RECOVERY vs CONCENTRATION

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

PURIFICATION OF dsRNA

In order to assess recovery of dsRNA, a 500bp fragment of dsRNA was synthesized using T7 RNA Transcription kit (Cat No. BIO-21072). 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 in accordance with the standard purification protocol. The purified dsRNA was subsequently resuspended in DEPC-treated water (Cat No. BIO-38030), and analyzed on a 3.5% agarose (RNase-free) gel (fig. 5). This experiment demonstrates that SureClean-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 and with Competitor Q's column-based purification method. The purified fragment was then resuspended, 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.

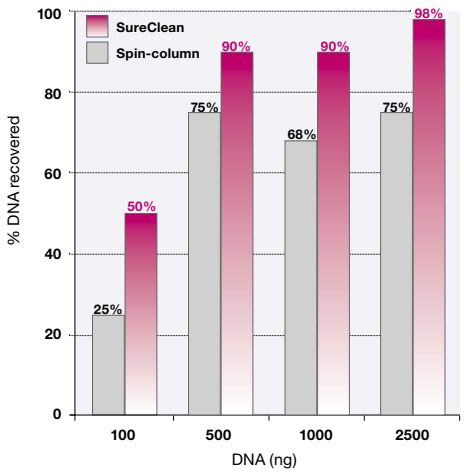

Figure 4. Recovery vs concentration. DNA purification using SureClean and Competitor's Q spin-column purification method. 30µl DNA solutions containing 100, 500, 1,000 and 2,500ng of plasmid DNA were purified using either SureClean or Competitor Q spin-columns.

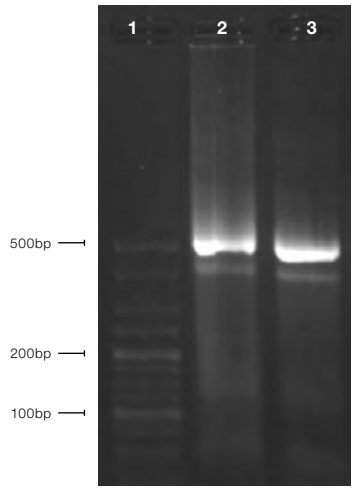

Figure 5. dsRNA recovery. A 500bp dsRNA fragment was synthesized using the T7 RNA Transcription kit, and then purified using Sureclean.
Lane 1. HyperLadder V
Lane 2. Unpurified dsRNA
Lane 3. dsRNA purified using SureClean

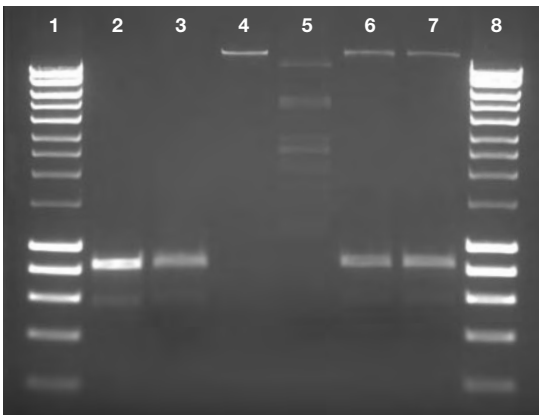

Figure 6. Enzyme Removal. An experiment was performed to remove the restriction endonucleases *Pst*I from a restriction digest and then demonstrate the absence of the enzyme in downstream applications.
Lane 1. HyperLadder I
Lane 2. ß-actin PCR fragment
Lane 3. ß-actin PCR fragment plus *Pst*I and *Pst*I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

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 2Kb fragment was amplified by PCR and subsequently purified using SureClean and Competitor U's single-tube purification method. The sequencing results obtained (fig. 7) indicate that SureClean efficiently increased the length of read of the purified PCR product.

B. CLONING

The ability of SureClean to enhance successful cloning was assessed. Three different-sized amplicons of 500bp, 1Kb and 2Kb 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, 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 Quick-Stick Ligase (Cat No. BIO-27028). The ligation reaction was then directly cloned into α-Select Gold-Efficiency Competent Cells (Cat No. BIO-85027). The data shown (fig. 8) demonstrate the suitability of SureClean as an important extra step in cloning the ligated PCR products.

