Article

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RAPID AMPLIFICATION OF RM-Yplex ASSAY

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Abstract

A multiplex PCR assay consisting of 13 Rapidly Mutating Y STR loci called RM-Yplex was developed. Platinum® Taq DNA polymerase was used to amplify the 13 Y STR loci in a single reaction in approximately 2.5 hours . In order to shorten the process with reliable results, several DNA polymerases were tested with the multiplex.

Phusion® Flash High Fidelity and Platinum® Taq DNA polymerases were investigated for conducting RM-Yplex assay at various PCR cycling conditions. Rapid, robust and efficient amplification of all markers within the multiplex was achieved. The amplification time was reduced from 2.5 hours to less than 28 minutes using Verti® PCR machine.

Keywords

Rapid PCR, Rapidly Mutating Y-STR, STR multiplex, DNA Polymerase

Introduction

Various commercial kits have been developed for DNA profiling to amplify wide range of short tandem repeats (STRs) loci on both sex and autosomal chromosomes. The commercial STR kits mostly have PCRs assays ranging between 1-3 hours. Faster PCR assays are therefore required to shorten the time of analysis which shall reduce the overall casework sample processing times within a forensic laboartory.

Rapid enzymes are high fidelity and their processivity allows them to incorporate nucleotides at a rate of 100 bases/second. The addition of sequence non-specific proteins that bind with the double stranded DNA can also accelerate the delivery of the enzyme to the template [1]. The implementation of enzymes with faster thermal cyclers would produce reliable results rapidly and efficiently. RM-Yplex was developed recently that used Platinum enzyme [2]. This assay had a duration of 2.5 hours therefore a rapid PCR was attempted.

Materials and Methods

The published RM-Yplex assay was used in all experiments [2]. A modified primer pair was further optimized with the primer set of the assay for DYS526a/b marker to resolve the imbalance problem observed within the two (a/b) regions earlier [2]. The conventional amplification was performed in 15 µl reaction volume containing 7 µl 1X Platinum® Multiplex PCR Master Mix (Life Technologies), 1.78 µl of the primer mix and 5.22 µl PCR grade water.

Phusion® Flash High Fidelity DNA polymerase (Thermo Scientific) reactions were performed in 20µl PCR volume, containing 10µl 1X Phusion® Flash Master Mix, 7.46 µl optimized primer mix and 0.54 PCR grade water, using the following cycling conditions:

- 98°C for 10 sec.
- 10 cycles: 98°C for 1 sec, 58°C for 5 sec, 72°C for 15 sec.
- 20 cycles: 98°C for 1 sec, 53°C for 5 sec, 72°C for 15 sec.
- 72°C for 1 min.

Amplifications were conducted on Veriti® thermal cycler (Life Technologies) using 200 µl PCR tubes.

The Taqman™ male DNA control (Life Technologies), two males and a female quantified DNA extracts were used throughout the experiments. The DNA input was kept constant at 1 ng in all PCR reactions.

1 µl of the amplified products were diluted into 8.5 µl of Hi-Di Formamide and 0.5 µl LIZ600 internal size standard (Life Technologies). Samples were then electro-kinetically injected at 1.6 kV for 10 seconds and separated on an 8 capillary ABI 3500 Genetic

Analyser (Life Technologies) using POP-6 Polymer (Life Technologies) on a 50cm capillary array. After data collection, genotyping was performed with GeneMapper® ID-X v1.2 (Life Technologies) using RM-Yplex bins and panels [2] with a threshold of 50 RFU.

Results and Discussion

A significant reduction in amplification time was achieved. The optimized rapid assay using Phusion® Flash High Fidelity DNA polymerase was only 28 minutes long (Fig 1). The results were compared with the conventional assay using Platinum® Taq DNA polymerase (Fig 2). The Phusion® Flash High Fidelity DNA polymerase lacks the 5′-3′exonuclease activity, therefore all the alleles differed in size by 1 bp when compared with the conventional RM-Yplex assay and were called as off ladders. This can be resolved easily by a re-amplification of the allelic ladder using the rapid assay.

Marker DYS403S1a/b exhibited an extra peak within the region DYF403a (Fig1). Therefore a further optimization needs to be held which could possibly require redesigning of the primers for this marker.

