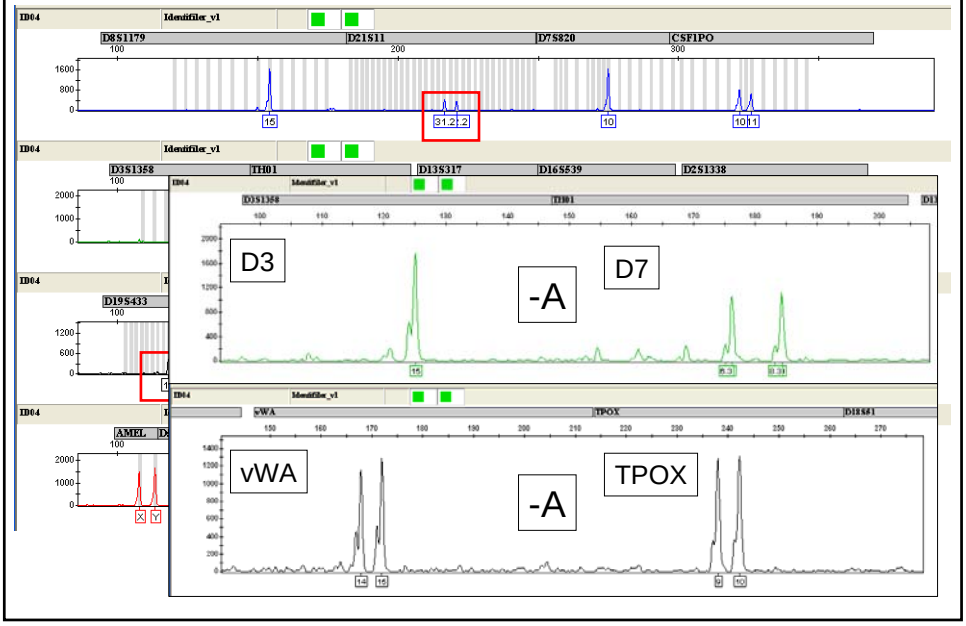


Some potential heterozygote imbalance issues?

Identifiler Rapid Cycling



Identifiler Rapid Cycling



Evaluation…

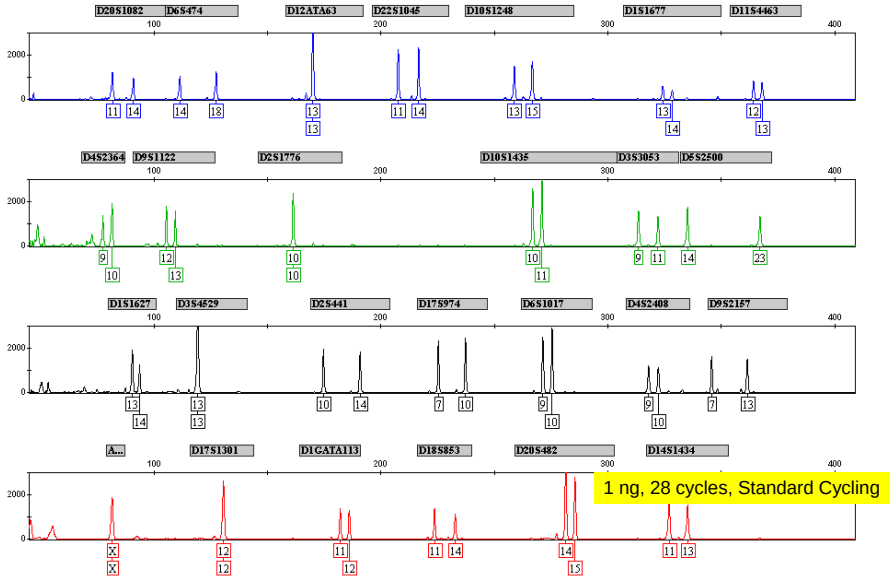
- The large 16 loci kits can be successfully amplified with a rapid thermal cycling protocol
- Additional polymerase is needed

- Which loci show imbalance?
- Limited by poorly adenylating loci?
- Can not alter primer concentrations/sequence
- Further work to be performed: sensitivity, stutter, drop out, etc

