A Fully Automated Workflow for High-Throughput Purification of Transfection-Grade Plasmid

Joshua Lee, Zoë Hermsen & Aaron Clausen

Zymo Research Corporation - 17062 Murphy Ave, Irvine, CA 92614

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BACKGROUND

Transfecting mammalian cells with plasmid DNA has become a critical tool for studying biological processes, developing therapeutics, gene editing, producing proteins, and generating recombinant viruses. While advances in DNA synthesis have simplified the construction of variant gene libraries for evaluating candidates of interest with transfection, purifying transfection-grade plasmid remains a major bottleneck for high-throughput screening. Traditionally, plasmid that meets the quantity, concentration, and low endotoxin requirements for mammalian cell transfection is prepared from large volumes of overnight *E. coli* culture using slow gravity flow anion-exchange columns and lengthy alcohol precipitation steps, making the process poorly suited for high-throughput and automated processing. To address this issue, Zymo Research developed a patented DNA purification method that is capable of purifying high amounts of transfection-grade plasmid from small volumes of overnight culture using silica coated magnetic beads. When combining this method with specialized magnetic particles that efficiently remove cellular debris following lysate neutralization, there is no need for centrifugation, making the plasmid purification process easy to completely automate using commercially available liquid handlers.

METHODS

- **Culturing *E. coli*** - 1 Liter of JM109 *E. coli* culture containing the mammalian luciferase expression vector pGL3-Control® (Promega) was grown overnight in LB medium to an OD_{600} of 2.67.
- **Plasmid Purification** - Plasmid was purified from 1 and 5 ml of overnight *E. coli* culture using Zymo Research's magnetic bead plasmid purification system or from 100 ml of overnight *E. coli* culture using Qiagen's EndoFree Maxi Kit following the manufacturer's suggested protocol.
- **Nanodrop Spectrophotometer Analysis** - Concentration and purity of eluted plasmid was assessed by measuring the absorbance at 260, 280 & 230 nm wavelengths using a Nanodrop Spectrophotometer (Thermo).
- **Gel Electrophoresis** - 5 µl of purified plasmid DNA from 5 ml of overnight culture were randomly selected across the plate and visualized on a 0.8 % agarose gel after running for 120 minutes at 100 volts.
- **Sequencing** - 10 µl of purified plasmid DNA from 5 ml of overnight culture were randomly selected across the plate and sent out for whole plasmid sequencing using Oxford Nanopore Technology (Plasmidsaurus).
- **Transfection** - 200 ng of plasmid purified from 5 ml of overnight culture using Zymo Research's magnetic bead system or from 100 ml of overnight culture using Qiagen's EndoFree Maxi Kit was transfected into HEK293T cells – a commonly used mammalian cell line for recombinant protein expression and virus production – using Lipofectamine 3000 (Invitrogen). The One-Glo Luciferase Assay (Promega) and an H1 Synergy Microplate Reader (BioTek) were used to detect the expression of the luciferase gene.

RESULTS

Recover highly concentrated plasmid from up to 5 ml of bacterial culture that is free from protein and salt contamination.

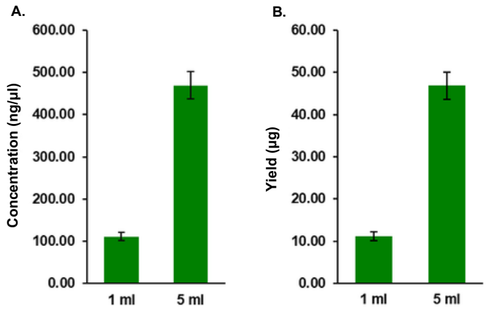

Figure 1. Average plasmid concentration (**A**) and yield (**B**) for 1 and 5 ml overnight *E. coli* culture inputs across the 96-well plates. Total plasmid yield and concentration was consistently high for both 1 and 5 ml cultures and plasmid yield from 5 ml of culture was approximately 5 times higher than that from 1 ml of culture, demonstrating that the plasmid purification system is robust even at higher culture loads.

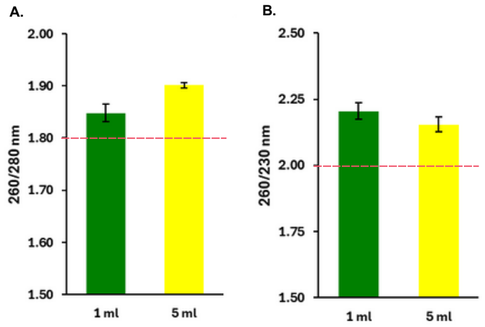

Figure 2. Average A260/A280 (**A**) and A260/A230 (**B**) ratios for 1 and 5 ml overnight *E. coli* culture inputs across the 96-well plates. A A260/A280 ratio above 1.8 indicates no protein contamination and a A260/A230 ratio above 2.0 indicates no salt contamination in the purified plasmid. These thresholds are represented by the red dotted lines on the graphs above. The A260/A280 (**A**) and A260/A230 (**B**) ratios revealed that plasmid purified from both 1 and 5 ml culture inputs were not contaminated with proteins or salts.

