GFX™ PCR DNA and Gel Band Purification Kit

PURIFICATION OF PCR PRODUCTS AND RESTRICTION FRAGMENTS

DOMINIQUE DUTSCHER SAS

The 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 (Fig 1). 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.

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, 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.
- **Reliability:** Consistent and successful removal of dNTPs and primers from PCR mixtures.
- **Reproducibilty:** High DNA recovery achieved with a capture buffer that contains a visual color indicator to ensure optimal pH for maximum DNA binding.

Designed for efficiency:

- Column holds up to 800 µL liquid volume (950 µL without a cap).
- Optimized columns fit into the 2 mL collection tube. The lid of the collection tube can be closed before centrifugation (Fig 2).
- Frosted area on both the columns and collection tubes for easy labeling.
- Addition of a visual color indicator to the capture buffer ensures optimal pH for maximum DNA binding and recovery.



Fig 1. The 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.

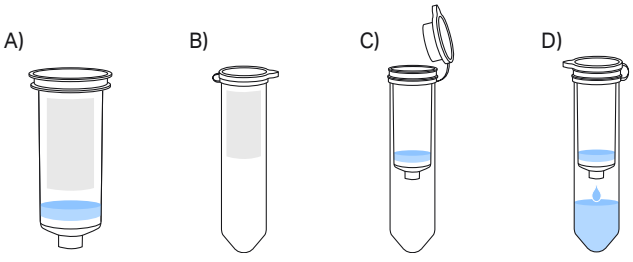


Fig 2. The MicroSpin™ column in the GFX™ kit has a maximum volume holding of up to 800 µL liquid volume (950 µL without a cap), a frosted area on the surface for easy labeling of samples and contains a blue colorcoded O-ring membrane seal (A). The GFX™ columns are optimized to fit 2 mL collection tubes that are supplied with the Kit (B, 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 MicroSpin™ column in the GFX™ kit. 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 3A). If the pH is > 7.5, DNA adsorption will be inefficient and the yield may be reduced. This 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. The pH indicator would be dark pink or red in color in this range (Fig 3B). 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 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 4). 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.

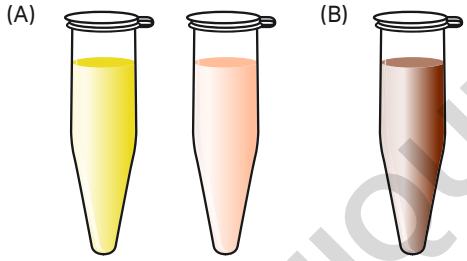


Fig 3. (A). A yellow or pale orange color indicates that the mixture of capture buffer and sample is at an optimal pH for efficient binding of DNA to the silica membrane; (B). A dark pink or red color shows that the pH of the mixture of capture buffer and sample is too high for efficient DNA adsorption to the silica membrane.

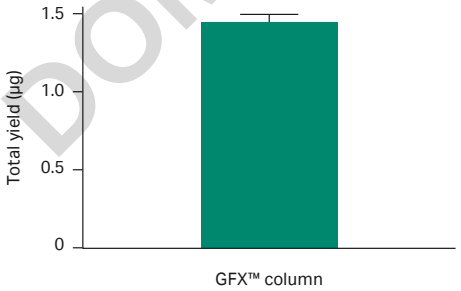


Fig 4. Yield of a gel-purified 910 bp PCR fragment (from the Tumour Protein p53 ORF) with the GFX™ PCR DNA and Gel Band Purification Kit. PCR DNA (1.5 µg) was loaded onto each column. The yield of purified DNA was calculated from A_{260} (mean of 6 samples) values.

A 910 bp PCR fragment from the Tumour Protein p53 open reading frame (ORF) was purified with the GFX™ PCR DNA and Gel Band Purification Kit 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 (Fig 4).

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}/A_{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 GFX™ PCR DNA and Gel Band Purification Kit routinely yields purified PCR fragments with A_{260}/A_{230} of 1.5 (Fig 5), indicating that it is suitable for downstream applications.

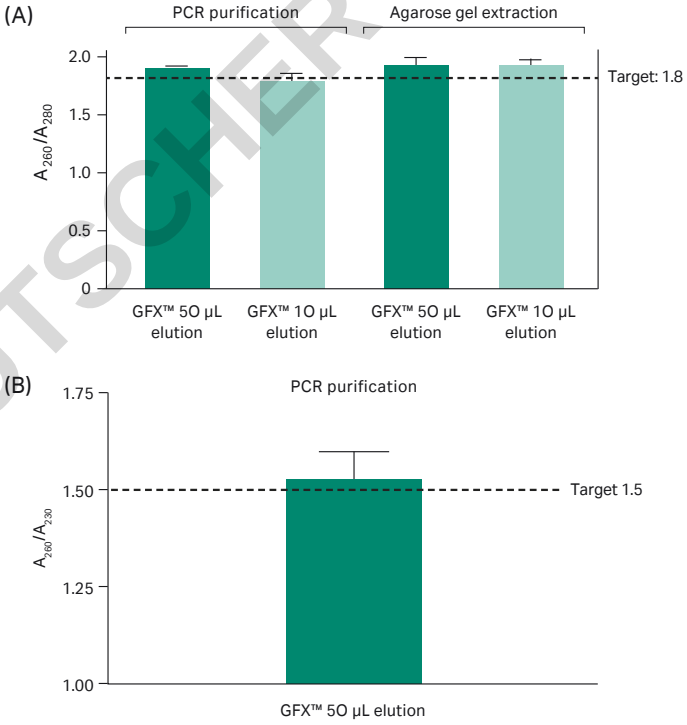


Fig 5. A 910 bp PCR fragment from the Tumor Protein p53 ORF was purified with the GFX™ PCR DNA and Gel Band Purification Kit. Both the PCR mixture in solution and PCR product embedded in an agarose gel were purified. (A) Comparative A_{260}/A_{280} readings for all PCR mixtures (eluted into 50 or 10 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.

