B198 The Development of a Nuclear Single Nucleotide Polymorphism (SNP) Probe Capture Assay for Massively Parallel Sequencing (MPS) of Degraded and Mixed DNA Samples

Nikhil Bose, BS, 2121 Glacier Drive, Unit 11, Davis, CA 95616; Katie Carlberg, MD, Children's Hospital Oakland Research Institute, 5700 Martin Luther King Junior Way, Oakland, CA 94609; George Sensabaugh, DCrim, University of CA, Berkeley, School of Public Health, 50 University Hall, MC 7360, Berkeley, CA 94720; Henry Erlich, PhD, Children's Hospital Oakland Research Institute, 5700 Martin Luther King Junior Way, Oakland, CA 94609; and Cassandra Calloway, PhD, 5700 Martin Luther King Junior Way, Oakland, CA 94609*

After attending this presentation, attendees will better understand a novel method of enrichment called probe capture that can be used to enrich targeted SNP regions in degraded DNA samples as well as DNA mixtures for MPS.

This presentation will impact the forensic science community by demonstrating that extremely fragmented DNA can be captured and sequenced by an SNP probe capture assay targeting a large number of SNP regions. Further, the SNP probe capture assay designed in this project introduces the combined usage of tri-allelic SNPs, tetra-allelic SNPs, lineage SNPs, and micro-haplotypes, thereby improving the capability of detecting mixtures even if the minor contributor percent is extremely small.

Mini Short Tandem Repeat (miniSTR) primer kits are used for STR analysis when DNA samples are degraded; however, in some cases such as mass disasters and missing persons, the DNA is extremely degraded and miniSTR primer binding sites may not be intact. In such cases, mitochondrial DNA (mtDNA) can be analyzed, but since mtDNA is maternally inherited and the "product rule" is not applicable, it is not as discriminatory as nuclear DNA analysis. SNPs can be viable markers for degraded DNA analysis since the region of interest is only a single base; however, primer extension assays used in conventional SNP typing methods have limited multiplexing capabilities and require intact primer binding sites. Recently, MPS methods have been shown to sequence up to 172 SNPs across multiple samples simultaneously with high read depth per sequence. Also, the method of targeted DNA library preparation using probe capture enrichment has proved successful in enriching extremely degraded mtDNA for MPS without the need of intact primer binding sites. Therefore, the goal of this project was to design and test a probe capture assay targeting forensically relevant nuclear DNA SNPs for MPS of degraded DNA samples and mixed DNA samples.

A total of 451 SNPs were selected in the design and development of this forensic SNP probe capture assay. These SNPs include 136 Identity Informative SNPs, 41 Ancestry Informative SNPs, 24 Phenotypically Informative SNPs, 25 X chromosome SNPs, 81 Y chromosome SNPs, 31 Tri-allelic SNPs, 39 Tetra-allelic SNPs, and 36 Micro-haplotypes. The custom probe panel was developed and tested for read depth, sensitivity, capturing size-selected and degraded DNA, and detecting two-person mixtures at different ratios. The results of the coverage test consisting of 16 samples at 25ng indicated that 448 SNPs out of 451 showed coverage ≥10X in each sample. Three SNPs dropped out consistently and were excluded in later studies. The sensitivity of this capture system was tested by determining the number of SNPs with a read depth of at least 10X for sample DNA amounts ranging from 50ng to 50pg. For amounts of 5ng and greater, the percent of SNPs covered (measure of sensitivity) was 100% for all SNPs, and the correct SNP genotype assignment was at least 99.78%. The percent of SNPs covered and percent of SNPs with correct genotype assignment decreased as the sample amounts reduced from 1ng to 50pg. Next,

the performance of the system was tested with size-selected DNA samples and mock-degraded DNA samples at varying sample amounts. With 0.5ng, size-selected sample DNA ≤75bp obtained coverage for 96.65% of all SNPs, and 95.09% of all SNPs obtained the correct genotype assignment. Mock-degraded samples at 10ng, 1ng, and 0.5ng with an average size of 150bp were sequenced. The coverage results for all SNPs were as follows: 100% for 10ng, 94.87% for 1ng, and 75.67% for 0.5ng. The mixture test contained samples at ratios ranging from 60:40 to 97.5:2.5. The X SNPs, Y SNPs, Tri-allelic SNPs, Tetra-allelic SNPs, and Micro-haplotypes detected 90% of the minor contributor SNPs for ratios between 60:40 and 90:10, and approximately 85% of minor contributor SNPs for ratios 95:5 and 97.5:2.5.

Based on these results, highly fragmented DNA samples can be successfully captured and sequenced for DNA samples fragmented to ≤75bp. Also, minor contributor ratios as low as 2.5% can be detected in mixtures. Therefore, it is expected that this system can be successfully applied to analyze highly degraded DNA obtained from mass disasters and missing person cases as well as mixed DNA samples.

Single Nucleotide Polymorphism, Probe Capture, Massively Parallel Sequencing