A89 Development of a Novel Human Mitochondrial DNA (mtDNA) Amplification Method for Use With Illumina® Next- Generation Sequencing Instrumentation

Brittania J. Bintz, MS, Western Carolina University, 111 Memorial Drive, NSB #231, Cullowhee, NC 28723; Mark R. Wilson, PhD, Western Carolina University, Forensic Science, Department of Chemistry/Physics, Cullowhee, NC 28723; Brandon C. Smith, BS, and Groves B. Dixon, BS, Western Carolina University, 111 Memorial Drive, NSB #231, Cullowhee, NC 28723*

After attending this presentation, attendees will gain understanding of the current methods used for library preparation using the Illumina® next-generation sequencing (NGS) platform. Additionally, attendees will learn of a novel method, developed in our laboratory, which enables researchers to bypass the recommended laborious and costly Illumina® library preparation method. This novel method allows researchers to utilize PCR to generate amplicons that can be sequenced directly. A high-fidelity TaKaRa™ enzyme with proofreading activity is used so that DNA is amplified accurately and efficiently. Data will be presented that illustrates the advantages of using TaKaRa™ versus a traditional polymerase enzyme such as Applied Biosystems® AmpliTaq Gold®.

This presentation will impact the forensic science community in terms of mtDNA sequence analysis. It is apparent that the field is moving in the direction of employing next-generation sequencing technologies for forensic mtDNA analysis. However, these techniques are often expensive, and laborious. A method to increase the efficiency and cost effectiveness of the Illumina® next-generation sequencing workflow, enabling the technology to be used more readily in the forensic laboratory has been developed. Laboratories wishing to adopt this novel method will no longer need to prepare libraries for sequencing by fragmenting gDNA, repairing ends and adding A overhang, ligating adapters and selecting for high quality DNA of interest. This method incorporates Illumina® adapters into the amplicons during PCR. Laboratories that are not quite ready to adopt next-generation sequencing technologies can still benefit from this amplification strategy. The TaKaRa™ high-fidelity enzyme produces significantly higher concentrations of amplicons during PCR of both pristine and compromised sample types than Applied Biosystems® AmpliTaq Gold® DNA polymerase. Use of the TaKaRa™ enzyme may enable laboratories to obtain mtDNA sequence data from difficult samples when AmpliTaq® Gold does not produce results.

Challenging forensic DNA samples, including bones and hair, often contain DNA that is degraded and/or is present in very low amounts. In order to obtain a reliable DNA profile, mitochondrial DNA (mtDNA) analysis is often utilized on these sample types. Studies employing newly emerging DNA sequencing technologies have been designed to interrogate amplified targets down to the single molecule level. While these technologies are capable of producing large quantities of usable sequencing data, they are laborious and peripheral instrumentation can be costly. For example, typical library preparation for the Illumina® GA_{IIx} platform includes DNA fragmentation (often using an expensive Covaris® DNA shearing instrument), end repair and addition of a single A overhang, adapter ligation and DNA selection. Additionally, multiplexing experiments that maximize the use of flow cell space require an expensive Paired-End Module (PEM) fluidics system coupled to the Illumina® $GAII_x$. The novel method was developed using forensically relevant sample types for human mtDNA amplicon generation for single-read DNA sequencing on the Illumina® $GA_{IIx,}$ which enables laboratories to obtain next-generation sequencing data using familiar protocols without the additional instrumentation described above. This method includes traditional PCR amplification of target DNA with TaKaRa™ high-fidelity DNA polymerase with 3' à 5' exonuclease proofreading activity. A high-fidelity enzyme was chosen in order to reduce misincorporation of bases during amplification which may have an impact on NGS sequencing results downstream. Flow cell adapter sequences and multiplexing index tags are included on the 5' end of the mtDNA hypervariable (HV) region-specific primers and are incorporated into the amplicon during PCR. Specifically, the amplification primers were designed with multiplexing tags directly 5' of the target specific primer sequence so that resulting sequences can be parsed by tag using Sequencher® software that contains a specific parsing algorithm. This amplification strategy produces equal or higher concentrations of amplicons than the current protocol employed in forensic laboratories. Further, these amplicons can also be sequenced using Sanger methods without any apparent hindrance from the extended primer sequences. Thus, this method enables forensic laboratories to adopt one mtDNA amplification protocol for multiple downstream sequencing technologies. Additionally, this library preparation proves to be more efficient and more cost effective than methods recommended by Illumina®.

Sequencing, MtDNA, Amplification

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