SureClean Plus

Column-Free Purification of DNA and dsRNA from PCR Reactions and Enzymatic Applications

Purification of nucleic acids is an essential technique for modern molecular biology. The removal of restriction enzymes, polymerases, dNTPs, primers and chemical inhibitors is an important factor for many downstream applications. As increasingly sensitive techniques are being developed, a higher demand is being placed on the ability to purify high-quality DNA. This application note reports on the performance of the Meridian reagent SureClean Plus, as a novel, column-free method for the purification of both DNA and dsRNA from PCR reactions or any enzymatic applications.

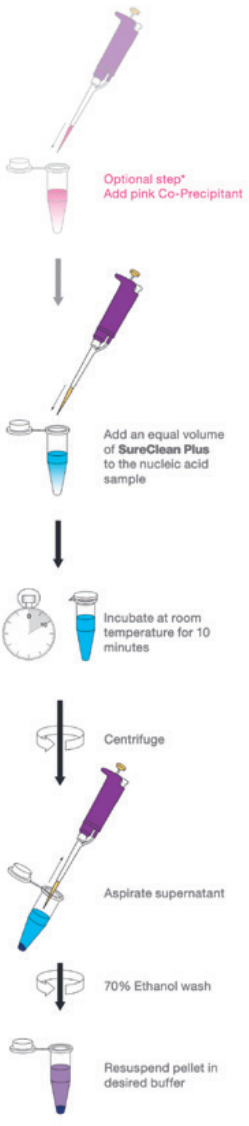

Fig 1. DNA and dsRNA purification using SureClean Plus.
The schematic diagram shows the simple, and cost-effective protocol used for purification of DNA using SureClean Plus.

REMOVAL OF PRIMERS FOLLOWING PCR REACTIONS

An important step in cloning PCR products is the removal of unincorporated dNTPs and primers, which are commonly over 25 bases in length, their presence can interfere with downstream applications and can have a detrimental effect on cloning experiments. To determine the cut-off point of fragment size by purification using SureClean Plus (Fig. 1), we took a DNA ladder containing a range of sizes from 25 to 500 bp, so as to compare SureClean Plus with two column-based purification methods. 30 µL of HyperLadder 25 bp (Cat. No. BIO-33031) was purified using the manufacturer's protocols. For each of the methods, DNA was resuspended in 30 µL of TE, of which 5 µL was loaded on to a 3.5% agarose gel. SureClean Plus efficiently purified DNA over 75 bp (Fig. 2). In contrast, the column-based method from supplier Q shows that following purification, DNA as small as 25 bp was still present. Conversely, the method from supplier X efficiently removes primers, however, it also removes any genuine PCR products of under 200 bases. The data in Fig. 2 shows that the level of recovery using suppliers Q and X is lower than with SureClean Plus.

RECOVERY VS SIZE

SureClean Plus has been specifically developed to ensure the maximal recovery of nucleic acids after purification. The experiment below demonstrates the recovery efficiency on a wide range of different sizes of DNA. 30 µL of HyperLadder 1 kb (Cat No. BIO-33025) was purified using SureClean Plus and the column-based methods of Suppliers Q and X. For each of the methods, DNA was resuspended in 30 µL TE. 5 µL was loaded on a 1% agarose gel (Fig. 3). These results clearly demonstrate the effectiveness of DNA recovery using SureClean Plus as compared with column-based purification methods.

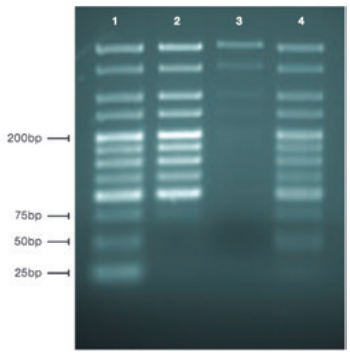

Fig 2. DNA purification using SureClean Plus and Suppliers Q & X spin-column purification methods.
30 µL of HyperLadder 25 bp (Cat No. BIO-33031) was purified using the manufacturer's protocols. For each of the methods DNA was resuspended in 30 µL TE, of which 5 µL was loaded on to a 3.5% agarose gel.
Lane 1 HyperLadder 25 bp
Lane 2 HyperLadder 25 bp purified using SureClean Plus
Lane 3 HyperLadder 25 bp purified using spin-columns from Supplier X
Lane 4 HyperLadder 25bp purified using spin-columns from Supplier Q

