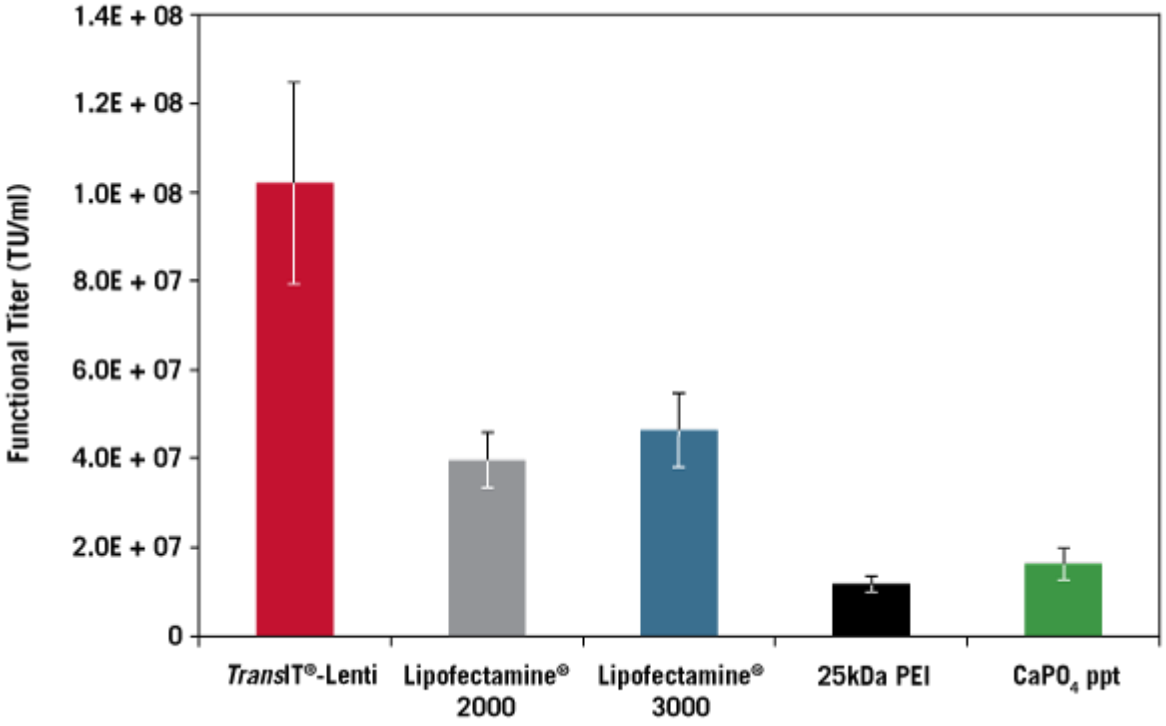
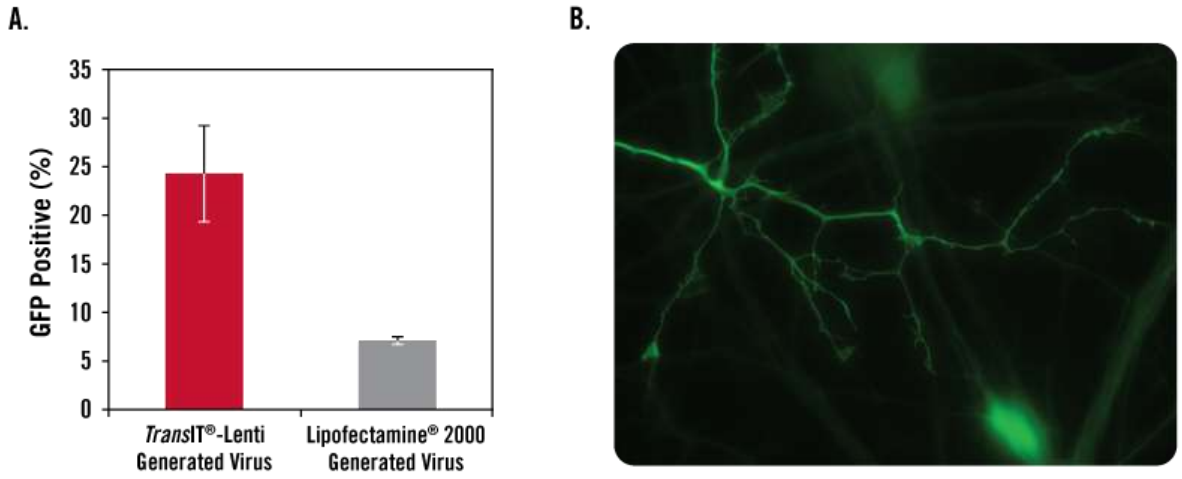
