illustra GFX PCR DNA and Gel Band Purification Kit

Introduction

The illustra™ GFX™ PCR DNA and Gel Band Purification Kit is designed for the rapid purification and concentration of DNA fragments ranging in size from 50 bp to 40 kb from PCR mixtures or agarose gel bands. This kit can be used to purify DNA from reaction volumes of up to 100 µl or DNA-containing agarose gel slices of up to 400 mg. The binding capacity is approximately 25 µg/column. The kit combines a chaotropic buffer with a glass-fiber matrix supported in a spin column for the purification of DNA from both solution and agarose gel. Typical recoveries range from 60 to 80% for DNA fragments from agarose gels to as high as 95% for PCR products from solution. DNA purity is exceptional—99.5% of contaminants are removed. We present a redesigned (Fig 1) and improved version of the versatile illustra GFX PCR DNA and Gel Band Purification Kit for the purification of DNA fragments.

illustra GFX PCR DNA and Gel Band Purification Kit delivers:

- **Versatility:** With a choice of two different input samples—either DNA in solution or DNA-containing agarose gel bands—and the flexibility to use two different elution buffers and an elution volume range of 10 to 50 µl to suit the requirements of any dowstream application
- **High quality:** Highly pure DNA products for downstream applications including cloning and sequencing
- **Flexibilty:** Functional equivalence to four Qiagen™ kits—QIAquick™ PCR Purification, MinElute™ PCR Purification, QIAquick Gel Extraction, and MinElute Gel Extraction
- **Reliability:** Consistent and successful removal of dNTPs and primers from PCR mixtures—unlike the Wizard™ SV Gel and PCR Clean-Up System (Promega) which cannot rid such PCR mixtures of dNTPs and primers
- **Reproducibilty:** High DNA recovery achieved with a capture buffer that contains a visual color indicator to ensure optimal pH for maximum DNA binding

Improvements to the kit include:

- Improved column design with increased maximum volume holding of up to 800 µl liquid volume (950 µl without a cap)
- Optimized columns that fit a new 2 ml collection tube. The lid of the collection tube can be closed (Fig 1d) before centrifugation
- Inclusion of a frosted area on both the columns and collection tubes for easy labeling
- Addition of a visual color indicator to the capture buffer to ensure optimal pH for maximum DNA binding and recovery

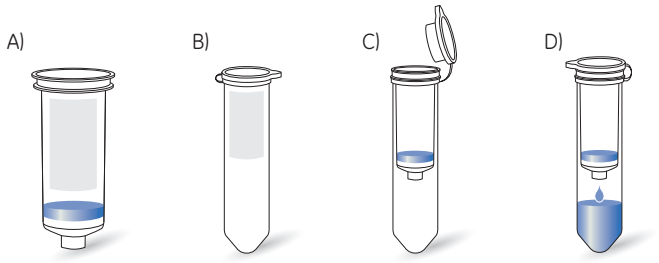

Fig 1. The new GFX MicroSpin column has an increased maximum volume holding of up to 800 µl liquid volume (950 µl without a cap), a frosted area on the surface for easy labeling of samples and contains a blue color-coded O-ring membrane seal (a). The GFX columns are optimized to fit new 2 ml collection tubes that are supplied with the Kit (b and c) with the ability to close the collection tube lid for centrifugation (d).

Addition of a pH indicator for easy determination of the optimal pH for DNA binding

The correct pH of the capture buffer plus sample mix is important for the efficient binding of DNA to the silica membrane of the GFX MicroSpin column. The capture buffer contains a pH indicator that changes color at different pH levels to visually indicate whether the capture buffer plus sample mix is at the optimal pH for DNA to bind to the silica membrane.

