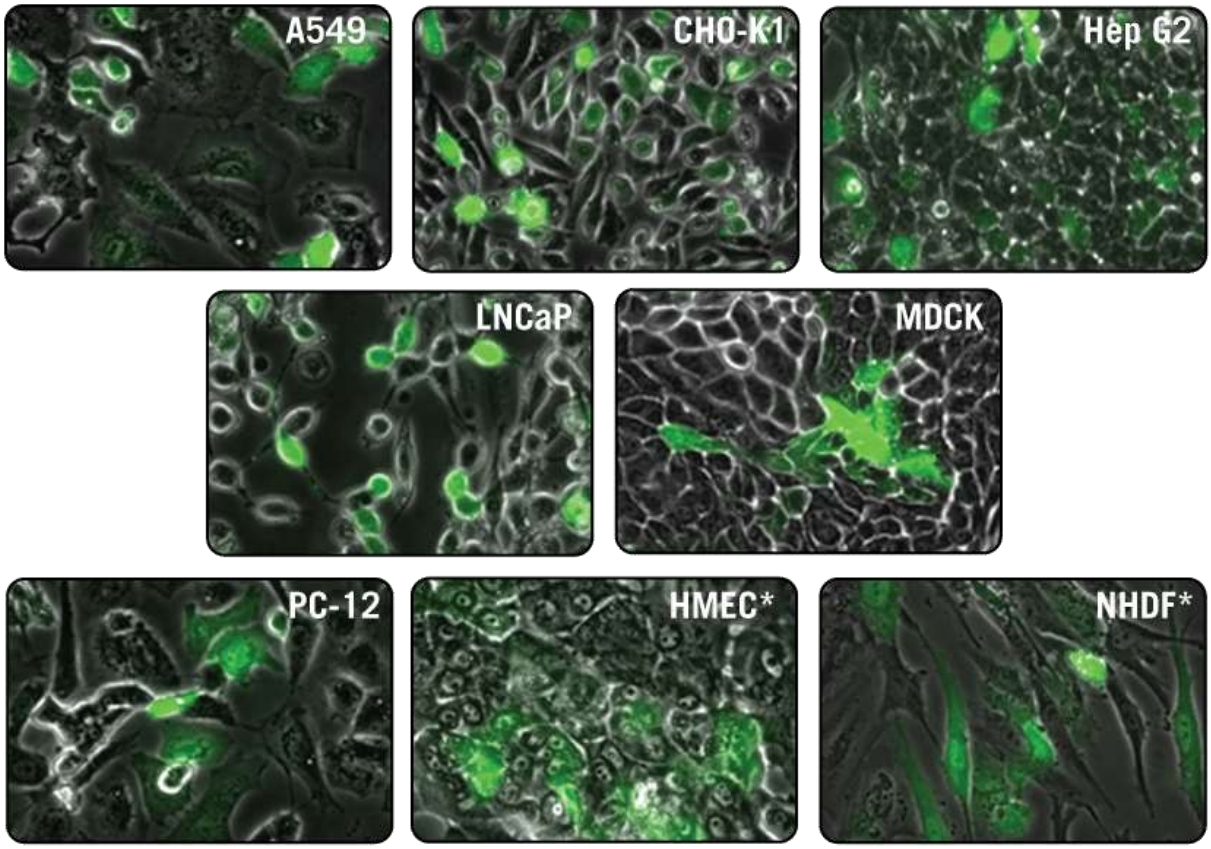
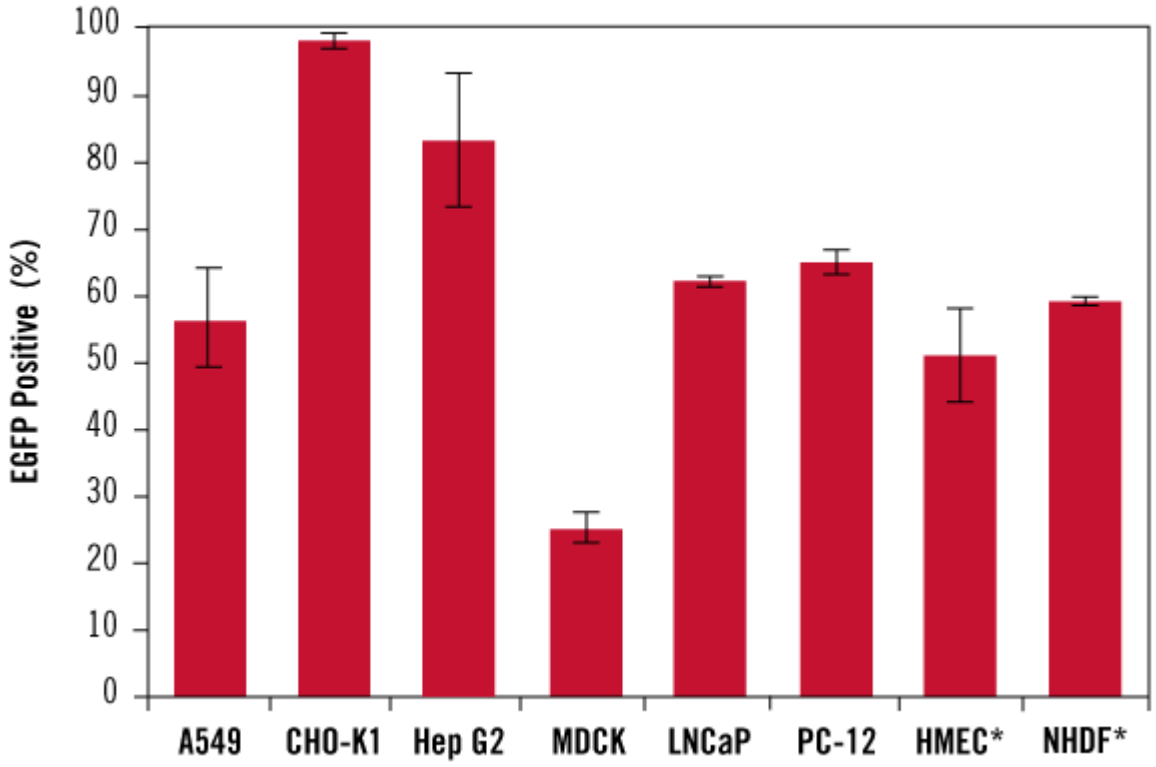
Figures and Data

