illustra GFX 96 PCR Purification Kit

Introduction

The illustra™ GFX™ 96 PCR Purification Kit uses glass-fiber matrix technology in a 96-well format for highly efficient purification of PCR products. DNA fragments from PCR are captured by the matrix in the presence of a chaotropic salt, and the contaminants are removed by washing the matrix with a buffered ethanol solution. The purified DNA product can be eluted with sterile water or a low-ionic strength buffer. The kit provides high yields of purified DNA for use in a variety of molecular biology applications including microarrays, fluorescent sequencing, labeling, hybridization, ligation, and transformation (1,2).

illustra GFX 96 PCR Purification Kit delivers:

- **Fast results:** Purification of 96 PCR products in as little as 15 min
- **High purity:** Over 99% removal of primers and dNTPs
- **Simpler purification:** Absence of hazardous organic extractions. Ethanol precipitation is not required to isolate purified DNA
- **High throughput:** Validated for use with the Macherey-Nagel NucleoVac™ 96 vacuum manifold
- **Convenience:** Filter plates, collection plates, and buffered solutions in a single kit

illustra GFX 96 PCR Purification Kit and QiaQuick™ 96 PCR Purification kits were used to purify PCR fragments for a comparative analysis of yield, purity, and performance of the purified PCR fragments in downstream applications such as restriction enzyme digestions, ligation, cloning, and sequencing.

Yield of purified PCR fragments

The percentage yield of purified PCR fragments recovered from each kit was determined by A_{260} measurements. Statistical analyses (Student's t-test with a p-value of 0.0015; 3 researchers, 6 samples each) show that the illustra GFX 96 PCR Purification Kit gave a greater percentage yield than the QiaQuick 96 PCR Purification Kit in this study (Fig 1).

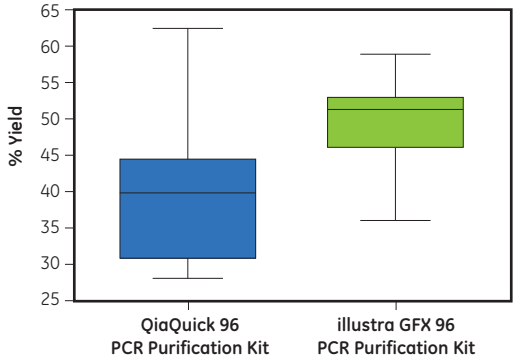

Fig 1. The illustra GFX 96 PCR Purification Kit showed an improved yield of purified DNA over the QiaQuick 96 PCR Purification Kit. A 910-bp PCR fragment from the Tumor Protein p53 ORF was purified with each kit. The percentage yield for each kit was determined with 1.5 µg of purified PCR product. Each kit was used according to the manufacturer's instructions.

Sample purity

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. The A_{260}/A_{280} value for samples purified with the illustra GFX 96 PCR Purification Kit was consistently at 1.8 (Fig 2).

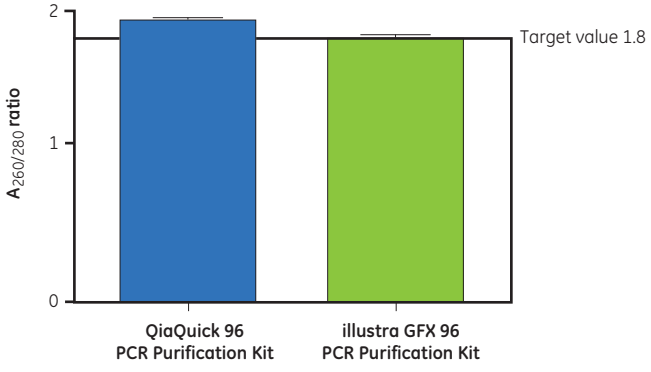

Fig 2. Spectrophotometric analysis of sample purity. A 2 µl aliquot of the purified 910-bp PCR fragment from the Tumor Protein p53 ORF was used to determine sample purity for each kit. Three different researchers used each kit to purify 6 different samples. Each kit was used according to the manufacturer's instructions.

Sequence analyses

Purified PCR samples were sent to the Qiagen™ Sequencing Service (QSS) for analysis with M13-forward (-20) and M13-reverse (-21) primers. All the sequencing reactions, dye terminator removal, and sequence analyses were performed by the QSS. Statistical analyses of Phred 20 (3, 4) read lengths (Student's t-test with a p-value of 0.3793) suggest that the performance of the illustra GFX 96 PCR Purification Kit is comparable to that of the QiaQuick 96 PCR Purification Kit.