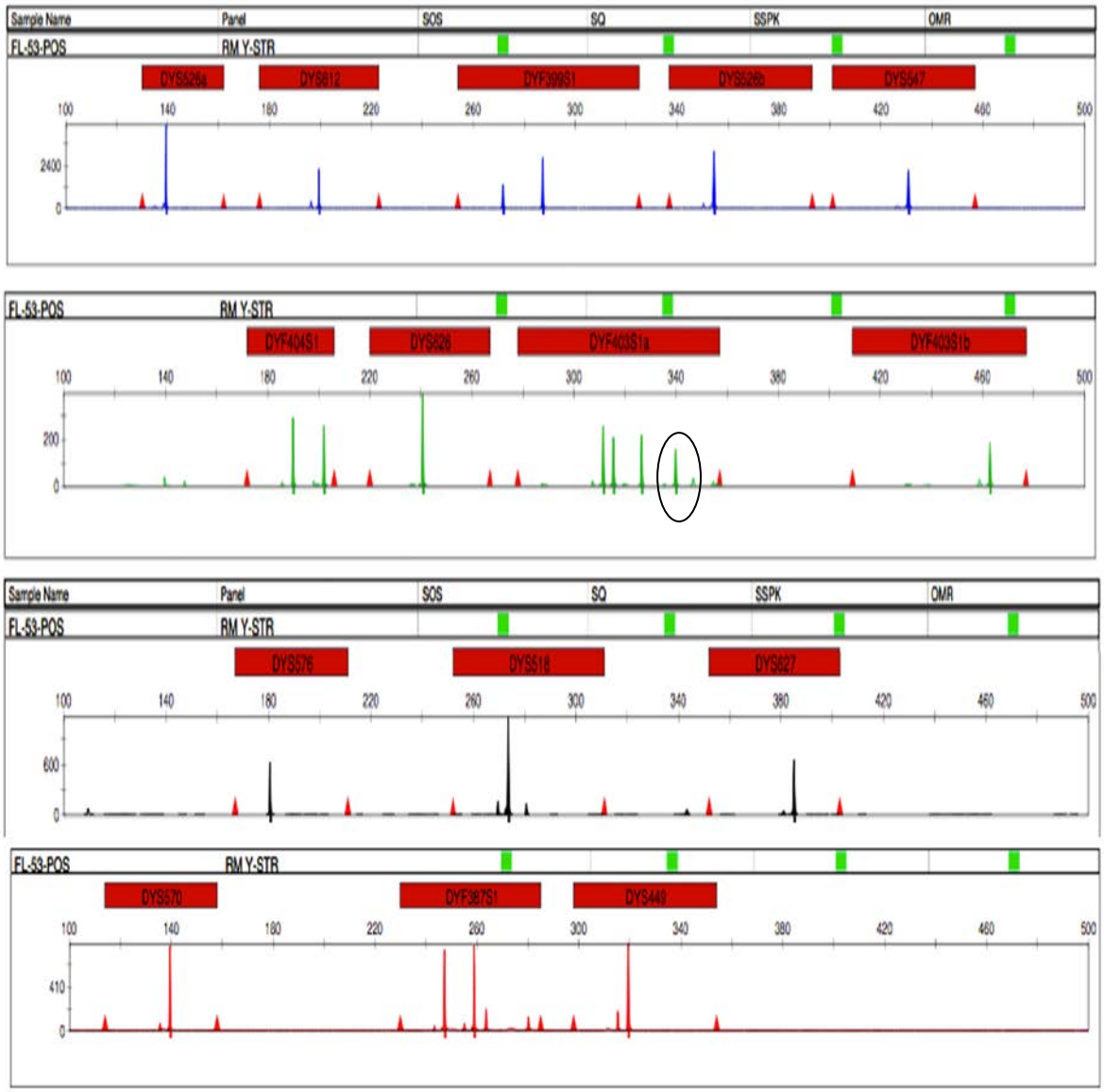

Fig1. Representative electropherogram showing the RM-Yplex profile of 1 ng of Taqman® male DNA control obtained using rapid PCR assay employing Phusion® Flash High Fidelity DNA polymerase . The four panels (top to bottom) represent 6-FAM™, Yakima Yellow ®, ATTO550® and ATTO565® dye-labeled peaks. The circle demonstrates the extra peak in DYS403a marker.