Visualization of High GFP Expression Using *Trans*IT-X2® Dynamic Delivery System
High GFP Transfection Efficiency in Multiple Cell Lines and Primary Cells Using *Trans*IT-X2® System
Functional Co-transfection of Plasmid DNA and siRNA Using *Trans*IT-X2® System
*Trans*IT-X2® Dynamic Delivery System Achieves Higher Knockdown than Lipofectamine® 2000
Effective miRNA Delivery Using *Trans*IT-X2® Yields Decreased Levels of PTK9 mRNA
High Efficiency CRISPR Genome Editing with *Trans*IT-X2®
*Trans*IT-X2® Outperforms Lipofectamine® CRISPRMAX™ for RNP Delivery



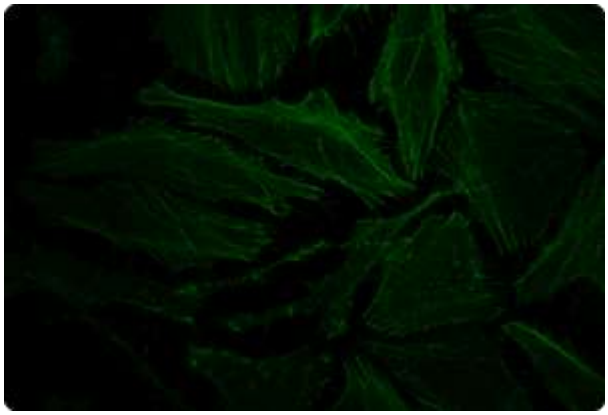
Visualization of High GFP Expression Using *Trans*IT-X2® Dynamic Delivery System. *Trans*IT-X2® Dynamic Delivery System was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, HepG2, LNCaP, MDCK, PC12, primary human mammary epithelial cells (HMEC) and normal human dermal fibroblasts (NHDF). Transfections were performed in 35 mm MatTek dishes using 4-8 µl of *Trans*IT-X2® to deliver 2 µg of DNA. Images (32X) were captured at 48 hours post-transfection using a Zeiss Axiovert S100 inverted fluorescence microscope.

