APPLICATIONS OF ION TORRENT PGM™ IN HUMAN IDENTIFICATION

Robert Lagacé, Sharon Chao Wootton, Reina Marie Langit, Walther Parson, Lori Hennessy
Life Technologies, HID, 850 Lincoln Center Drive, Foster City, California 94404, USA

The field of human identification has been dominated by capillary electrophoresis-based (CE) STR fragment analysis. There has also been a minor effort to sequence the hypervariable regions I/II of the mitochondrial genome by CE. The low throughput of CE sequencing makes it difficult to incorporate complex DNA testing into routine procedure for criminal labs. Next-generation DNA sequencing technologies have advanced dramatically in recent years, although the high costs to setup and operate these technologies have slowed adoption by criminal labs. With the recent launch of the Ion Torrent PGM™, applications of more complicated contents can be designed for the forensic community to take advantage of the low cost and high throughput features that the PGM™ provides.

The whole 16 kb mitochondrial genome can be sequenced on one chip on PGM™. If sequenced on CE, 64 separate reactions would be necessary (assuming 500 bp amplicons and forward/reverse sequences). We can simultaneously sequence whole mitochondrial genomes from 25-50 individuals on one 316 chip. It is also possible to combine many currently used STR kits such as Identifiler, YFiler, NGM Select, as well as phenotypic SNPs, autosomal SNPs, Y SNPs, and Indel markers into one testing kit.

To test the feasibility of this idea, we have built an assay system in which we designed 32 PGM™ A fusion adaptors with a short sequence tag made of different combinations of nucleotides attached to the A adaptor (barcode). We have amplified the whole mitochondrial genome with 2 PCRs each yielding overlapping 8-9kb amplicons. The two PCR products were then combined, sheared, and ligated to P1 and A-fusion barcoded adaptors. The PCR products from each individual can then be pooled and sequenced on the PGM on one chip. Additionally, for more compromised samples, we have created a 2 PCR mitochondrial mini amplicon system consisting of 2 multiplexes of 5 primer sets spanning the mitochondrial control region.

To demonstrate feasibilty of the SNP assay, we have constructed a panel of 103 autosomal and 35 Y chromosome SNPs selected from publically available datasets. A single PCR multiplex for ~200 bp amplicons covering the 138 SNP loci has been generated using the AmpliSeq™ Designer pipeline. The PCR products were ligated to P1 and A-fusion barcoded adaptors. Barcoded libraries from 32 individuals were pooled and sequenced on one chip on the PGM™ and compared to reference genotypes. ⌘