Transfecting Primary Cells using RJH Bioscience Reagents

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

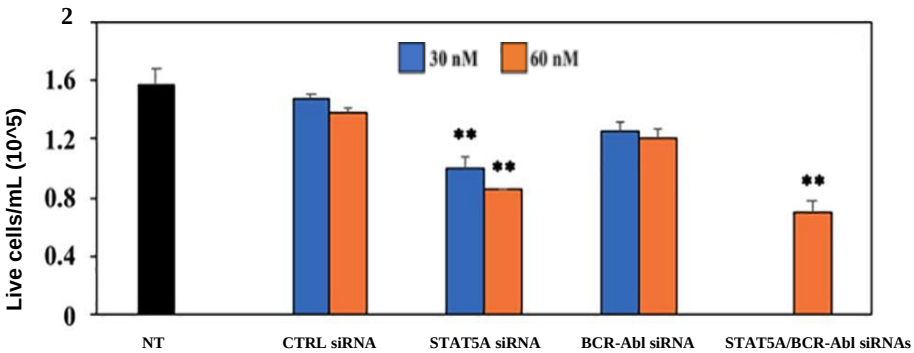
We are developing delivery systems to implement nucleic acid therapeutics in a clinical setting. Our goals are to realize the therapeutic potential of nucleic acid involved in RNAi (siRNA) and transgene expression (pDNA and mRNA). Partnerships are actively sought for various preclinical and clinical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

R&D Services

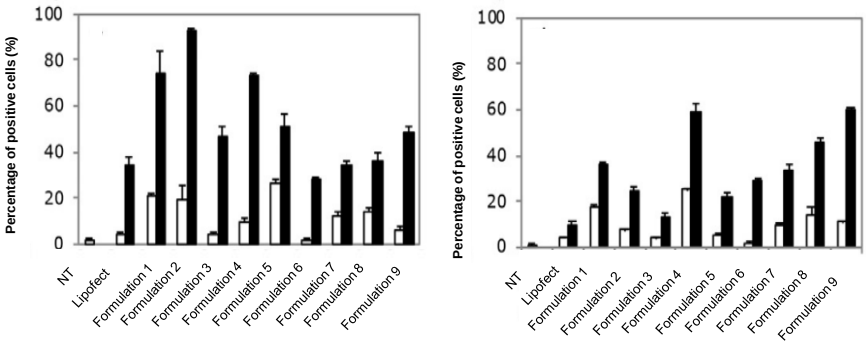
We offer high quality research and development services associated with transfection optimization and construct validation. Our goal is to provide the best delivery materials for your cargo and cell of choice. Our services are assessed and initiated by a quote request via the screening services page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

Applications in Primary Leukemic Cells

Suspension-growing leukemic cells are known to have poor transfection efficiencies due to minimal surface area and limited endocytosis. RJH reagents provide consistent and high transfection efficiencies in these cell types, ensuring that the delivery of nucleic acids in primary cells is possible.



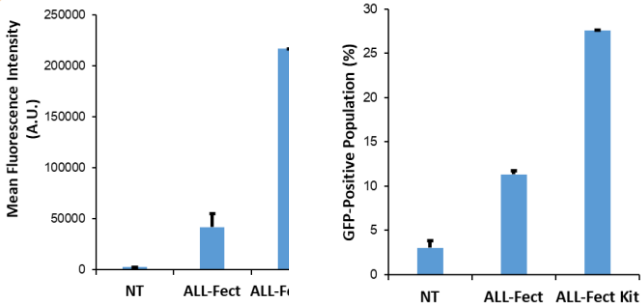
Effect of siRNA treatment on proliferation of BCR-Abl(+) leukemia patient cells. Cells were treated for 3 days with 30 and 60 nM siRNA with a transfection reagent:siRNA ratio of 6:1. The siRNAs used were control, STAT5A, and BCR-Abl specific siRNAs. Live cells were counted by trypan blue exclusion assay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



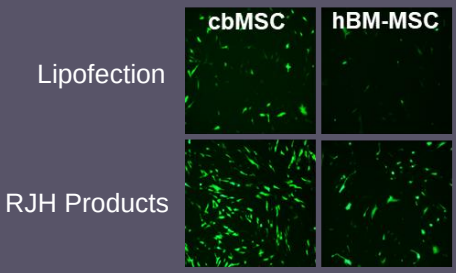
Transfection of siRNA into primary chronic myeloid leukemia cells. FAM-labeled siRNA was delivered to fresh cells from 2 patient donors (left and right graphs). The cells were transfected with Lipofectamine™ 2000 (Lipofect) or different RJH formulations at a reagent:FAM-siRNA ratio of 9:1 and at siRNA concentration of 60 nM. Cells were harvested for flow cytometry analysis 2 days after transfection. Percentage of siRNA-positive cells are shown as the average of 3 replicates. Data from DOI: 10.1016/j.jconrel.2019.08.018.