* indicates primary cell types

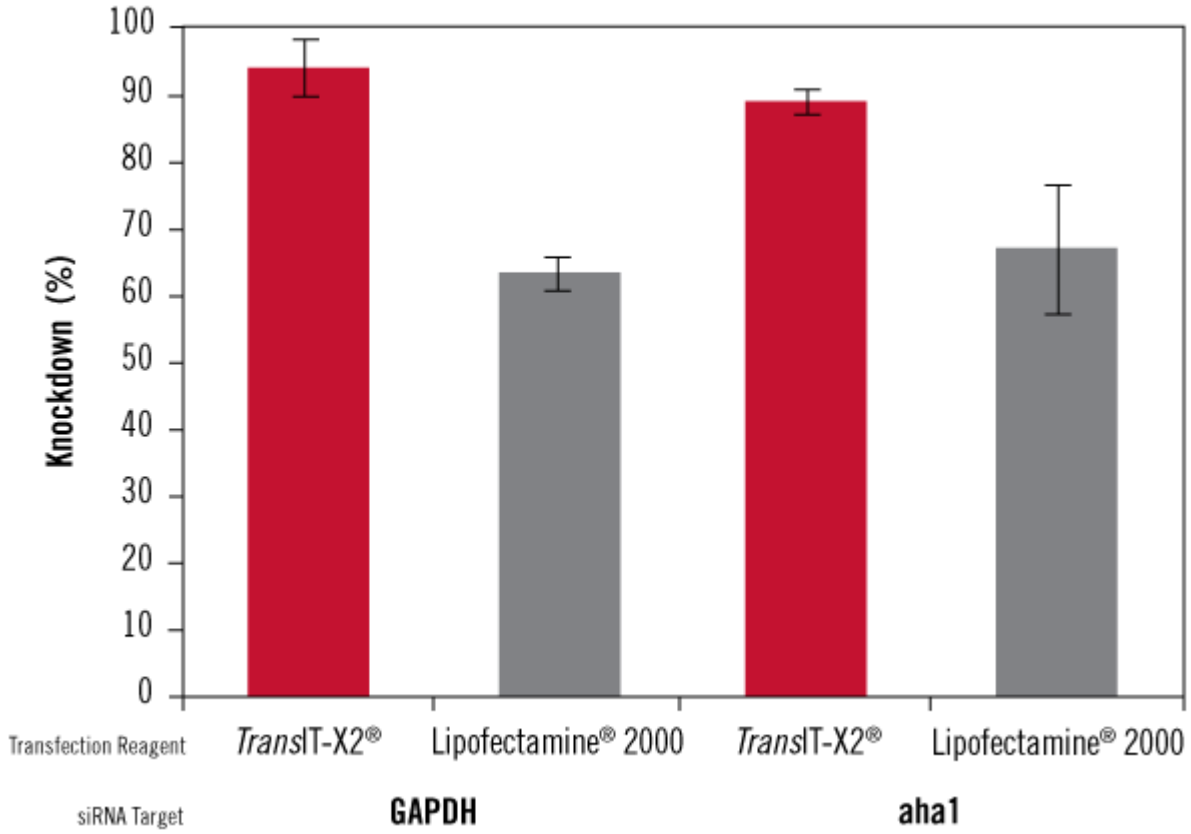


High GFP Transfection Efficiency in Multiple Cell Lines and Primary Cells Using *Trans*IT-X2® Dynamic Delivery System. *Trans*IT-X2® Dynamic Delivery System was used to transfect plasmid DNA encoding EGFP into A549, CHO-K1, Hep G2, MDCK, LNCaP, PC-12, primary human mammary epithelial cells (HMEC) and normal human dermal fibroblasts (NHDF). Transfections were performed in 96-well plates using 0.2-0.4 µl of *Trans*IT-X2® to deliver 0.1 µg of DNA (2:1, 3:1 or 4:1 reagent: DNA ratio). Triplicate wells were assayed 48 hours post-transfection using guava easyCyte™ 5HT Flow Cytometer.