Further Evaluation of Our Loci

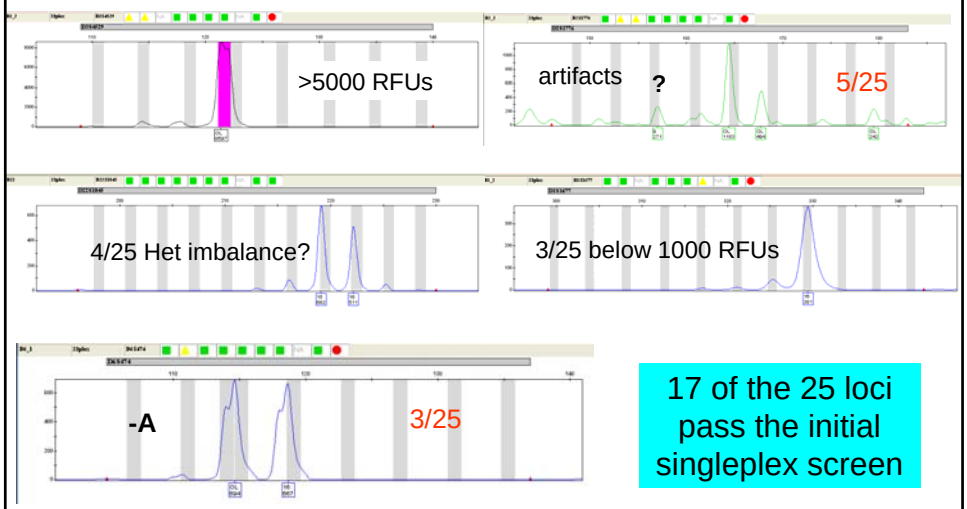
- We currently have an autosomal 26plex assay working in our lab
- Amplified (25/26) each locus in singleplex under rapid cycling conditions
- Evaluate each locus for signal, adenylation and artifacts
- Rank and test candidate loci in a rapid multiplex

26plex Presented at AAFS 2008



Becky Hill presentation at 60th Annual Meeting of the American Academy of Forensic Sciences (Washington, DC), February 21, 2008, "Development of a New Autosomal STR Multiplex with Additional Loci to Benefit Human Identity Testing" [.pdf]

Singleplex Evaluation



17 of the 25 loci pass the initial singleplex screen

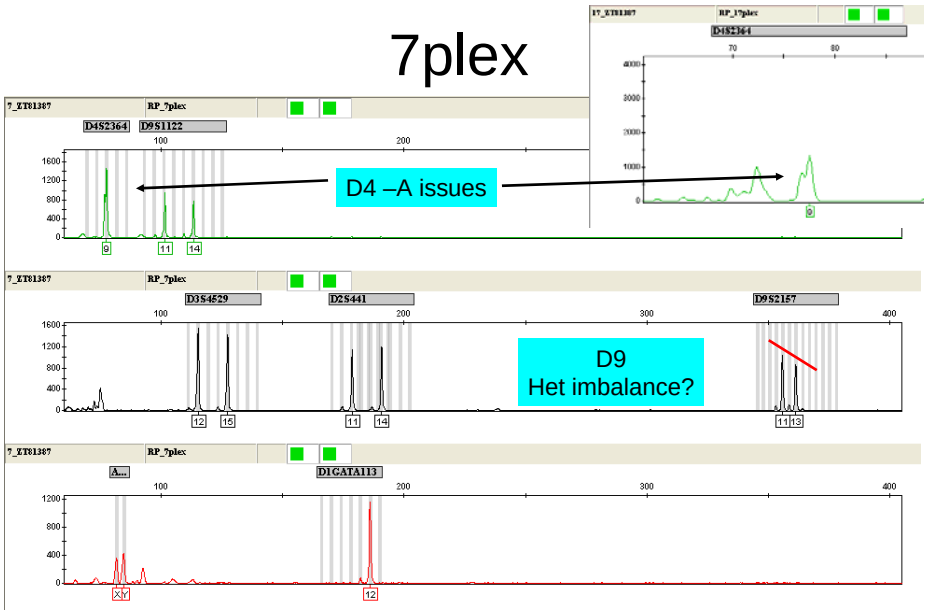
Testing 4 Multiplexes

- After singleplex evaluation 4 multiplexes were tested (empirical balancing)
  - 17plex
  - 14plex
  - 12plex
  - 7plex

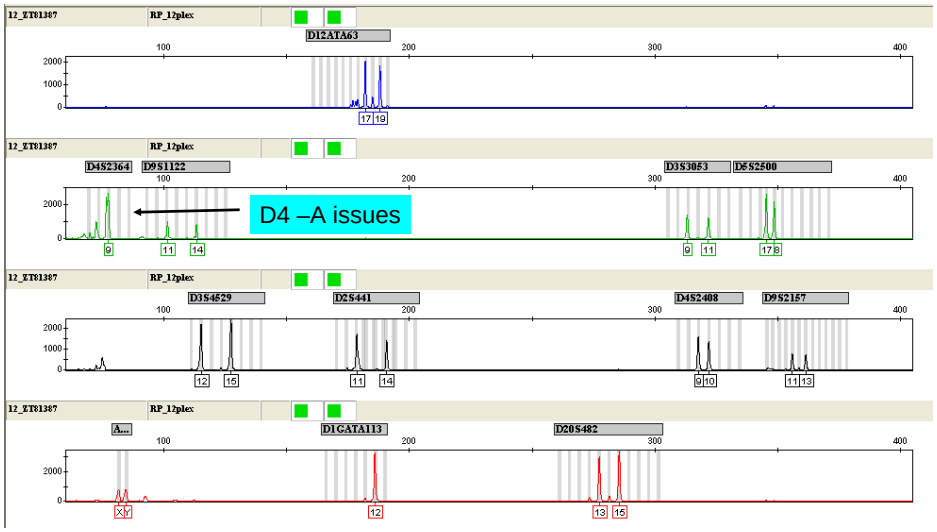
  } Subset of the 17plex

- Run under rapid cycling conditions
- 1 ng DNA, 28 cycles, PyroStart + 1 U SpeedSTAR