Applications with Mesenchymal Stem Cells

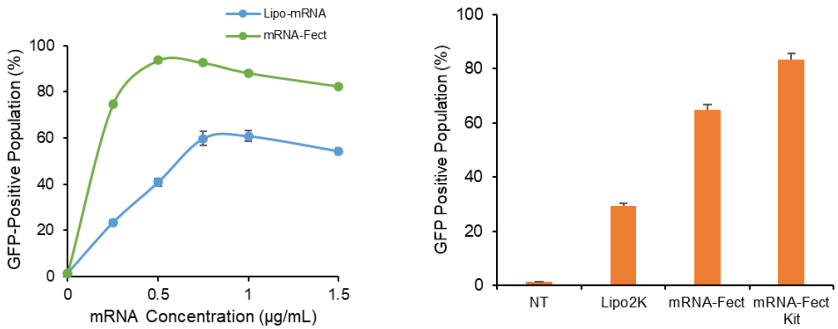
Primary mesenchymal stem cells (MSCs) are attachment-dependent cells that are derived from various tissues and actively explored for clinical utility. They display good endocytosis activity, but still poses challenges to delivery and expression of transgenes. RJH transfection reagents have been optimized to delivery pDNA and mRNA cargo to these types of cells.



Transfection of pDNA in human bone marrow-derived MSCs. GFP expressing pDNA was delivered into hBM-MSC's using RJH products All-Fect and All-Fect kit (with Trans-Booster). Transfection efficiency was determined by flow cytometry where the mean fluorescence in the cells (left) and the percentage of population expressing GFP (Right) was determined.



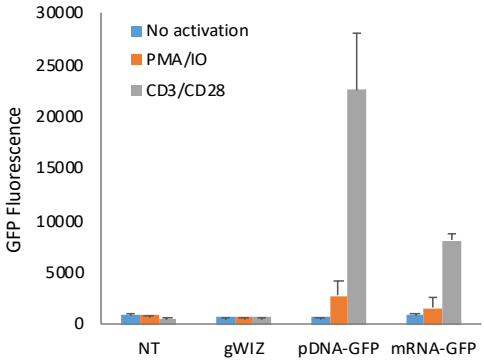
Transfection of pGFP in primary MSC. Cord Blood derived MSCs (cbMSC) and human bone marrow derived MSCs (hBM-MSC) were transfected with a GFP expressing plasmid complexed with an RJH reagent (ALL-Fect) or a leading lipofection product. Cells were visualized via fluorescent microscopy.



mRNA delivery in hBM-MSCs. Left. GFP expression via mRNA was determined at varying concentrations when complexed to mRNA-Fect (RJH Biosciences) or Lipofectamine™ 2000 (Invitrogen). Right. GFP expression via mRNA when delivered by RJH products mRNA-Fect and the mRNA-Fect kit (that includes Trans-Booster). Transfection efficiency for both experiments was determined by flow cytometry where the percent of population expressing GFP (Right) was determined.

Delivery of Nucleic Acids to Peripheral Blood Mononuclear Cells (PBMCs)

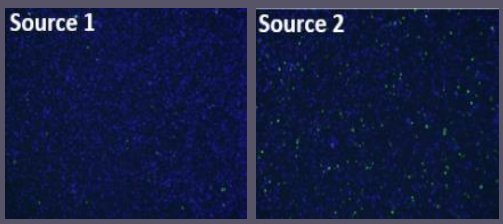
Several products from RJH can provide superior transfection in easily accessible PBMCs. Our products allow for nucleic acids to be delivered for many applications that require gene silencing or transgene expression.

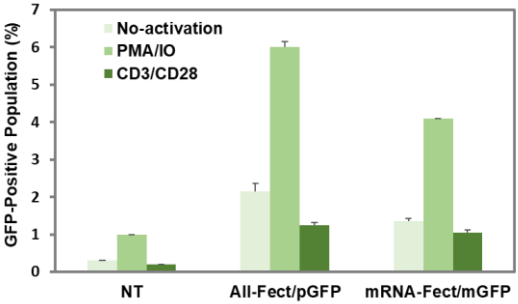


Peripheral Blood Mononuclear Cell (PBMC) Transfections. Transfection efficiency was determined using PBMCs from a donor. The cells were used without any activation or activation with PMA/IO or CD3/CD28 antibodies (Immunocult™). No transfection group (NT) or transfection of with a non-expressing pDNA (gWIZ) did not give any fluorescence in cells. A GFP coding pDNA (pDNA-GFP) and mRNA (mRNA-GFP) gave readily detectable fluorescence, by using the RJH ALL-Fect kit. Mean GFP fluorescence was determined by flow cytometry 2 days post transfection.

