The impact of whole genome amplification on forensic testing

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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.

Here we describe the use of QIAGEN's REPLI-g technology for forensic identitiy testing, utilizing a novel method for whole genome amplification (WGA) termed multiple displacement amplification (MDA). 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.

Very small amounts of sample
(e.g., forensic trace samples)

→

WGA
"Immortalization"
Create more sample DNA

→

"Unlimited" Analyses
(e.g., STR typing, sequencing, SNP genotyping, clinical studies)

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.

Schematic Representation of REPLI-g Amplification

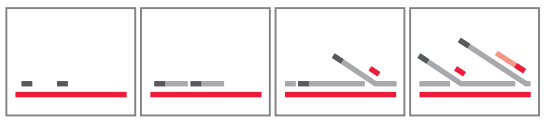


Figure 1 Phi 29 DNA polymerase moves along the DNA template strand displacing the complementary strand. The displaced strand becomes a template for replication allowing high yields of high-molecular-weight DNA to be generated.

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.

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 µg per 50 µl 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).

Uniform DNA Yield from Various Amounts of Template

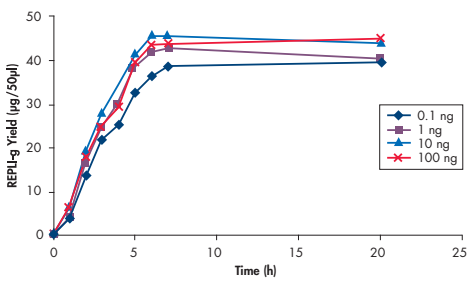


Figure 2 Various amounts of human genomic DNA were amplified in a standard REPLI-g Midi reaction and aliquots taken at the indicated timepoints. The yield of amplified DNA from a 50 µl reaction was approximately 40 µg, regardless of the amount of starting material.

High-Molecular-Weight DNA

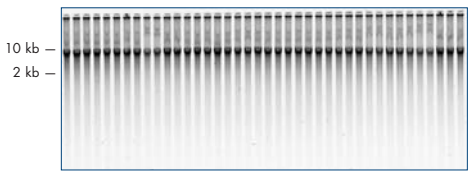


Figure 3 Agarose gel (1%) electrophoresis of 40 DNA samples amplified using REPLI-g whole genome amplification and visualized by SYBR® Green Staining.

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.

Highly Representative Amplification Using REPLI-g Technology

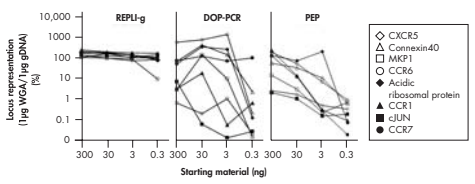


Figure 4 The relative representation of eight loci was determined using real-time quantitative PCR for DNA amplified using REPLI-g technology, DOP-PCR, and PEP. Locus representation was determined by comparison to 1 µg of unamplified control DNA. © 2002 National Academy of Sciences, U.S.A.

STR Analysis of Amplified DNA

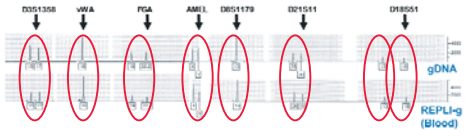
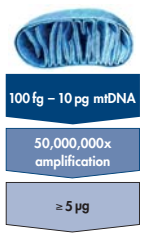


Figure 5 DNA from human whole blood purified using the QIAamp DNA Blood Mini Kit was either directly used in an AmpFLSTR® Profiler Plus™ STR assay or amplified using the REPLI-g Midi Kit prior to direct (without prior cleanup) STR analysis. The REPLI-g amplified DNA showed exactly the same STR profile as the purified genomic DNA with full profiles obtained for both DNA samples.

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.

Highly Efficient mtDNA Amplification

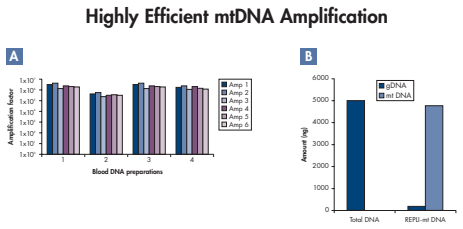


Figure 6 A The relative amplification of mitochondrial DNA amplified using the REPLI-mt Kit in 4 different blood spot samples was determined using 6 different real-time, quantitative PCR assays. B The relative representation of nuclear genomic DNA versus mitochondrial DNA in a sample prior to and after amplification using the REPLI-mt Kit. Typical unamplified DNA preparations comprise approximately 0.1 % of mtDNA (i.e., 99.9 % nuclear DNA).

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 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.

Improved Amplification of Damaged DNA Using the REPLI-g Damaged DNA Protocol

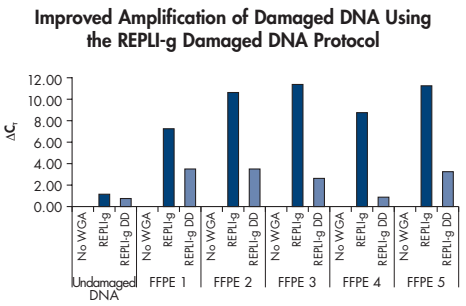


Figure 7 Comparison of unamplified DNA (**No WGA**) with DNA amplified using either the standard REPLI-g protocol (**REPLI-g**) and the optimized protocol for damaged DNA (**REPLI-g DD**). The amplification success of 5 different formaline-fixed parafin embedded tissue samples (FFPE) relative to undamaged reference DNA was determined using quantitative, real-time PCR. For all damaged DNA samples a significant increase in sensitivty due to whole genome amplification could be shown. Note: A ΔC_T of 11 represents a C_T value of 40 (i.e., unamplifyable DNA).

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