B107 Report of Results From an Interlaboratory Study of the HVI/HVII mtDNA Linear Array Assay

Cassandra D. Calloway, MS, Michael Grow, BS, Natahsa Stankiewicz, BS, Jim Chou, BS, Rebecca Reynolds, PhD, and Henry Erlich, PhD, Roche Molecular Systems, 1145 Atlantic Avenue, Alameda,CA*

The participant will gain knowledge of results from an interlaboratory study of the HVI/HVII mtDNA linear array assay.

Over the past several years, the authors have been developing a rapid method of analysis of sequence variation in HVI and HVII utilizing the established technologies of PCR amplification and immobilized probe hybridization. The original linear array for mitochondrial DNA sequence analysis was comprised of 17 sequence-specific oligonucleotide (SSO) probes. To increase the value of this assay, a primer pair for the HVI region was incorporated into the PCR amplification reaction and added 18 additional probes to detect sequence variation in four regions of HVI and at 2 additional heteroplasmy hotspots (16093 and 189). The "C-stretch" probe and the intensity control probe from the original array were removed. The final version of the HVI/HVII linear array assay consists of 2 primer pairs for co-amplification of HVI and HVII PCR products and 33 probes immobilized in 31 lines for detection of sequence variation at 18 positions spanning both hypervariable regions. This assay was sent to 10 laboratories for beta site testing and the results from this testing will be presented here.

The beta study was designed as a 3 part initial training study and followed by independent study whereby up to 1,000 arrays and reagents were provided. For study 1A the participant was provided 11 DNAs and PCR products and asked to amplify the DNAs, compare the yields on a gel to the provided DNAs, and type them with the linear arrays. The goal of this part of the study was to ensure the participant could amplify DNA without introducing any contaminants and successfully type and interpret the arrays. For study 1B the participant was provided 15 DNAs varying in concentration and including some samples with mixtures. The goal of this study was to demonstrate the participant could successfully quantitate the amount of PCR product, dilute the product, or increase the yield by further amplification cycles if necessary, and successfully type and interpret the arrays. This study included several heteroplasmic samples as well as samples which were intentionally mixed to look like contamination in order to be a challenging interpretation test. For study 1C the participant was provided with 2 hairs from a single individual and was asked to extract the hairs, amplify the DNA, and compare the yield and mitotype to a provided extract from the same individual. This study was designed to test the participant's ability to successfully extract hairs without introducing contamination. For further practice, the participant was asked to extract 5 hairs from 2 additional individuals. Results from the initial training studies will be presented as well as data from the independent studies.

mtDNA, HVI/HVII Linear Array, Beta Testing

 ** Presenting Author*