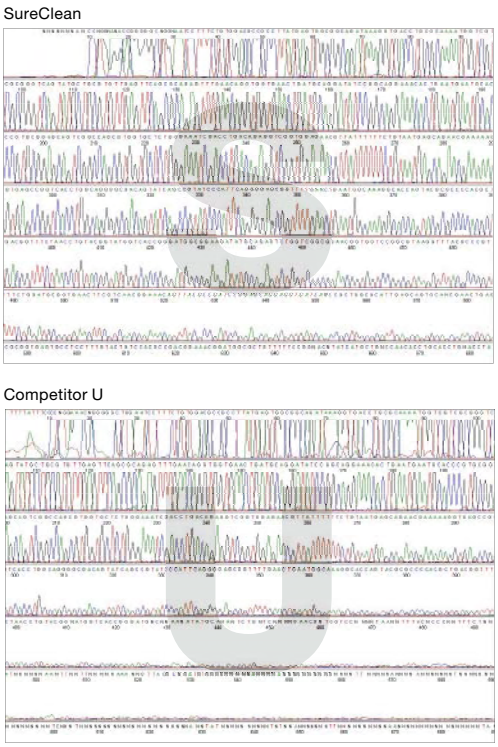

Figure 7. Sequencing results of a PCR fragment purified by using Sureclean and Competitor U's single-tube purification method. Chart showing the length of read from a sequencing reaction, using SureClean (S) and competitor U's single-tube method (U).

C. TRANSFECTION

A vector containing GFP was prepared with and without SureClean as a final clean-up step after plasmid preparation. 3µg of the GFP vector was transiently transfected into CHOK1 cells at a 1:4 ratio of DNA:PlasFect reagent (Cat No. BIO-46026) 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.

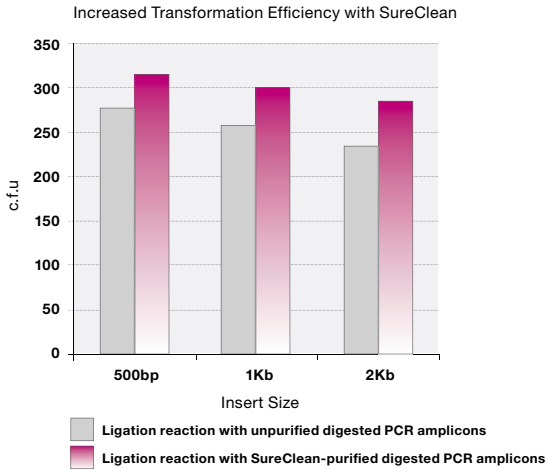

Figure 8. Transformation efficiency of ligation reactions performed with unpurified and SureClean-purified DNA. Three PCR amplicons, amplified from λ DNA were digested using the restriction endonucleases *EcoR*I and *Hind*III. The restriction digests were split into two. One half of the reaction was purified using SureClean whereas the other half was not purified, and both the SureClean-purified and the unpurified PCR fragments were ligated into an *EcoR*I/*Hind*III plasmid vector (3:1 insert:vector ratio) using Quick-Stick Ligase (Cat No. BIO-27028). The chart above shows an increase in colony-forming units (c.f.u) when the SureClean-purified PCR amplicons for the ligation reaction are used.

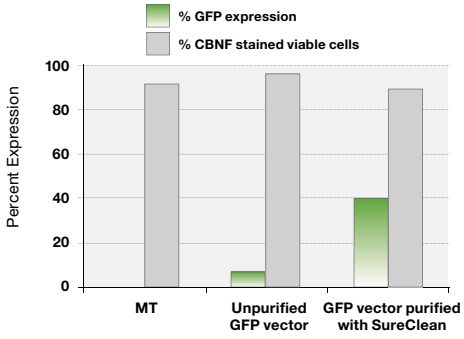

Figure 9. Transfection efficiency of SureClean-purified and unpurified GFP vector in CHOK1 cells. 3µg of a GFP vector, either purified with SureClean after plasmid preparation or not purified was transiently transfected into CHOK1 cells at a 1:4 ratio of DNA:PlasFect (Cat No. BIO-46026) and incubated 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.