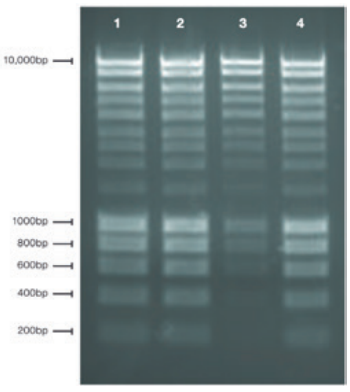

Fig 3. DNA purification using SureClean Plus and Suppliers Q & X spin-column purification methods.
30 µL of HyperLadder 1 kb (Cat No. BIO-33025) was purified using the manufacturer's protocols. For each of the methods DNA was resuspended in 30 µL TE, of which 5 µL was loaded on to a 1% agarose gel.
Lane 1 HyperLadder 1kb
Lane 2 HyperLadder 1kb purified using SureClean Plus
Lane 3 HyperLadder 1kb purified using spin-columns from Supplier X
Lane 4 HyperLadder 1kb purified using spin-columns from Supplier Q.

RECOVERY VS CONCENTRATION

In order to compare recovery rates with different DNA concentrations, solutions containing increasing amounts of DNA were purified using SureClean Plus and Supplier Q's spin-column purification (Fig. 4). Unlike most column-based methods, SureClean Plus maximizes recovery with nucleic acid solutions of low, medium or high concentrations.

PURIFICATION OF dsRNA

In order to assess recovery of dsRNA, a 500 bp fragment of dsRNA was synthesized using T7 RNA Transcription. This technique utilizes the synthesis of two single-stranded complimentary RNA fragments, which are annealed to each other by heating to 65°C for 5 minutes and cooling slowly. Following the annealing step, the double-stranded fragment was purified using SureClean Plus in accordance with the standard purification protocol. The purified dsRNA was subsequently resuspended in DEPC-treated water, and analyzed on a 3.5% agarose (RNase-free) gel (Fig. 5). This experiment demonstrates that SureClean Plus-purified the dsRNA with a good recovery rate.

REMOVAL OF RESTRICTION ENDONUCLEASES

Restriction enzyme digestion is a common method requiring downstream purification of DNA and the removal of enzyme. In order to test this, a fragment of the ß-actin gene was amplified from human genomic DNA and then digested using *Pst*I enzyme (the ß-actin fragment contained no *Pst*I sites). After a 2-hour incubation at 37°C, the gene was purified with SureClean Plus and with Supplier Q's column-based purification method. The purified fragment was then resus-pended, following which λ DNA and *Pst*I buffer were added. Following a 2-hour incubation period at 37°C, no apparent digestion of the λ DNA was observed (Fig. 6), so confirming the complete removal of the *Pst*I restriction enzyme.

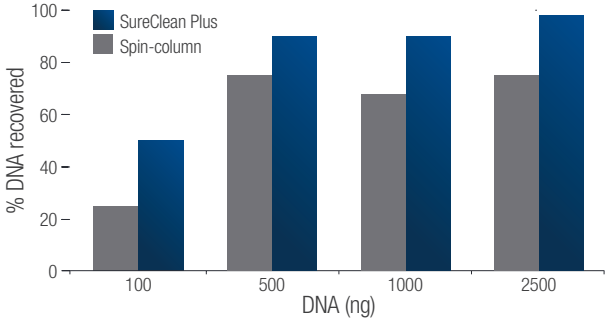

Fig 4. Recovery vs Concentration. DNA purification using SureClean Plus and Supplier's Q spin-column purification method.
30 µL DNA solutions containing 100, 500, 1,000 and 2,500 ng of plasmid DNA were purified using either SureClean Plus or Supplier Q spin-columns.

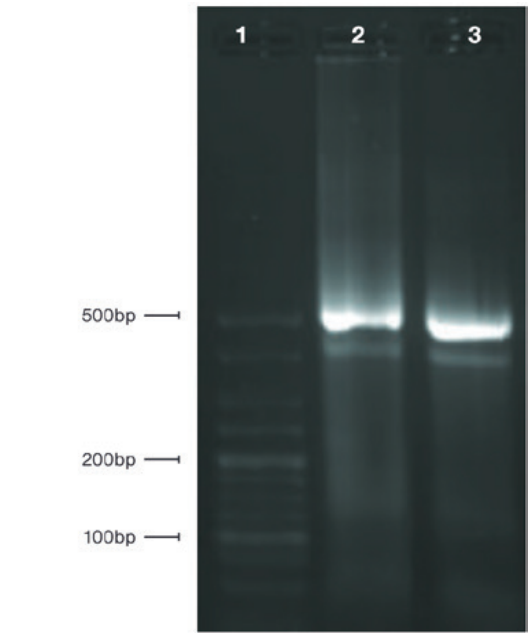

Fig 5. dsRNA recovery. A 500 bp dsRNA fragment was synthesized using the T7 RNA transcription kit, and then purified using SureClean Plus.
Lane 1 HyperLadder 25 bp
Lane 2 Unpurified dsRNA
Lane 3 dsRNA purified using SureClean Plus

