Overview of Whole Genome Amplification (WGA) with Qiagen REPLI-g kits & protocol for WGA using the REPLI-g ultra fast kit.

- *If you want to skip the intro and read directly the protocol that I used, go to page 4. It is worth though to have a look at least at the second figure, in particular at the right scheme since it's about the ultra-fast procedure.*

Here is a brief overview of the general procedure, taken from the Qiagen web-site:

Whole Genome Amplification (WGA)

REPLI-g principle

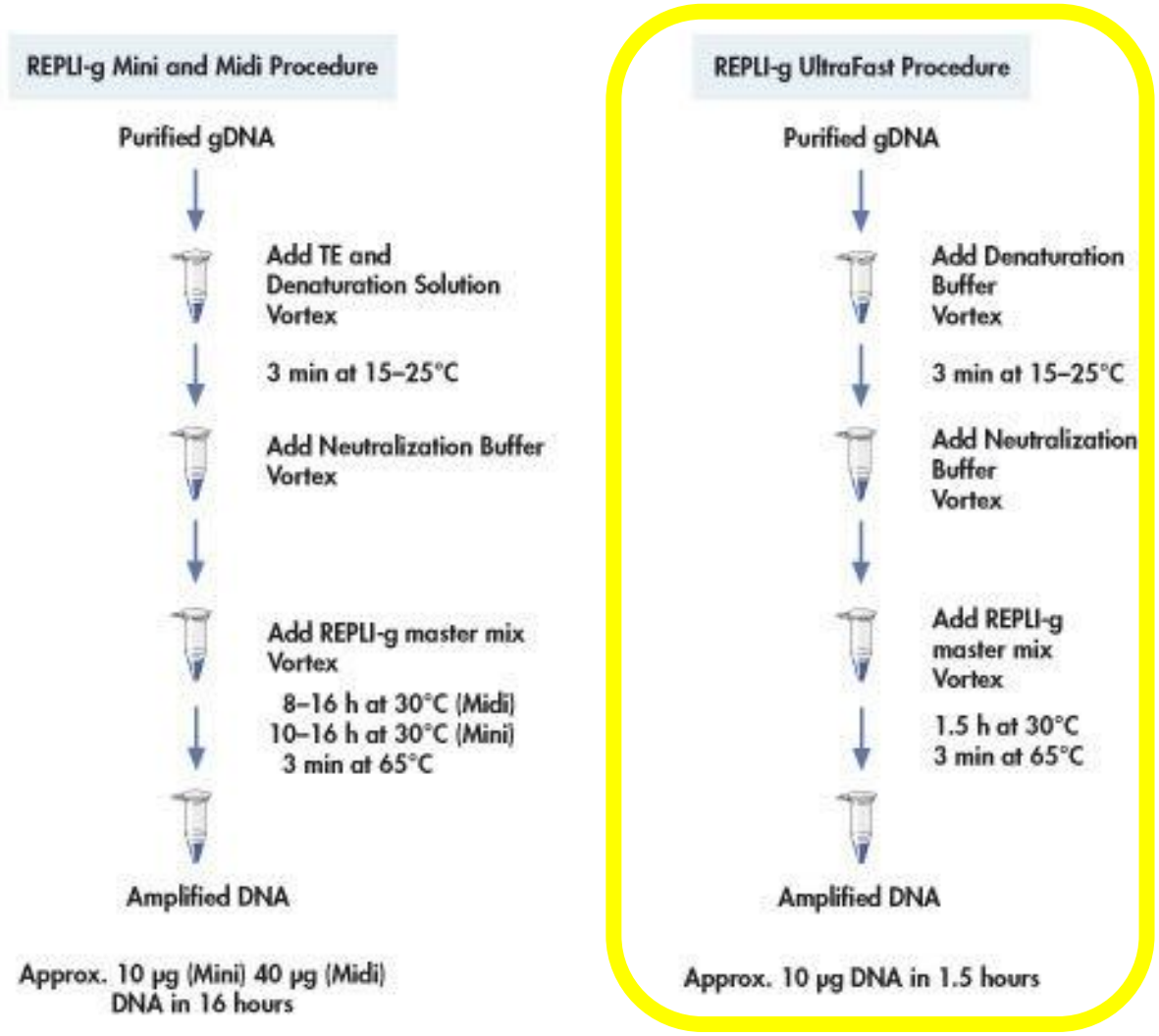
The REPLI-g procedure involves the binding of random hexamers to denatured DNA followed by strand displacement synthesis at a constant temperature using the enzyme Phi29 DNA polymerase. Additional priming events occur on each displaced strand leading to a network of branched DNA structures (see figure "Schematic representation of REPLI-g DNA amplification").