For efficient binding of DNA to the silica membrane, the mixture of capture buffer and DNA sample requires a pH ≤ 7.5. The pH indicator will appear yellow or pale orange in color within this range (Fig 2a). If the pH is > 7.5 (which

can occur if the agarose gel electrophoresis buffer is not refreshed, is incorrectly prepared, or if the pH of the sample exceeds the buffering capacity of the capture buffer), DNA adsorption will be inefficient and the yield may be reduced. The pH indicator would be dark pink or red in color in this range (Fig 2b). If the pH of the binding mixture is > 7.5 it can be adjusted by the addition of a small volume of 3 M sodium acetate pH 5.0 before loading onto the GFX MicroSpin column. If the pH indicator is yellow or pale orange in color, the mixtue of capture buffer plus the DNA sample is at optimal pH for efficient DNA binding to the silica membrane. The dye indicator neither interferes with DNA binding nor does it affect yield (Fig 3) and it is completely removed during the wash step. In addition, using a colored as opposed to a clear binding mixture allows easy visualization of any unsolubilized agarose. Complete solubilization is necessary to obtain maximum yields.

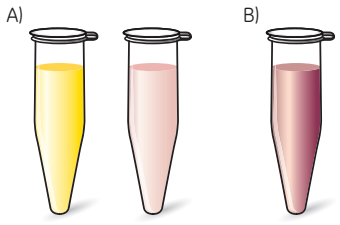

Fig 2. (A). A yellow or pale orange color indicates that the mixture of capture buffer and sample is at an optimal pH for efficient binding of DNA to the silica membrane; (B). A dark pink or red color shows that the pH of the mixture of capture buffer and sample is too high for efficient DNA adsorption to the silica membrane.

A 910 bp PCR fragment from the Tumour Protein p53 open reading frame (ORF) was purified with the QIAquick gel extraction Kit and both old and new versions of the illustra GFX PCR DNA & Gel Band Purification Kit (the old version of the Kit contains the current MicroSpin column and capture buffer without a pH indicator; the new version of the Kit contains the new MicroSpin column and a capture buffer with a pH indicator) according to the standard protocol. DNA from a PCR sample was purified from agarose gel bands (300 mg) and eluted into a volume of 50 µl. A Mann-Whitney t-test p value of 0.6635 showed that there was no significant difference in yield between the three kits (Fig 3).

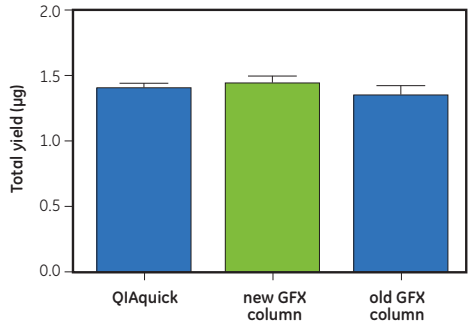

Fig 3. Yield comparison of a gel-purified 910 bp PCR fragment (from the Tumour Protein p53 ORF) with the illustra GFX PCR DNA & Gel Band Purification Kit (old and new versions) and the QIAquick gel extraction Kit. PCR DNA (1.5 µg) was loaded onto each column. The yield of purified DNA was calculated from A_{260} (mean of 6 samples) values. Each kit was used according to standard instructions.

Optical density values at 260 and 280 nm can be used to assess the purity of a DNA sample in solution. An A_{260}/A_{280} value of 1.8 is indicative of a highly pure DNA sample whereas values greater than 2 suggest the likelihood of DNA fragmentation or the presence of other contaminants. Another measure of purity is the A_{260}/A_{230} ratio (nucleic acid to salt ratio). An $A_{260/230}$ value of less than 1.5 indicates the presence of salt at levels that could affect downstream applications. Use of the extra wash buffer in the illustra GFX PCR DNA and Gel Band Purification Kit routinely yields purified PCR fragments with A_{260}/A_{230} of 1.5 (Fig 4), indicating that it is suitable for downstream applications.

