RJH BIOSCIENCES

Applications of RJH Reagents in Suspension-Growing Cells

Index

About RJH Biosciences

We develop novel transfection reagents that deliver different types of nucleic acids to a range of mammalian cells in culture, while tailoring the transfection agents further to act as delivery vehicles for preclinical models and clinical therapy involving nucleic acids. Our reagents display exceptional activities on specific types of cells, while acting broadly for delivery of different types of nucleic acids.

Transfection Reagents

We offer specific and broadly acting transfection reagents to modify cells with DNA and RNA. The reagents are polymeric in nature and have been optimized for a variety of cell types and applications involving cell culture (*in vitro*) and animal models (*in vivo*). We offer reagents tailored for primary and suspension cells, as well as adherent cell lines.

Clinical Development

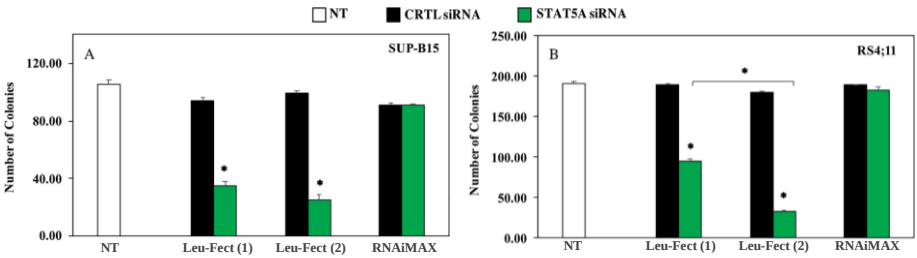
We are developing delivery systems to implement nucleic acid therapeutics in a clinical setting. Our goals are to realize the therapeutic potential of nucleic acid involved in RNAi (siRNA) and transgene expression (pDNA and mRNA). Partnerships are actively sought for various preclinical and clinical programs.

R&D Services

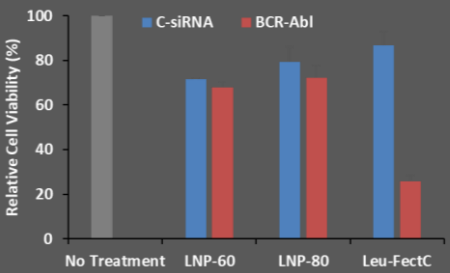
We offer high quality research and development services associated with transfection optimization and construct validation. Our goal is to provide the best delivery materials for your cargo and cell of choice. Our services are assessed and initiated by a quote request via the screening services page on our website or by a simple email.

Delivery of siRNA in Leukemia Cells

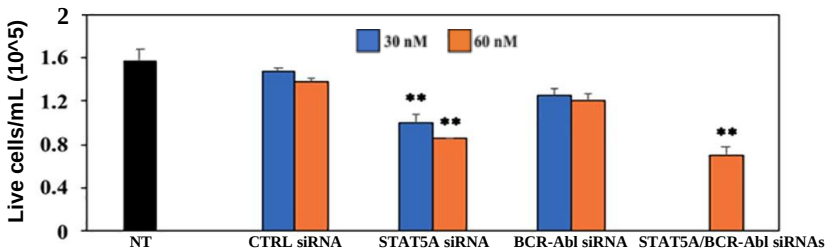
RJH Bioscience reagents are well tailored to provide delivery of siRNA into suspension growing cells and producing high efficiency transfections. We have tested our products extensively for use in leukemia cells to validate their therapeutic potential in *in vitro* and in animal studies.



Growth inhibition of leukemic cell lines transfected with control and STAT5A siRNA complexes. Colony counts in agarose gels were performed two weeks after the treatment of cells at 60 nM siRNA concentration and at RJH reagent:siRNA ratio of 6, (RNAiMAX:siRNA ratio: 2.5:1 as recommended by the manufacturer). (A) SUP-B15 cells and (B) RS4;11 cells (n = 3). * p<0.05. Data from DOI: journal.pone.0251719(1). NT: No treatment.