DOMINIQUE DUTSCHER SAS

Ligation and cloning

DNA purified from PCR mixtures and agarose gel bands was used for ligation and cloning reactions. Blue-white screening 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 (Fig 6).

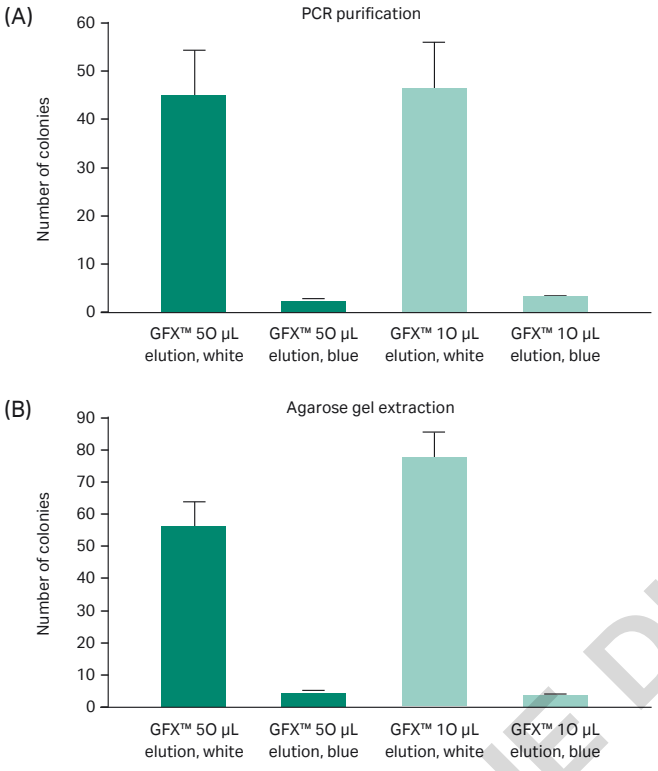


Fig 6. Ligation and cloning experiments with DNA purified from (A) PCR mixtures and; (B) DNA-containing agarose gel bands. A 910 bp PCR fragment from the Tumor Protein p53 ORF was purified with the kit according to the standard instructions. Both the PCR mixture in solution and PCR product embedded in 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.

Sequencing analysis

PCR mixtures purified with the GFX™ PCR DNA and Gel Band Purification Kit 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 (Fig 7).



Fig 7. 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.

Analysis of purified PCR sample composition

DNA markers of a known concentration were repurified with the GFX™ PCR DNA according to the standard protocol into an elution volume of 50 µL each (Fig 8).

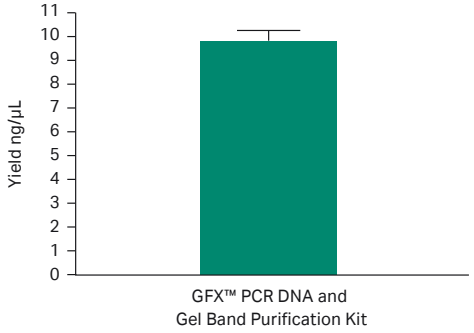


Fig 8. Yield of repurifed DNA markers with the GFX™ PCR DNA and Gel Band Purification Kit. Fifty nanograms (50 ng) of marker was purified from the supplier's stock of 10 ng/µL. The yield of repurified DNA ladder was calculated from A_{260} (mean of 6 samples) values. The kit was used according to the standard instructions.

Summary

The GFX™ PCR DNA and Gel Band Purification Kit was used to purify DNA from both PCR mixtures and DNA-containing agarose gel bands. The yield and purity of the samples as determined by primer and dNTP content was measured and the data shows that the GFX™ PCR DNA and Gel Band Purification Kit is well suited for purification of DNA fragments directly from PCR reactions or as bands embedded in agarose gel, providing versatility and simplification for the user, with added pH indicator to ensure optimal conditions are met for purification.

DOMINIQUE DUTSCHER SAS

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).

Ordering information

Product	Quantity	Product code
GFX™ PCR DNA and Gel Band Purification Kit	10 purifications	28903466
	100 purifications	28903470
	250 purifications	28903471

DOMINIQUE DUTSCHER SAS

cytiva.com

For local office contact information, visit cytiva.com/contact

Cytiva and the Drop logo are trademarks of Life Sciences IP Holdings Corp. or an affiliate doing business as Cytiva. GFX and MicroSpin are trademarks of Global Life Sciences Solutions USA LLC or an affiliate doing business as Cytiva.

Qiagen is a trademark of Qiagen GmbH. Any other third party trademarks are the property of their respective owners.

© 2020–2022 Cytiva

CY24228-13Jan22-DF