Characterization of Human Remains

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Abstract

Hard tissues like bones, teeth, nails, and hair are sometimes the only samples left for study in situations involving missing people and large-scale disasters. It may have been difficult to genotype these remnants in the aftermath of extreme environmental exposure. However, a more recent technology known as Massively Parallel Sequencing (MPS) could improve upon our current techniques by typing different and more markers in a single analysis, and consequently improving the power of discrimination. Short Tandem Repeat analysis (STR) via Capillary Electrophoresis (CE) is still the gold standard for DNA typing. In this study, bone and tooth samples that had been subjected to a variety of DNA insults (cremation, embalming, decomposition, thermal degradation, and fire) were evaluated and sequenced using the Precision ID chemistry and a custom AmpliSeqTM STR and iiSNP panel on the Ion S5TM System, the ForenSeq DNA Signature Prep Kit on the MiSeq FGXTM system, as well as the GlobalFilerTM. The findings showed that conventional CE-based genotyping operated as anticipated, resulting in a partial or complete DNA profile for every sample, and that both sequencing chemistries and platforms were able to recover enough STR and SNP information from the majority of the same difficult samples. Considering the degree of damage to some samples, run measures such as profile completeness and mean read depth delivered good results with each system. For both MPS systems, the majority of sample insults—aside from decomposed—produced around the same amount of alleles. Similar markers resulted in perfect agreement between the two platforms.

Keywords: Massively parallel sequencing • Ion S5™• Missing persons • Human remains • Challenged remains

Introduction

Global issues include cases of missing persons, unidentified human remains, and major extinctions. Many migrants and refugees have lost their lives trying to cross borders or oceans, or have disappeared as a result of human trafficking. Skeletal remains (bone, teeth) are typically the only samples available for DNA analysis when identifying human remains in missing person's investigations. However, due to their biological make-up, environmental exposure (humidity, temperature, UV light, and microbes), DNA damage and/or degradation, the presence of inhibitors, and the potential for contamination or comingled remains, some samples are more difficult to treat than others. Currently, the method most frequently employed to study such remains is the combination of Short Tandem Repeat (STR) locus amplification and Capillary Electrophoresis (CE). The most common usage of STRs is due to their strong discriminating ability. The power of discrimination may be reduced by the possibility that these substantially damaged samples lack sufficient DNA fragment lengths to produce complete CE-based STR profiles [1].

As a result, other techniques and genetic markers are being investigated in case they are more suitable for samples with poor typing abilities. Some degraded samples may still be used for Single Nucleotide Polymorphisms (SNPs) using Massively Parallel Sequencing (MPS). Large sample multiplexing, improved mixture deconvolution, and the simultaneous analysis of various marker types (e.g., Identity-Informative SNPs (iiSNPs), Ancestry-Informative SNPs (aiSNPs), STRs, and Phenotypic-Informative SNPs (piSNPs)) are just a few of the promising features that MPS demonstrates. In comparison to STR analysis alone, the simultaneous use of multiple marker systems (STRs and SNPs) can increase the powers of discriminating and the success of typing with difficult samples. Additionally, MPS can find sequence variations in the amplicons of these markers, sometimes exposing SNPs in STR repeat regions and previously unreported microvariants that were not picked up by CE technology [2].

14 cadavers were obtained from the Applied Anatomical Research Center (AARC) at Sam Houston State University in Huntsville, Texas, for the collection of bone (N=19) and tooth (N=5) samples (33 cm window slices). These samples were subjected to a variety of abuses, including as fire, decomposition, embalming, cremation, and thermal degradation. The remains were thermally deteriorated in an oven at 232°C for 45 minutes, incinerated in a 900°C oven for 2.5 hours, embalmed in 30% glutaraldehyde for 880 days, surface exposed for 12 months–18 months, and then ignited with gasoline in a house (a pretend arson scenario) and burnt until they self-extinguished. As stated in Zeng et al., bone portions were cleaned, chipped, and powdered. With a sterile toothbrush and 10% bleach, teeth were cleaned before being brushed with 70% ethanol and rinsed with DI H_2O. The SPEX CertiPrep 6750 Freezer/Mill Cryogenic Grinder was used to powder the teeth after they had been individually wrapped in big task wipes, lightly smashed with a hammer, and ground. Using a TD technique, three samples of each of the 300 mg bone and teeth powders were extracted. Before the cadavers were subjected to any abuse, reference buccal swabs were collected (burning, de- composition, etc.). The AutoMate ExpressTM Forensic DNA EXtraction System and PrepFiler Express (Thermo Fisher Scientific) were then used to extract the reference swabs' DNA in accordance with the manufacturer's instructions [3].