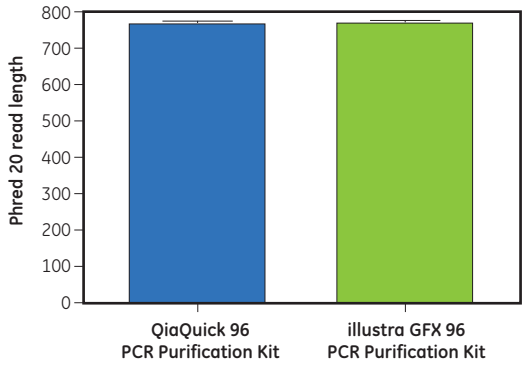

Fig 3. Comparable Phred 20 read lengths for the illustra GFX 96 PCR Purification Kit and QiaQuick 96 PCR Purification Kit. An aliquot of the purified 910-bp PCR fragment from the Tumor Protein p53 ORF was sequenced. Each purification kit was used according to the manufacturer's instructions.

Restriction enzyme digestion

Purified DNA samples (200 ng) from the PCR product were used for restriction enzyme digestions. HpaII was used for the restriction enzyme digests because the DNA substrate contained appropriate cleavage sites and in addition, HpaII is salt-sensitive to > 50 mM KCl. PCR mixtures normally contain high concentrations of salt, and ineffective removal of the salt can have adverse effects on downstream applications. Control reactions without restriction enzyme were prepared. The results were analyzed by agarose gel electrophoresis. All purified PCR samples were completely digested with the HpaII enzyme (Fig 4).

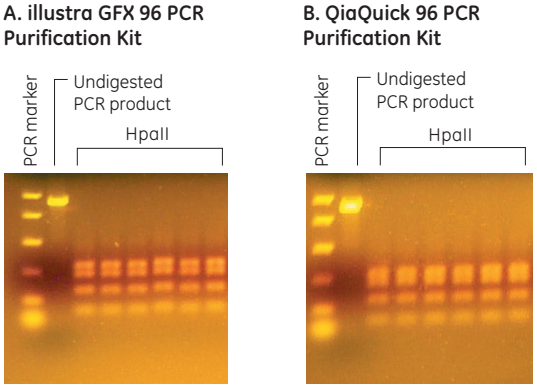

Fig 4. Complete HpaII digestion of PCR products purified with: (A) illustra GFX 96 PCR Purification and; (B) QiaQuick 96 PCR Purification kits. An aliquot of the purified 910-bp PCR fragment from the Tumor Protein p53 ORF was digested once. Each purification kit was used according to the manufacturer's instructions.

Ligation and cloning

DNA was purified from PCR products with the GFX 96 PCR Purification and the QiaQuick 96 PCR Purification kits. The ability of the purified DNA to undergo successful ligation reactions into a pUC18-based vector was tested with Qiagen's PCR Cloning plus™ Kit. The PCR product was amplified from an ampicillin-resistant plasmid but kanamycin was used as the selectable marker during the cloning step to eliminate the risk of plasmid carryover. Recombinant clones were selected using blue/white colony selection. A one-way ANOVA was carried out on the number of colonies and the p-value was < 0.0001, indicating that the means were significantly different. However, since any cloning experiment depends on so many variables, it is fair to conclude that both kits produced comparable cloning results. The ligation and cloning experiments were carried out by three different researchers with 6 replicates per researcher (Fig 5).

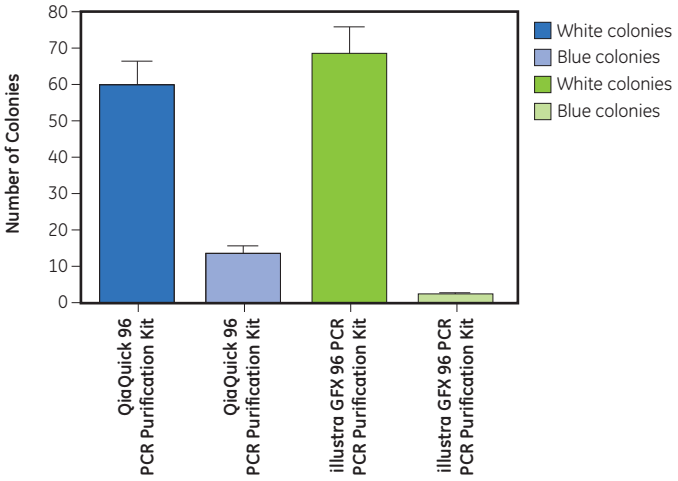

Fig 5. Ligation and cloning results depicting improved or comparable blue/white colony ratios with the illustra GFX 96 PCR Purification Kit.