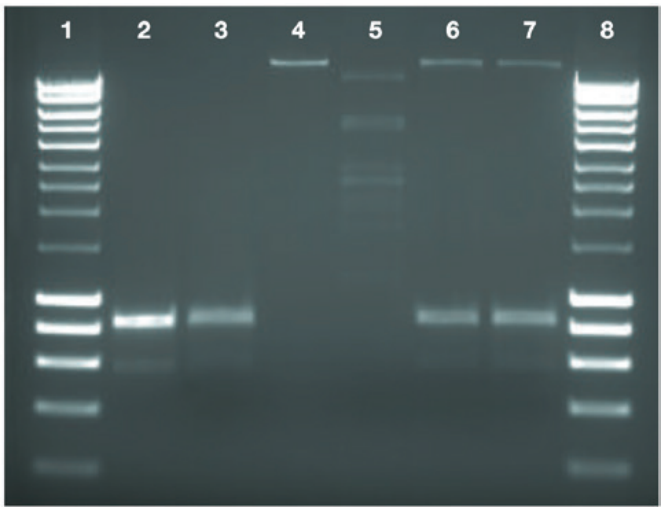

Fig 6. Enzyme Removal. An experiment was performed to remove the restriction endonucleases *Pst*I from a restriction digest and then demonstrate the absence of the enzyme in downstream applications.
Lane 1 HyperLadder 1 kb
Lane 2 ß-actin PCR fragment
Lane 3 ß-actin PCR fragment plus *Pst*I and *Pst*I buffer
Lane 4 20 ng λ DNA
Lane 5 20 ng λ DNA plus *Pst*I and *Pst*I buffer
Lane 6 ß-actin PCR fragment plus *Pst*I and *Pst*I buffer, purified using SureClean Plus. λ DNA and *Pst*I buffer were then added and incubated for 2 hours
Lane 7 ß-actin PCR fragment plus *Pst*I and *Pst*I buffer, purified using Supplier Q's column based purification method. λ DNA and *Pst*I buffer were then added and incubated for 2 hours
Lane 8 HyperLadder 1 kb

DOWNSTREAM APPLICATIONS

A. Sequencing

PCR products are used for numerous downstream applications, including sequencing. The length of read of a sequencing reaction depends on the purity of the DNA sample. In the following experiment, a 2 kb fragment was amplified by PCR and subsequently purified using SureClean Plus and Supplier U's single-tube purification method. The sequencing results obtained (Fig. 7) indicate that SureClean Plus efficiently increased the length of read of the purified PCR product.

B. Cloning

The ability of SureClean Plus to enhance successful cloning was assessed. Three different-sized amplicons of 500 bp, 1 kb and 2 kb DNA, were amplified using PCR from DNA. Following digestion of the PCR products with the restriction enzymes *EcoR*I and *Hind*III, the reaction was split into two halves. One half was further purified using SureClean Plus, whilst the other was left untreated. Each of the three amplicons was subsequently ligated into an *EcoR*I/*Hind*III plasmid vector (3:1 insert:vector ratio), using Ligase. The ligation reaction was then directly cloned into Competent Cells. The data shown (Fig. 8) demonstrate the suitability of SureClean Plus as an important extra step in cloning the ligated PCR products.

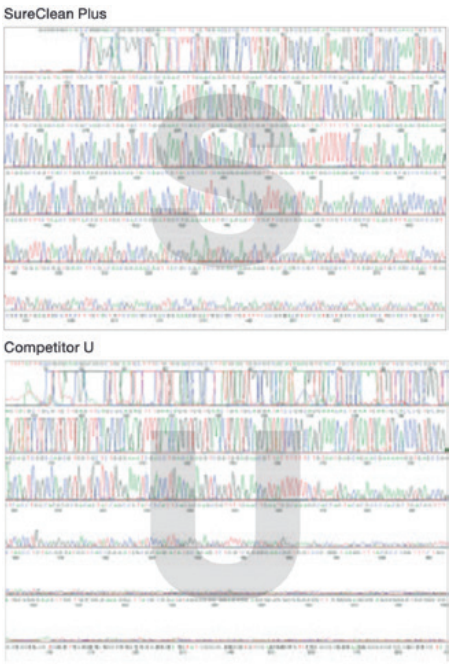

Fig 7. Sequencing results of a PCR fragment purified by using SureClean Plus and Supplier U's single-tube purification method.
Chart showing the length of read from a sequencing reaction, using SureClean Plus (S) and Supplier U's single-tube method (U).