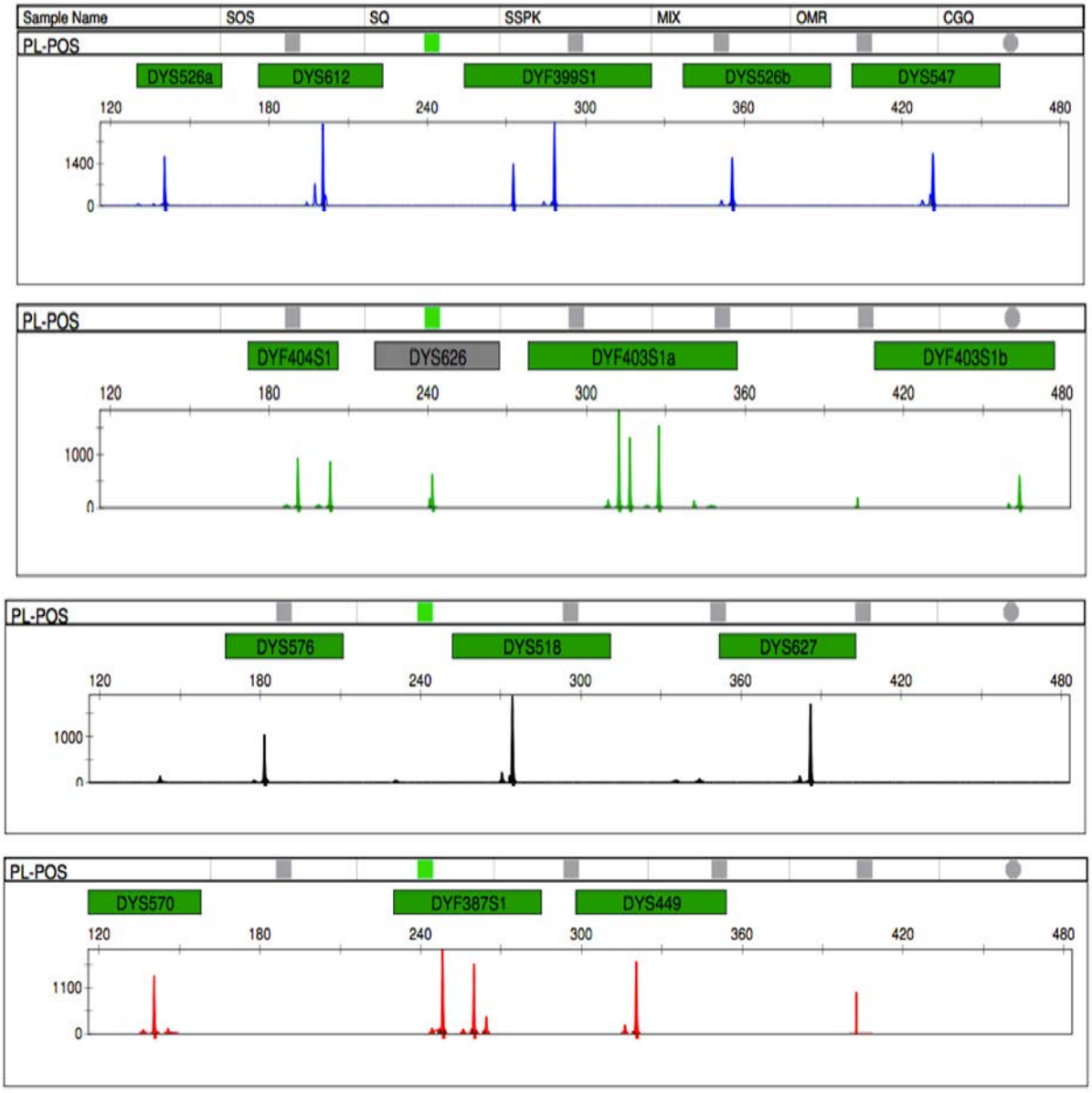

Fig2. Representative electropherogram showing the RM-Yplex profile of 1 ng of Taqman® male DNA control obtained using the conventional PCR employing Platinum® DNA Polymerase with the new primer set for DYS526 a/b marker. The four panels (top to bottom) represent 6-FAM™, Yakima Yellow ®, ATTO550® and ATTO565® dye-labeled peaks.

Despite higher amounts of primers than used in the conventional assay, the assay resulted in more robust amplification without the need to modify other parameters such as cycle numbers, $MgCl_2$ concentrations, buffer, enzyme concentrations or annealing temperatures [3]. Further optimization to minimize the intra-color imbalance obtained with the rapid assay, is possible by altering PCR primer concentrations.

Conclusion

A robust and reliable rapid RM-Yplex assay has been developed. The design, testing and optimization of the multiplex through careful primer titration resulted in a rapid multiplex to amplify 13 RM Y-STR markers in under 28 minutes. It was interesting that time wise, 30 cycles of the rapid assay were equivalent to four cycles of the conventional assay.

The obvious advantage was a significant reduction of time required for completing for the assay, which reduces the overall processing times and generates robust results.

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Conflict of Interest Statement

None

The Role of the Funding source

None

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