# Rapid Amplification of Commercial STR Typing Kits

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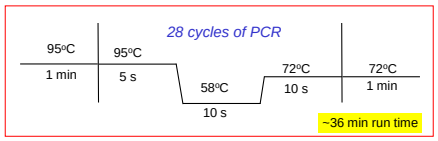
Forensic DNA typing is currently conducted in approximately 8 to 10 hours. The process includes DNA extraction, quantitation, multiplex PCR amplification, and fragment length detection. Today's commercial multiplex short tandem repeat (STR) typing kits are not optimized for rapid PCR thermal cycling. Current protocols require approximately 3 hours for amplifying a multiplex containing 15 STR loci plus amelogenin. With the continuing development of miniaturization technologies such as microfluidic and micro-capillary devices, there is a desire to reduce the overall time required to type DNA samples. Such miniature devices could be used for initial screening at a crime scene, at a border, and at airports. There is also the benefit of reducing the required PCR amplification time for labs typing single-source reference samples. Surveys of fast processing polymerases working in combination with rapid cycling protocols have resulted in the development of a 'rapid' PCR amplification protocol[1]. Results are obtained in less than 36 minutes run on a standard peltier-based thermal cycler employing a heating rate of 4ºC/s. Capillary electrophoresis characterization of the PCR products indicates good peak balance between loci, strong signal intensity and minor adenylation artifacts. Genotyping results are concordant with standard amplification conditions utilizing a standard 3 hour (non-rapid) thermal cycling procedure. The rapid assay conditions are robust enough to routinely amplify 0.5 ng of template DNA (with 28 cycles). Further work in this area with various 'non-standard' thermal cyclers and fast polymerases has resulted in decreasing the amplification time to less than 20 minutes for 16 loci.

[1]Vallone, P.M., Hill, C.R., Butler, J.M. (2008) Demonstration of rapid multiplex PCR amplification involving 16 genetic loci. *FSI Genetics* 3(1): 42-45.

## Initial Work with Developing Rapid PCR Methods

- Amplification of 16 loci contained in Identifiler STR kit (in 36 min)
- 750 pg of DNA template (single source sample), 28 cycles, GeneAmp 9700 thermal cycler heating rate = 4ºC/s
- Combined DNA polymerases
  - SpeedStar (Takara)
  - PyroStart (Fermentas)
- 1 min hot start and 1 min post PCR cycling soak
- 2 µL of Identifiler STR primer mix, 10 µL total reaction volume
- 100% concordance versus standard protocol for 60 samples
- Conclusions: with the use of faster polymerases rapid multiplex amplification of STRs is possible
- Published - Vallone, P.M., Hill, C.R., Butler, J.M. (2008) Demonstration of rapid multiplex PCR amplification involving 16 genetic loci. *FSI Genetics* 3(1): 42-45

### Rapid PCR Thermal Cycling Profile

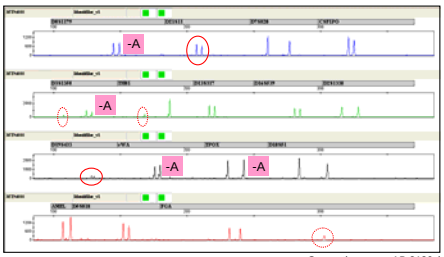



Maximum heating rate of ~4ºC/s on a GeneAmp 9700 (Applied Biosystems)

General thermal cycler parameter characteristics:
- Fast hot start polymerases (~1 min)
- Minimal hold times during cycling
- Minimal post cycling soak
- Utilize maximum heating/cooling ramp rate of thermal cycler

**The above thermal cycling profile is a suggested starting point for further optimization…**

### Identifiler Profile Amplified in 36 Minutes




Separation on an AB 3130xl

- All 16 loci amplified
- Lower signal intensity than a standard amplified profile
- Low signal for some loci (D19S433, D21S11)
- Non-specific artifacts (FGA, VIC/NED dye channel – 'noise')
- Incomplete adenylation for some loci (D8S1179, D3S1358, vWA, TPOX)

### Goals for Continued Work

- Improve interlocus balance
  - –D19S433 & D21S11 (for the Identifiler kit)
- Reduction of incomplete adenylation artifacts
- Test additional commercial STR kits
- Determine more efficient polymerase combinations
- Test alternative thermal cyclers
- Test faster cycling times

### Commercial DNA Polymerases

A three component polymerase 'cocktail' was found to give slightly improved results for the Identifiler kit (improved signal for D19S433 & D21S11)

0.5 x master mix PyroStart (Fermentas) ($0.14 USD/rxn)
0.5 x master mix Premix Ex Taq (Takara) ($0.22 USD/rxn)
0.25 µL = 1.25 units of SpeedStar (Takara) ($1.09 USD/rxn)

This optimized cocktail was tested on various kits and on various thermal cyclers (10 µL PCR reaction volume)

Continually testing and evaluating new polymerases

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***Additional funding from the FBI was used to purchase rapid thermal cyclers and the evaluation of forensic DNA typing as a potential biometric***

*Poster available for download from STRBase*
*http://www.cstl.nist.gov/biotech/strbase/pub_pres/ValloneISFG2007poster.pdf*

