High-throughput isolation of plasmid DNA suitable for mammalian cell transfection

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Abstract

A high-throughput plasmid DNA isolation system for the automated isolation of plasmid DNA was developed. The system is uniquely suited for the isolation of high-quality plasmid DNA from bacterial cells using either a vacuum- or centrifuge-based protocol. Plasmid DNA isolated is of high quality as evidenced by the absence of RNA, extremely low genomic DNA contamination, high supercoiled to nicked form ratios, low endotoxin levels, as well as reliable performance in all downstream applications such as restriction digestion, PCR, sequencing, transformation of bacterial cells, and most importantly transfection of mammalian cells.

Introduction

The PureLink™ 96 HQ Plasmid DNA Purification Kit is based on a modified alkaline cell lysis procedure and selective plasmid DNA binding to glass microfiber (GF) filters. Plasmid DNA is subjected to stringent wash procedures after binding to a silica-based matrix and is efficiently eluted with a low salt buffer or water in a final step after which it can be used directly in all relevant downstream applications.

The PureLink™ 96 HQ Plasmid DNA Purification Kit has been validated on robotic workstations such as the Biomek FX (Beckman-Coulter) and the Genesis (Tecan USA) liquid handling platforms. The automated protocols result in consistently high DNA yield and quality (Figure 1) with low genomic DNA and non-detectable RNA contamination (data not shown). DNA was mainly in supercoiled form.

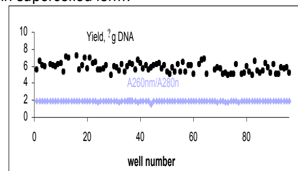


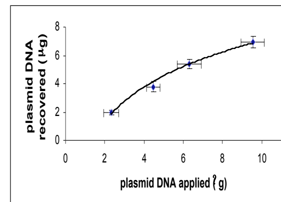
Figure 1: Well-to-well variation analysis of 96 plasmid DNA samples isolated using the PureLink™ 96 HQ kit (vacuum protocol) on the Beckman FX. The average yield of isolated plasmid DNA (1.3 x 10^9 *E. coli* cells/ well) was 5.8 µg ± 10.3% and the average ratio A260nm/A280nm: 1.86 ± 0.04.

Well Capacity and DNA Recovery

Pre-purified plasmid DNA (pUN1L6A) was mixed with Neutralization/Binding buffer and isolated using the PureLink™ 96 HQ kit. 2-15 µg plasmid DNA was applied to each well. The elution was performed with 150 µl Elution Buffer.

The average recovery for the plasmid DNA was 75- 80% in all cases (Figure 2), demonstrating a well binding capacity greater than 10 µg. Recovery reached 90% when 2 µg DNA were applied.

The well-to-well variability (CV%) remained below 10%, with no apparent dependence on the initial amount of DNA that was applied (data not shown). From the 150 µl elution volume, 118.7 µl with a CV % of less than 5% were routinely recovered using vacuum filtration.



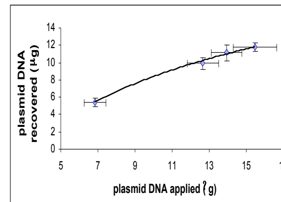


Figure 2: Well binding capacity for plasmid DNA. DNA concentration (A260nm) as well as volume recovery were determined using the SpectraMax 384 spectrophotometer.

Mammalian cell transfection

Plasmid DNA (pcDNA3.1™/His/*lacZ*) purified with the PureLink™ 96 HQ or an anion-exchange based 96-well plate kit was used for transfection of two mammalian cell lines, GripTite™ MSR 293, and Huh-7 cells using Lipofectamine™ 2000.

DNA isolated with either PureLink™ 96 HQ or the anion exchange kit showed similar toxicity levels with the %CV for the PureLink™ 96 HQ samples slightly above the expected 10% (data not shown).

Endotoxin levels for the DNA isolated with PureLink™ 96 HQ were confirmed (Pyrotell®-T reagent) at low levels (<100 EU/µg DNA).

Higher reporter gene (β-galactosidase) expression levels in the GripTite™ 293 MSR cell line were seen compared to Huh-7 cells (Figure 3). This was expected since it is known that GripTite™ 293 cell line is less sensitive to the presence of endotoxin. The higher variation observed with the DNA isolated with PureLink™ 96 HQ agree well with the higher variation in the toxicity values for the same samples.

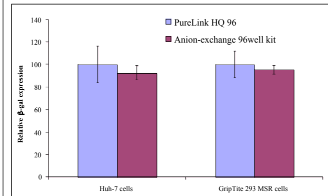
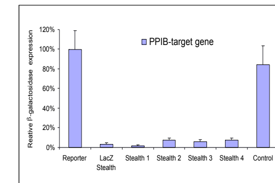


Figure 3: Transfection efficiency using plasmid DNA isolated with PureLink™ 96 HQ (n=96) or an anion-exchange based 96-well kit (n=27). Transfection efficiency, i.e. β-galactosidase activity, was determined using the Tropix Galacto-Star™ System, (chemiluminescent β-galactosidase assay) 24 h post-transfection. Results were expressed as β-galactosidase (ng) activity per well. Relative transfection efficiencies are shown as percentages relative to the efficiency obtained with the PureLink™ 96 HQ kit (100%) for each cell line.

Stealth™ RNAi Target Screening

pSCREEN-iT™/ lacZ-DEST, a Gateway®-adapted reporter vector, was used to clone the target gene (e.g. PPIB: cyclophilin B or CDK2: cyclin dependent kinase). PureLink™ 96 HQ Plasmid DNA kit (centrifugation protocol) was used to isolate the screening expression vectors from *E. coli* cultures. Target screening was performed with co-transfection of the purified vector with the corresponding Stealth™ RNAi molecule at 2 nM concentration in GripTite™ 293 MSR cells using Lipofectamine™ 2000. (Figure 4). Target RNA knockdown was assessed by measuring β-galactosidase reporter readout 24 h post transfection.

Consistently high quality plasmid DNA was isolated with PureLink™ 96 HQ kit and was used in Stealth™ RNAi target screening.



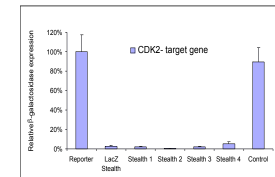


Figure 4: Stealth™ RNAi Target Screening using expression vectors isolated with PureLink™ 96 HQ kit. β-galactosidase expression levels for different Stealth RNAi are shown relative to expression when cells are transfected only with the expression construct, Reporter, (=100%). Control represents non-target specific RNAi molecule; LacZ Stealth represents LacZ-specific Stealth RNAi.

Results and Conclusions

- Quick and efficient isolation of high-quality plasmid DNA from *E. coli* cells
- PureLink™ Binding Plate well capacity: 10 µg
- Low genomic DNA and no detectable RNA contamination
- High quality of isolated plasmid DNA with either the centrifugation or the vacuum filtration protocol
- Compatible with Biomek FX and Tecan Genesis liquid handling stations
- Isolated plasmid DNA suitable for use in automated, fluorescent sequencing with >98% accuracy of base identity for a 600-base read (data not shown)
- Low endotoxin content and high supercoiled to nicked forms ratio provide consistent high transfection efficiencies when DNA is introduced into most of the commonly used mammalian cell lines
- High quality of isolated plasmid DNA successfully used in Stealth™ RNAi target screening experiments

Acknowledgements

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