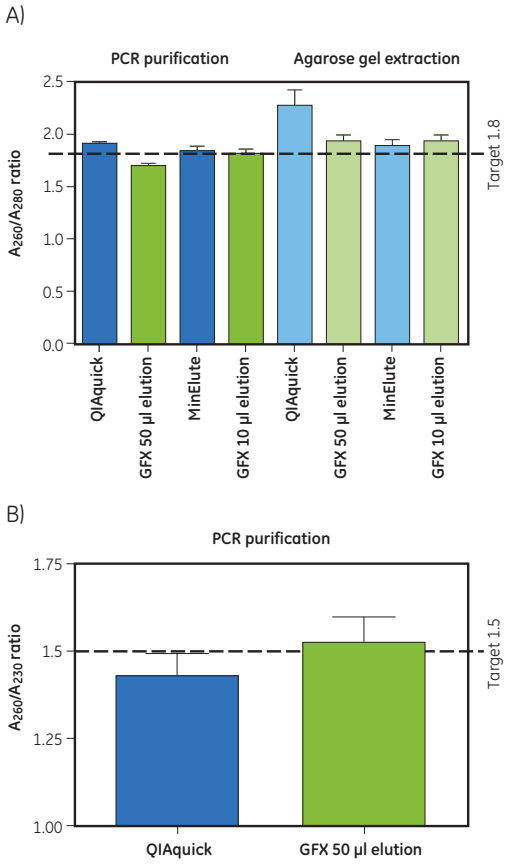

Fig 4. A 910 bp PCR fragment from the Tumor Protein p53 ORF was purified with the kits indicated above. Both the PCR mixture in solution and PCR product embedded in an agarose gel were purified. (A) Comparative A_{260}/A_{280} readings for all the samples tested including PCR mixtures (eluted into 50 or 10 µl) and agarose gel band extractions (eluted into 50 or 10 µl); (B) A_{260}/A_{230} for purified PCR samples with a 50 µl elution volume. For each elution volume, three different researchers purified three different samples with each kit. For purification, 1.5 µg and 0.75 µg of the appropriate sample was loaded onto the column and eluted into 50 and 10 µl of elution buffer, respectively.

Ligation and cloning

DNA purified from PCR mixtures and agarose gel bands was used for ligation and cloning reactions. Blue/white colony selection with β-lactamase was used to identify recombinant clones. White colonies indicate insertion of the PCR fragment into a pUC-based plasmid, disrupting the β-lactamase open reading frame. Blue colonies indicate re-ligation of the plasmid without disruption of the β-lactamase open reading frame,

and non-insertion of the PCR fragment. Statistical analysis using a one-way ANOVA showed no significant difference in cloning efficacy between the kits and researchers ($p > 0.05$) for both PCR purification and agarose gel band extractions (Fig 5).

