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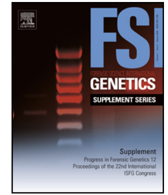

Validation of a multiplex system with 20 multi-Indels for forensic purposes

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ABSTRACT

Multi-Indel polymorphism has been considered as a useful supplementary tool for human identification. Compared with the conventional single Indel marker, it has showed obvious advantage in improving the discrimination power. Previously, we established a novel multiplex system with 20 multi-Indels witch contains 43 single InDel markers. Currently, validation studies were performed with species specificity, sensitivity, mixture study and artificially degraded samples, according to the FBI/SWGDAM guideline. The results showed (a) no fluorescent signal for forensically relevant animals was obtained, (b) complete and accurate profiles were detectable as DNA amount was 0.3 ng, and (c) the reliable results of typing 20 multi-Indels could be obtained from degraded DNA. The mixture of two unrelated individuals in a ratio up to 3:7 could be recognized with all alleles. In conclusion, this study demonstrated that the multiplex system with 20 multi-Indels can be used as a useful supplementary method for human identification and paternity testing.

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1. Introduction

Insertion/deletion polymorphisms (Indels) are length polymorphisms generated by insertions or deletions of one or more nucleotides in the genome. Indels showed potential advantages as low mutation rates, short amplified fragments and the improved application in the analysis of degraded samples [1–3]. However, the discrimination power of Indels is relatively low. Previously, a multiplex system with 20 multi-Indel markers consist of 43 Indel loci from dbSNP database was constructed. In the present work, the validation study of the novel 20 multi-Indel multiplex system was carried out [4]. Compared with the conventional single Indel marker, multi-Indel not only revealed potential advantages in the analysis of degraded samples, but also in improving the discrimination power. In the present work, species specificity, sensitivity, mixture study and artificially degraded samples were performed in accordance with the standards of the Scientific Working Group on DNA Analysis Methods (FBI/SWGDAM) to assess the multiplex system [5].

2. Materials and methods

Blood samples were obtained from 20 unrelated individuals (10 males and 10 females), aged 20–30 years, in Chinese Han population and 7 forensically relevant animal species (dog, cat, chicken, pig, rabbit, rat and duck). Genomic DNA was extracted from samples used the salting-out based method. The DNA concentration was quantitated by the NanoDrop1000 spectrophotometer and analyzed by NanoDrop 2.4.7c software (NanoDrop Technologies Inc., Wilmington, DE). 9947A human control DNA (Promega, USA) was used for detection the sensitivity of the multiplex system, with a series of DNA inputs of 10 ng, 5 ng, 3 ng, 1 ng, 0.5 ng, 0.3 ng and 0.1 ng. Non-human DNA samples from 7 forensically common animal species (10 ng DNA each from dog, cat, chicken, pig, rabbit, rat and duck) were subjected to PCR amplification using the multiplex system, and each species there were three individuals. For mixture studies, mixtures of two individual samples (male 9948 and female 9947A) were examined at various ratios (9:1, 4:1, 3:7, 2:1, 1:1, 0:1) as the total DNA amount of 1 ng, and the tests were performed three times to guarantee the accuracy. The multiplex PCR amplifications were carried out in a GeneAmpPCR systems 9600 (Applied Biosystems, Foster City, CA, USA), in a total volume of 25 μl, included 2 μl DNA template (1 ng/μl), 12.5 ml of Multiplex PCR Mix (Qiagen, German), 0.03–1.50 mM of PCR primers and water. The PCR reaction was performed as following conditions: an initial hot start of 5 min at 95, followed by 28 cycles (94 °C for 30 s, 60.4 °C for 30 s, 72 °C for 30 s); 72 °C for 20 min. With ever PCR amplification, ddH_2O was used as

