



QIAGEN® plasmid purification technologies for high-throughput transfection screening using automated and manual processing

Different manual and automated purification technologies were examined to study the effects of DNA quantity and purity on transfection efficiency. Human cells (293 and Huh-7) and Chinese Hamster Ovary cells (CHO-K1) were transfected with plasmid DNA isolated using either MagAttract® 96 miniprep technology on a BioRobot® 8000 workstation, QIAprep® 96 Turbo technology on a BioRobot 3000 workstation, or QIAwell® Ultra technology using a manual procedure.

Transfection, the introduction of foreign molecules such as DNA into a recipient eukaryotic cell, has become a powerful tool for the study and control of gene expression, mutational analysis, and the investigation of regulatory elements and cell signaling. Transfection screening is a high-throughput application used to screen large numbers of DNA sequences for specific activities in cultured cell lines. This approach is widely used in the drug discovery process and necessitates the use of miniprep chemistries in 96-well format and laboratory automation. Transfection efficiency is influenced by parameters such as the vector construct and cell-line used, the amount, purity, and quality of DNA used for transfection, and the transfection method.

Anion-exchange purification technologies for mini- to large-scale plasmid purification deliver ultrapure DNA that is optimal for transfection. Several QIAGEN® DNA purification technologies are available in 96-well format (Table 1). The suitability of these technologies, together with automated high-throughput plasmid purification technologies were evaluated for transfection of different cell lines using Effectene® Transfection Reagent.

Table 1. DNA purity using QIAGEN technologies

Technology	Principle	DNA purity
MagAttract	Silica adsorption	High
QIAprep Turbo	Silica adsorption	High
QIAwell Ultra	Anion-exchange	Ultrapure



Materials and methods

Plasmid DNA (pCMV β) was purified from *E. coli* DH 5 α TM using MagAttract 96 Miniprep technology on the BioRobot 8000 workstation and QIAprep 96 Turbo 96-well technology on the BioRobot 3000 workstation. A manual procedure using QIAwell technology was also used. DNA purified using each technology was pooled and yields determined by absorbance and densitometric gel analysis (Table 2). Endotoxin levels were measured using the Kinetic-QCLTM test kit.

Table 2. Yields and endotoxin activity of plasmid DNA purified using QIAGEN technologies

Technology	DNA yield/well ($\mu\text{g}/\mu\text{l}$)		Endotoxin level (EU/ μg)
	Absorbance	Gel analysis	
QIAwell Ultra	14.1	11.0	0.2
QIAprep Turbo	12.4	8.1	50
MagAttract	2.1	1.5	24054

Huh-7 cells, and CHO-K1 cells (2×10^4 per well) and human 293 cells (4×10^4 per well) were seeded into 96-well plates 1-day before transfection. Transfections using 0.1 μg DNA measured by gel analysis and 1–4 μl of Effectene Reagent per well were performed according to the standard protocol. After an incubation of approximately 6 hours, complexes were removed from the cells and fresh medium was added. Transfection efficiency was measured two days after transfection using a β -galactosidase assay (1).

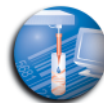
Results

Human 293 cells showed comparable transfection efficiencies with DNA purified using all three purification technologies (Figure 1). Transfection efficiencies using CHO-K1 cells and QIAwell, or QIAprep purified DNA showed comparable results (Figure 2). Transfection efficiency was reduced using MagAttract purified DNA.

Transfection efficiency was clearly influenced by DNA quality using the sensitive cell line Huh-7 (Figure 3). QIAwell purified DNA delivered the highest transfection efficiencies in all cell lines tested. QIAprep purified DNA showed slightly reduced transfection efficiencies compared to QIAwell purified DNA. MagAttract purified DNA showed very low transfection efficiencies in Huh-7 cells.

References:

1. Hall C.V., P.E. Jacob, G.M. Ringold, F. Lee. 1983. Expression and regulation of *Escherichia coli lacZ* gene functions in mammalian cells. *J. Mol. Appl. Genet.* **2**: 101.



