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 microwell- 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 polymorphisms working in combination with rapid PCR cycling protocols have resulted in the development of a ‘rapid’ PCR amplification protocol. 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 10 minutes for 16 loci. Vallone, P.M., Hill, C.R., Butler, J.M. (2008) Demonstration of rapid multiplex PCR amplification involving 16 genetic loci. P decrypted (31): 42-45.

Goals for Continued Optimization of Rapid PCR Protocol

• Improve inter-plate stability
• D19S433 & D21S11 (for the Identifiler kit) Reduced 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 provide improved results for the Identifiler kit (increased signal for D19S433 & D21S11).

0.6 μM master mix PyroStart (Fermentas) ($0.14 USD/rxn)
0.25 μM forward primer (Tsakara) ($0.22 USD/rxn)
0.25 μL of 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 = 20 min

SmartCycler (Cepheid)

• Heating rate: 15°C/s
• Heating mechanism: Air chamber (pivoting rotor)
• Tube format: 0.1 mL – 72 tube/rotor
• 28 cycles = 36 min

Rotor-Gene Q (Qiagen)

• Heating rate: 15°C/s
• Heating mechanism: Air chamber (pivoting rotor)
• Tube format: 0.1 mL – 72 tube/rotor
• 28 cycles = 36 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

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)

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