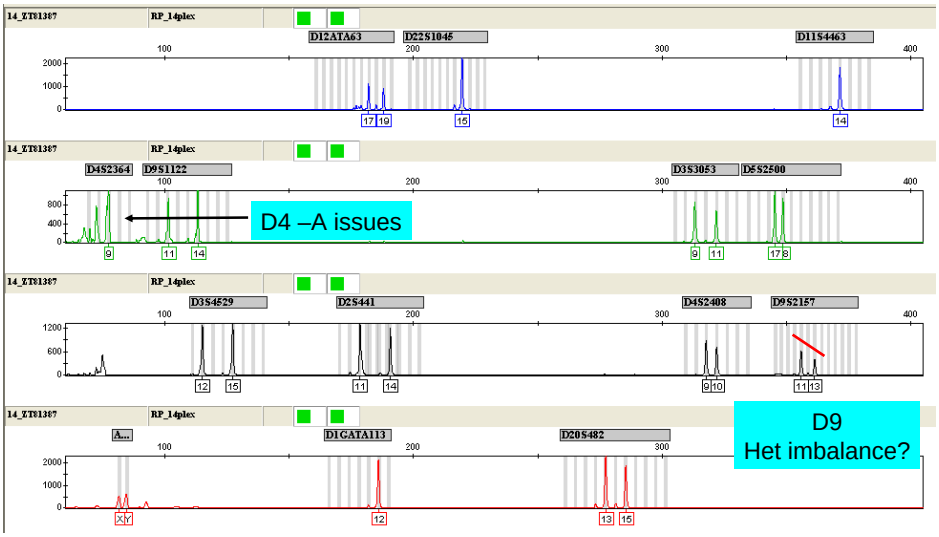
7plex



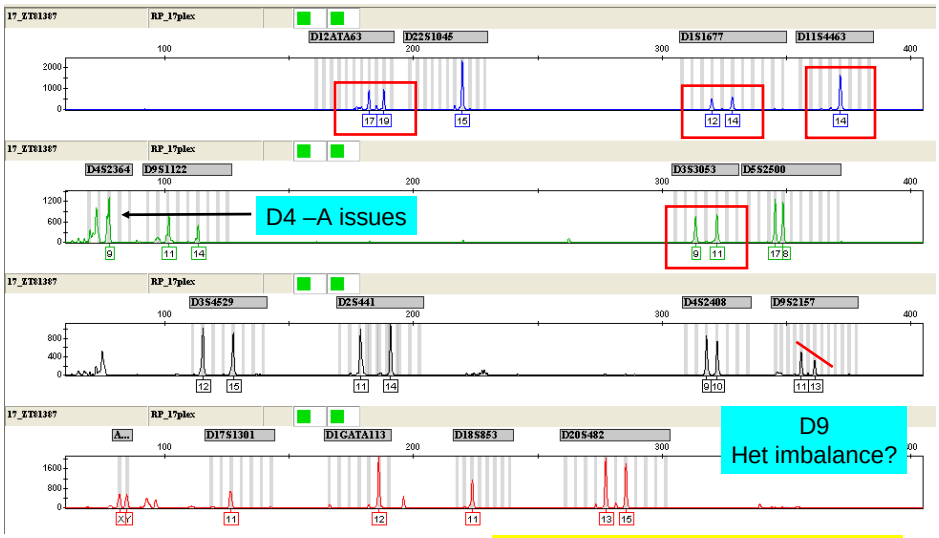
12plex



14plex



17plex



Rapid Multiplex Concordance

- Results for the rapid multiplexes were compared with previously run assays (Standard cycling – TaqGold)
- N = 16 samples
- D4S2364 adenylation issues/artifacts
- D9S2157 severe het imbalance – allele drop out in 2 samples (13,13 vs 13,14) and (7,7 vs 7,11)
- Further evidence that heterozygote imbalance does not directly track with amplicon size

Conclusions

- A large multiplex (17plex) can be amplified in 36 min on an AB 9700 cycler
- Evaluate for adenylation efficiency and heterozygote imbalance
- Larger multiplexes require additional polymerase to obtain complete profiles

- More rigorous testing of our larger multiplexes
- Test other/faster thermal cycling platforms
- Commercial primer mixes can be used – still needs further investigation

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