DEVELOPMENT OF A RAPID DNA TYPING APPROACH FOR FASTER FORENSIC SUSPECT SCREENING

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Applying DNA extraction-free, faster PCR amplification and electrophoresis technologies, we developed a rapid DNA typing approach for faster forensic analysis. This approach reduces three quarters of current standard forensic DNA typing analysis time, so it is very suitable for faster suspect screening.

Nowadays, forensic DNA typing has become one of the main means of criminal investigation and court trail evidence. With the adoption of DNA typing analysis, it is more and more used in the daily use of suspects screening and conviction. With the urgent need for protecting innocent people and solving criminal case as soon as possible, how to speed up the DNA typing analysis becomes the main research interest.

In collaboration with Wuxi AGCU ScienTech Incorporation, We have developed a rapid DNA-STR typing approach using DNA extraction-free, rapid PCR amplification and faster electrophoresis analysis technologies.

1. Rapid detection kit development.

1.1 Selection of polymorphism STR loci

We chose six STR loci and a Amelogeninin locus which have high power of discrimination, they are: D3S1358, D13S317, D8S1179, D21S11, vWA, D5S818.

1.2 Design and selection of primers

We decided to use multi-PCR amplification system and through the function of BLAST and other species genome data in GENEBANK to eliminate the non-specificity of PCR amplification.

The primers were synthesized and purified by *AGCU ScienTech Incorporation*, using HPLC to test the purity, the value showed above 99%, A_{260}/A_{280} was more than 1.2.

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Seven loci were divided into three groups; the principle of grouping was that there was no interference between primers on the results of specificity during the process of PCR amplification.

panel 1(blue): Amel, D3S1358, D13S317
panel 2(green): D8S1179, D21S11
panel 3(yellow): vWA, D5S818

1.3 Establishment of PCR reaction system

PCR was performed in a 25μl reaction mixture containing 2.5 mM Mg^{2+},0.25 mM dNTPs, 10 mM Tris-HCl, 10 mM TC, 0.008% NaN_3, 0.3 mg/ml DPB, 5.0 U Q-Taq.

The PCR reaction was performed at 94℃ for 1 min for the first cycle and then 28~30 cycles of 98℃ for 5 s, 59℃ for 15 s, 72℃ for 10 s. The final cycle was 10 min at 72℃ and 4℃ forever to ensure complete extension for all PCR reaction.

1.4 Allelic ladders assembly

The peak value of Allele Ladder was no less than 400 RFU. The ratio between minimum and maximum peak value within the same locus was more than 60%. The average peak ratio of different loci within one color was more than 60% and also more than 60% between colors. The value of nonspecific peak can't exceed that of the adjacent allele by 20%.

We prepared six allelic ladders reference materials through PCR amplification, which are D3S1358, D13S317, D8S1179, D21S11, vWA, D5S818.

1.5 Expressmarker 7 STR fluorescence kit assembly

Raw material	Characteristics	Quantity	Quality demand
EX7 Primers(fluorescence primers mixture)	Light pink liquid	1000 μl×1 tube	Be able to amplify objective bands using standard PCR.
EX Reaction Mix(reaction buffer)	Colorless liquid	1000 μl×2 tube	Be able to amplify objective bands using standard PCR.
Reaction Mix B(reaction buffer)	Colorless liquid	300 μl×1 tube	Be able to amplify objective bands using standard PCR.
Q-Taq(hotstart)	Sticky liquid	200 μl×1 tube	Be able to amplify objective bands using standard PCR.
Control DNA(Standard DNA template solution)	Colorless liquid	20 μl×1 tube	0.5ng/μl, ABI3130 genetic analyzer shows correct peak positions of allelic using general PCR，when usage amount is 1μL /25μL, sum of peaks is more than 1000.
sdH_2O(ultrapure water)	Colorless liquid	1500 μl×1 tube	Millipore pure water apparatus shows resistivity no less than 18.2 MΩ·cm, TOC no more than

			5 ppb; pH 6.5～7.3 measured by precise pH test paper.
EX7 Allelic Ladder(allelic genotyping standard solution)	Colorless liquid	25 µl×1 tube	ABI3130 genetic analyzer shows correct peak positions of every allelic and peak value is more than 400.
AGCU Marker SIZ-500 (AGCU internal standard solution)	Colorless liquid	250 µl×1 tube	ABI3130 genetic analyzer shows correct peak positions of every fragments and value is more than 200.

2. Rapid running gel research

An innovative real inter-cross linking polymer network was formed when we performed random copolymerization (acrylamide-N, N- dimethyl acrylamide) in a solution, which contains poly (N, N-dimethyl acrylamide). The resulting new capillary electrophoresis running gel—EX-Q20 possessed both separating effect and self-coating function, in which the traditional capillary electrophoresis running gel could not have.

3. Application of rapid screens

3.1 Sample preparation

FTA paper which absorbs suspect bloodstain sample was punched into a round piece of paper in 1.2 mm diameter by BSD600-DUET punching apparatus (BSD, Australia), then the round papers was put into 96- well plates for rapid PCR amplification directly.

Meanwhile, parallel experiments were performed with a Sinofiler kit on extracted DNA samples using the Chelex method, the kit was produced by AB Corporation.

3.2 PCR condition

PCR was carried out in a 25µl reaction in 10µl EX Reaction mix, 1.5µl Reaction Mix B, 1.0µl Q-Taq, 5µl EX7 Primers and 7.5µl ddH_2O.

The PCR reaction was performed at 94℃ for 1 min for the first cycle and then 30 cycles of 98℃ for 5 s, 59℃ for 15 s, 72℃ for 10 s. The final cycle was 5 min at 72℃ to ensure complete extension for all PCR reaction.

Parallel experiment was performed according to manual provided by the Sinofiler kit.

3.3 Electrophoresis analysis

Mix 0.5µl PCR products or Allelic Ladders of Express marker 7 STR with 0.25µl AGCU Marker SIZ-500 and 9.25µl deionized formamide, 95℃ denature for 3 min, then ice-bath for 3 min, electrophoresis analysis, running gel is EX-Q20, electrophoresis parameter Instrument Protocol is AGCU_E5.

Parallel experiment was performed according to the manual provided by the Sinofiler

kit.

4. Results analysis

4.1 Extraction time

Use direct PCR amplification technology, no time was spent on DNA extraction. While in parallel experiment, DNA extraction took about one hour.

4.2 PCR time

Using Expressmarker 7 kit on an ABI 9700 model PCR thermal cycler, the PCR amplification took 57 minutes; on an ABI Veriti model the amplification needed 35 minutes.

In parallel experiment, on an ABI 9700 model PCR thermal cycler, the PCR amplification took three hours and nine minutes, on an ABI Veriti model needed two hours and fifty minutes.

4.3 Electrophoresis time

Electrophoresis analysis for 16 samples running at one time using POP4 gel took 45 minutes, while using EX-Q20 gel only needed 20 minutes instead.

4.4 Genotype results

In parallel experiment, from a common locus in one sample, the genotype results obtained either from Expressmarker7 or Sinofiler kit were exactly the same.

In conclusion, the established approach of rapid DNA-typing analysis is very suitable for identifying suspects quickly. Since this method only needs 1-1.5 hours from samples to final typing results for 16 samples at one time, which costs just about a quarter of current standard analysis time.