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<AT>A study of degraded skeletal samples using ForenSeq DNA Signature™ Kit

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<ABS-HEAD>Abstract

<ABS-P>Recent advances in massively parallel sequencing (MPS) has become a very promising technology for massive genetic sequencing [1]. In this study Illumina ForenSeq™ DNA Signature Prep Kit was tested to determine if MPS offers a more comprehensive evaluation of degraded samples than the traditional fragment analysis/capillary electrophoresis based method. The Illumina® ForenSeq™ DNA Signature MPS Kit, includes 200 genetic loci [2]. The use of NGS would therefore reduce the analysis time and augment the identification of human remains. In this context we aimed to analyse the hard tissue degraded samples using Illumina® ForenSeq™ DNA Signature MPS Kit. These samples had given partial profiles with dropout at several loci with GlobalFiler™ kit previously. The MPS kit showed that it is highly sensitive, aids in higher allele recovery for STR loci and provides valuable information about biogeographic ancestry, identity and phenotypic features from a single analysis. The work resulted in highly successful amplification and sequencing of 30 degraded bone/teeth samples using MPS method.

<KWD>Keywords: MPS; ForenSeq™; MiSeq FGX; GlobalFiler™; Autosomal STR; Forensic; Qatar.

<H1>1. Introduction

Recent advances in massively parallel sequencing (MPS), provides advantages to analyse DNA from skeletal human remains, particularly old ones due to enhanced sensitivity and as many more markers can be analysed simultaneously. The Illumina® ForenSeq™ DNA Signature Kit, includes autosomal STRs, Y STRs, X STRs, Identity SNPs and Ancestry SNPs [1]. In this work we investigated the performance of the ForenSeq™ DNA Signature kit, Illumina on a set of degraded skeletal DNA samples from Serbia. In the forensic context, the major advantage of these markers is their possible usage with highly degraded DNA as in disaster victim identification and forensic samples. The introduction of massively parallel sequencing (MPS) has alleviated or resolved the limitations associated with capillary electrophoresis based fragment analysis, thereby ushering a new era in forensic casework analysis [1].

<H1>2. Materials and Methods

<H2>2.1 Typing Success on (Bones samples)

Human DNA extracts from different cases were used to assess the degradation using GlobalFiler™ kit [3].Samples included, bone & teeth. All samples were previously extracted using organic solvent extraction method, or on Automate Express using PrepFiler™ kit, and quantified using Quantifiler® Trio kit.

<H2>2.2 GlobalFiler™ PCR Amplification & Capillary Electrophoresis

Reaction setup and thermal cycling were performed according to manufacturer's instructions. Amplified samples were prepared for fragment analysis and capillary electrophoresis by adding 1µl amplified product to 8.7 µl Hi-Di™ Formamide (Thermo Scientific Fisher) and 0.3 µl LIZ-600 size standard (Thermo Scientific Fisher). All samples were analysed on ABI 3500 genetic analyser.

<H2>2.2 ForenSeq DNA Signature Prep Kit (Beta version)

The beta version of the ForenSeq DNA Signature Prep Kit provides PCR primer mixes for the targeted amplification of 58 STRs (i.e., 27 autosomal STRs, 24 Y-STRs, and 7 X-STRs) and 94 identity informative SNPs (iSNPs) with the option to include 56 ancestry informative SNPs (aSNPs) and 22 phenotypic informative SNPs (pSNPs) depending on the primer mix used [2]. The sizes of the targeted amplicons for SNPs range from 64 to 231 base-pairs (bps), and the sizes of the targeted amplicons for STRs range from 61 to 430 bps. A primer mix containing a pair of tagged oligos for each target sequence was mixed with the DNA sample. PCR cycles linked the tags to the copies of each target to form DNA templates consisting of the regions of interest flanked by universal primer sequences. The tags were used to attach indexed adapters, which were then

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amplified using PCR, purified, pooled into a single tube, and then sequenced. This process was done within a single reaction with integrated indexing to support sequencing of 30 samples in a single run including the positive and negative controls. The samples were sequenced on Illumina Miseq FGx.

