Mirus

Selecting the Best Transfection Method

When to Use Transfection Reagents, Viral Transduction or Electroporation

INTRODUCTION

The uses of nucleic acid delivery have expanded from recombinant gene expression to a diversity of other experimental applications, including gene knockdown with small interfering RNAs (siRNA) and genome editing with CRISPR ribonuclear protein complexes (RNPs). With these advances, three approaches have become a mainstay for delivering nucleic acid cargo into mammalian and insect cells: chemical transfection, viral transduction, and electroporation (Table 1). We will highlight the primary benefit associated with each method to help you identify the best approach for a given experimental application and available laboratory resources. No single delivery method is ideal for all situations, but researchers may routinely employ a suboptimal approach for the sake of familiarity or to avoid any start-up costs associated with new methods. In addition to describing these three methods, we will introduce the Mirus Bio *Trans*IT® transfection reagents and Ingenio® EZporator® Electroporation System, which are both easy to use and cost-effective.

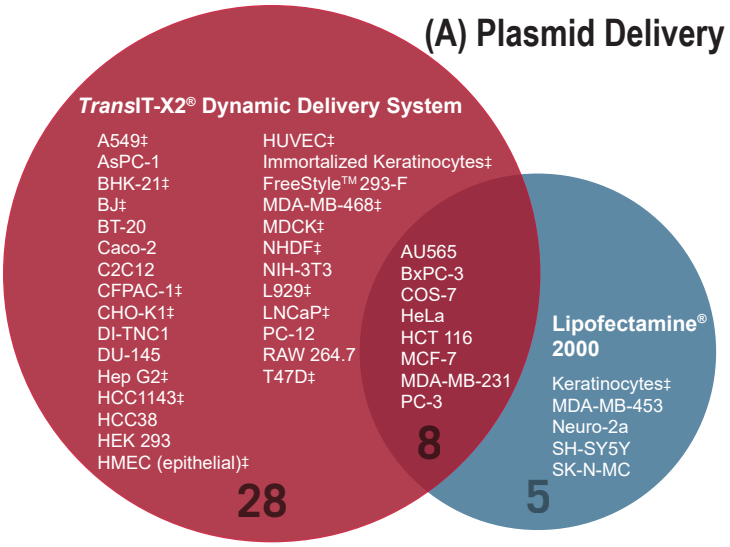
TABLE 1. Features of Common Gene Delivery Techniques.

	Chemical Transfection	Viral Transduction	Electroporation
Related Materials	chemical reagents, e.g. *Trans*IT® reagents, calcium phosphate, cationic lipids and polymers, nanoparticles	viral vector-producing or packaging cell line	electroporator, cuvettes, electroporation buffer
Mechanism of Action	condensation and complexation of cargo, mediation of charge interactions between cargo and cell surface, endocytosis	depends on viral vector, chemical reagents, entry of packaged cargo via viral infection of cells	permeabilization of cell membrane via applied electrical field
Process	add transfection complex mixture to cells	transfect cells to produce viral vectors, harvest and purify vectors, infect cells	suspend cells in buffer and apply electrical pulse
Time Required	minimal	several days	minimal
Primary Cost	chemical reagents	time, chemical reagents	up-front purchase of electroporator
Primary Benefit	convenience	efficient, targeted delivery to both *in vitro* and *in vivo* systems, including quiescent cells	delivery of diverse cargo to hard-to-transfect cell types

CONVENIENCE: CHEMICAL TRANSFECTION

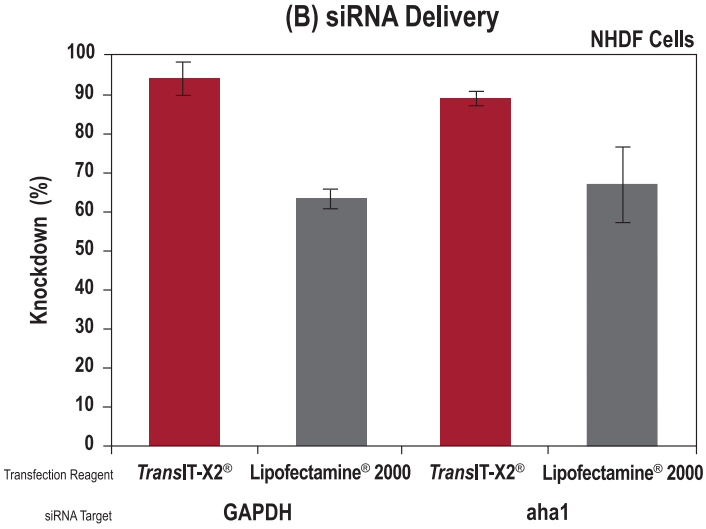
Complexing nucleic acids with chemical transfection reagents to deliver them into cells is generally the quickest method of delivery. For many common cell types, chemical transfection is highly efficient and nontoxic. No specialized materials are required aside from the chemical transfection reagent itself. Chemical transfection is often the most convenient delivery method for plasmids and oligonucleotides for gene expression and knockdown studies.

