POWERSEQ™ AUTO/Y SYSTEMS PROTOTYPE: POPULATION DATA, SENSITIVITY AND MIXTURES STUDIES ON MASSIVELY PARALLEL SEQUENCING (MPS) PLATFORM

Rodrigo Moura Neto[1], Rosane Silva[2], Isadora Mello[2], Victor Dias[2], Jonathan King[3], Bruce Budowle[3,4]

[1]Instituto de Biologia

[2]Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro

[3]Center for Human Identification, University of North Texas Health Science Center

[4]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University

Forensic DNA typing relies on length-based separation of PCR products of STRs for routine casework. Massively parallel sequencing (MPS) elucidates an additional level of STR motif variation. Also, MPS may combine different marker types into one single reaction, reducing both the amount of sample used and turn-around-time of analysis. Therefore, MPS methodologies are being considered as an additional tool in forensic genetic casework. The PowerSeq™ Auto/Y System (Promega Corporation), a multiplex forensic kit for MPS, enables analysis of the 22 autosomal STR markers (plus Amelogenin) from the PowerPlex® Fusion 6C kit and 23 Y-STR markers from the PowerPlex® Y23 kit. Population Data were generated (with 0.5 ng input of DNA) from 145 individuals from an admixed sample from Rio de Janeiro, Brazil. For the Sensitivity Studies, 1ng, 0.25ng, 0.0625ng and 0.0156ng of DNA input were analyzed in triplicate. For Mixture Studies, 1 ng of total gDNA from a male and female sample 1 at 1:1, 1:4, 1:9, 1:19 and 1:49 proportions were analyzed in triplicate. All samples were amplified according to the manufacturers' recommended protocol. Library preparation was performed with the TruSeq® PCR-free DNA HT kit. Individual libraries were quantified using PowerSeq™ Quant MS System, barcoded and normalized to 4nM. Pooled libraries were sequenced (2x300bp pair-end) on the Illumina MiSeq®, using MiSeq Reagent kit V3. Raw data (FastQ) were generated for each indexed sample and analyzed using STRaitRazor v2s. The resultant population data showed the greatest increase in Expected Heterozygosity, from length-base to sequence-base, of 16.5% at the D5S818 locus. The overall random match probability decreased from 5.9E-28 to 7.6E-33. The Sensitivity Studies showed that 98% of full autosomal profiles could be obtained from ≈15pg of input DNA. Full Y-STR profiles were detected in all samples even at 0.0156ng of input DNA. The total amount of reads in Read 2 was the same as Read 1, at 0.0156ng, and 85%, at 1ng of DNA input. The Mixtures Studies revealed that a full profile, male or female, at 1:19 dilution, was obtained for both autosomal and Y-STR markers. At a dilution of 1:49, 97% of the alleles of sample Male:Female, and 91% of the alleles of sample Female:Male could be detected for the autosomal markers, for Read 1. For Read 2, the minor contributor alleles also were detected in both Male:Female and Female:Male 1:49 mixtures at 89% and 83% completeness, respectively. Comparison of autosomal allele and Y STR haplotype frequencies were made to U.S. populations.