B30 The Impact of Whole Genome Amplification on Forensic Testing

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The goal of this presentation is to educate investigators and analysts about the applicability of isothermal whole genome amplification methods to obtain usable DNA from previously inaccessible samples. Data will be presented on getting usable STR profiles from compromised DNA.

This presentation will impact the forensic community and/or humanity by outlining the adoption of whole genome amplification methods into standard forensic investigation process. Data will be presented.

Introduction: The availability of DNA from trace samples can be limited. A reliable technology capable of accurate replication of high-quality genomic DNA from limited samples enabling reliable STR typing is required. The replicated DNA must be identical to the original genomic DNA template and ideally replication of genomic DNA should also be possible directly from the forensic sample without extraction of DNA.

Much effort has been invested by forensic scientists in the optimization of PCR-based generic amplification methods for genomic DNA amplification such as degenerate oligonucleotide-primed PCR (DOP-PCR) or primer extension preamplification (PEP). However, these PCR-based methods generate non-specific amplification artifacts and give incomplete coverage of loci.

The use of QIAGEN's REPLI-g technology for forensic identity testing, utilizing a novel method for whole genome amplification (WGA) termed multiple displacement amplification (MDA) is described. This technique is capable of accurate in vitro DNA replication of whole genomes, without sequence bias, yielding DNA suitable for direct use in STR typing.

Normalized yields from a variety of samples: Various samples can be used in a REPLI-g MDA reaction, including purified genomic DNA and fresh or dried blood. Typical DNA yields from a REPLI-g Mini Kit in vitro DNA replication reaction are approximately 10 ìg per 50 ìl reaction. A uniform concentration of amplified DNA is usually achieved regardless of the quantity of template DNA (Figure 2). Obtaining uniform DNA yields from varying template concentrations is particularly important for STR typing applications, enabling subsequent analysis without the need to measure or adjust DNA concentration. The average product length is typically greater than 10 kb, with a range between 2 kb and 100 kb (Figure 3).

Whole Genome Amplification (WGA) of human mitochondrial DNA: A single mitochondrion contains several copies of mtDNA and each cell in the human body contains hundreds to thousands of mitochondria. This effectively means that there are hundreds to thousands of mtDNA copies in a human cell compared to just 2 copies of nuclear DNA located in that same cell. Thus, forensic scientists make use of enhanced sensitivity by characterization of mtDNA in situations where nuclear DNA is significantly degraded, or present in very small quantities.

In situations where a reference sample cannot be obtained (e.g., from a long deceased or missing individual), a mtDNA reference sample can be obtained from any maternal relation.

The newly developed REPLI-mt Kit contains DNA polymerase, buffers, and reagents for whole genome amplification from small samples of human mitochondria genome using multiple displacement amplification (MDA). The technology allows uniform amplification of the whole mitochondrial genome with minimal nuclear DNA contamination. Typical DNA yields are approximately 5 µg per 50 µl reaction.

Method: The REPLI-g MDA method is an isothermal genome amplification utilizing a uniquely processive DNA polymerase capable of replicating 100 kb without dissociating from the genomic DNA template (Figure 1). The DNA polymerase has a 3 >_ 5 exonuclease proofreading activity to maintain high fidelity during replication and is used in the presence of exonuclease-resistant primers to achieve high yields of DNA product. In addition, the REPLI-g amplification enzyme is significantly more tolerant against inhibition than Taq DNA polymerase.

Two novel technologies have been developed for the amplification of highly compromised material and for specific amplification and enrichment of human mitochondrial DNA. Genomic DNA becomes damaged on exposure to the environment, as is often the case with crime scene samples. mtDNA analysis is applied in forensic science to enhance sensitivity in situations where nuclear DNA is significantly degraded or where insufficient quantity is available. The application of these new WGA techniques will further enhance the usefulness of WGA to forensic sciences.

Minimum sequence bias: PCR-based methods (e.g., DOP-PCR and PEP) generate nonspecific amplification artifacts, give incomplete coverage of loci, and generate DNA fragments significantly less than 1 kb long that will lead to reduced amplification success and poor results when using large STR amplicons. In contrast, REPLI-g provides highly uniform amplification across the entire genome, with no sequence bias.

Amplification of fragmented or damaged DNA

Biological samples exposed to the environment often yield compromised DNA. The degree of DNA damage depends on a number of factors, including environmental conditions such as UV irradiation, pH, and the method of sample processing prior to DNA isolation (e.g., homogenization). Different types of DNA damage can occur; the most prominent being chemical or enzyme-induced fragmentation.

The novel REPLI-g damaged DNA technology allows whole genome amplification of fragmented or otherwise damaged DNA previously isolated from biological samples. It enables highly uniform amplification

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across the entire genome (depending of the degree of damaging) and is suitable for use with DNA >_ 300 bp in size. REPLI- g damaged DNA amplification is a two step process: an initial processing reaction preparing the damaged DNA for whole genome amplification and a second amplification reaction.

Whole Genome Amplification, Low Copy Number, Casework Evidence

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