Merged DAPI-stained and green fluorescent micrographs of CD3/CD28 activated PBMCs (from two separate donor sources) 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)

CD3/CD28 Activated PBMC + mGFP/mRNA-Fect

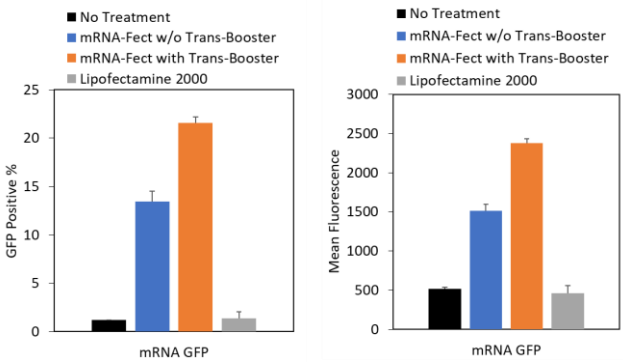




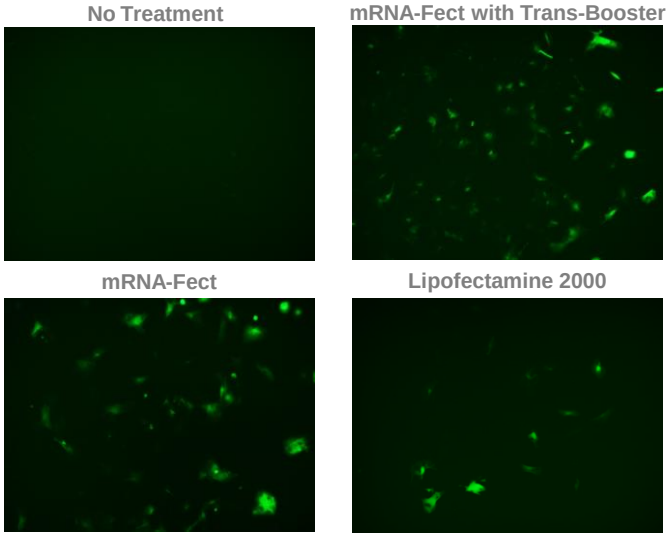
GFP-expressing mRNA transfection in PBMCs using ALL-Fect and mRNA-Fect. Quantification by flow cytometry shows the percentage of GFP-positive cells. High efficiency mRNA delivery is achieved under optimally after CD3/CD28 activation.

Mouse Cells as a Preclinical Model

Beyond working with the primary cells *in vitro*, our products are effective for modification of primary cells from preclinical models. Transfecting cells from mice are particularly important before progressing to animal models. Transfection reagents from RJH Biosciences are designed to provide you with the best materials for your preclinical experiments.



GFP Expression in Mouse Bone Marrow (BM) Cells by mRNA. The cells were harvested from bone marrows of NCG mice and attached cells were used for transfection after 2 weeks. An mRNA expressing GFP was complexed with transfection reagents Lipofectamine™ 2000, mRNA-Fect (RJH Biosciences) and mRNA-Fect with the Trans-Booster reagent (mRNA-Fect Kit; RJH Biosciences). Percentage of cells containing GFP (left) and the mean fluorescence of the population (right) was determined 2 days post transfection via flow cytometry.

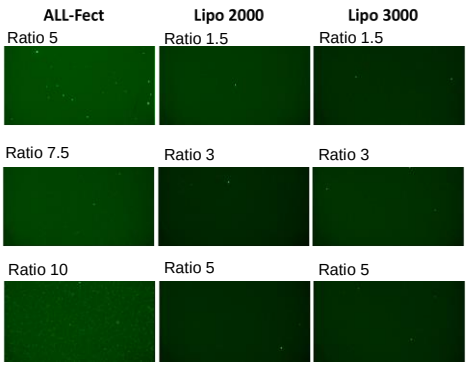


GFP Expression in Mouse Bone Marrow (BM) Cells by mRNA. mRNA expressing GFP was complex with transfection reagents Lipofectamine™ 2000, mRNA-Fect (RJH Biosciences) and mRNA-Fect with the Trans-Booster reagent (mRNA-Fect kit; RJH Biosciences). GFP containing cells were determined via microscopy.