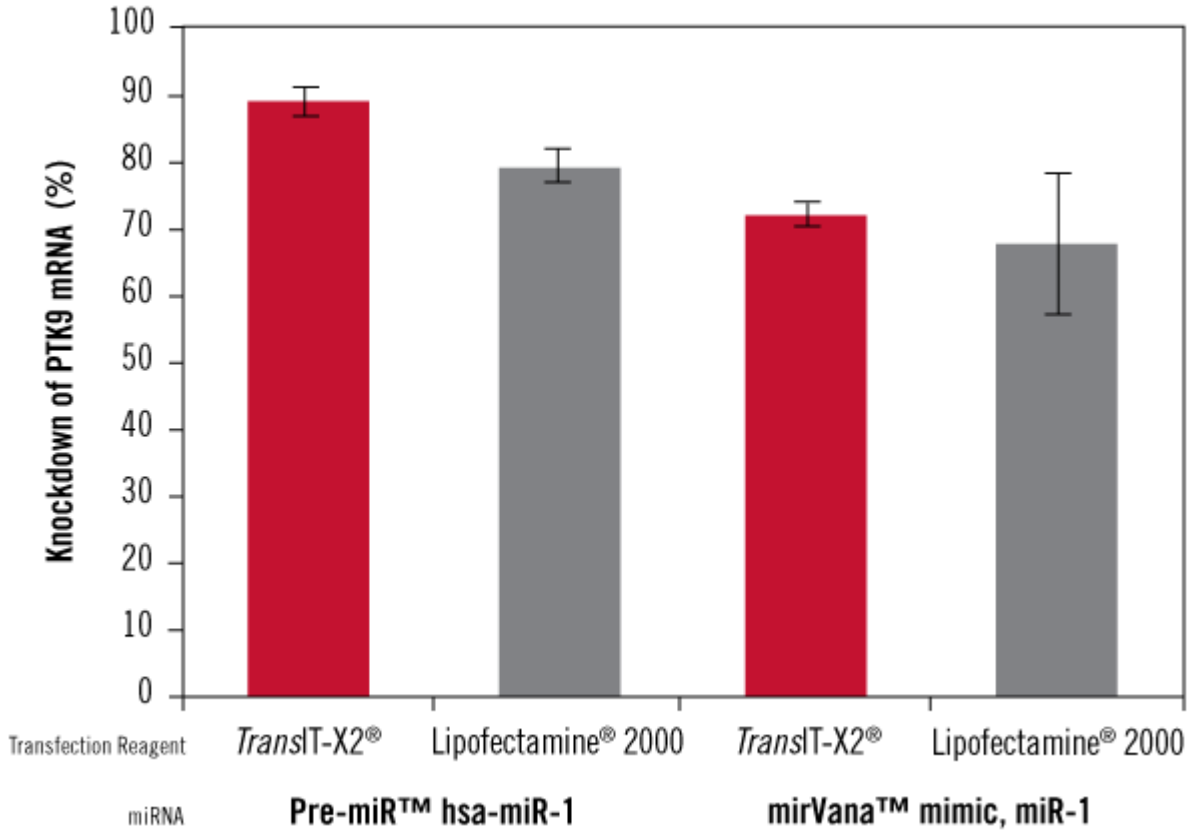
*indicates primary cell types



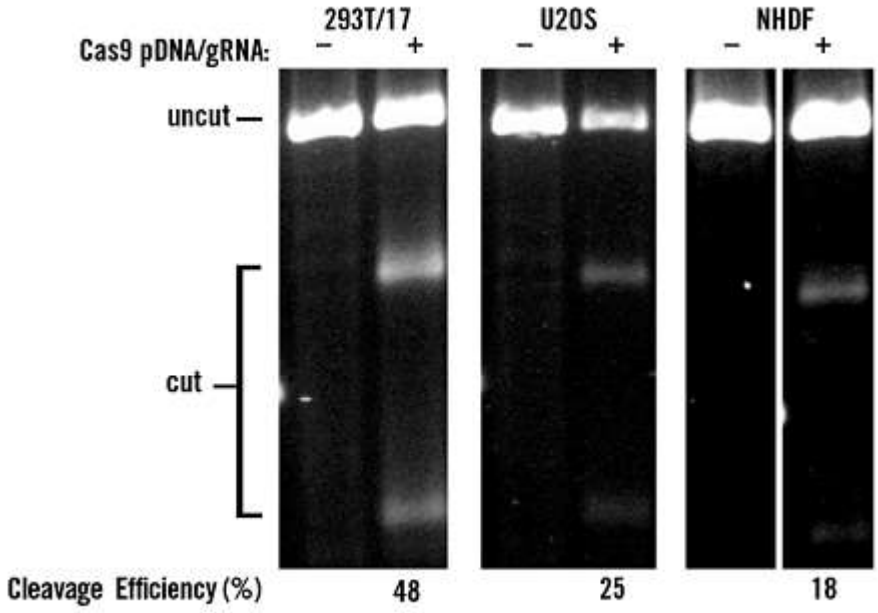
Functional Co-transfection of Plasmid DNA and siRNA Using the *Trans*IT-X2® Dynamic Delivery System. *Trans*IT-X2® Dynamic Delivery System was used to transfect plasmid Cy®5 labeled DNA encoding nuclear YFP and Cy®3 labeled siRNA into HeLa cells. Transfection was performed in 6-well plates with Poly-L-Lysine (PLL) coated coverslips using 4 µl of *Trans*IT-X2® to deliver 2 µg of DNA and 25 nM siRNA (2:1 reagent:DNA ratio). Actin cytoskeleton was stained using Alexa Fluor® 350 Phalloidin. Images (63X) were captured at 24 hours post-transfection using a Nikon A1R confocal microscope. Image key: yellow (nuclear YFP), blue (Cy®5 labeled DNA), red (Cy®3 labeled siRNA), green (actin cytoskeleton).



