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Rapid Amplification of Commercial STR Typing Kits

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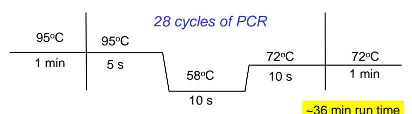
Forensic DNA typing is currently conducted in approximately 8 to 10 hours. The process includes DNA extraction, quantitation, multiplex PCR amplification, fragment length detection, and data interpretation. Today's commercial multiplex short tandem repeat (STR) typing kits are not optimized for rapid 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 4°C/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 = 4°C/s)
- Combined DNA polymerases
  - SpeedStar (Takara Bio Inc., Shiga, Japan)
  - PyroStart (Fermentas, Glen Burnie, MD)
- 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 ~4°C/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 PCR 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)

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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

Goals for Continued Optimization of Rapid PCR Protocol

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

Commercial DNA Polymerases

A three component DNA polymerase 'cocktail' was found to give improved results for the Identifiler kit (increased 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 STR typing kits and on various thermal cyclers (10 µL PCR reaction volume)

Testing Four Thermal Cyclers

GeneAmp 9700 (Applied Biosystems)

- Heating rate: 4°C/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)

25 µL PCR rxn volume

- Heating rate: 10°C/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

Mastercycler pro (Eppendorf)

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

Rotor-Gene Q (Qiagen)

- Heating rate: 15°C/s
- Heating mechanism: Air chamber (spinning rotor)
- Tube format: 0.2 mL – 72 tube/rotor
- 28 cycles = 36 min

Testing Additional Commercial STR Kits

Yfiler, MiniFiler and Promega S5 STR typing kits were also tested under rapid cycling conditions. Below are examples of their amplification performance on a GeneAmp 9700. Improvements were not observed using the other three thermal cyclers.

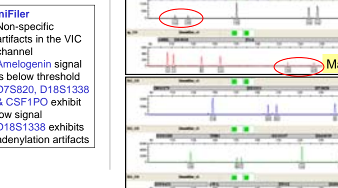
Amplification times were approximately 36 minutes of the GeneAmp 9700 using the 3 polymerase cocktail 10 µL rxn vol, 1 ng of DNA, and 28 cycles (the annealing temperature was set to 1 degree below the prescribed temperature for each STR typing kit)

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 signal 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	Avg Signal	AB 9700 Rank	Avg Signal	SmartCycler Rank	Avg Signal	Mastercycler Rank	Avg Signal	Rotor-Gene Rank	Avg Signal
1	D13S317	0.141	7	0.068	10	0.055	15	0.019	1	0.096
2	D3S1358	0.111	10	0.040	9	0.058	13	0.023	3	0.087
3	TH01	0.104	1	0.168	2	0.110	8	0.069	5	0.068
4	D8S1179	0.083	13	0.027	12	0.048	9	0.057	15	0.040
5	D16S539	0.074	3	0.105	5	0.073	5	0.092	4	0.084
6	D21S11	0.069	15	0.020	16	0.015	12	0.028	14	0.042
7	TPOX	0.055	2	0.108	1	0.113	2	0.113	10	0.061
8	D2S1338	0.051	4	0.099	6	0.064	3	0.102	7	0.062
9	D7S820	0.050	11	0.033	7	0.062	7	0.079	13	0.050
10	D19S433	0.048	16	0.016	15	0.020	14	0.020	16	0.023
11	vWA	0.047	8	0.065	4	0.090	4	0.100	6	0.067
12	D5S818	0.038	14	0.026	13	0.047	11	0.031	8	0.062
13	CSF1PO	0.037	6	0.068	8	0.061	6	0.089	9	0.061
14	D18S51	0.036	5	0.082	3	0.099	1	0.122	2	0.095
15	FGA	0.030	12	0.028	14	0.032	16	0.012	11	0.052
16	AMEL	0.027	9	0.048	11	0.052	10	0.043	12	0.051

