REPLI-g® Mini and Midi Kits

For highly uniform whole genome amplification from small or precious samples

REPLI-g Kits provide highly uniform whole genome amplification from a variety of small or precious sample types. The simple and robust method is capable of accurate amplification of genomes, and generates DNA suitable for direct use in a range of genotyping assays. The unique REPLI-g Mini and Midi Kit chemistry enables the same protocol and reaction volumes to be used with each kit, enabling easy reaction scale up or down depending on your specific needs.

Benefits of REPLI-g Mini and Midi Kits include:

- Advanced technology — easy and fast reaction setup
- Reproducible amplification — from a variety of starting materials
- More data from your precious samples — amplify sufficient DNA for all future research
- Robust and reliable protocol — for consistent results you can trust

High fidelity whole genome amplification

REPLI-g technology provides highly uniform DNA amplification across the entire genome, with minimal sequence bias (1). The method is based on Multiple Displacement Amplification (MDA) technology, which carries out isothermal genome amplification utilizing a uniquely processive DNA polymerase capable of replicating up to 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.

Schematic Representation of REPLI-g Amplification

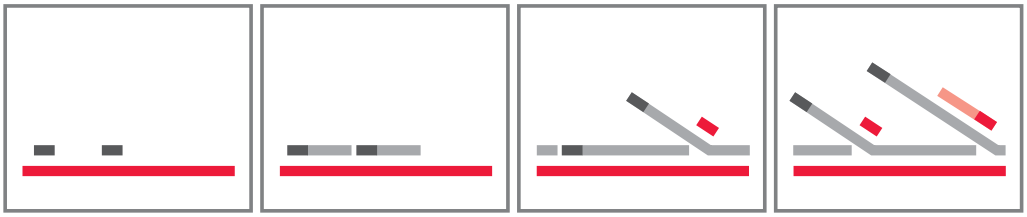

Figure 1 Phi 29 DNA polymerase moves along the DNA template strand displacing the complementary strand. The displaced strand becomes a template for replication allowing high yields of high-molecular-weight DNA to be generated.

High yields from a variety of samples

Various clinical and non-clinical research samples can be used, including genomic DNA, fresh or dried blood, fresh or frozen tissue, and cells. Heavily degraded genomic DNA may not serve as a good template for amplification. Typical DNA yields per 50 µl reaction are up to 10 µg (Mini Kit) and 40 µg (Midi Kit) (Figure 2). A uniform yield of amplified DNA is usually achieved regardless

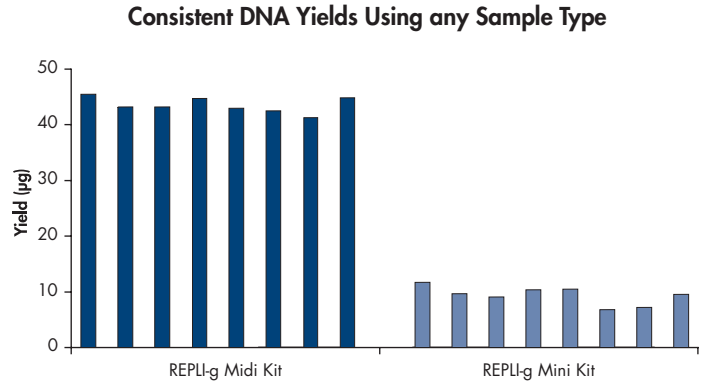

Figure 2 Various starting materials including genomic DNA, and heparin- and EDTA-preserved whole blood were amplified using REPLI-g Midi and Mini Kits. Typical yields of 40 µg (Midi Kit) and 8–10 µg (Mini Kit) were obtained.

