Automated High Throughput Transient Transfection

Abstract 2 of the paper:

"Implementation of an Automated High-Throughput Plasmid DNA Production Pipeline"

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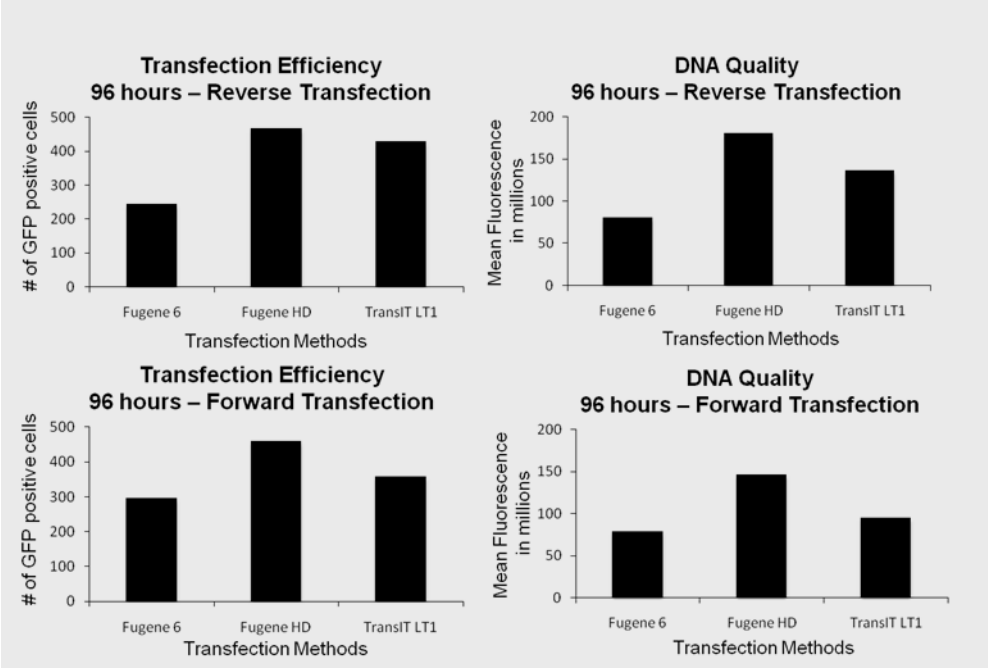
This paper highlights a new technology for fully automated, small-scale plasmid purification. This technology integrates with mature technologies for completely automated transient transfection of mammalian cells and high throughput mammalian protein purification.

Automated small-scale-plasmid DNA purification is a novel technology enabling a workflow that integrates seamlessly with high throughput, automated transient transfection through to high throughput, automated protein purification.

Automation of transient transfection begins with manual experiments to determine the optimal ratio of DNA to lipid, and the optimal ratio of the DNA:lipid complex to cells. A typical, finalized manual protocol is the following. Mix 6 µL of 10 ng/µL DNA with 10 µL 2.3 mM 25 kDa linear PEI and 84 µL DMEM-based medium to make up 100 µL of the lipid DNA complex. Incubate the DNA:lipid complex at 4°C for 10 minutes and then add to 850 µL of Expi293. These cells are obtained from seeding cells at 2 x 106 cells/mL and incubation at 37°C, 8% CO_2 with shaking for 2 hours. The transfection reaction expresses protein with incubation for 7 days at 37°C, 8% O_2, 80% humidity with shaking.

To automate this transfection procedure, each manual step is evaluated and, if necessary, modifications are made to conform to the constraints of automated liquid handling. However, these parameters used for automation are along the lines stated above. Liquid handling instrumentation utilizes a variety of pipette tip volumes. Ideally, automated transient transfection would employ 200 or 300 µL volume pipette tips to form the DNA:lipid complex as well as transfer the complex to the cell cultures. In addition, the instrument should be able to utilize 1 mL pipette tips, which would be used to aliquot Expi293 cell stocks to individual wells of a deep well plate. The transfer volumes and fluid flow rates used for automated transient transfection is well within the limits of standard liquid handing instrumentation. The final consideration for automating transient transfection is determined by the desired throughput. Liquid handling automation is available with pipette heads equipped with different options including 4, 8, 12, and 96 at-a-time pipetting channel heads.



The quality of the purified plasmid was demonstrated by transfection into COS7 cells using three different transfection reagents by both forward and reverse methods. Transfections were carried out as per manufacturer's suggested protocols. Ninety six hours after transfection, GFP positive cells were counted and mean fluorescence calculated for each method using an incuCyte instrument. As shown by the graph, all three methods yielded normal number of transfected cells as well as normal mean fluorescence with plasmid purified with pipette tip columns.

High throughput transient transfection of mammalian cells for high throughput protein production requires automating all of the key steps within several processes including steps upstream to transient transfection. These steps include transformation, colony picking, plasmid purification and normalization, transient transfection of mammalian cells, protein expression and protein purification. Each step of automation was examined and assayed to measure throughput and quality of delivered product.

Procedure for high throughput mammalian protein production

A. Automated Plasmid Production

1) Setup *E. coli* cultures. Aliquot 1.4 mL TB to a 2 mL deep-well plate with square wells and round bottom
2) Innoculate medium with single colony of interest using a colony picker
3) Seal the plate with a Thompson seal
4) Incubate at 37 °C with shaking at 300 rpm for 16 hours
5) Harvest cells when OD600 reaches 18
6) Spin plate for 15 minutes at 1,000 rpm
7) Decant spent media
8) Place plate of cell pellets on liquid handling robot deck and setup for Lysate Direct PhyTip Column purification
9) Measure Plasmid DNA concentration and normalize to 15 ng/µL

B. Automated Transient Transfection

1) Dispense 850 µL Expi293F cells at 2.0e6 cells/mL to each well of a 2 mL deep well plate
2) Incubate at 37 °C, 8% CO_2 with shaking at 1,000 rpm with 3 mm orbital diameter for 2 hours
3) Dilute 20 µL plasmid DNA, corresponding to 1 µg, with 80 µL of a solution consisting of DMEM medium supplemented with 25 kDa PEI at 0.23 mM.
4) Using automation mix a volume of 85 µL three times. Incubate at 4 °C for 10 minutes. Then add to the cells.
5) Incubate at 37 °C, 8% CO_2, 80% humidity with shaking.
6) Add feed reagents 24 hours post transfection as per manufacturer's recommendations.

C. Automated Mammalian Protein Purification

1) Spin cell cultures at 5k RPM for 15 minutes
2) Transfer supernatant to fresh 2 mL Deep Well Plate
3) Use 1 mL PhyTip columns packed with 20 µL of Protein A resin to purify proteins
4) Load PhyTip columns and equilibrate in 1 mL with 1 cycle of processing. While maintaining the tip of the PhyTip column 1 mm above the bottom of the well,one cycle consists of aspirating actual volume less 50 µL at 0.5 mL/minute, pause 20 seconds, dispense actual volume less 50 µL at 0.5 mL/minute, and pause 20 seconds.
5) Capture sample using 4 cycles at 0.25 mL/minute
6) Wash in 1 mL Wash 1 buffer using 1 cycle
7) Wash in 1 mL Wash 2 buffer using 1 cycle
8) Elute using 60 µL Elution buffer. For any volume less than 250 µL, aspirate and dispense an additional 230 µL. Use 4 cycles.
9) Neutralize by adding 15 µL of neutralization buffer and mix with 1 cycle of 75 µL.