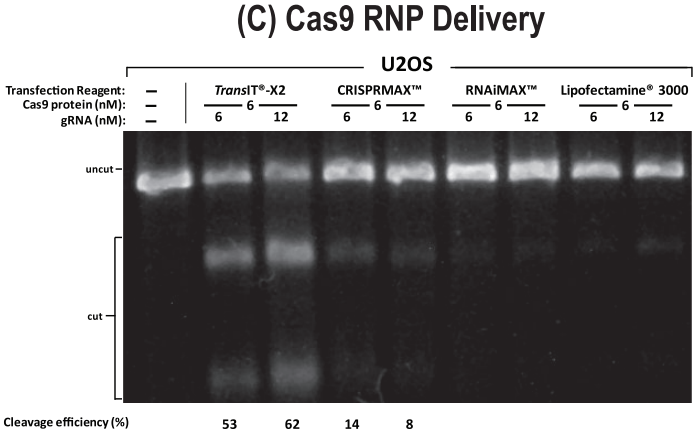
Some formulations, like the *Trans*IT-X2® Dynamic Delivery System, can also be used to complex with Cas9 RNPs for CRISPR-mediated gene editing (Figure 1). Mirus Bio also offers turnkey solutions for cell type-specific transfections, transfection of mRNA and oligos, and reagents for protein production in high-density suspension CHO and HEK 293 cell cultures. Depending on the cell type, chemical transfection may be the most convenient nucleic acid delivery method.



‡ Cell types with >2-fold luciferase expression in head-to-head comparisons.

FIGURE 1. *Trans*IT-X2® Dynamic Delivery System Outperforms Lipofectamine™ for Multiple Applications. (A) In a head-to-head comparison of plasmid DNA expression in 41 different cell types, the *Trans*IT-X2® Dynamic Delivery System outperformed Thermo Scientific Lipofectamine™ 2000 for most of the cell types tested. (B) Greater knockdown with siRNAs was also observed in some cell lines, like primary normal human dermal fibroblasts (NHDF). (C) In addition to plasmid and oligonucleotide delivery, the *Trans*IT-X2® Dynamic Delivery System can also complex with Cas9 RNPs for CRISPR-mediated gene editing studies. The *Trans*IT-X2® Dynamic Delivery System was used to deliver Cas9 RNPs into U2OS cells, and a T7E1 mismatch assay was used to measure cleavage efficiency at 48 hours post-transfection.





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*Trans*IT-X2® Dynamic Delivery System:
https://www.mirusbio.com/sample?filter=6010