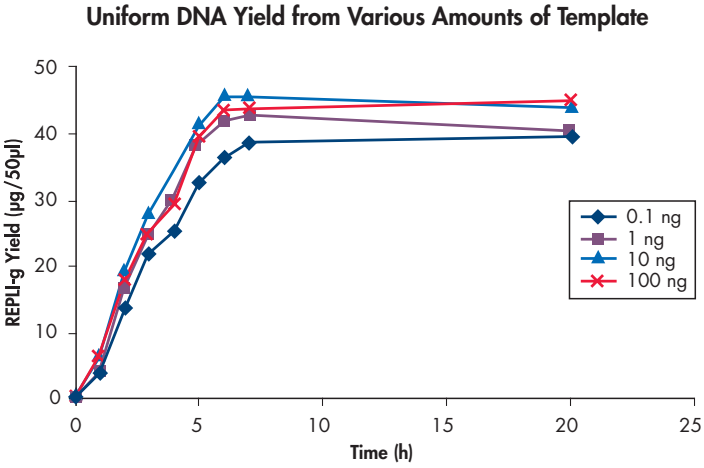

Figure 3 Various amounts of human genomic DNA were amplified in a standard REPLI-g Midi Kit reaction and aliquots taken at the indicated timepoints. The yield of amplified DNA from a 50 µl reaction was approximately 40 µg, regardless of the amount of starting material.

of the quantity of template DNA (Figure 3). Obtaining uniform DNA yields from varying template concentrations is particularly important for high-throughput applications, enabling subsequent genetic 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. The length of the REPLI-g amplified DNA allows complex restriction enzyme analysis and long-range PCR to be carried out.

Highly representative amplification

Traditional methods of genomic DNA amplification include the time-consuming process of creating EBV-transformed cell lines followed by whole genome amplification using random or degenerate oligonucleotide-primed PCR. However, PCR-based methods (e.g., DOP-PCR and PEP) can generate nonspecific amplification artifacts, give incomplete coverage of loci, and generate DNA less than 1 kb long that cannot be used in many downstream applications (2). In contrast, REPLI-g provides highly uniform amplification across the entire genome, with minimal locus bias (Figures 4 and 5).

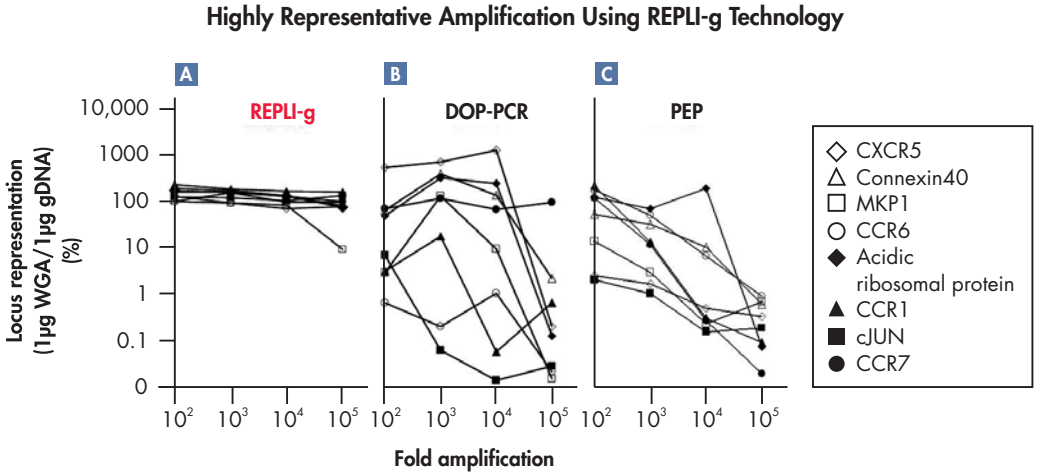

Figure 4 The relative representation of eight loci was determined using real-time quantitative PCR for DNA amplified using A REPLI-g technology B DOP-PCR and C PEP. Locus representation was determined by comparison to 1 µg of unamplified control DNA (2). © 2002 National Academy of Sciences, U.S.A.

