

APPLICATION OF DIRECT AMPLIFICATION OF DNA FROM BLOODSTAIN SAMPLES USING PowerPlex®21 SYSTEM

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【Key words】 forensic biological evidence; direct amplification; scene bloodstain samples; DNA

Objective: Evaluate the feasibility of direct amplification of DNA from bloodstain samples using the PowerPlex® 21 System. **Method:** The bloodstain samples were processed by two methods: direct amplification and magnetic separation. The successful rate of two amplification methods was evaluated. **Result:** For bloodstain samples, no significant difference in successful rate was found between the two methods. **Conclusion:** The PowerPlex® 21 System is a 21-locus, fast-amplification STR system with robust and sensitive performance for rapid DNA typing, which can be used to directly amplify casework daily samples.

Bloodstain samples are the most ordinary sample type that forensic laboratories handle daily. As DNA technology continues to play an increasingly important role in criminal investigation, to find a fast and effective way to meet the evolving needs of the human identification community, such as extracting DNA quickly and accurately, shortening processing time, especially when dealing with serious cases, is an important issue forensic laboratories are faced with. Recently, some STR kits, such as Identifiler®Plus^[1], AGCU17+1^[2], Goldeneye 20A^[3], 6+1 STR kit^[4], DNA Typer™ 15 plus^[5], PowerPlex®18D^[6], Identifiler®Direct^[7] and PowerPlex®16HS^[8] were reported as direct amplification kits. Direct amplification of bloodstain samples using the PowerPlex® 21 System has not been verified. Using the PowerPlex® 21 System, a direct amplification feasibility study was carried out on bloodstains from casework, by comparison with amplification of purified DNA using magnetic beads, which may provide effective reference for daily cases.

1 Materials and Methods

1.1 Materials

152 bloodstain samples were chosen randomly from ordinary scene samples, including blood stains from swab, yarn, clothing, crime tools, FTA® card and so on. The samples were stored at room temperature for less than one month.

1.2 Instruments and Reagents

Freedom EVO 100-4(TECAN Corporation, Switzerland); EQ1000 DNA extraction kit (Eastwin Life Sciences, Inc.); AB-9700 PCR Thermal Cycler (ABI Corporation, USA); PowerPlex®21 System (Promega Corporation, USA); ABI-3130xl Genetic Analyzer (ABI Corporation, USA).

1.3 Methods

1.3.1 DNA

Divide samples into two aliquots, one aliquot for direct amplification and another aliquot for magnetic beads-based purification. For magnetic beads-based purification, DNA from bloodstains was extracted by using EQ1000 DNA extraction kit on Tecan Freedom EVO 100/4 Liquid Handler. Direct amplification of DNA from storage card punches: create appropriate disks from storage card samples with a manual punch tool into a 96-well reaction plate. The size of ordinary blood samples was chosen according to the blood quantity of substrate and was generally 2*2.0 mm disks.

1.3.2 DNA Amplification

Amplification of extracted DNA or direct amplification, was performed using the PowerPlex® 21 System on Applied Biosystems GeneAmp PCR System 9700 Thermal Cycler. Amplification extracted DNA reactions contained 2µl Master Mix, 2µl Primer Pair Mix, 2µl template DNA and 4µl ddH₂O generally. The reaction volume of direct-amplification was 10µl, with 2µl of Master Mix, 2µl of Primer Mix, 6µl of ddH₂O and punches instead of template DNA. Add 0.5ng 2800M control DNA as positive control into a reaction well, and pipet 1ul H₂O instead of template DNA as negative control into a reaction well.

1.3.3 Detection of Amplified Products

Add 1.5µl amplified products from PCR mixtures of the two amplification methods into mix of CC5Internal Lane Standard 500 and Hi-Di™ formamide, respectively. Denature the mixtures at 98°C for 5 minutes. Detect these amplified products using Applied Biosystems 3130x/Genetic Analyzer. Analyze data using Genemapper®ID-X software.

2 Results

Complete and sharp DNA profiles with excellent inter-loci balance can be generated by amplifying purified DNA samples and unpurified DNA samples (direct amplification) using the PowerPlex®21 System. There was no allele drop out for the two methods. The results showed excellent concordance when analyzing amplified products from extracted DNA and direct-amplification of DNA (Figure 1 and 2). There is no significant difference in success rate between the two amplification methods.