Purified plasmid is consistently supercoiled, free from host genomic DNA & RNA, and suitable for sequencing.

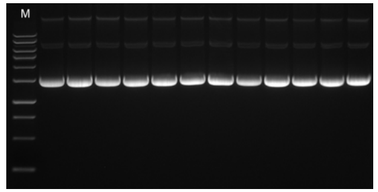

Figure 3. Visualization of the purified plasmid post agarose gel electrophoresis confirmed that the recovered plasmid was predominantly supercoiled and free of host genomic DNA and RNA. M: 1 kb DNA Ladder (Zymo Research).

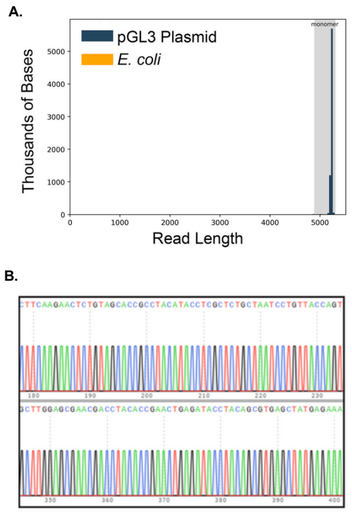

Figure 4. Recovered plasmid DNA is free of *E. coli* genomic DNA and suitable for Oxford Nanopore whole plasmid sequencing. An example of a read length histogram (**A**) and a sequencing chromatogram (**B**) for the purified plasmid from one of the wells.

Purified plasmid consistently produces high luciferase expression in HEK293T cells that is comparable to traditional endotoxin-free, anion-exchange plasmid purification techniques.

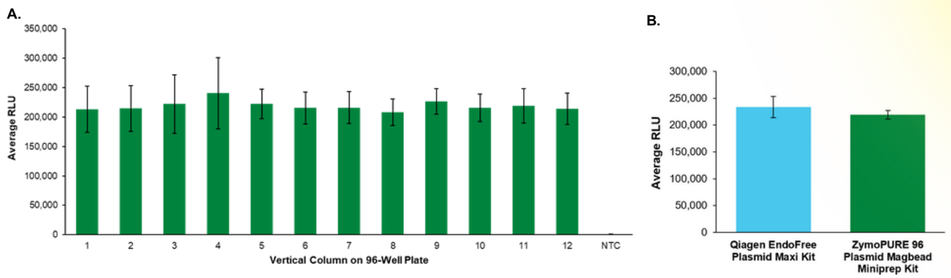

Figure 5. HEK293T cells (Passage 6) were plated on a 96-well TC plate at 10K cells/well and incubated 48 hours prior to transfection (70 % confluency). Bar graph representations of relative light units (RLU) averaged for each vertical column in the 96-well HEK293T culture plate (**A**) and averaged across the entire 96-well HEK293T culture plate (**B**). *NTC (No Transfection Control) wells had low RLU and plasmid transfection into HEK293T cells resulted in consistently high luciferase expression that was similar to plasmid produced with a traditional anion-exchange endotoxin-free plasmid purification method (**B**).

ZymoPURE 96 Plasmid Magbead Miniprep system results in superior concentrations and yields compared to other commercially available magnetic bead-based kits.

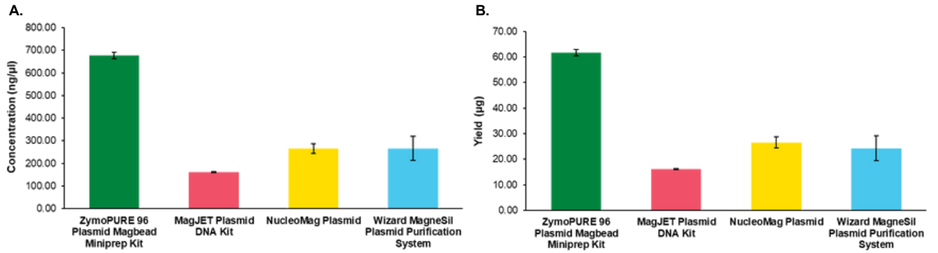

Figure 6. Plasmid DNA was purified from 5 ml of the same overnight *E. coli* culture using Zymo Research's magnetic bead system or several popular magnetic bead-based kits from other suppliers. Average plasmid concentration (**A**) and yield (**B**) achieved with the ZymoPURE 96 Plasmid Magbead Miniprep Kit was superior to other commercially available magnetic bead-based plasmid purification solutions. Shown are means ± SDEV of 8.

CONCLUSIONS

In successfully integrating magnetic bead technology with a high-yield and low endotoxin plasmid prep system, this method enhances the efficiency of using transfection for large-scale screening, accelerating discovery using recombinant plasmid DNA technology.

Contact
Joshua Lee: jlee@zymoresearch.com