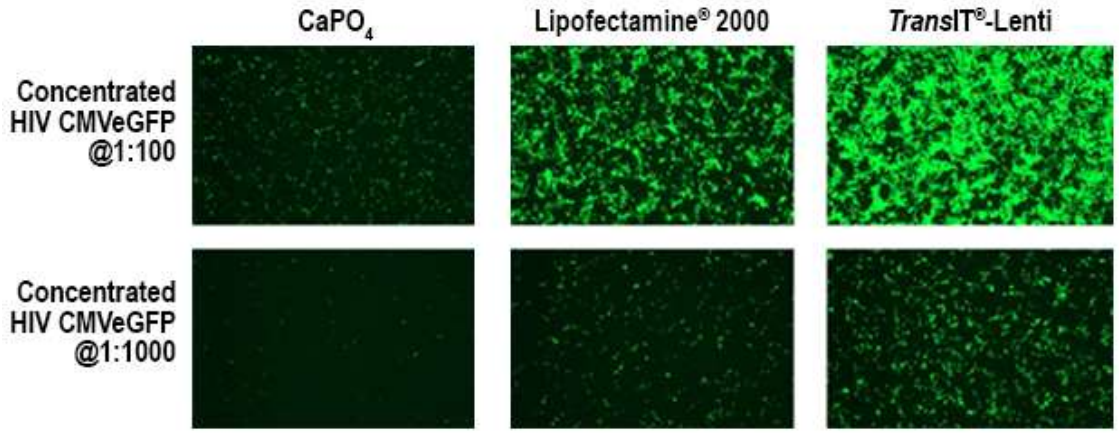
Figures and Data



