RAPID AND DIRECT IDENTIFICATION OF STR LOCI USING MULTIPLEX FLUORESCENT PCR AMPLIFICATION SYSTEMS

Zhou Huaigu[1], Xia Zhifang[2], Chen Ronghua[1], Tao Li[2], Ping Yuan[1], Zhang Chen[1], Liu Yanan[1], Chen Xin[1], Shao Yihong[2], Zheng Weiguo[2]
[1]Institute of Forensic Science, Shanghai Municipal Public Security Bureau; Key Laboratory of Forensic Evidence and Scene Technology, Ministry of Public Security ; Shanghai Key Laboratory of Crime Scene Evidence, China, 200083;
[2] AGCU ScienTech Incorporation, Wuxi, 214174, China

Since British Genetist Alec Jeffreys proposed DNA finger printing in 1985, Forenic DNA analysis technology has made great progress, on the one hand, the analysis speed has improved greatly, namely from 6-8 weeks to a few hours now ; on the other hand, with the progress of PCR Technology, Fluorescent Labeling and Electrophoresis, broadened the amplification of forensic human identification, improved the reliability of DNA-STR typing. Currently, DNA-STR typing has become one of the most practical and technology-driven technique. DNA- typing results have been accepted routinely and is one of the key evidence resources for court decisions.

DNA-STR Typing Technology, usually includes DNA extraction and purification, PCR amplification and electrophoresis. DNA extraction takes more than one hour, PCR amplifications need 3-4 hours and electrophoresis costs over one hour, so the whole DNA typing process takes 6-8 hours. Hence to minimize the time needed to obtain DNA-typing results has become a hot topic in Forensic Science research. Currently the focuses have been fast DNA extraction and electrophoresis. For fast DNA extraction, there were reports that whole blood could be amplified directly. For STR commercial kits, Promega, ABI and AGCU Scientech Inc. all have direct PCR amplification kits which can amplify bloodstain directly without the need of DNA extraction, and eliminating the DNA extraction process. For faster electrophoresis, the research interest has been on using microfluidic technology. On the hand, there were very limited reports on Faster PCR amplification research.

Current amplification system consists of 14 loci and one sex locus, with all 13 CODIS loci included. This amplification system has been modified to fulfill the requirements of fast and direct STR loci fluorescent multiplex PCR amplification.

Materials and Methods

DNA samples

Regular blood stain papers 2-9 years retained, 10 pieces each, 8 pieces of one year retained regular blood stain paper, total 88 samples.

Instruments

3130XL genetic analyzer (ABI,USA), 9700 PCR Amplifier (ABI, USA), Veriti Amplifier (ABI, USA), Lifepro Amplifier (Bori Sicence Tech, Hangzhou China), BSD600-DUET Paper Hole Puncher (BSD, Australia).

Samples preparations

SpeedMarker STR loci fluorescent multiplex PCR amplification system, BSD600-DUE THole Puncher (Diameter 1.2mm), one piece of punched blood stain paper in each well on a 96-well plate and perform amplification in situ.

IdentifilerKit (ABI, USA) Parallel Tests, BSD600-DUE THole Puncher (Diameter 3.2mm),

two pieces of punched blood stain paper in each well on a 96-well plate, ReadyAmpTM Genomic DNA Purification System (Promega,USA) 200µl, 2% AmpliTaq Gold K 5µl, 56℃incubation, 90 min, 99℃ denature 30min, centrifuge, take supernatant for PCR.

PCR Reactions
PCR reaction volume 25 25µl, include Reaction Mix A 10µl, Reaction Mix B 1.5µl, Primers 5µl, Q-Taq enzyme 1.0µl, 5U/µl), sdH_2O 7.5µl.
Initial denaturing 1 min at 94˚, thermo-cycling, 5 s 98˚, 15 s 59˚, 10 s 72˚, 28 cycles, end extention 5 min, 72˚, stored at 4˚.

Parallel tests using AB Identifiler kits were carried out followed the manufacturer's recommendation.

STR loci identification were achieved using ABI genetic analyzer 3130XL Electrophoresis conditions for the rapid and direct STR loci fluorescent multiplex PCR amplification system: 0.5µl PCR amplified reaction was mixed with Allelic Ladder, 0.25µl AGCU Marker SIZ-500 and 9.25 mL of HiDi Formamide. The samples were heated at 95˚ for 3 min and snap-cooled on ice for 3 min before injection. All the injection and electrophoresis parameters followed Instrument Protocol AGCU_E5.

Results and Discussion
Amplification Time
This rapid and direct STR loci fluorescent multiplex PCR amplification system, the PCR amplification time was achieved in 57 minutes on ABI 9700; 35 minutes on ABI Veriti and 45minutes on Lifepro.

The standard Identifiler kit achieved similar amplification results in 3 hours and 9 minutes on ABI 9700; 2 hours and 50 minutes on ABI Veriti and 2 hours and 59 minutes on Lifepro.

Tying Results
Of 88 samples, with the exception of one failed to get any amplification result, all 87 samples achieved accurate STR tying. Each and every locus showed one peak (homozygous) or two peaks for heterozygous. The RFP of each peak is above 100 and their DNA fragment mobility is within 0.5bp of the corresponding alleic ladder.

Parallel testing results
DNA-STR typing patterns achieved using the rapid and direct STR loci fluorescent multiplex PCR amplification systems, were identical compared with the parallel results from standard Identifiler kits.

Discussion
With rapid progress of globalization, people are able to move around the world quickly and freely, some criminals are able to commit a crime in one country in the morning and then go shopping in another country in the afternoon. To protect citizens' well-being, it becomes more important that rapid detection and identification of criminals be used in deterring, preventing and solving crimes.

This method describes applying a rapid and direct STR loci fluorescent multiplex PCR amplification system to achieve faster human identification. The PCR amplification time was achieved within one hour using ABI 9700, which equals to 30% regular Identifiler amplification time: it was further reduced to about 20% of that time on ABI Veriti and 25% on Lifepro, hence the DNA-STR typing speed was greatly improved.

Our results showed that DNA-STR typing patterns achieved using the rapid and direct STR loci fluorescent multiplex PCR amplification systems were clear and accurate compared with the results from standard Identifiler kits. This further illustrated this rapid and direct STR loci fluorescent multiplex PCR amplification system was designed properly and it fullfills the requirements of forensic science.

With optimization and commercialization of this rapid and direct STR loci fluorescent multiplex PCR amplification system, this could be used as a new and powerful weapon for the forensic community.