The Potential of HUMACTBP2 (SE33) as a DNA Screening Locus

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Abstract

The viability of using the highly polymorphic DNA locus, HUMACTBP2 (SE33), as a screening locus on heavily bloodstained evidence prior to full profile DNA testing was assessed. Genotyping at the SE33 locus was conducted on 160 Caucasians from the Lehigh Valley region of Pennsylvania. Results showed that the population conformed to Hardy-Weinberg equilibrium and that the locus had an observed heterozygosity of 0.944.

Sensitivity, mixture and degradation studies were conducted. Typing results were obtained with as little as 0.0625 ng of DNA and the minor component of a two-component mixture was genotyped in a 9:1 ratio in one sample. In two degradation studies, bloodstains were applied to different substrates and exposed for 2 months and 1.5 months, respectively, of northeastern Pennsylvania weather. Genotypes were obtained on more than half of the substrates containing bloodstains stored in the ambient environment for at least one month.

INTRODUCTION

Forensic laboratories examining biological evidence are often faced with decisions on which and how many evidentiary samples to perform DNA analysis on. In most instances, laboratories perform testing on the requisite number of genetic markers to generate a full DNA profile. Usually this is the most efficient way of processing a case, but in instances where profuse bleeding from multiple donors is involved, the process can be time consuming, costly, and it may not be necessary to generate a full 16 loci profile for every sample. In these types of cases, it is often necessary to test numerous stains to find the one most probative stain for DNA testing. A faster and more cost effective method would be to simply screen heavily bloodstained evidence with a single DNA locus to determine if the stain is probative and warrants a full DNA profile. The human beta-actin related pseudogene (HUMACTBP2; SE33), a highly polymorphic STR locus, has been identified as a microsatellite that shows potential as a means of screening the probative value of bloodstains ([1]). SE33 is localized on chromosome 6 ([2]), consists of 32 alleles ranging between 227-316 bp including a variety of microvariants, and is comprised of a complex repeat sequence of AAAG ([3]). The comparatively low rate of stutter and high degree of polymorphism ([1]) make it an ideal candidate for forensic applications. SE33 is currently one of five loci included in Germany's DNA database ([4]).

MATERIALS AND METHODS

GENOTYPING OF TEST SAMPLES

DNA was extracted from each sample using 5% Chelex® (Bio-Rad Laboratories, Hercules, CA) ([5]). Quantitation of DNA in each sample was achieved with the QuantiBlot Human DNA Quantitation Kit® (Applied Biosystems, Foster City, CA) ([6]). The HUMACTBP2 (SE33) locus was then amplified using the PowerPlex® ES Monoplex System, SE33 (Promega Corporation, Madison, WI) according to the recommendations of the manufacturer ([7]). The ABI 2700 thermal cycler (Applied Biosystems, Foster City, CA) was used for amplification using AmpliTaq Gold® DNA Polymerase (Applied Biosystems) at an activity of 5u/µl with the following parameters: initial incubation of 95°C for 11 minutes and 96°C for 2 minutes; 10 cycles of 94°C for 1 minute, 60°C for 1 minute, 70°C for 1.5 minutes; 20 cycles of 90°C for 1 minute, 60°C for 1 minute, 70°C for 1.5 minutes; and extension at 60°C for 30 minutes. The amplified products were analyzed with an ABI Prism 310 Genetic Analyzer (Applied Biosystems) and genotyped using GeneMapper® ID version 3.2 software (Applied Biosystems).

DATABASING

Allele frequencies were determined from buccal swabs collected from 160 individuals from the Lehigh Valley section of Pennsylvania and genotyped. Allele frequencies were tabulated and the genotype distribution analyzed for Hardy-Weinberg equilibrium (Table I) using the Arlequin program ([8]). A total of 28 alleles were observed, ranging from 12 to 36 repeat units. No deviation from Hardy-Weinberg equilibrium was observed. Polymorphism information content (PIC), power of discrimination (DP), probability of a match (Pm), power of exclusion (EP), and observed heterozygosity (Ho) were calculated using the POWERSTATS program ([9]) (Table2).

Figure 1

Table 1: Allele frequencies of HUMACTBP2 (SE33) in a Caucasian population from the Lehigh Valley Region of Pennsylvania.