High Functional Titers with *Trans*IT®-Lenti Transfection Reagent. Adherent 293T/17 cells were transfected in a 6-well plate with pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix powered by MISSION® (1:1 ratio, 2 µg/well) with the following reagents: *Trans*IT®-Lenti (3:1, vol:wt), Lipofectamine® 2000 (3:1), Lipofectamine® 3000 (3:1:1), 25 kDa PEI (6:1), or CaPO4 precipitation (4 µg pDNA/well). The supernatant was harvested, filtered (0.45 µm), and titered using 293T/17 cells. Lentivirus transductions were performed in the presence of 8 µg/ml *Transduce*IT™ and GFP expression was measured 72 hours post-transduction using guava easyCyte™ 5HT Flow Cytometer. Error bars represent triplicate transfection complexes titered individually. Functional titers were calculated using virus dilutions with less than 20% GFP positive cells.

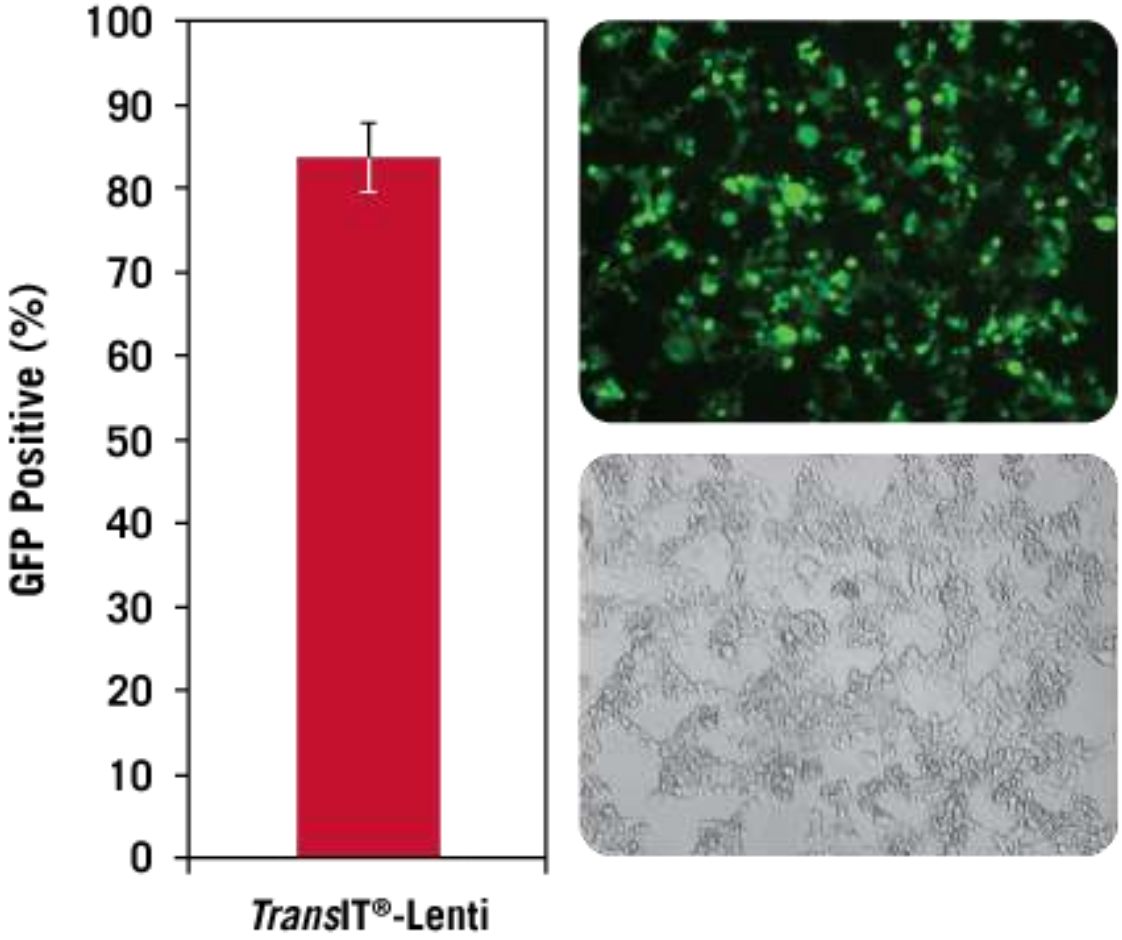


High Transduction Efficiency with Unconcentrated Lentivirus Using *Trans*IT®-Lenti. (A) Lentivirus was produced with the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt) or Lipofectamine® 2000 using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix powered by MISSION®). The supernatant was harvested, filtered (0.45 µm), and frozen. Lentivirus transductions were performed 5 days post-plating with iCell® Motor Neurons (Cellular Dynamics International). For both*Trans*IT-Lenti and Lipofectamine® 2000, one microliter of unconcentrated supernatant was added per well of a 96-well plate. GFP efficiency was measured 72 hours post-transduction using guava easyCyte™ 5HT Flow Cytometer. Error bars represent the SEM of duplicate wells. (B) iCell® Motor Neurons were plated in Ibidy 35mm dishes and transduced withlentivirus produced using the *Trans*IT®-Lenti Transfection Reagent and MISSION® vectors. Images were captured at 72 hours post-transduction with a Zeiss Axiovert S100 inverted fluorescence microscope using a 63X objective under oil.