## Testing Thermal Cyclers

### GeneAmp 9700 (Applied Biosystems)

- Heating rate: 4ºC/s
- Heating mechanism: Peltier block (Al)
- Tube format: 0.2 mL - 96 well plate
- 28 cycles = 36 min (for the general rapid thermal cycling profile shown on the left)

### SmartCycler (Cepheid)

- Heating rate: 10ºC/s
- Heating mechanism: heating plates and air circulating fan
- Tube format: proprietary 25 µL tubes
- 16 reactions per instrument (ability to run 16 independent thermal cycling profiles)
- Can also be used for real time PCR
- 28 cycles = 20 min

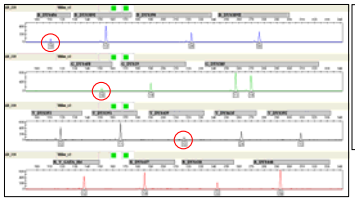
25 µL PCR rxn volume

### Mastercycler pro (Eppendorf)

- Heating rate: 6ºC/s
- Heating mechanism: Peltier block (Ag)
- Tube format: 0.2 mL - 96 well plate
- 28 cycles = 19 min

## Testing Additional Commercial STR Kits

Yfiler, MiniFiler and Promega S5 kits were also tested under rapid cycling conditions. Below are examples of their performance on a GeneAmp 9700. No improvements were observed using the other tow rapid cyclers.
Amplification times were approximately 36 minutes of the GeneAmp 9700 using the 3 polymerase cocktail, 10 µL vol, 1 ng of DNA, and 28 cycles



**Yfiler**
- All loci are present
- DYS456, DYS458, & DYS439 exhibit the lowest signal
- Note: Results are poor (> 50% allele drop out) on the SmartCycler and Mastercycler pro cyclers (data not shown)

**MiniFiler**
- Non-specific artifacts in the VIC channel
- Amelogenin is below threshold
- D7S820, D18S1338 & CSF1PO exhibit low signal
- D18S1338 exhibits adenylation artifacts

**PowerPlex S5**
- Amelogenin signal is low
- D18S51 signal is low & exhibits adenylation artifacts

## Testing Cyclers with Identifiler

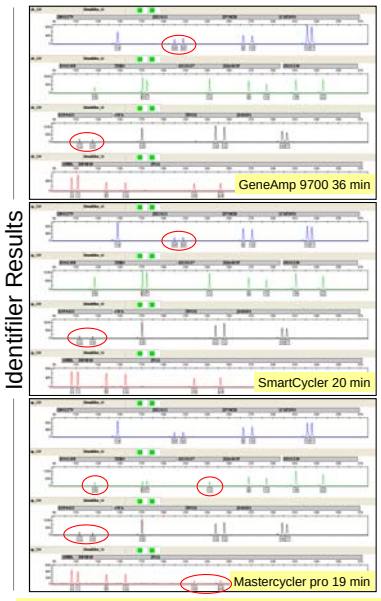
| Idfiler Rank | Standard Conditions Locus | Mean Signal | AB 9700 Rank | Mean Signal | SmartCycler Rank | Mean Signal | Mastercycler Rank | Mean Signal |
|---|---|---|---|---|---|---|---|---|
| 1 | D13S317 | 0.141 | 7 | 0.068 | 10 | 0.055 | 15 | 0.019 |
| 2 | D3S1358 | 0.111 | 10 | 0.040 | 9 | 0.058 | 13 | 0.023 |
| 3 | TH01 | 0.104 | 1 | 0.168 | 2 | 0.110 | 8 | 0.069 |
| 4 | D8S1179 | 0.083 | 13 | 0.027 | 12 | 0.048 | 9 | 0.057 |
| 5 | D16S539 | 0.074 | 3 | 0.105 | 5 | 0.073 | 5 | 0.092 |
| 6 | D21S11 | 0.069 | 15 | 0.020 | 16 | 0.015 | 12 | 0.028 |
| 7 | TPOX | 0.055 | 2 | 0.108 | 1 | 0.113 | 2 | 0.113 |
| 8 | D2S1338 | 0.051 | 4 | 0.099 | 6 | 0.064 | 3 | 0.102 |
| 9 | D7S820 | 0.050 | 11 | 0.033 | 7 | 0.062 | 7 | 0.079 |
| 10 | D19S433 | 0.048 | 16 | 0.016 | 15 | 0.020 | 14 | 0.020 |
| 11 | vWA | 0.047 | 8 | 0.065 | 4 | 0.090 | 4 | 0.100 |
| 12 | D5S818 | 0.038 | 14 | 0.026 | 13 | 0.047 | 11 | 0.031 |
| 13 | CSF1PO | 0.037 | 6 | 0.068 | 8 | 0.061 | 6 | 0.089 |
| 14 | D18S51 | 0.036 | 5 | 0.082 | 3 | 0.099 | 1 | 0.122 |
| 15 | FGA | 0.030 | 12 | 0.028 | 14 | 0.032 | 16 | 0.012 |
| 16 | AMEL | 0.027 | 9 | 0.048 | 11 | 0.052 | 10 | 0.043 |
| | ideal | 0.063 | | | | | | |

Average heterozygote peak balance was similar for all cyclers > 0.88

*The **mean signal** is the normalized fraction of the total signal (RFUs) in the electropherogram. This metric provides a quantitative measure of the multiplex balance and individual locus performance.*

For the GeneAmp 9700 and SmartCycler D21S11 & D19S433 exhibit the weakest signal while FGA, D13S317, D3S138, & D19S433 are weak for the Mastercycler. However, despite signal imbalance all loci peaks are above the 50 RFU detection threshold.