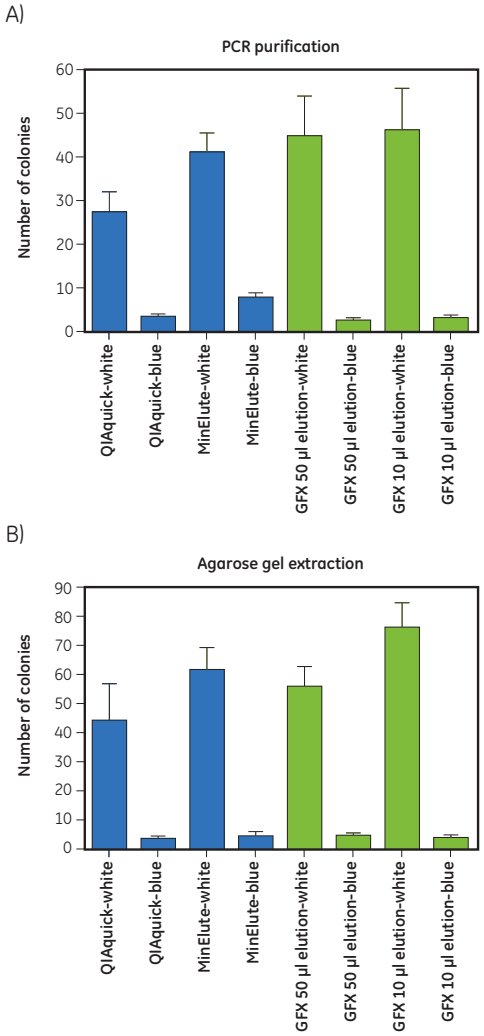

Fig 5. Ligation and cloning experiments with DNA purified from (A) PCR mixtures and; (B) DNA-containing agarose gel bands. A 910 bp PCR fragment from the Tumor Protein p53 ORF was purified with each kit. Both the PCR mixture in solution and PCR product embedded in an agarose gel were purified. For the ligation reaction, 65 ng of each purified sample was used. Two different volumes (20 and 100 µl) of the transformation mixture were plated out to aid colony counting. Each kit was used according to the manufacturer's instructions.

Sequencing analysis

PCR mixtures purified with the illustra GFX PCR DNA and Gel Band Purification Kit and the QIAquick and MinElute PCR purification kits were used as templates for DNA sequencing reactions. Purified PCR samples were sent to the Qiagen™ Sequencing Service (QSS) for analysis with M13-forward (-20) and M13-reverse (-21) primers. We found no significant difference in Phred 20 (1, 2) scores ($p > 0.05$) between the QIAquick PCR Purification and the illustra GFX PCR DNA and Gel Band Purification kits for an elution volume of 50 µl. Similarly, there was no significant difference between the illustra GFX PCR DNA and Gel Band Purification and the MinElute PCR Purification kits for a 10 µl elution volume (Fig 6).

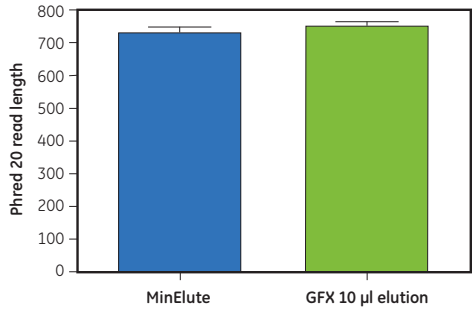

Fig 6. Phred 20 read length obtained for purified PCR samples. An aliquot of the purified 910-bp PCR fragment from the Tumor Protein p53 ORF was sequenced. Each purification kit was used according to the manufacturer's instructions.

Analysis of purified PCR sample composition

DNA markers of a known concentration were repurified with the illustra GFX PCR DNA and Gel Band Purification Kit and the Wizard SV Gel and PCR Clean-Up System according to the manufacturers' protocol into an elution volume of 50 µl each. A Mann-Whitney t-test p value of 0.1797 showed that there was no significant difference in yield between the two kits (Fig 7).

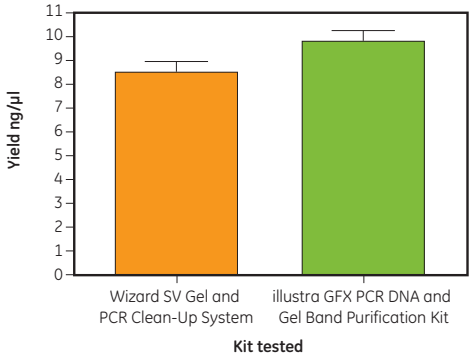

Fig 7. Yield comparison of repurifed DNA markers with the Wizard SV Gel and PCR Clean-Up System and the illustra GFX PCR DNA and Gel Band Purification Kit. Fifty nanograms (50 ng) of each marker was purified from the supplier's stock of 10 ng/µl. The yield of repurified DNA ladder was calculated from A_{260} (mean of 6 samples) values. Each kit was used according to the manufacturer's instructions.