Summary

The illustra GFX 96 PCR Purification Kit provides improved or comparable results to that of the QiaQuick 96 PCR Purification Kit according to criteria such as ligation and cloning, sequencing analyses, and restriction enzyme digestion. With regards to sample purity and yield, illustra GFX 96 PCR Purification Kit outperforms Qiagen's QiaQuick 96 PCR Purification Kit.

References

1. Marko, M.A., *et al.*, A procedure for the large-scale isolation of highly purified plasmid DNA using alkaline extraction and binding to glass powder. *Anal. Biochem.* 121, 382 (1982).2.
2. Vogelstein, B. and Gillespie, D. Preparative and analytical purification of DNA from agarose, *Proc. Natl. Acad. Sci. USA* **76**, 615–619 (1979).
3. Ewing, B. *et al.*, Base-Calling of Automated Sequencer Traces Using Phred.I. Accuracy Assessment. *Genome Res.* **8**, 175-185. (1998)
4. Ewing, B and Green, P. Base-calling of automated sequencer traces using Phred. II. Error probabilities. *Genome Res.* **8**, 186-194. (1998).

Ordering information

illustra GFX 96 PCR Purification Kit (2 × 96-well plates)	28-9034-37
illustra GFX 96 PCR Purification Kit (10 × 96-well plates)	28-9034-45

Asia Pacific: Tel: +65 6275 1830 Fax: +65 6275 1829 **Australasia** Tel: + 61 2 9899 0999 Fax: +61 2 9899 7511 **Austria** Tel: 01/57606-1619 Fax: 01/57606-1627 **Belgium** Tel: 0800 73 888 Fax: 02 416 82 06 **Canada** Tel: 1 800 463 5800 Fax: 1 800 567 1008 **Central, East, & South East Europe** Tel +43 1 972720 Fax: +43 1 97272 2750 **Denmark** Tel: 45 16 2400 Fax: 45 16 2424 **Eire** Tel: 01494 544000 Fax: 0044 1494 542010 **Finland & Baltics** Tel: +358-(0)9-512 39 40 Fax: +358 (0)9 512 39 439 **France** Tel: 01 6935 6700 Fax: 01 6941 9677 **Germany** Tel: (089) 96281 660 Fax: (089) 96281 620 **Greater China** Tel: +852 2100 6300 Fax: +852 2100 6338 **Italy** Tel: 02 27322 1 Fax: 02 27302 212 **Japan** Tel: +81 3 5331 9336 Fax: +81 3 5331 9370 **Latin America** Tel: +55 11 3933 7300 Fax: +55 11 3933 7304 **Middle East & Africa** Tel: +30 210 9600 687 Fax: +30 210 9600 693 **Netherlands** Tel: 0800 82 82 82 1 Fax: 0800 82 82 82 4 **Norway** Tel: 815 65 555 Fax: 815 65 666 **Portugal** Tel: 21 417 7035 Fax: 21 417 3184 **Russia & other C.I.S. & N.I.S** Tel: +7 (495) 956 5177 Fax: +7 (495) 956 5176 **Spain** Tel: 93 594 49 50 Fax: 93 594 49 55 **Sweden** Tel: 018 612 1990 Fax: 018 612 1910 **Switzerland** Tel: 0848 8028 12 Fax: 0848 8028 13 **UK** Tel: 0800 616928 Fax: 0800 616927 **USA** Tel: +1 800 526 3593 Fax: +1 877 295 8102

www.gelifesciences.com/illustra

GE Healthcare UK Limited
Amersham Place
Little Chalfont
Buckinghamshire
HP7 9NA
UK

imagination at work

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala
Sweden

GE Healthcare Europe, GmbH
Munzinger Strasse 5, D-79111 Freiburg
Germany

GE Healthcare Bio-Sciences Corp.
800 Centennial Avenue, P.O. Box 1327
Piscataway, NJ 08855-1327
USA

GE Healthcare Bio-Sciences KK
Sanken Bldg., 3-25-1, Hyakunincho
Shinjuku-ku, Tokyo 169-0073
Japan.