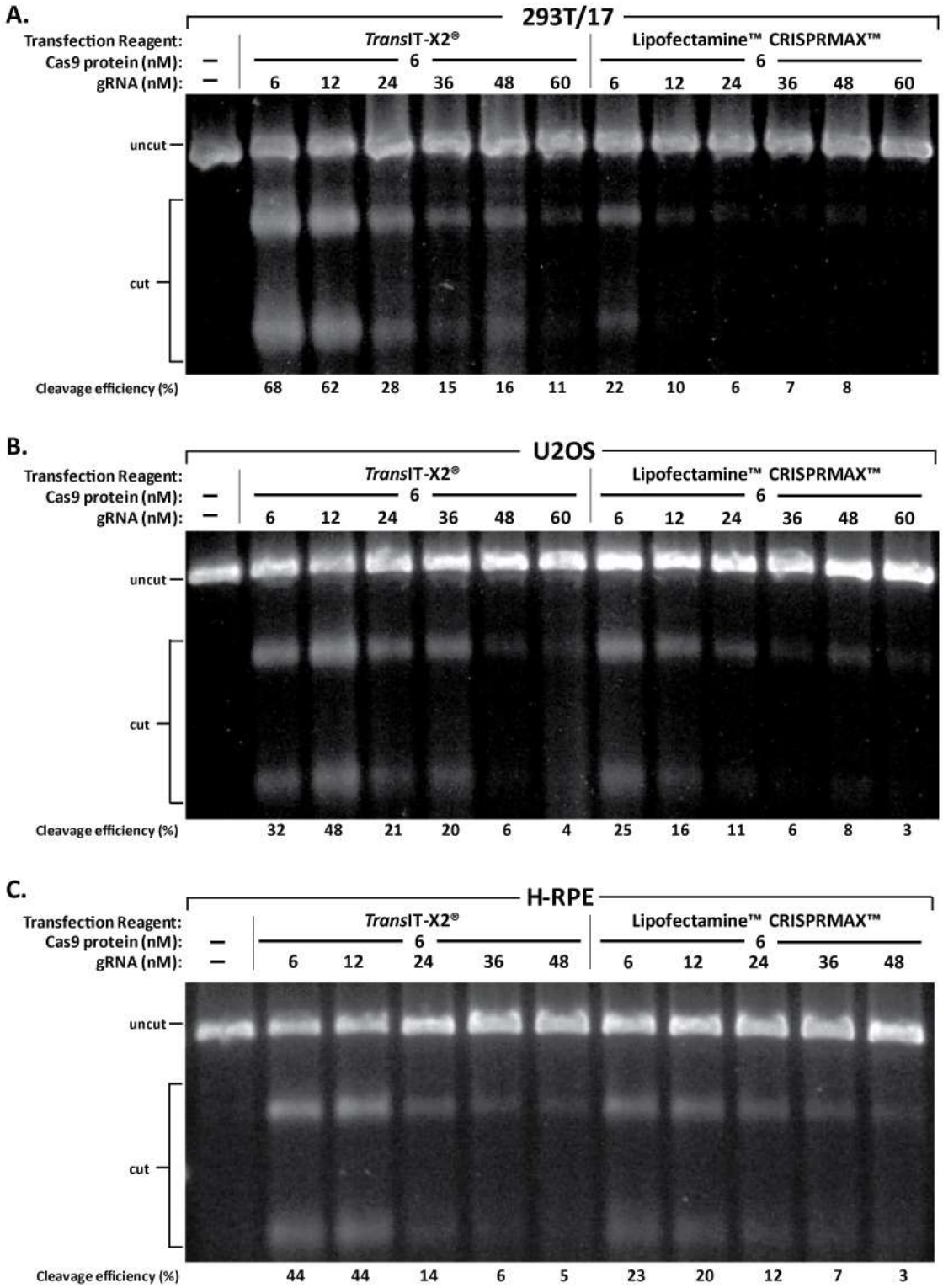
***Trans*IT-X2® Dynamic Delivery System Achieves Higher Knockdown than Lipofectamine® 2000.** *Trans*IT-X2® Dynamic Delivery System and Lipofectamine® 2000 Transfection Reagent were used to transfect siRNA targeting endogenous proteins – GAPDH and AHA1 or to deliver a non-targeting siRNA control in normal human dermal fibroblasts (NHDF). Cells were transfected in a 6-well plate using 4 µl of *Trans*IT-X2® or 6 µl of Lipofectamine® 2000 and 25 nM siRNA according to each manufacturer's protocol. The amount of GAPDH or AHA1 mRNA was measured relative to 18s rRNA levels using qRT-PCR and then normalized to the mRNA levels of the non-targeting control, 48 hours post-transfection. Error bars represent the standard deviation of triplicate wells.