**Assay conditions:** 28 cycles, 1 ng DNA, 10 µL PCR reaction volume
Thermal cycling profile (as shown on left)
Optimized 3 polymerase cocktail (described on left)
Employing maximum heating and cooling rate of each cycler
Detection threshold of 50 RFUs

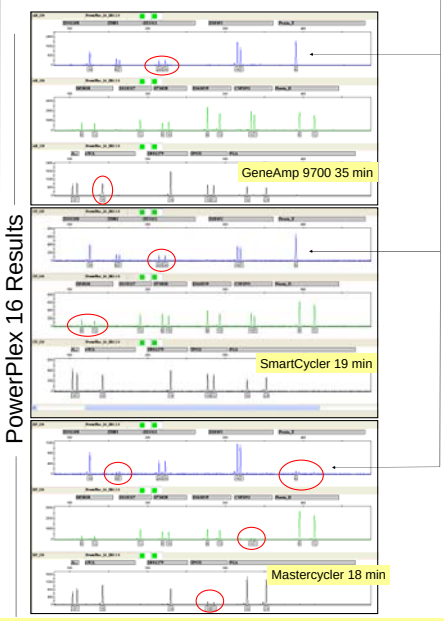



## Testing Cyclers with PowerPlex 16 (PP16)

| PP16 Rank | Standard Conditions Rank | Mean Signal | AB 9700 Rank | Mean Signal | SmartCycler Rank | Mean Signal | Mastercycler Rank | Mean Signal |
|---|---|---|---|---|---|---|---|---|
| 1 | vWA | 0.033 | 15 | 0.031 | 14 | 0.031 | 7 | 0.058 |
| 2 | FGA | 0.037 | 11 | 0.039 | 6 | 0.066 | 2 | 0.106 |
| 3 | AMEL | 0.040 | 8 | 0.051 | 3 | 0.080 | 6 | 0.068 |
| 4 | TPOX | 0.050 | 9 | 0.046 | 4 | 0.073 | 14 | 0.020 |
| 5 | D21S11 | 0.054 | 16 | 0.022 | 16 | 0.025 | 10 | 0.043 |
| 6 | TH01 | 0.055 | 12 | 0.037 | 12 | 0.043 | 13 | 0.020 |
| 7 | D8S1179 | 0.059 | 7 | 0.052 | 11 | 0.049 | 11 | 0.038 |
| 8 | Penta_E | 0.060 | 4 | 0.090 | 7 | 0.065 | 16 | 0.009 |
| 9 | D3S1358 | 0.064 | 10 | 0.045 | 9 | 0.058 | 8 | 0.055 |
| 10 | Penta_D | 0.066 | 1 | 0.136 | 1 | 0.192 | 1 | 0.251 |
| 11 | D7S820 | 0.067 | 6 | 0.056 | 8 | 0.059 | 5 | 0.069 |
| 12 | D13S317 | 0.072 | 14 | 0.032 | 13 | 0.034 | 9 | 0.053 |
| 13 | D5S818 | 0.076 | 13 | 0.035 | 15 | 0.026 | 12 | 0.028 |
| 14 | D16S539 | 0.081 | 3 | 0.113 | 2 | 0.081 | 4 | 0.070 |
| 15 | CSF1PO | 0.092 | 5 | 0.088 | 10 | 0.050 | 15 | 0.013 |
| 16 | D18S51 | 0.096 | 2 | 0.127 | 5 | 0.068 | 3 | 0.102 |
| | ideal | 0.063 | | | | | | |

Average heterozygote peak balance was similar for all cyclers > 0.88
For the GeneAmp 9700 and SmartCycler D21S11, vWA & D5S818 exhibit the weakest signal while CSF1PO, Penta E, TH01 & TPOX are weak for the Mastercycler. Penta E proved quite robust on the 9700 and SmartCycler, but extremely poor on the Mastercycler.

**Assay conditions:** 28 cycles, 1 ng DNA, 10 µL PCR reaction volume
The PP16 rapid thermal cycling profile was modified to emulate prescribed PP16 parameters: Denaturing at 94ºC for 10 cycles followed by denaturing at 90ºC for 18 cycles (annealing temp 59ºC)
Optimized 3 polymerase cocktail (as shown on left)
Employing maximum heating and cooling rate of each cycler
(no incremental ramping as in the prescribed PP16 thermal cycling protocol)




Conclusion: The 16 loci in each kit can be detected under the stated rapid amplification conditions. Improved interlocus balance and reduction of non-specific artifacts may have to be addressed by varying PCR primer concentrations and/or sequence.