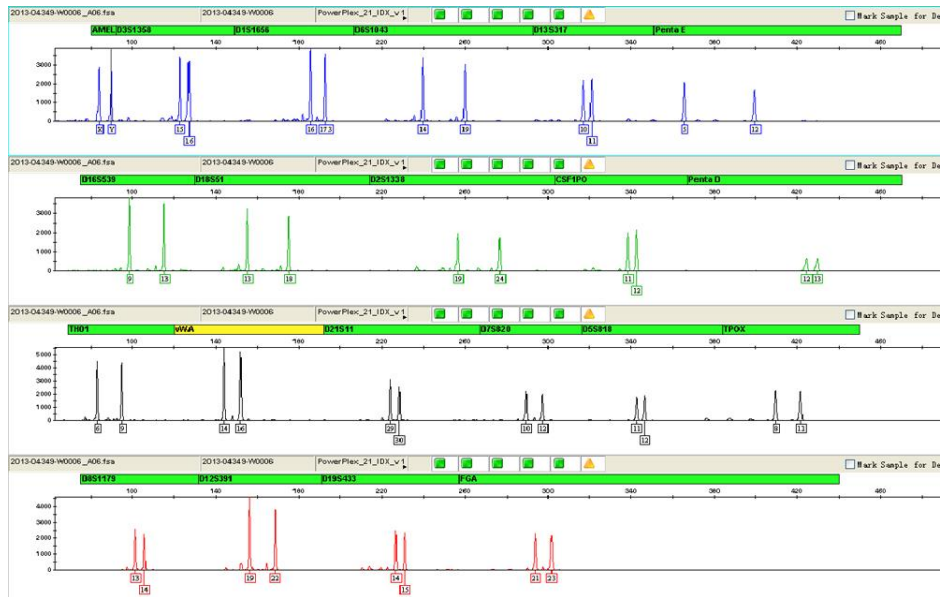


Fig. 1 DNA profiles of magnetic separation

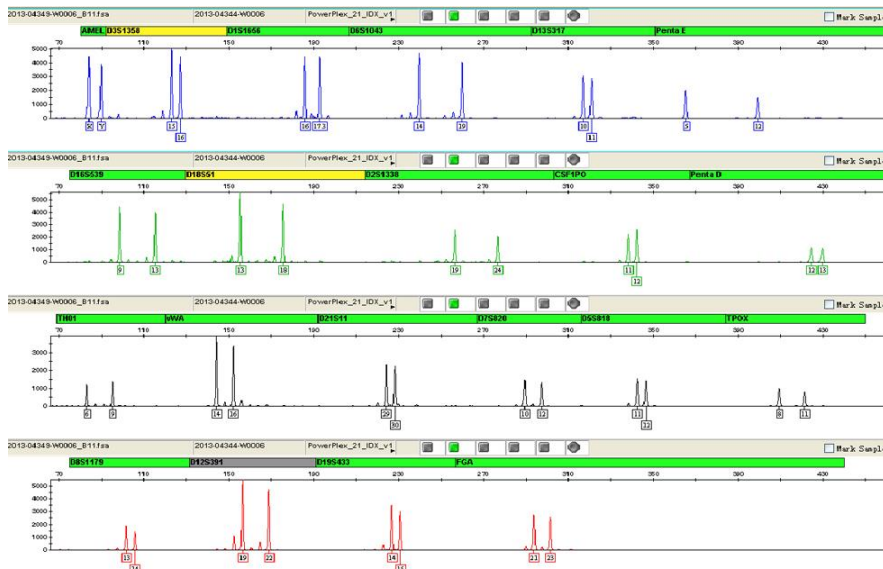


Fig.2 DNA profiles of direct-amplification

3 Discussion

Direct amplification kits can directly amplify unpurified DNA samples, by removing the DNA extraction process. Blood samples chosen in this study were from various substrates, such as blood spot on the weapon or on the ground, yarn, cotton and so on, which accounted for most of all case samples.

This study illustrates the feasibility of direct amplification from scene bloodstain samples using the PowerPlex® 21 System. The additional loci in the PowerPlex® 21 System, compared with other STR kit used in the lab such as PowerPlex® 16 HS System and AmpFLSTR® Identifiler® Direct PCR Amplification Kit, are helpful in increasing system efficiency and ensuring the reliability of the identification results of the caseworks notably. Meanwhile, with the combination of reduced sample

preparation time and rapid amplification cycling technology, the amplification time is successfully reduced to less than 1.5 hours. Additionally, PowerPlex® 21 System has superior inhibitor tolerance of tannic acid, hematin and humic acid. For direct-amplification of DNA, the cycle number is 26; and 28 cycles for amplification extracted DNA. As most of forensic DNA labs in China use 1/2 reaction volume, the direct-amplification quantity of unpurified DNA samples is significantly less than that of purified DNA samples, about 1/4 to 1/5 of that of purified DNA samples. Therefore, the size of ordinary blood samples was chosen according to the type of substrate generally. In a word, the PowerPlex® 21 System can directly amplify ordinary bloodstain samples, by removing the DNA extraction process, and brought down the detection efficiency notably.

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