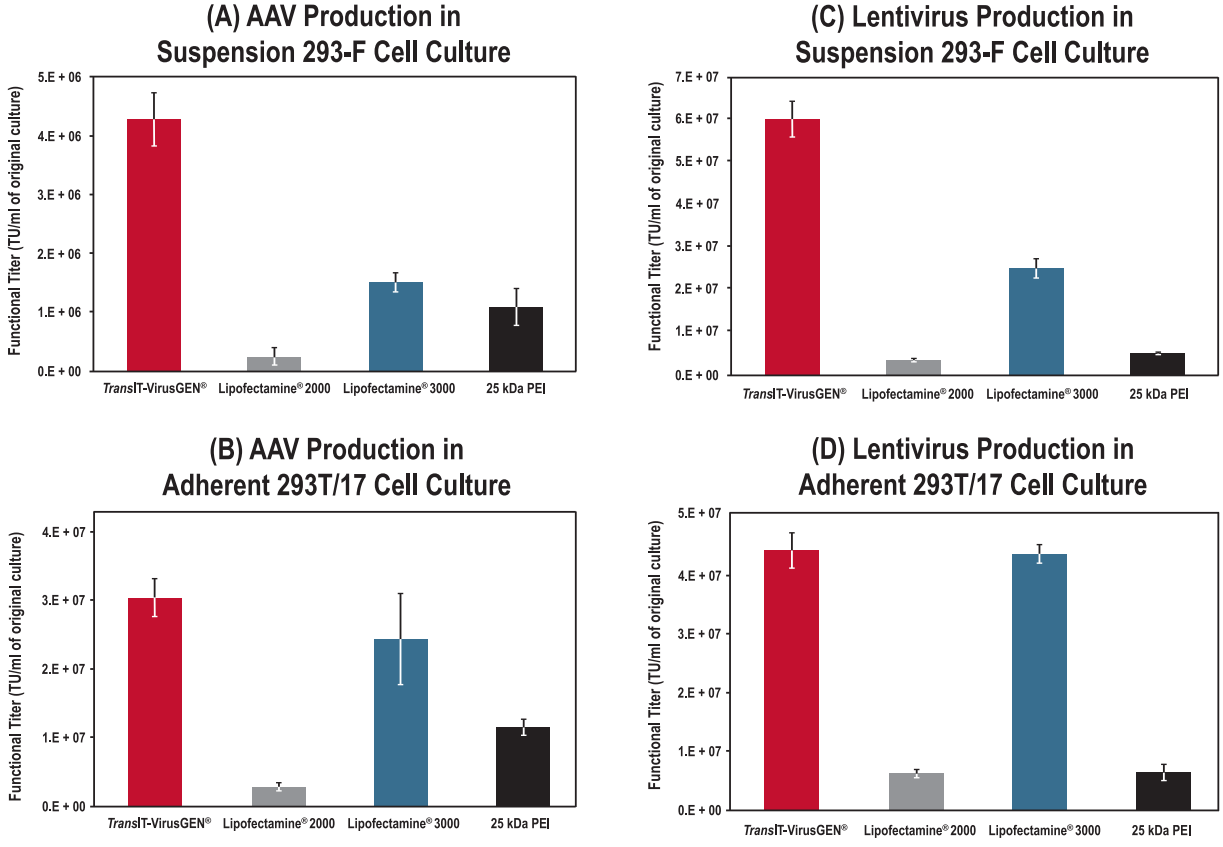
TARGETED EFFICIENCY: VIRUS TRANSFECTION

Virus-mediated expression via transduction with lentivirus (LV) or adeno-associated virus (AAV) is a valuable solution for some non-dividing cells and cells refractory to transfection with chemicals or chemical reagents. Additionally, recombinant viruses can be pseudotyped for cell-type specific infection and gene expression.

Though AAV and LV can both be used for *in vivo* and *in vitro* experiments, the small size of AAV (~20 nm) allows for more efficient spread in tissue than LV (>100 nm). However, the small size of AAV also limits its packaging capacity to 4.7 kb compared to LV (~10.2 kb). Expression of AAV can persist for weeks in non-dividing cells, while integration of lentiviral genomes can be used to generate stable cell lines.

Vectors are usually produced via transient co-transfection of packaging plasmids with a plasmid encoding the gene of interest. As shown in Figure 2, high titer production of LV or AAV with the *Trans*IT-VirusGEN® Transfection Reagent provides large quantities of virus for multiple viral transductions and difficult-to-transfect cells.

FIGURE 2. *Trans*IT-VirusGEN® Transfection Reagent for Higher AAV and LV Titers. Transfection with *Trans*IT-VirusGEN® Transfection Reagent yields higher titers per transfection compared to other reagents, which saves time and resources, eliminating the need for multiple transfections to produce the required amounts of AAV and LV in both suspension (A, C) and adherent (B, D) HEK 293 cells.



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FLEXIBILITY: ELECTROPORATION

Establishing a platform for viral vector production may be too time-consuming for some experimental applications. Electroporation is a quick, non-viral alternative for delivering diverse molecular cargo to difficult-to-transfect cells. Unlike the other two methods, efficient delivery is less limited by the size of the gene of interest or payload. For studies in difficult-to-transfect cells like primary T cells (Figure 3), consider an electroporation delivery approach. The Ingenio® Electroporation product line offers a universal solution for multiple cell types and cuvettes compatible with most conventional electroporators.

Or, choose the cost-effective Ingenio® EZporator® Electroporation Device to support your entire electroporation workflow.

Our expertise in transfection has helped Mirus Bio craft a comprehensive offering for nucleic acid delivery. Our team of dedicated scientists are happy to help with your transfection and electroporation needs.

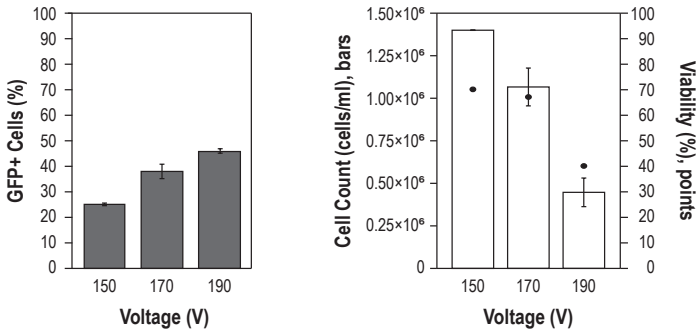

FIGURE 3. The Ingenio® Electroporation System Balances Efficiency and Viability. Primary human T cells were electroporated with an eGFP reporter plasmid and assessed after electroporation by flow cytometry.

Did you know you can demo the EZporator® Electroporation Device?

Visit: www.mirusbio.com/EZporator/demo

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