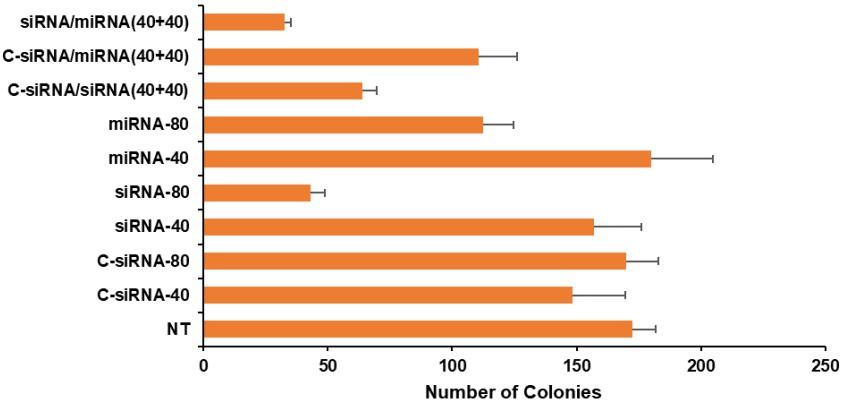
Effect of BCR-Abl siRNA treatment on proliferation of K562 cells. Cells were treated with siRNA concentration of 60 and 80 nM formulated with the clinical Lipid Nanoparticle Formulation (LNP) DLin-MC3-DMA and with 60 nM Leu-Fect C formulation (RJH Biosciences) at Leu-Fect C:siRNA ratio of 6:1. Cell viability was determined by trypan blue exclusion assay after 3 days.



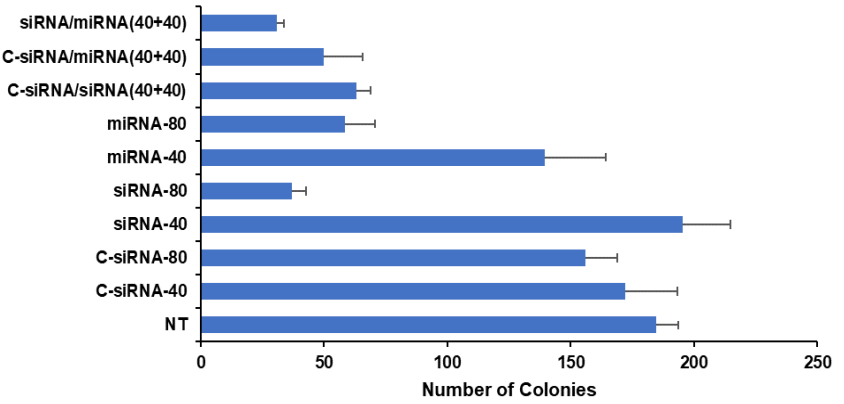
Effect of siRNA treatment on proliferation of BCR-Abl(+) patient cells. Cells were treated for 3 days with 30 and 60 nM siRNA with transfection reagent:siRNA ratio of 6:1. The siRNA used were control (scrambled), STAT5A, and BCR-Abl specific siRNAs. Live cells were counted by trypan blue exclusion assay (n=3); ** p<0.01. Data from DOI: 10.1371/journal.pone.0251719

Delivery of microRNA in Leukemia Cells

RJH products can support co-delivery of different nucleic acids in hard-to-transfect cells. There is a large therapeutic potential in combining different RNA and/or DNA reagents for treating diseases. Our reagents can provide high efficiency transfection irrespective of the nucleic acid cargo.



Colony Forming Unit (CFU) assay for K562-WT cells. Colony Forming Unit (CFU) assay for K562-IMR cells treated with the following: a control double-stranded RNA (C-RNA), a specific siRNA against an oncogene, and a proprietary miRNA. The nucleic acid concentrations were 40 or 80 nM, or a combination of 40+40 nM. Cells were transfected with RJH product LeuFect A/nucleic acid complexes for 24 hours and then transferred to methyl-cellulose gels for further cultivation for 2 weeks. CFUs were counted at the end of 2-week culture period (in triplicate).



