

Tips for Optimal Primer Design for Amplification of Non-Human Mitochondrial DNA using the REPLI-g® Mitochondrial DNA Kit

The REPLI-g Mitochondrial DNA Kit can be used for amplifying mitochondrial DNA from humans as well as other species. To enable this, the REPLI-g mt Reaction Buffer no longer contains human primers. Instead, human primers are provided in a separate tube labeled "REPLI-g Human mt Primer Mix". When the kit is used to amplify mitochondrial DNA from other species than humans, users can substitute the REPLI-g Human mt Primer Mix with an appropriate primer mix of their choice.

The efficiency and specificity of amplification is, in part, dictated by the sequence of the primers. The success of the amplification reaction using the REPLI-g Mitochondrial DNA Kit is greatly enhanced when the primers used for amplification are designed according to the following rules:

- Select 10 to 20 different primers from the mitochondrial genome of interest.
- Ensure that half of the primers hybridize to one strand, while the second half hybridizes to the complementary strand.
- Select an almost uniform distance between primer hybridization sites within the mitochondrial genome.
- Use primers that are 8 to 14 nt long.
- Use a phosphorothioate (PTO) backbone at the 3' end of the primers. We recommend including phosphorothioate modifications between the last 3 bases of the 3' end of the primers: 5'-N-N-N-PTO-N*N*N-3'.
- Ensure that the concentration of primers is optimized to be in the range of 1 to 10 μ M.

Please be aware that for other REPLI-g products the primers are included in the master mix and cannot easily be exchanged.

User-Developed Protocol

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor. Safety data sheets (SDS) for any QIAGEN product can be downloaded from www.qiagen.com/safety.

The REPLI-g Mitochondrial DNA Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

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