



## QIAGEN Supplementary Protocol:

### Purification of REPLI-g<sup>®</sup> amplified DNA using the QIAamp<sup>®</sup> DNA Mini Kit

This protocol is designed for the purification of REPLI-g amplified DNA using the QIAamp DNA Mini Kit. The quality of REPLI-g amplified DNA purified using this protocol has been proven by use in Affymetrix<sup>®</sup> arrays.

**IMPORTANT:** Please consult the "Safety Information" and "Important Notes" sections in the *QIAamp DNA Mini Handbook* before beginning this procedure. For safety information on the additional chemicals mentioned in this protocol, please consult the appropriate material safety data sheets (MSDSs) available from the product supplier.

#### Equipment and reagents to be supplied by user

- 1.5 or 2 ml microcentrifuge tubes
- Microcentrifuge
- Pipet tips (pipet tips with aerosol barriers for preventing cross-contamination are recommended)
- Vortexer
- Deionized, nuclease-free water
- Optional: TE buffer (10 mM Tris·Cl; 1 mM EDTA, pH 8.0) for elution (step 9)
- 96–100% ethanol

#### Important points before starting

- All centrifugation steps should be carried out at room temperature (15–25°C).

#### Handling of QIAamp spin columns

Owing to the sensitivity of nucleic acid amplification technologies, the following precautions are necessary when handling QIAamp spin columns to avoid cross-contamination between sample preparations:

- Carefully apply the sample or solution to the QIAamp spin column. Pipet the sample into the QIAamp spin column without wetting the rim of the column.
- Change pipet tips between all liquid transfers. The use of aerosol-barrier pipet tips is recommended.
- Avoid touching the QIAamp membrane with the pipet tip.
- After all pulse-vortexing steps, briefly centrifuge the 1.5 ml microcentrifuge tubes to remove drops from the inside of the lid.

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- Wear gloves throughout the entire procedure. In case of contact between gloves and sample, change gloves immediately.

### **Spin protocol**

- Close the QIAamp spin column before placing it in the microcentrifuge. Centrifuge as described in the protocol.
- Remove the QIAamp spin column and collection tube from the microcentrifuge. Place the QIAamp spin column in a new collection tube. Discard the filtrate and the collection tube. Note that the filtrate may contain hazardous waste and should be disposed of appropriately.
- Open only one QIAamp spin column at a time, and take care to avoid generating aerosols.
- For efficient parallel processing of multiple samples, fill a rack with collection tubes to which the QIAamp spin columns can be transferred after centrifugation. Used collection tubes containing the filtrate can be discarded, and the new collection tubes containing the QIAamp spin columns can be placed directly in the microcentrifuge.

### **Centrifugation**

QIAamp spin columns will fit into most standard 1.5–2 ml microcentrifuge tubes. Additional 2 ml collection tubes are available separately.

Centrifugation of QIAamp spin columns is performed at 6000 x g (8000 rpm) in order to reduce centrifuge noise. Centrifuging QIAamp spin columns at full speed will not affect DNA yield. Centrifugation at lower speeds is also acceptable, provided that nearly all of each solution is transferred through the QIAamp membrane. All centrifugation steps should be carried out at room temperature (15–25°C).

## Things to do before starting

- Equilibrate REPLI-g amplified DNA to room temperature (15–25°C).
- Equilibrate Buffer AE or 1x TE buffer, pH 8.0 to room temperature for the elution step (step 9).
- Mix Buffer AL\* thoroughly by shaking before use. Buffer AL is stable for 1 year when stored at room temperature.
- Buffer AW1\* is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) as indicated on the bottle and in Table 1. Buffer AW1 is stable for 1 year when stored closed at room temperature.

**Table 1. Preparation of Buffer AW1**

Kit size	AW1 concentrate (ml)	Ethanol (ml)	Final volume (ml)
50	19	25	44
250	95	125	220

Buffer AW2<sup>†</sup> is supplied as a concentrate. Before using for the first time, add the appropriate amount of ethanol (96–100%) to Buffer AW2 concentrate as indicated on the bottle and in Table 2. Buffer AW2 is stable for 1 year when stored closed at room temperature.

**Table 2. Preparation of Buffer AW2**

Kit size	AW2 concentrate (ml)	Ethanol (ml)	Final volume (ml)
50	13	30	43
250	66	160	226

\* Contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach.

<sup>†</sup> Contains sodium azide as a preservative. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

## Procedure

1. **Place 50  $\mu$ l REPLI-g amplified DNA into a 1.5 ml microcentrifuge tube.**
2. **Add 150  $\mu$ l nuclease-free water. Mix by vortexing and centrifuge briefly.**
3. **Add 200  $\mu$ l Buffer AL to the sample. Mix by vortexing for 15 s and centrifuge briefly.**

In order to ensure efficient binding of the DNA to the QIAamp spin column membrane, it is essential that the REPLI-g amplified DNA and Buffer AL are mixed thoroughly to yield a homogeneous solution.