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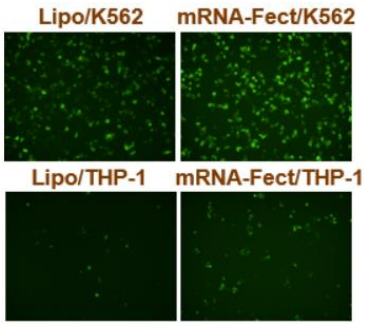
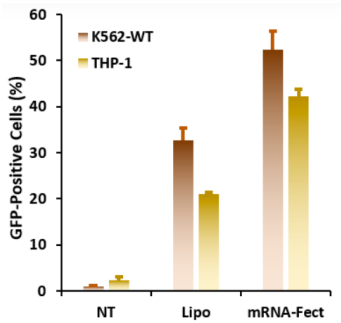
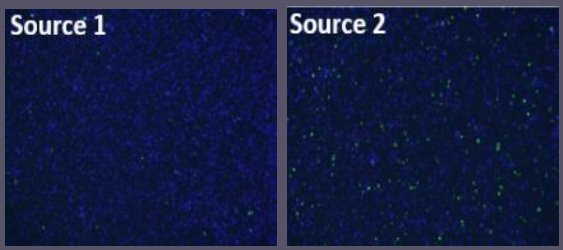
Delivery of mRNA in PBMCs

RJH Product mRNA-Fect enables high efficiency mRNA transfection to suspension-growing peripheral blood mononuclear cells (PBMCs). Extensive testing of RJH reagents has shown superior delivery of mRNA payload, especially in difficult-to-transfect primary cells.

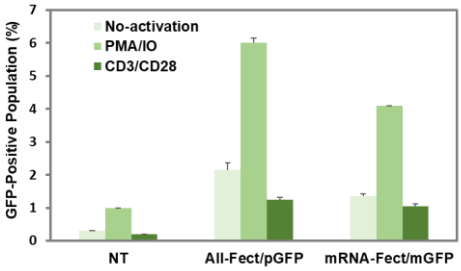


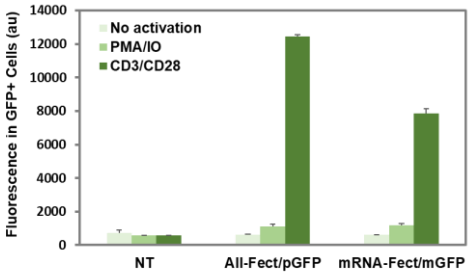


Transfecting GFP-expressing mRNA in lymphocytic cell lines (K562 and THP-1) using mRNA-Fect and a competing lipofection reagent. GFP expression was visualized 48 hours after transfection using fluorescent microscopy (left) and the proportion of GFP-expressing cells was quantified by flow cytometry (right).



Merged DAPI-stained and green fluorescent micrographs of CD3/CD28 activated PBMCs (from 2 separate donors) transfected with mGFP using mRNA-Fect. mRNA-Fect shows highly efficacious transfection in these difficult-to-transfect primary, suspension-growing cells with minimal toxicity. Lipofection reagents were not effective in PBMCs (not shown)