Thermo Fisher Scientific's GlobalFilerTM PCR Amplification Kit was used on a ProFlexTM 96-well PCR System in accordance with the manufacturer's instructions to undertake PCR amplification of STRs. DNA target input was 0.8 ng, whereas the entire 15 L of extract was amplified for samples with low template concentrations (0.05 ng/L). Thermo Fisher Scientific's 3500TM Genetic Analyzer with POP-4TM polymer and a 36 cm capillary array was used for separation and detection. GeneMapperTM ID-X v. 1.4 and an internal Excel workbook were used to examine the data. Allele peaks were assigned using an analytical threshold of 150 RFUs and a stochastic threshold of 600 RFUs typical peak height (APH). It was determined by adding the peak heights at all of the sample replicates' loci and dividing by the number of replicates. The Average Peak Height Ratios (APHR) were determined by adding the peak height ratios for each locus for the sample replicates and dividing by the number of repetitions. The peak height ratio of a locus was given a value of zero if allele or locus dropout occurred. Utilizing three replicates for each sample, the Standard Deviation (SD) was computed [4].

Discussion

Libraries were created using Primer MiX A and the ForenSeqTM DNA Signature Prep Kit from Verogen, Inc. in San Diego, California, USA, in accordance with the manufacturer's instructions. Primer MiX A focuses on 94 iiSNPs, 24 Y-STRs, 7 X-STRs, and 27 autosomal STRs. Those containing more DNA than 0.2 ng were standardised to 0.2 ng, while samples with less DNA (0.01 ng–0.1 ng) were used neat (5 L maximum input). According to the manufacturer's procedure, normalised sample libraries were pooled in equal volumes together with a positive 2800 M template control

from the ForenSeqTM DNA Signature Prep Kit and a negative control (nuclease-free H_2O). On a MiSeq FGXTM (Illumina, San Diego, CA, USA) device, sequencing was carried out in accordance with the manufacturer's instructions while using the MiSeq FGXTM Reagent Kit (Verogen) [5].

The proportion of present alleles to the total expected alleles was used to calculate the number of reportable alleles. The total number of alleles in each panel was used to calculate the expected number of alleles. 44 alleles were produced in entire female profiles and 46 alleles in full male profiles[6]. To different degrees of profile completeness, all bone and tooth samples amplified with the GlobalFilerTM PCR Amplification Kit produced a STR profile. Across the samples, reportable alleles ranged from 10±3 to full profiles. The samples of the thermally damaged teeth provided complete profiles. The profiles from the embalmed and cremated samples were entirely and almost entirely complete, respectively. Profiles from mock arson burned samples ranged from 27±6 reportable alleles to full profiles [7].

APH varied between 5154 RFUs and 1952 Relative Fluorescence Units (RFUs) and 210578 and 8846 RFUs among all samples. In general, the trend seen with STR profile completeness was consistent with the pattern reported across the sample types when the APH was taken into account [8]. The disintegrated remains provided the lowest APHs, ranging from 5154 1952 RFUs to 50751 ± 21380 RFUs, whereas the thermally damaged samples produced the greatest APHs, ranging from 65684 ± 18897 RFUs to 210578 8846 RFUs. Profile completeness and APH both exhibited a decreasing trend from thermally degraded to decomposed samples, and so did APHRs. All samples' APHRs ranged from 8% to 25% to 87% to 11%, with APHRs below 70% found in little under half (46%) of the samples. The decomposed remnants provided the least balanced profiles, ranging from 8% to 25% to 44% to 42% APHRs, while the thermally damaged teeth ranged from 71% to 19% to 87% to 11%. The total number of alleles that fell out at each locus across all samples was used to calculate allele dropout. Comparing the allelic dropout to a reference sample allowed for this determination. For CE samples, complete profiles were acquired. The thermally deteriorated (five samples) and embalmed samples did not show any allelic dropout, but the cremated sample (one sample) showed one dropout event at the DYS391 locus. The largest percentage of allele dropout occurred in the decomposed samples (six samples), where 48% of alleles were lost. The burned samples (eleven samples) showed 11% allelic dropout. As one might anticipate, as the locus' size increased, so did the number of allelic dropout events [9].

Conclusion

Overall, compared to the CE-based kit, MPS produced more genetic data in 22 samples and generated genetic data from tested samples. Additionally, more alleles will increase the discrimination capacity of the system. For some more challenging samples, CE did produce a usable DNA profile for identification based only on the 20 CODIS core loci, but the greater number of loci included in MPS multiplexes allowed for more genetic information to be obtained from the majority of samples, with the exception of the decomposed remains. The findings indicate that while CE-based approaches were more reliable for detecting skeletal samples, MPS may retrieve more probative information from the majority of samples. While MPS kits for forensic use have only been available for less than five years, CE chemistry has significantly advanced over the past 25 years. Performance could be improved by tweaking the chemistry and design of MPS panels, though.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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