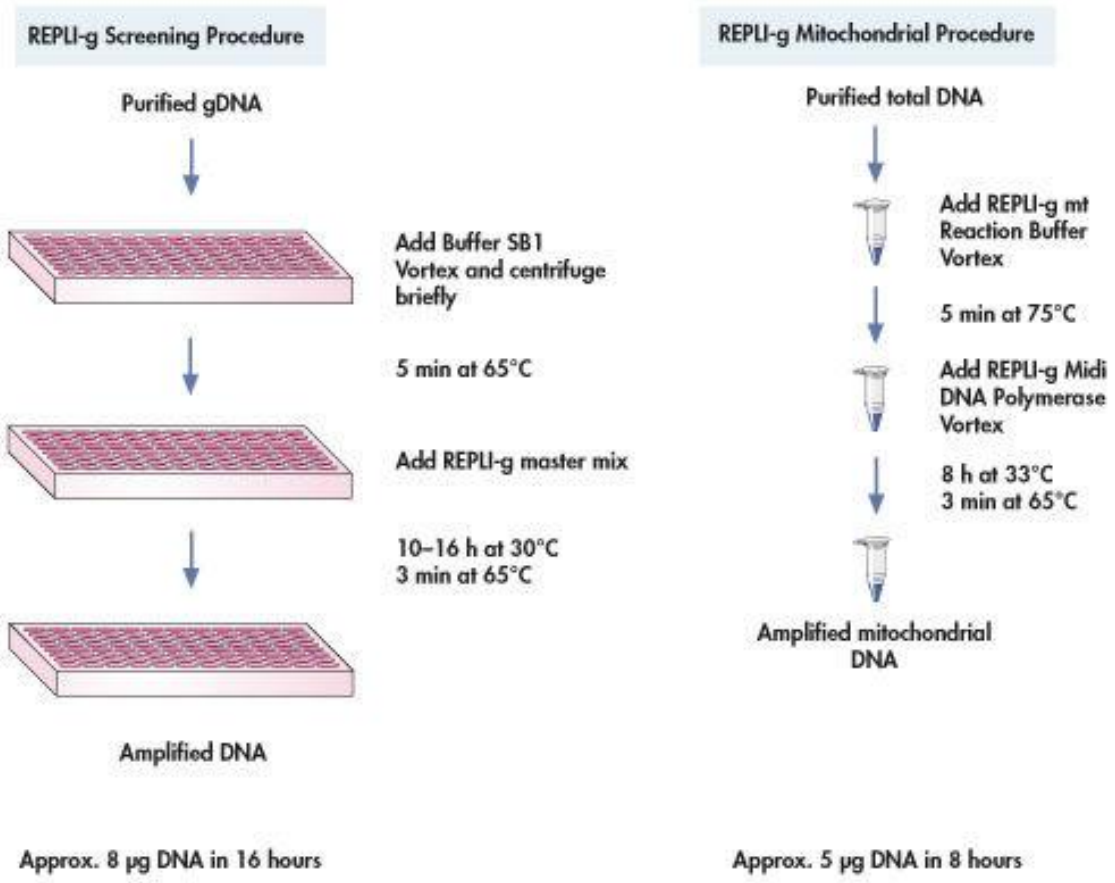
Phi29 DNA polymerase does not dissociate from the genomic DNA template allowing the generation of DNA fragments up to 100 kb without sequence bias. The enzyme has a 3'→5' exonuclease proofreading activity and provides error rates 100 times lower than Taq DNA polymerase-based methods (see PCR-based WGA).

Schematic representation of REPLI-g DNA amplification. 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.

REPLI-g procedure

REPLI-g Kits provide streamlined protocols, requiring minimal hands on time.





Working with fragmented DNA

Multiple Displacement Amplification (MDA) requires average genomic DNA fragment sizes of approximately 2 kb in order to amplify DNA without introducing any bias. Fragmented or low quality DNA can be used as long some DNA fragments are above 2 kb in length. This is because randomly fragmented DNA should contain multiple intact copes of each locus. However, to ensure accurate locus representation, the starting amount of template DNA should be increased (see figure "Effect of increasing template amount for fragmented DNA samples" .

For more information about all the Qiagen products for WGA see:

http://www.qiagen.com/products/wholegenomeamplification/default.aspx

and

http://www.qiagen.com/mc/repli-g/default.aspx?gaw=Replig&gkw=QIAGEN%20whole%20genome%20amplification

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WGA Protocol using the REPLI-g ultra fast kit.

Few details first…

The starting material is 10ng of purified genomic DNA, preferably eluted in TE. If you have 40ng or more of starting DNA it is ideal, but I tried with 10ng (or even less) and it worked for some samples. You should quantify the amount of starting DNA with Qubit 2.0 Fluorometer.

The protocol says that the expected yield is around 7-10ug but it's likely to have a much lower yield.

If the kit is new:

Reconstitute Buffer DLB by adding 500ul H2O. Mix and spin down. Store it in the -20C.

Procedure

1. Briefly thaw and vortex all the reagents except the Ultra-fast DNA polymerase, which should be thaw on ice just before use.

2. Prepare Buffer D1 (denaturation buffer) and N1 (neutralization buffer) as follows:

BUFFER D1

Component	Vol x1 (ul)	x10 (ul)
Reconstituted buffer DLB	0.125	1.25
H2O	0.875	8.75
Total volume	1	10

Mix and spin down.

BUFFER N1

Component	Vol x1 (ul)	x10 (ul)
Stop solution	0.2	2
H2O	1.8	18
Total volume	2	20

Mix and spin down.

Note: as you can see, the volumes of some reagents to make buffers D1 and N1 are very small. If you have less samples, you can still prepare enough buffers for 10 samples and store the left over in the -20.

3. Place 1ul template DNA into a small PCR tube (or strip).

4. Add 1ul Buffer D1, vortex and spin down.

5. Incubate samples 3 min at 15-25C.

6. Add 2ul Buffer N1, vortex and spin down.

7. Prepare the Polymerase master mix as follows:

Master Mix

Component	Vol x1 (ul)	x10 (ul)
REPLI-g UltraFast Reaction buffer	15	150
REPLI-g UltraFast DNA Polymerase	1	10
Total volume	16	160

Mix gently and spin down.

8. Add 16ul of master mix to the denatured samples from step 6.

9. Incubate at 30C for 1.5 h.

10. Inactivate Polymerase holding the sample at 65C for 3 min.

11. Quantify samples with Qubit 2.0 Fluorometer.

12. Store samples at -20C.