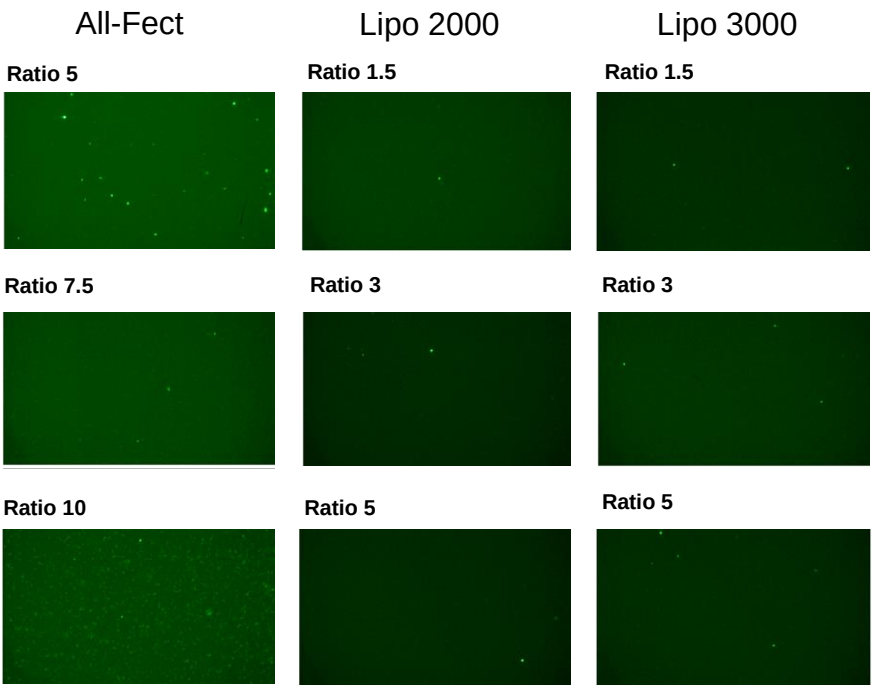




GFP-expressing mRNA transfection in PBMCs using ALL-Fect and mRNA-Fect. Quantification by flow cytometry shows the percentage of GFP-positive cells (left) and extent of GFP fluorescence per cell (right). High efficiency mRNA delivery is achieved under optimally after CD3/CD28 activation (right), while PMA/IO gave auto-fluorescent cells (left) with little transfection.

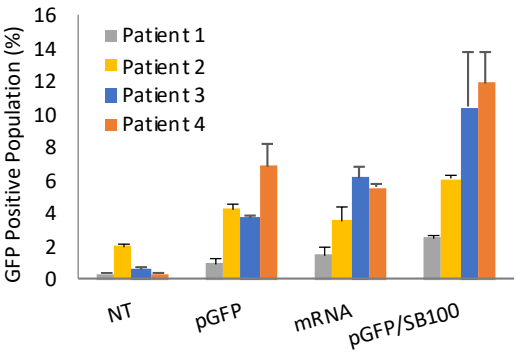
Delivery of Plasmid DNA in PBMCs

Plasmid DNA and similar nucleic acid reagents are the technology of choice for many users in transgene expression. At RJH Biosciences, we can provide reagents for delivery of many pDNA types and are continually validating our products for different pDNA architectures and sizes.



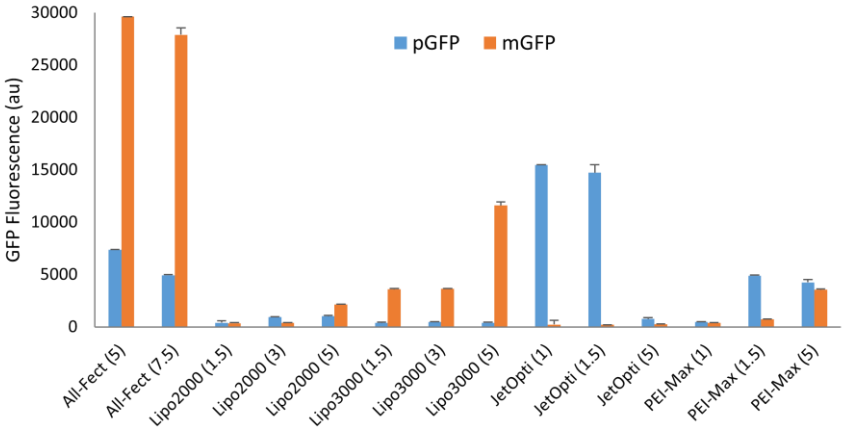
Transfection of PBMCs using RJH and other commercial products. Transfection reagents complexed with GFP expressing DNA were ALL-Fect (left), Lipofectamine™ 2000 (middle) and Lipofectamine™ 3000 (right). GFP expression was visualized after 2 days via fluorescence microscopy.

Peripheral Blood Mononuclear Cell (PBMC) Transfections. Transfection efficiency was determined using samples (PBMCs from whole blood) from four sources. Variance among the donor PBMCs was determined with no transfection group (NT) or transfection of pDNA encoding a GFP SB transposon, GFP expressing mRNA, and a complete GFP SB transposon system (transposon donor and transposase coded by a pDNA mixture). Percentage of cells expressing GFP was determined by flow cytometry 2 days post transfection.