Simple one-tube procedure

The simple and robust method is capable of accurate genome amplification from small quantities of isolated target genomic DNA, or directly from whole blood, dried blood cards, buffy coat, and tissue culture cells.

The sample material is lysed and the DNA is denatured by adding lysis buffer. This gentle alkaline denaturation results in uniform DNA denaturation enabling amplification across the whole genome with minimal sequence bias. In contrast, heat denaturation can damage the template DNA, resulting in lower and less consistent locus representation (Figure 6). After neutralization, a master mix (including REPLI-g DNA Polymerase) is added and the isothermal amplification reaction proceeds overnight at 30°C.

Applications

REPLI-g amplified genomic can be used in a variety of downstream applications, including:

- SNP (Figure 7) and STR (Figure 8) genotyping analysis
- RFLP and Southern blot analysis
- Comparative genome hybridization (1, 2)

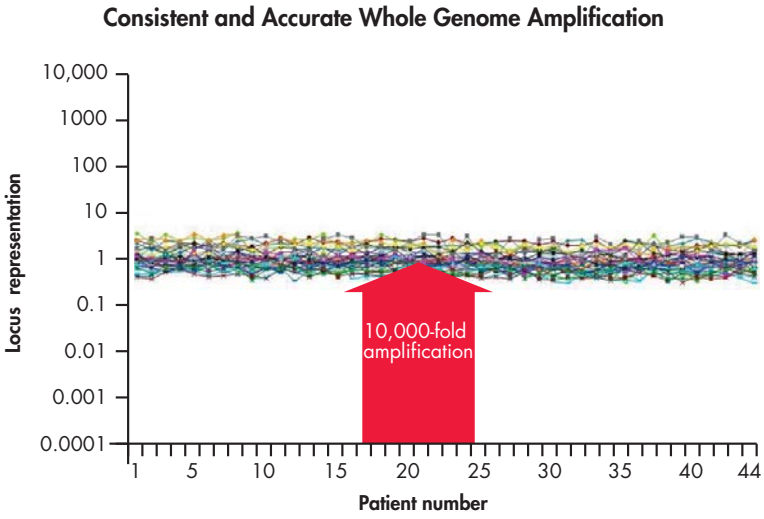

Figure 5 Real-time PCR was performed on 47 human loci (2 loci on each autosomal pair, 2 loci on the X chromosome[s], and 1 locus on the Y chromosome) from 44 different samples amplified using REPLI-g technology. Each sample was amplified approximately 10,000-fold with a maximum bias of representation between the loci being only 6-fold.

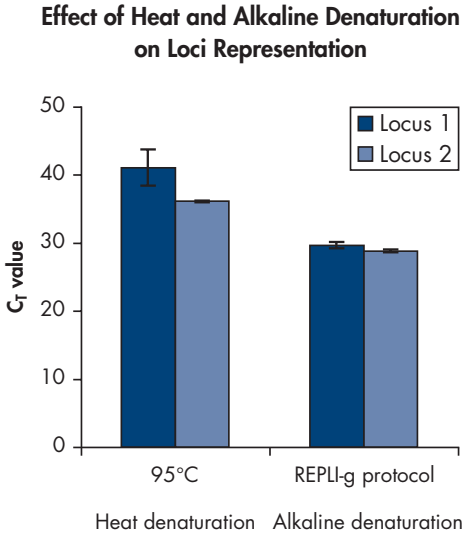

Figure 6 Genomic DNA samples (10 ng) were denatured using heat (95°C) or the standard REPLI-g Kit alkaline lysis protocol. After amplification using REPLI-g DNA Polymerase the C_T values of 2 loci were compared between samples. The low C_T values of loci amplified using the REPLI-g Kit alkaline lysis protocol indicate better locus representation, meaning there has been no loss of sequence information at these loci.