Effective miRNA Delivery Using *Trans*IT-X2® Dynamic Delivery System Yields Decreased Levels of PTK9 mRNA. *Trans*IT-X2® Dynamic Delivery System and Lipofectamine® 2000 Transfection Reagent were used to transfect Pre-miR™ hsa-miR-1 miRNA Precursor or mirVana™ miRNA mimic, miR-1, both known to decrease PTK9 mRNA levels. A Pre-miR™ negative control was also transfected to assess baseline mRNA levels. T47D cells were transfected in a 12-well plate using 3 µl of *Trans*IT-X2® or Lipofectamine® 2000 and 50 nM miRNA according to each manufacturer's protocol. The amount of PTK9 mRNA was measured relative to 18s rRNA levels using qRT-PCR and then normalized to the mRNA levels of the negative control, 48 hours post-transfection. Error bars represent the standard deviation of triplicate wells.



Efficient Genome Editing with Cas9 Plasmid DNA + Guide RNA Oligonucleotides. HEK293T/17, U2OS and NHDF cells were co-transfected with 0.5 µg of Cas9 encoding pDNA (MilliporeSigma) and 50nM PPIB targeting 2-part gRNA (Dharmacon) using *Trans*IT-X2® Dynamic Delivery System (2 µl/well of a 24-well plate, Mirus Bio). A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.



***Trans*IT-X2® Outperforms Lipofectamine® CRISPRMAX™ for RNP Delivery.** Ribonucleoprotein (RNP) complexes composed of PPIB targeting 2-part gRNA (IDT) and Cas9 protein (PNA Bio) were delivered into HEK293T/17 (**A**), U2OS (**B**), and primary H-RPE (**C**) cells using TransIT-X2 Dynamic Delivery System (1 µl/well, Mirus Bio) or Lipofectamine® CRISPRMAX™ (1.5 µl/well and 1 µl/well of Lipofectamine® Cas9 Plus™ Reagent, ThermoFisher) in a 24-well format according to the manufacturers' protocol. Varying levels of gRNA (6 nM – 60 nM) were tested with 6 nM Cas9 protein. A T7E1 mismatch detection assay was used to measure cleavage efficiency at 48 hours post-transfection.