Plasmid-mediated gene silencing

Using short interfering RNA (siRNA) is the method of choice for gene silencing. The high-purity and variety of available modifications, combined with high transfection efficiencies make this an ideal tool for target confirmation, screening, and other high throughput projects. However, certain applications are suited to plasmid-mediated gene silencing, where target cells or organisms are transformed with plasmid DNA containing an RNA polymerase (pol) promoter that initiates transcription of a hairpin, or duplex, RNA. These hairpin, or duplex RNA are processed in vivo and direct gene silencing by mechanisms similar to siRNA.

Plasmid-based gene silencing:

Advantages	Disadvantages
Increased duration of silencing — in vivo propagation of the principal gives effective silencing, even of genes encoding proteins with long half-lives	**More hands-on time** — cloning, selecting, propagating and purifying vectors is tedious and provides multiple sources of error
Transfection is more stable — stable clones may be maintained and single-cell clones can be screened	**Vector maintenance** — sequencing of hairpin inserts is often required to check orientation and to ensure absence of sequence mutation or reorganization
	Purification of vector is critical — even small amounts of endotoxin and other contaminants can cause cytopathic effects, non-specific inhibition of protein translation, and other experimental artefacts

QIAGEN-quality plasmid purification and transfection for gene silencing

Critical factors for successful plasmid-based gene silencing include both purity of DNA and the efficiency of transfection. Levels of cytotoxicity following transfection depend not only on the cell type and transfection reagent used, but also on the purity of the plasmid DNA. Many contaminants of DNA, such as endotoxin, are capable of triggering non-specific effects, such as knock-down of gene expression through mechanisms similar to the PKR pathway and the interferon response.

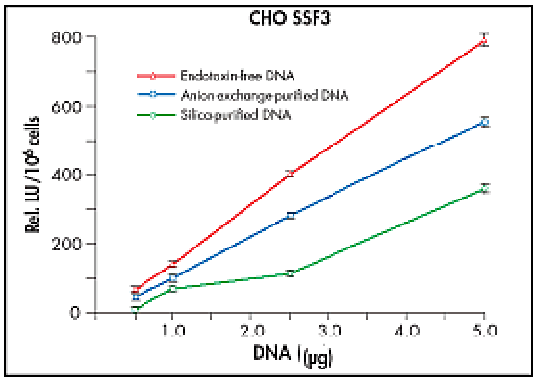
Non-specific knock down of gene expression and protein translation can result in meaningless gene silencing data. Using proven purification technologies to ensure high DNA quality, and minimal contamination with salts, proteins, and bacterial endotoxin helps to ensure optimal transfection results and specific gene silencing.

In order to maximise transfection efficiency and minimise cytotoxicity, it is most important to match transfection and purification technologies to the cell type. QIAGEN provides a range of manual and automated plasmid purification technologies and kits as well as a choice of transfection reagents and the QIAGEN Transfection Resource Book to ensure optimal gene silencing in either robust or sensitive cultured cells.

A range of DNA purities from plasmid minipreps

Technology	DNA yield/well (µg/µl) Absorbance	Gel analysis	Endotoxin level (EU/µg)
QIAwell Ultra	14.1	11.0	0.2
QIAprep Turbo	12.4	8.1	50
MagAttract	2.1	1.5	2405.4

DNA Purity Affects Transfection Efficiency



Effect of the amount and quality of DNA (a luciferase reporter plasmid) on transfection efficiency in CHO SSF variants grown in suspension under serum-free conditions. Each point represents the mean of three independent experiments; bars represent standard deviations; ***Rel. LU****: relative light units. Data kindly provided by M. O. Zang-Gandor, Novartis AG, Basel, Switzerland.*

Find out more about QIAGEN solutions for plasmid-mediated gene silencing in sensitive and primary cultured cells and standard cell lines.