Allele	Frequency (n=160)
12	0.015
14	0.018
15	0.021
16	0.061
17	0.067
18	0.073
18.2	0.003
19	0.067
19.2	0.003
20	0.037
20.2	0.009
21	0.037
21.2	0.003
22	0.006
22.2	0.040
23.2	0.037
24.2	0.037
25.2	0.027
26.2	0.055
27.2	0.082
28.2	0.091
29.2	0.055
30.2	0.043
31.2	0.030
32	0.003
32.2	0.040
33	0.012
33.2	0.006
34	0.012
34.2	0.003
36	0.006
Total HWE*	p>0.05
He	0.945

*Test for Hardy-Weinberg equilibrium
He, expected heterozygosity

Figure 2

Table 2: Population genetics and forensic data of HUMACTBP2 (SE33) from Table 1.

PIC	DP	*Pm*	EP	Ho
0.94	0.989	0.011	0.888	0.944

PIC, polymorphism information content; DP, power of discrimination, *Pm*, probability of match; EP, power of exclusion, *Ho*, observed heterozygosity, Ho, expected heterozygosity

SENSITIVITY AND MIXTURE STUDIES

Samples were amplified at concentrations ranging from 0.03125ng to 5ng in duplicate. The lowest concentration producing reliable typing results was 0.0625ng (one of two samples) and it was determined that 0.5ng was the optimal quantity of DNA for amplification.

Mixture studies were also performed to determine the maximum ratio at which the minor component can be detected. Two sets of two-component DNA mixtures were prepared in the following ratios: 1:19, 1:15, 1:9, 1:4, 1:2, 1:1, 1:4, 1:9. 1:15 and 1:19. In both mixtures, the minor component could not be determined in any mixture with a ratio of 1:15 or 1:19. The minor component was obtained in one of the sets at a 1:9 ratio and in both sets at 1:4, 1:2 and 1:1.

DEGRADATION STUDIES

Two degradation studies were performed. In the first, equal amounts of 10 bloodstains were respectively applied to denim, leather, silk, cotton and polyester blend substrates. The blood was allowed to dry on the substrates at room temperature for 4 days. The bloodstains were then placed outside from February to April in Northeastern Pennsylvania weather, which included rain, snow, sun and wind during this time. Samples were then collected once a month and genotyped to determine the degradation rate of SE33. Results were compared with bloodstains concurrently stored at room temperature.

In a second study, equal amounts of 4 bloodstains were respectively applied to denim, cotton, polyester blend and acrylic substrates and allowed to dry at room temperature for 4 days. The bloodstains were then placed outside from October to December in Northeastern Pennsylvania weather, which included rain, sun and wind. Samples were collected at 1 week and six weeks of exposure and genotyped to determine the degradation rate of SE33. Results were compared with bloodstains concurrently stored at room temperature.

Results from the first study indicated a pattern of increasing degradation with time of environmental exposure. After one month, only 3 substrates (silk, cotton, and leather) produced SE33 genotypes. DNA was not detected on either the denim or polyester blend substrates. After the second month, only bloodstains from the leather substrate produced SE33 genotypes. The silk and cotton substrates yielded no quantifiable DNA. Genotypes were determined from bloodstains on all substrates stored at room temperature for 2 months.

Results from the second study produced similar results. SE33 genotypes were obtained on bloodstains from all substrates at the 1-week collection. However, at the 6- week collection, SE33 genotypes were obtained on only the bloodstains from the acrylic and cotton substrates. No quantifiable DNA was found on the denim and polyester blend substrates after 6 weeks of exposure.

DISCUSSION OF RESULTS

The adherence to Hardy-Weinberg equilibrium and the high values calculated for PIC (0.94), DP (0.989), EP (0.888), and Ho (0.944) and the low value obtained for Pm (0.0.11) demonstrates that SE33 is an ideal locus for a DNA screening procedure. The data were found to be consistent with a previous population study of Caucasians from North America ($_{10}$).

Results from sensitivity, mixture and degradation studies do not negate the potential of SE33 to be used as a screening locus. Genotyping at the SE33 locus was found to perform optimally at a DNA concentration of 0.5ng, which is in the concentration range of most forensic DNA applications. Results from the mixture study indicate that the minor component can be detected at the 10% level (9:1). This percentage is only slightly higher than that stated for other loci ($_{11}$). Other work has indicated that the minor component can be detected at the 5% level, although it depends on the combination of genotypes in the mixture ($_{11}$). Although the size of alleles from the SE33 locus is comparatively high in comparison with certain other STR loci (e.g. VWA, TH01), results from the degradation studies show that SE33 is also adequately stable when exposed to environmental factors. Genotypes were obtained on more than half of the substrates containing bloodstains stored in the ambient environment for one month. This time interval will typically exceed the time in which most physical evidence is collected at a crime scene.

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