Transfection Efficiencies with Effectene Reagent and 293 Cells

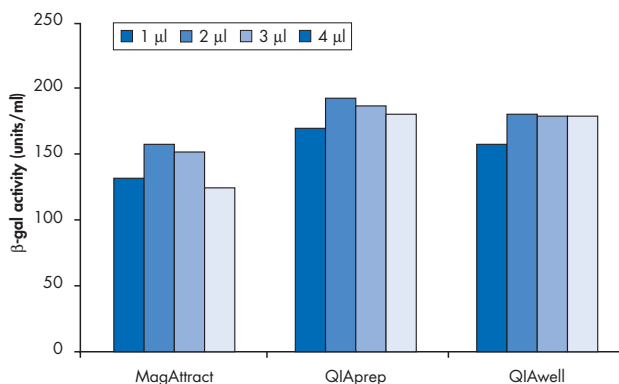


Figure 1. Colored bars correspond to different volumes of Effectene Reagent used for transfection: 1 μ l, 2 μ l, 3 μ l, 4 μ l. Values are means based on 2 independent experiments.

Transfection Efficiencies with Effectene Reagent and CHO-K1 Cells

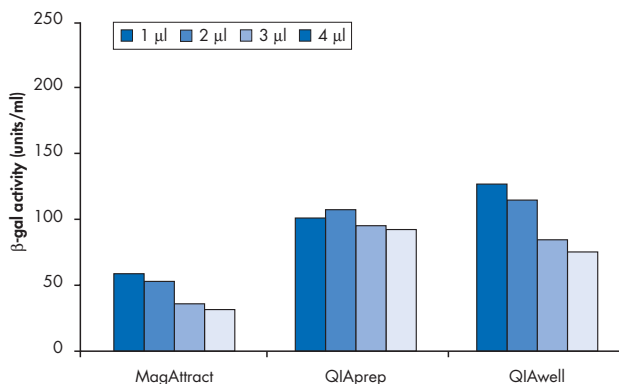


Figure 2. Colored bars correspond to different volumes of Effectene Reagent used for transfection: 1 μ l, 2 μ l, 3 μ l, 4 μ l. Values are means based on 3 independent experiments.

Transfection Efficiencies with Effectene Reagent and Huh-7 Cells

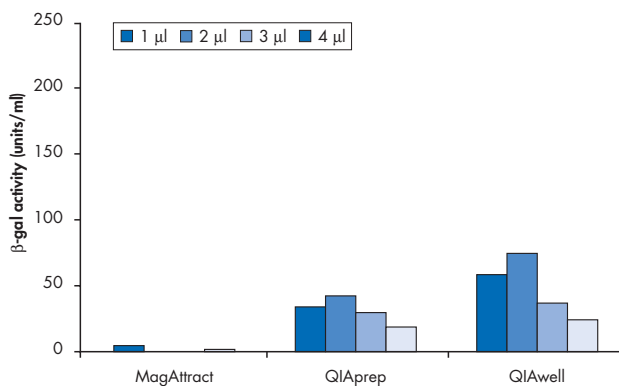


Figure 3. Colored bars correspond to different volumes of Effectene Reagent used for transfection: 1 μ l, 2 μ l, 3 μ l, 4 μ l. Values are means based on 2 independent experiments.



Conclusions

Generally, ultrapure or ultrapure and endotoxin-free DNA should be used for transfection, but this can present cost problems for high-throughput projects such as transfection screening. Using Effectene Reagent, highly pure miniprep DNA is suitable for transfection screening when using robust cell lines, although ultrapure DNA should be used when working with sensitive cell lines. The transfection technology used also influences the efficiency of transfection screening.

- For primary cells and sensitive cells such as Huh-7, ultrapure miniprep DNA isolated using QIAwell Ultra anion-exchange technology should be used.
- QIAprep Turbo silica-membrane technology is suitable for purification of plasmid DNA for use in transfection screening of less sensitive cell lines such as CHO-K1, and robust cell lines such as 293.
- DNA purified using MagAttract 96 Miniprep magnetic-bead technology is suitable for transfection of very robust cell lines but not sensitive cell lines such as Huh-7.
- Automating purification of plasmid DNA using BioRobot workstations accelerates high-throughput transfection screening projects.

QIAprep®, QIAwell®, MagAttract®, BioRobot®, Effectene®, (QIAGEN); Kinetic-QCL™ (BioWhittaker Inc.); DH 5α™ (Life Technologies Inc.).

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