Purification of control PCR mixtures

Both the illustra GFX PCR DNA and Gel Band Purification Kit and the Wizard SV Gel and PCR Clean-Up System (Promega) were used to purify a control PCR mixture containing primers, standard PCR buffer, dNTP mix, *Taq* DNA polymerase, and magnesium chloride, but no template. The comparative spectra (Fig 8) suggests that certain components of the PCR mixture could not be removed with the Wizard SV Gel and PCR Clean-Up System whereas the illustra GFX PCR DNA and Gel Band Purification Kit was capable of removing these components of the PCR mixture. Primers and dNTP mixtures are some of the components that could affect the A_{260} readings and it appears that the Wizard SV Gel and PCR Clean-Up System is incapable of removing both of these components.

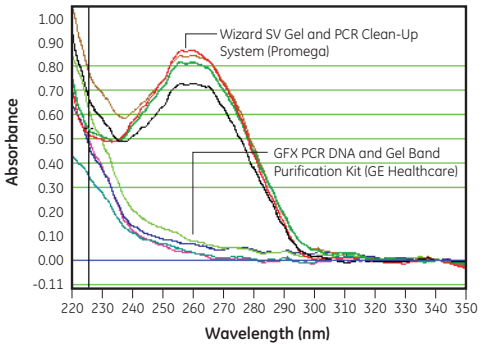

Fig 8. Spectra of purified PCR samples (prepared with no template). Distilled water was substituted for PCR mixtures and purified with the Wizard SV Gel and PCR Clean-Up System (Promega). There was negligible difference in the A_{260} values between the blank and purified distilled water suggesting that neither buffer nor column material leaked into the final eluate (data not shown). Each kit was used according to the manufacturer's instructions.

Spectrophotometric analysis (A_{260} measurements) of the eluates were measured and the result (Fig 9) indicates that primer and dNTP leaked into the final product with the Wizard SV Gel and PCR Clean-Up System.

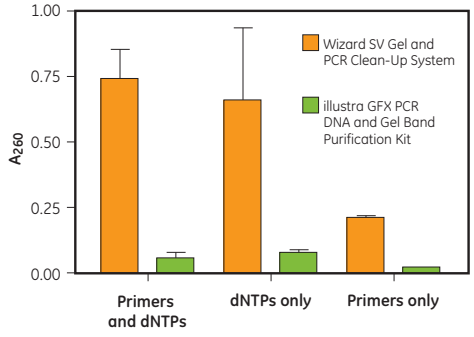

Fig 9. Comparative A_{260} values for eluates of the illustra GFX PCR DNA and Gel Band Purification Kit and the Wizard SV Gel and PCR Clean-Up System (Promega). The application of distilled water to both columns ruled out the possibility of membrane fragmentation or any other extraneous contribution to the observed A_{260} readings (data not shown). Samples containing (i) primers only, (ii) dNTPs only and, (iii) a mixture of primers and dNTPs were applied to both columns and the A_{260} values for each eluate was measured.

Summary

The illustra GFX PCR DNA and Gel Band Purification Kit was used to purify DNA from both PCR mixtures and DNA-containing agarose gel bands in comparison with equivalent kits from Promega and Qiagen. The yield and purity of the samples as determined by primer and dNTP content was measured and the data shows that the illustra GFX PCR DNA and Gel Band Purification Kit outperformed the Wizard SV Gel and PCR Clean-Up System (Promega).

Purified samples from both Qiagen and GE Healthcare kits were used for sequencing, ligation, and cloning reactions. The data shows that the illustra GFX PCR DNA and Gel Band Purification Kit is functionally equivalent to four kits from Qiagen: QIAquick PCR Purification, MinElute PCR Purification, QIAquick Gel Extraction, and MinElute Gel Extraction kits.

Ordering information

Product	Code No
illustra GFX PCR DNA and Gel Band Purification Kit (100 purifications)	28-9034-70
illustra GFX PCR DNA and Gel Band Purification Kit (250 purifications)	28-9034-71

References

1. Ewing, B and Green, P. Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Res.* **8**, 186-194. (1998).
2. Ewing, B. *et al.*, Base-Calling of Automated Sequencer Traces Using Phred.I. Accuracy Assessment. *Genome Res.* **8**, 175-185. (1998).

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