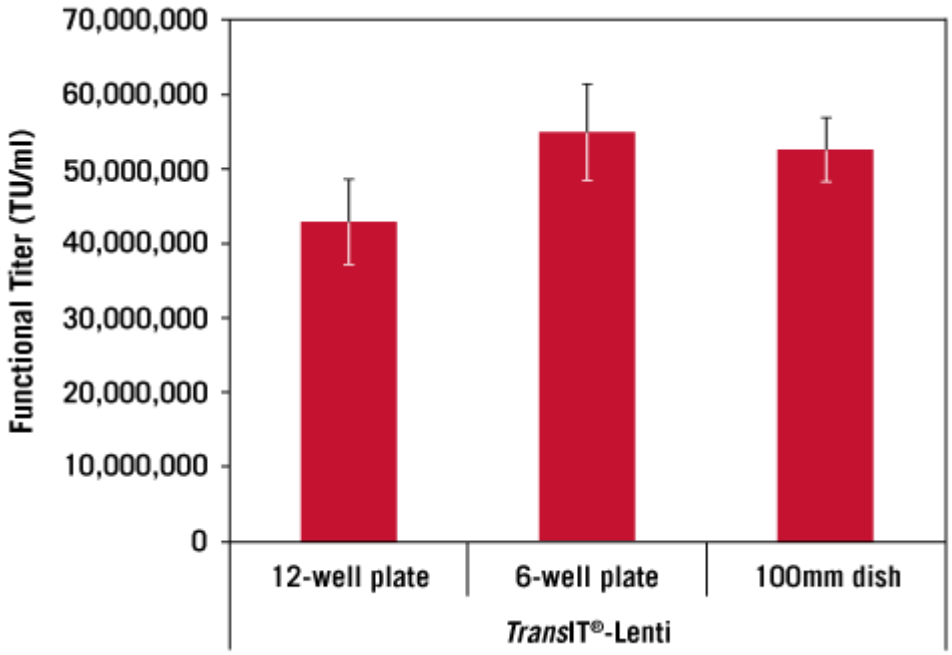


Comparison of $CaPO_4$, Lipofectamine® 2000 or *Trans*IT®-Lenti Generated Lentivirus. HIV CMVeGFP Virus was produced in HEK 293FT cells using either $CaPO_4$, Lipofectamine® 2000 or *Trans*IT®-Lenti Transfection Reagent per the manufacturer's protocol. Lentivirus was collected 48 hours post-transfection and concentrated by prolonged centrifugation at 9,000 x g. HT1080 cells were infected with a 1:100 or 1:1000 dilution of each concentrated lentivirus. Images (above) were captured 48 hours post-transduction.

Data courtesy of Jeremy Coffin, University of Iowa Viral Vector Core



High Efficiency Transfection with *Trans*IT®-Lenti Transfection Reagent. Adherent 293T/17 cells were transfected in a 6-well plate format using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix) using the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt). GFP efficiency was measured at 48 hours post-transfection using guava easyCyte™ 5HT Flow Cytometer. Error bars represent five transfection complexes. Images were captured at 48 hours post-transfection (10X objective) using a Zeiss Axiovert S100 inverted fluorescence microscope. The observed cell rounding and cell-cell fusion is due to high expression of the vesicular stomatitis virus G protein (VSV-G) for pseudotyping the recombinant lentivirus.



Lentivirus Production is Scalable. Adherent 293T/17 cells were transfected in a 12-well, 6-well or 100 mm plate format using the MISSION® vectors (pLKO.1-puro-CMV-TurboGFP™ transfer vector and the Lentivirus Packaging Mix at a 1:1 ratio) and the *Trans*IT®-Lenti Transfection Reagent (3:1, vol:wt). The supernatant was harvested, filtered (0.45 µm), and titered using 293T/17 cells. Lentivirus transductions were performed in the presence of 8 µg/ml *Transduce*IT™ and GFP expression was measured 72 hours post-transduction using guava easyCyte™ 5HT Flow Cytometer. Error bars represent triplicate transfection complexes titered individually. Functional titers were calculated using virus dilutions with less than 20% GFP positive cells.