4. **Add 200  $\mu$ l ethanol (96–100%) to the sample. Mix by vortexing for 15 s and centrifuge briefly.**
5. **Carefully apply the mixture from step 4 to the QIAamp spin column (in a 2 ml collection tube) without wetting the rim, close the cap, and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp spin column in a clean 2 ml collection tube (provided), and discard the tube containing the filtrate.**

Close each spin column in order to avoid aerosol formation during centrifugation.

Centrifugation is performed at 6000 x g (8000 rpm) in order to reduce noise. Centrifugation at full speed will not affect the yield or purity of the DNA. If the sample has not completely passed through the column after centrifugation, centrifuge again at higher speed until the QIAamp spin column is empty.

6. **Carefully open the QIAamp spin column and add 500  $\mu$ l Buffer AW1 without wetting the rim. Close the cap and centrifuge at 6000 x g (8000 rpm) for 1 min. Place the QIAamp spin column in a clean 2 ml collection tube (provided), and discard the collection tube containing the filtrate.**
7. **Carefully open the QIAamp spin column and add 500  $\mu$ l Buffer AW2 without wetting the rim. Close the cap and centrifuge at full speed (20,000 x g; 14,000 rpm) for 3 min.**
8. **Place the QIAamp spin column in a new 2 ml collection tube (not provided) and discard the collection tube with the filtrate. Centrifuge at 20,000 x g (14,000 rpm) for 1 min.**

- 9. Place the QIAamp spin column in a clean 1.5 ml microcentrifuge tube (not provided), and discard the collection tube containing the filtrate. Carefully open the QIAamp spin column and add 200  $\mu$ l Buffer AE or 1x TE buffer, pH 8.0. Incubate at room temperature (15–25°C) for 1 min, and then centrifuge at 6000 x g (8000 rpm) for 1 min.**

Incubating the QIAamp spin column loaded with Buffer AE or 1x TE buffer, pH 8.0 for 5 min at room temperature before centrifugation generally increases the yield of REPLI-g amplified DNA.

A second elution step with a further 200  $\mu$ l Buffer AE may increase yields.

Volumes of more than 200  $\mu$ l should not be eluted into a 1.5 ml microcentrifuge tube because the spin column will come into contact with the eluate, leading to possible aerosol formation during centrifugation.

Elution with volumes of less than 200  $\mu$ l increases the final DNA concentration in the eluate significantly, but reduces the overall DNA yield. Eluting with 2 x 100  $\mu$ l instead of 1 x 200  $\mu$ l does not increase elution efficiency.

For long-term storage of REPLI-g amplified DNA, eluting in Buffer AE or 1x TE, pH 8.0 and storing at –20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

### **DNA yield and purity**

The typical yield of REPLI-g amplified genomic DNA after purification using the QIAamp DNA Mini Kit is 25–40  $\mu$ g.

The yield of REPLI-g amplified DNA after cleanup is influenced by several factors including the amount of DNA amplified, which in turn is influenced by the quality of genomic DNA used. For example, enzyme inhibitors as well as the use of fragmented genomic DNA decrease the yield of the REPLI-g reaction. Highly degraded samples tend not to be amplified evenly across the genome. In general, an average genomic DNA fragment size of 2 kb is the minimum requirement for high-quality whole genome amplification, assuming no portions of the genome are degraded to the point where information will be missing. At least a small portion of 10 kb fragments or larger need to be present in the genomic DNA sample for even amplification of the entire genome.

The recovery of REPLI-g amplified DNA is slightly lower compared to other DNA samples due to the high DNA concentrations reached during the REPLI-g reaction and the high molecular weight of REPLI-g amplified DNA. In addition, DNA recovery is drastically affected if no ethanol was added in step 4 or to Buffers AW1 or AW2 (see “Things to do before starting”). DNA recovery is also affected by the pH. The pH required for binding REPLI-g amplified DNA to the QIAamp column is <7.5, while the elution buffer should be between pH 8.0–8.5.

Measurement of REPLI-g amplified DNA cannot be performed by OD measurement without cleanup as residual amounts of primers and nucleotides would drastically overestimate the concentration REPLI-g amplified DNA. After cleanup the concentration of REPLI-g amplified DNA can be determined by measuring the absorbance at 260 nm ( $A_{260}$ ) in a spectrophotometer. To ensure significance, readings should be between 0.15 and 1.00. An absorbance of 1 unit at 260 nm corresponds to 50  $\mu$ g of DNA per ml ( $A_{260}=1 \rightarrow 50 \mu\text{g/ml}$ ). Before quantification of purified REPLI-g amplified DNA, the DNA must be diluted 1:10 (e.g., place 15  $\mu$ l purified REPLI-g DNA into a reaction tube and add 135  $\mu$ l TE buffer). DNA concentration can be determined without prior cleanup by fluorescence reading using Quant-iT™ PicoGreen® dsDNA reagent (Life Technologies Corp., cat no. P7581). Follow instructions recommended in the REPLI-g Handbooks.

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](http://www.qiagen.com) or can be requested from QIAGEN Technical Services or your local distributor.

Material safety data sheets (MSDS) for any QIAGEN product can be downloaded from [www.qiagen.com/Support/MSDS.aspx](http://www.qiagen.com/Support/MSDS.aspx).

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