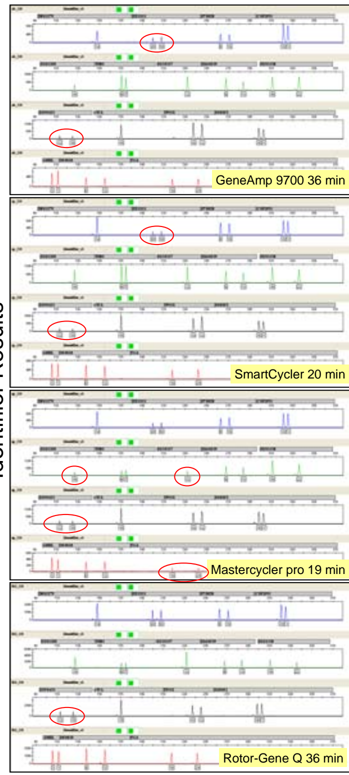
Average heterozygote peak balance was similar for all cyclers > 0.88

*The **avg signal** stated in the table above 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. D19S433 is the weakest locus for the Rotor-Gene Q. Despite interlocus 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

Identifiler Results



Testing Cyclers with PowerPlex 16 (PP16)

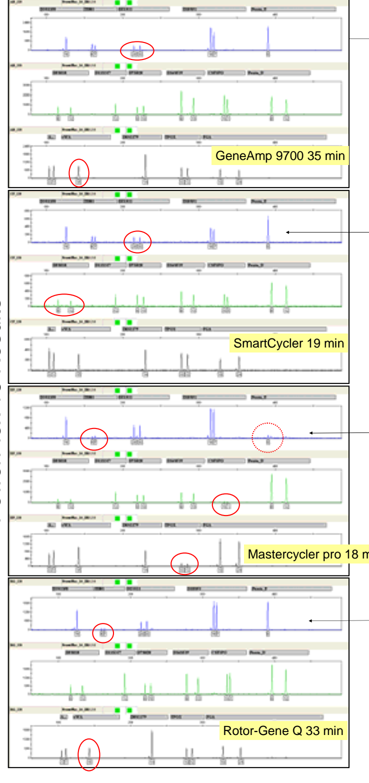
PP16 Rank	Standard Conditions Rank	Avg Signal	AB 9700 Rank	Avg Signal	SmartCycler Rank	Avg Signal	Mastercycler Rank	Avg Signal	Rotor-Gene Rank	Avg Signal
1	vWA	0.033	15	0.031	14	0.031	7	0.058	15	0.027
2	FGA	0.037	11	0.039	6	0.066	2	0.106	9	0.051
3	AMEL	0.040	8	0.051	3	0.080	6	0.068	14	0.033
4	TPOX	0.050	9	0.046	4	0.073	14	0.020	12	0.041
5	D21S11	0.054	16	0.022	16	0.025	10	0.043	13	0.034
6	TH01	0.055	12	0.037	12	0.043	13	0.020	16	0.006
7	D8S1179	0.059	7	0.052	11	0.049	11	0.038	8	0.062
8	Penta_E	0.060	4	0.090	7	0.065	16	0.009	6	0.063
9	D3S1358	0.064	10	0.045	9	0.058	8	0.055	7	0.062
10	Penta_D	0.066	1	0.136	1	0.192	1	0.251	1	0.165
11	D7S820	0.067	6	0.056	8	0.059	5	0.069	5	0.064
12	D13S317	0.072	14	0.032	13	0.034	9	0.053	10	0.049
13	D5S818	0.076	13	0.035	15	0.026	12	0.028	11	0.041
14	D16S539	0.081	3	0.113	2	0.081	4	0.070	3	0.091
15	CSF1PO	0.092	5	0.088	10	0.050	15	0.013	4	0.080
16	D18S51	0.096	2	0.127	5	0.068	3	0.102	2	0.133

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. vWA and TH01 are weak for the Rotor-Gene Q. Penta E proved quite robust on the 9700, SmartCycler and Rotor-Gene Q, 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 94°C for 10 cycles followed by denaturing at 90°C for 18 cycles (annealing temp 59°C)
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)

PowerPlex 16 Results



Conclusion: The 16 loci in each STR 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.

Poster available for download from STRBase:http://www.cstl.nist.gov/biotech/strbase/pub_pres/VallonePromega2009poster.pdf