<H1>3. **Results**

The GlobalFiler™ results for the selected 30 samples showed partial profiles for 22 samples out of which 10 samples had a dropout of 6 or more loci (DI of >6). Despite the degraded nature of samples a high intensity of reads was obtained for all samples. In majority of samples all loci were correctly called, however in the highly degraded samples a few loci dropped out A total of 62.5% of all possible loci were observed to have amplified, of which 80% were <150 bp in fragment size. Of all the markers amplified the highest proportion present were (100%) Phenotypic SNPs, (98.6%) for Autosomal STRs & X-STRs, (98.6%) identity SNPs & Ancestry SNPs (Fig. 1 A-E).

<H1>4. Discussion

For the majority of sequencing work, depth of coverage refers to the average coverage per base across a run, whereas targeted sequencing for forensic applications relies on the actual amplicon or base count for comparison between alleles/loci and for the accurate identification of the present variants. In our study, the depth of coverage for the degraded bone samples found at 95.6% and the range of read depth was 20000 to 4000000 reads, which means that the MPS based analysis were highly sensitive. In this study, for all 30 bone samples, the STR allele recovery using MPS was higher than the GlobalFiler™ kit. Positive and negative controls performed as expected.

Ancestry-informative SNP results were obtained for all ancestry SNP markers for 20 samples, 10 samples had a range of 31 to 54 ancestry SNPS respectively. Using the ancestry-informative SNP data, the major population bio-ancestry bone samples was determined to be Western European Ancestry (86% of samples), followed by Admixed American (7%), and East Asia (7%) since the bone cases were from Belgrade Serbia. The phenotypic SNPs provided blue eye colour predictions and the most likely hair colour was blond or dark blond/brown for majority of the samples in line with the expected phenotypic features[4]. The results of the Haplogroup Predictor online tool (Athey, 2015) for the male bone samples Y-STR haplotypes predicted to belong to haplogroup I2a (50%) followed by E1B1B (18%), G2A (15%), R1b (14%), J2A (2%) and R1A (1%).

<H1>5. Conclusion

In this work Forensic Signature DNA Kit analyses of degraded bone samples indicated that the allele recovery for auSTRs was significantly higher than the GlobalFiler™ kit. All samples sowed concordant results for GlobalFiler™ and ForenSeq™ Kit. MPS data showed concordant and superior quality auSTR profiles. The availability of information about the visible phenotypic traits along with identity & ancestry information and traditional STR data can tremendously enhance the value of the MPS analyses in forensic casework. This work has shown clear advantages of MPS analyses of degraded human remains in terms of the amount of relevant information gained for forensic casework and mass disaster victim identification.

<H1>5. Conflict of Interest

None.

<H1>6. Supplementary Tables and Figures

None

<H1>7. Acknowledgment

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<REF>References

<BIBL>

[1] Børsting, C. and Morling, N.;1; Next generation sequencing and its applications in forensic genetics. *Forensic Sci. Int. Genet.* 2015; <DOI> http://dx.doi.org/10.1016/j.fsigen.2015.02.002</DOI>

[2] Illumina, Inc. Forenseq™ DNA Signature Prep Guide, February 2016 (Part#15049528 Rev. D) http://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/forenseq/forenseq-dna-signature-prep-guide-15049528-d.pdf.

[3] Martín, P., De Simón, L. F., Luque, G., Farfán, M. J. & Alonso, A.;1; 2014. Improving DNA data exchange: Validation studies on a single 6 dye STR kit with 24 loci. *Forensic Science International: Genetics,* 13, 68-78.

[4] Walsh, S., Liu, F., Wollstein, A., Kovatsi, L., Ralf, A., Kosiniak-Kamysz, A., Branicki, W. & Kayser, M.;1; 2013. The HIrisPlex system for simultaneous prediction of hair and eye colour from DNA. *Forensic Science International: Genetics,* 7, 98-115.

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<Figure>Figure 1. (A & B) Results of 30-bone samples run on FGx-MiSeq showing a cluster density of 866 K/mm2. A total 95% of clusters generated run passed filters, which showed that the reaction was highly successful. The average intensity of the reads was 200000. (C) DNA Profile of a degraded bone sample (DI = 13) showing a dropout of 7 auSTR loci using GlobalFiler™ kit. MPS results for various markers from the same sample show high efficacy and enhanced discrimination power of Illumina® ForenSeq™ DNA Signature Prep kit. (D) Phenotype estimation of a Serbian bone sample showing a high prediction for blond hair and blue eyes. The sample was predicted to have an ancestry of Eastern European population correctly. (E) Pie chart showing the Y Haplogroup predictions for the 30 bone and teeth samples using Haplogroup predictor online tool.

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