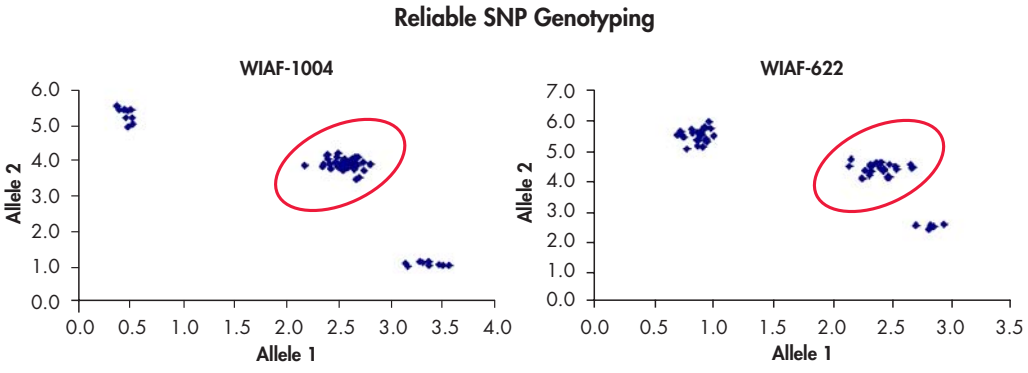

Figure 7 DNA amplified using REPLI-g technology, without subsequent purification, was subjected to SNP genotyping at 2 randomly selected loci (WIAF-1004 and WIAF-622) using a TaqMan® analysis. Tight clusters of alleles allow reliable determination of genotyping of homo- and heterozygote genotypes.

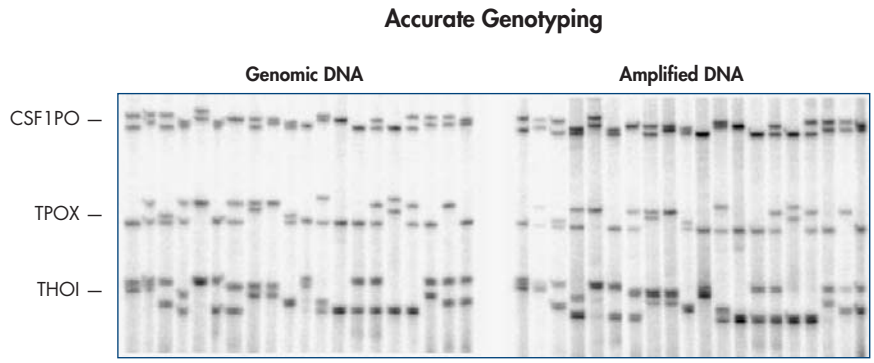

Figure 8 20 DNA samples amplified using REPLI-g technology, without subsequent DNA purification were subjected to genotyping analysis using 3 STR loci (CSF1PO, TPOX, and THOI). Results were compared to those obtained for unamplified genomic DNA. The DNA was separated by polyacrylamide gel electrophoresis and visualized by silver staining. A lane with one band represents a homozygote, while a lane with two bands represents a heterozygote for the specific STR locus.

References

1. Hosono, S. et al. (2003) Unbiased whole-genome amplification directly from clinical samples. Genome Res. **13**, 954.

2. Dean, F.B. et al. (2002) Comprehensive human genome amplification using multiple displacement amplification. Proc. Natl. Acad. Sci. USA **99**, 5261.

Ordering Information

Product	Contents	Cat. no.
REPLI-g Mini Kit (25)	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl whole genome amplification reactions	150023
REPLI-g Mini Kit (100)	DNA Polymerase, Buffers, and Reagents for 100 x 50 µl whole genome amplification reactions	150025
REPLI-g Midi Kit (25)	DNA Polymerase, Buffers, and Reagents for 25 x 50 µl whole genome amplification reactions	150043
REPLI-g Midi Kit (100)	DNA Polymerase, Buffers, and Reagents for 100 x 50 µl whole genome amplification reactions	150045
REPLI-g Human Control Kit (25)	Human control DNA for 25 x 50 µl whole genome amplification reactions	150090

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