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negative control. Meanwhile, 1 ng human DNA sample, 9947A, was detected as the positive control. The amplified products were separated detected by a 3130 Genetic Analyzer (Applied Biosystems, USA) according to the manufacturer's instructions. The raw data was analyzed by GeneMapper ID v3.2 software (Applied Biosystems) with the bin-set for the 20 plex [4]. Artificially degraded DNA samples were prepared in a reaction to test stability of the multiplex system, which contained 10 mg DNA, 10 DNase I reaction buffer and sterile water as 200 ml total volume. The DNA was digested for 0 min, 2.5 min, 5 min, 10 min, 20 min, 40 min, 60 min, 90 min, 120 min, 180 min, 270 min and 10 h by DNase I (TaKaRa Biotechnology (Dalian) Co., Ltd.) in the amount of 0.035 U. 13 ml products were removed at each digested time out. To inactivate DNase, the digested products were added 3 ml of 25 mM EDTA and then incubated at 80 °C for 10 min. After digestion, 1 ml degraded DNA sample within DNA fragmentation was detected by agarose electrophoresis (1% agarose, 120 V, 80 min, ethidium bromide detection).

3. Results and discussion

3.1. Species specificity

To validate the ability of the multiplex system detecting DNA from nonhuman species, the forensic relevantly species that may exist in criminal cases were tested. The common feeding animals like dog, cat, chicken, pig, rabbit, rat and duck were used to evaluate the species specificity. No fluorescent signal for nonhuman DNA was observed. The results showed all the loci in the system possessed highly human species specificity.

3.2. Sensitivity

In the forensic routine cases, we always obtain various amounts of DNA samples, which are ordeal the sensitivity of the detection system. To decide the lower limits amount of DNA sample for a full profile (20 multi-Indel alleles) with peak heights above 50 RFU, sensitivity studies of the multiplex system were performed using 9947A that were diluted to from 10 ng to 0.1 ng of DNA were amplified and detected. Reproducibly complete and accurate profiles were obtained when input DNA amount down to 0.3 ng. As the decrease in amount of DNA, partial profiles were observed with alleles dropped out. Hence, the lower limit of DNA template for this system was sensitive to 0.3 ng. The results showed the pull up peak, when the high amount of DNA was put in.

3.3. Mixture study

Mixtures that contain more than one individual sample are very common in forensic casework. To evaluate mixture detection performance, two mixture series at the ratios: 1:0, 9:1, 4:1, 3:7, 1:1, 0:1 were created and distributed. Each mixture was amplified a total quantity of 1 ng DNA for 30 cycles. Most of the loci were detected with all mixture ratios. When at 3:7 ratio of 9947A and 9948, all loci of the mixture were detected and no allele dropped out. As the mixture ratio increased, the total number of alleles detected decreased. Therefore, the lower limit mixture ratio of DNA template for this system was 3:7.

3.4. Degraded DNA study

In many situations in forensic casework, if DNA is exposed to environmental contaminants, as elements or fire, degradation could occur by the oxidative processes, bacterial or biochemical. In order to evaluate the performance of the system in cases of poor quality DNA, a degradation study was carried out. Purified genomic DNA was incubation with DNase I at 37 °C in a time course manner to create a DNA degradation model. After each time of incubation, the average fragment length of DNA was examined on agarose gel. Longer incubation times and further degradation we would obtain smaller average fragments.

The degraded DNA sample at the time point of 180 min and 600 min of incubation were analyzed with the 20 multi-Indels assay, more than 90% of alleles and less than 20% of alleles could be detected, respectively. The data showed that the 20 multi-Indels could obtain reliable results from degraded DNA.

4. Conclusion

In this study, the validation of the 20 multi-Indels was performed with the revised validation guidelines issued according to the FBI/SWGDAM guideline. The multiplex system with 20 multi-indels was sensitive to 0.3 ng of input DNA, suited for the analysis of degraded DNA and enabled the detection of a second DNA source in a sample. Additionally, good performance was acquired in the test of species specificity.

In conclusion, our validation study demonstrated that the 20 multi-Indels assay was proved to be a robust and efficient supplement tool for forensic application as paternity testing and human identification.

Conflict of interest

None.

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