C. Transfection

A vector containing GFP was prepared with and without SureClean Plus as a final clean-up step after plasmid preparation. 3 µg of the GFP vector was transiently transfected into CHOK1 cells and incubated for 24 hrs. Transfection efficiency was determined by flow cytometry on an Agilent 2100 Bioanalyzer. The data obtained (Fig. 9) illustrate an increased transfection efficiency of the vector, following further purification of DNA using SureClean Plus.

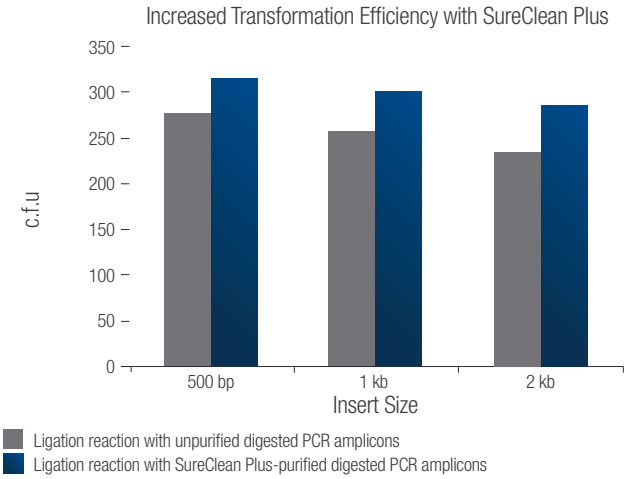

Fig 8. Transformation efficiency of ligation reactions performed with unpurified and SureClean Plus-purified DNA.
Three PCR amplicons, amplified from λ DNA were digested using the restriction endonucleases *EcoR*I and *Hind*III. The restriction digests were split into two. One half of the reaction was purified using SureClean Plus whereas the other half was not purified, and both the SureClean Plus-purified and the unpurified PCR fragments were ligated into an *EcoR*I/*Hind*III plasmid vector (3:1 insert:vector ratio) using Ligase. The chart above shows an increase in colony-forming units (c.f.u) when the SureClean Plus-purified PCR amplicons for the ligation reaction are used.

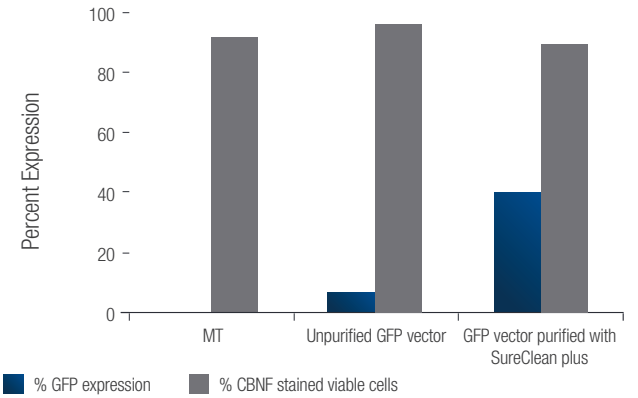

Fig 9. Transfection efficiency of SureClean plus-purified and unpurified GFP vector in CHOK1 cells.
3 µg of a GFP vector, either purified with SureClean Plus after plasmid preparation or not purified was transiently transfected into CHOK1 cells and incubated for 24 hours, The transfection efficiency was determined by flow cytometry on an Agilent 2100 Bioanalyzer and the data are shown on a bar graph with the % of GFP-expressing cell population and % of CBNF-stained viable cells. MT is the mock transfection.

SUMMARY

Many of today's commercially available methods for purification of DNA rely on chaotropic salts and expensive spin-columns. These can lead to low recovery rates, DNA duplex denaturation and excessive cost for the researcher. In contrast, SureClean Plus provides column-free purification without organic solvents, resulting in high quality and purity of both DNA and dsRNA with negligible loss of product.

In addition, SureClean Plus is a universal purification system, efficiently removing primers/primer-dimers, restriction enzymes, unincorporated nucleotides and inhibitors of downstream applications, and conversely exhibiting almost no loss of product (recovery rates of up to 98%). SureClean Plus enables the researcher to resuspend the purified nucleic acid in any buffer and volume of choice, thus permitting the purification process to be tailored specifically to suit the experiment.

SureClean Plus enables standard 50 µL reactions to be purified in a single tube at approximately one-third of the cost of suppliers' spin-columns. When smaller reaction volumes are used, savings are even higher. For applications in which visual recognition of the DNA pellet is desired, SureClean Plus is supplied with a pink co-precipitant.