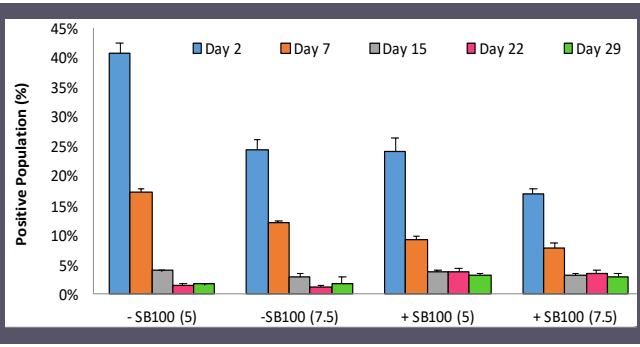
Delivery in Immune Cells

Modification of immune cells is a major endeavor for developing novel therapies for a range of diseases. Transfection reagents are urgently needed to deliver DNA and RNA molecules for effective engineering of these cells. RJH reagents are particularly tailored for the immune cells.

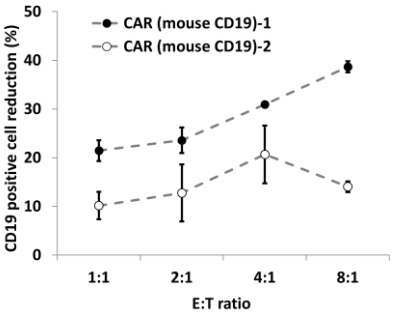


Comparison of transfection efficiencies using RJH reagents and other commercial reagents. A pDNA and mRNA expressing GFP reporter were transfected in Jurkat T-cells using RJH Product All-Fect, Lipofectamine 2000/3000, jetOPTIMUS, or PEI MAX. Various reagent:DNA ratios (w/w) were used as recommended by the manufacturers. Percentage of GFP positive cells was obtained with flow cytometry 3 days post transfection.

Longevity of transfection in T-cells. A SB transposon system (SB100) that includes expressible GFP was transfected into Jurkat T-cells using the ALL-Fect transfection reagent (RJH Biosciences) at different transfection reagent:pDNA ratios (in brackets). Percentage of cells expressing GFP was determined by flow cytometry at several time points over the span of 29 days.

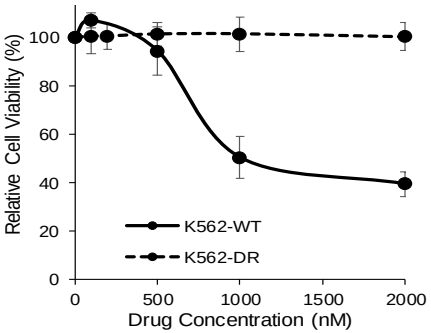


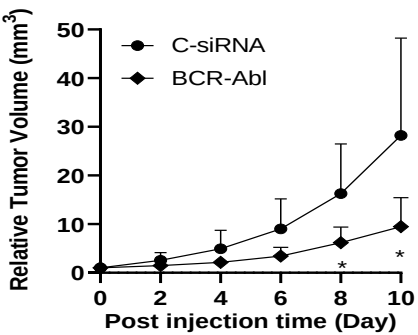
Cell killing assay with co-culturing of effector (CAR-modified Jurkat) and target (mouse WEHI) cells. Reduction in the percentage of CD19(+) WEHI cells when co-cultured with Jurkat T-cells (modified with 2 different pDNAs coding for anti-hCD19 CARs) was determined at different effector:target (E:T) ratios. The percentage of remaining cells was accessed 3 days after initiation of co-culture by flow cytometry and CD19 antibody staining.



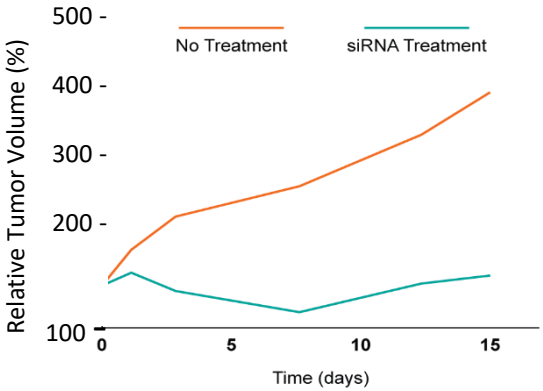
In Vivo Applications

Beyond cell culture applications, RJH reagents are functional in animal models. Our products are effective at every stage of preclinical development, providing customers a reliable system to validate their nucleic acid technologies in animal models involving suspension cells.





Tumor volume reduction using siRNA in a drug-resistant K562 tumor model compared to treatment of drug-resistant and wild-type K562 cells with imatinib. Left. K562 cells were treated with TKI imatinib at different drug concentrations. Percent cell viability was determined by the MTT Assay. Drug-resistant cells do not respond to imatinib while wild-type cells readily respond to the imatinib. **Right.** Control and BCR-Abl targeting siRNA were complexed with a RJH transfection reagent and administered subcutaneously in the vicinity of subcutaneous drug-resistant K562 tumors. Tumor volume was assessed externally at different time intervals. The use of RJH transfection reagent for siRNA delivery provided a significant therapeutic response in drug-resistant tumors.

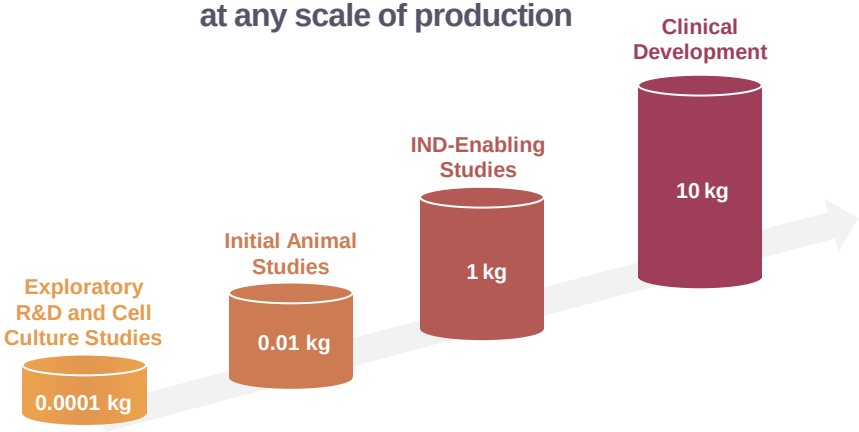


Tumor volume reduction using siRNA in a wild-type K562 mouse model. Tumors were treated with a siRNA:RJH transfection reagent complex and assessed in a drug-resistant K562 mouse models. Mice were injected with the complex intraperitoneally. As a control, this was compared to no tumor treatment. Tumor volume was assessed externally by calibers at different time intervals.

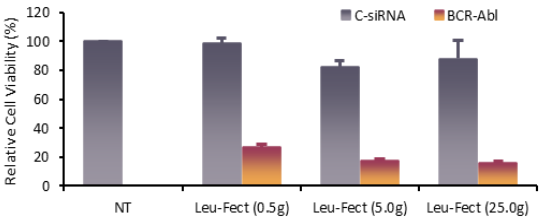
Producing RJH Reagents at Large Scale

RJH reagents provide high efficiency nucleic acid delivery to a wide array of cell types as well as in animal models. The process to prepare the reagents are readily scaled-up to prepare reagents with reproducible performance over a wide range of scales.

RJH Biosciences can service your transfection needs at any scale of production

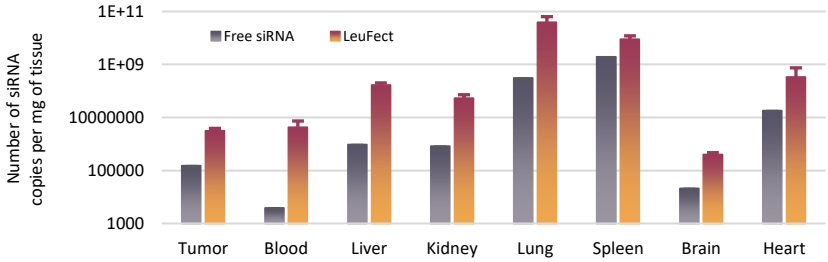


Performance of Leu-Fect Prepared at Different Scales



Testing the efficacy Leu-Fect reagent produced at different scales. Leu-Fect reagent from 500 mg, 5 g and 25 g scales was used to deliver BCR-Abl siRNA to K562 cells. Leu-Fect successfully transfected the cells and induced equivalent cell killing in all cases.

siRNA Delivery to Different Organs with RJH Reagents



Biodistribution of siRNA. Leu-Fect reagent from 25g scale was used to deliver a specific siRNA by intravenous injection. Organ levels of the siRNA was determined with a ddPCR.

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