Comparing RJH to Commercial Reagents

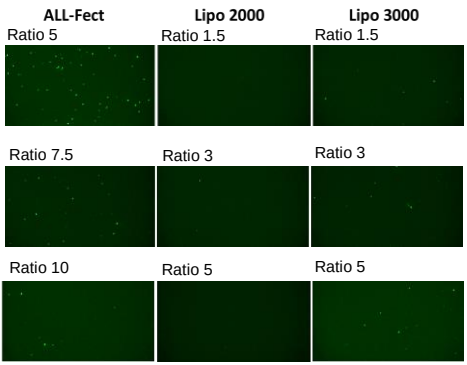
At RJH Biosciences, we continually compare our products to competitors to ensure we are providing the best reagents possible for researchers. This also helps with the first steps of optimization, saving our customers time and resources with their transfection experiments.

pDNA Transfection

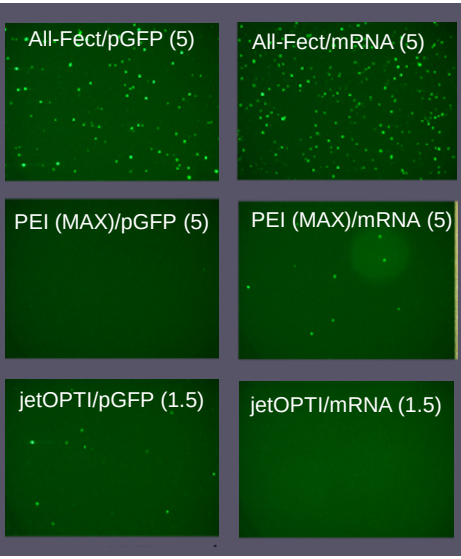


Transfection of pDNA into PBMCs using RJH reagents and competitor products. The transfection reagents complexed with the GFP expressing DNA were ALL-Fect (left), Lipofectamine™ 2000 (middle) and Lipofectamine™ 3000 (right). GFP expression was visualized after 2 days.

mRNA Transfection



Transfection of mRNA into PBMCs using RJH reagents and competitor products. Transfection reagents complexed with GFP expressing mRNA were ALL-Fect (left), Lipofectamine™ 2000 (middle) and Lipofectamine™ 3000 (right). GFP expression was visualized after 2 days.

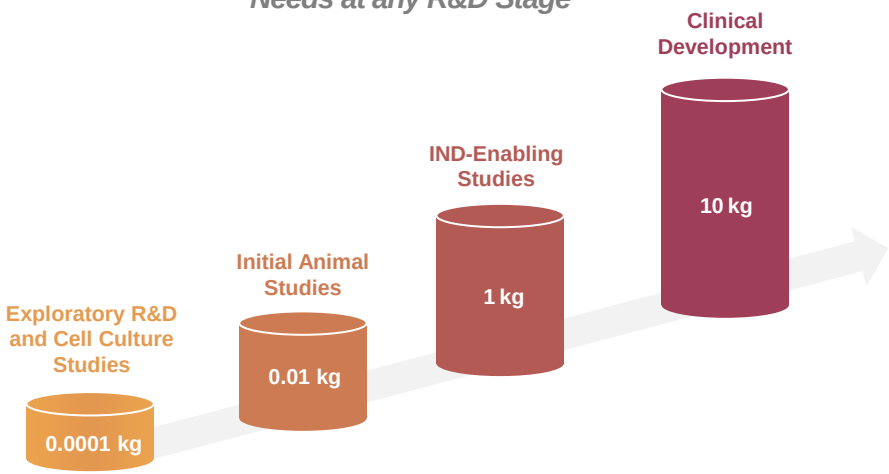


Transfection of PBMCs using RJH and other commercial products. Transfection reagents complexed with the GFP expressing plasmid (pGFP) and mRNA at indicated ratios (in the brackets, as recommended by respective manufacturers). The transfection reagents were ALL-Fect from RJH Biosciences, PEI (MAX) (from Polysciences), and jetOPTIMUS (from Polyplus). GFP expression was visualized after 2 days via fluorescence microscopy.

Upscaling Delivery and Applications

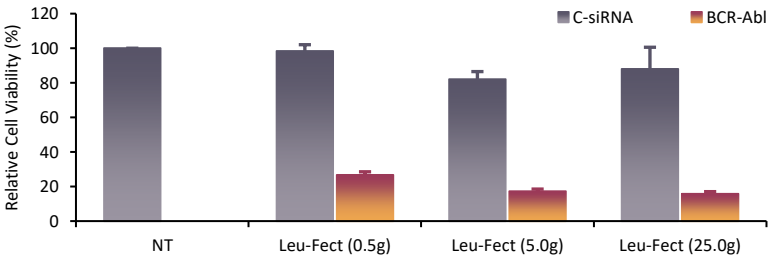
Access to large amounts of transfection reagents for preclinical and clinical studies is imperative for commercial success. RJH Biosciences prepares its reagents at a wide range of volumes without the loss of performance. We can provide materials over a wide range of scales.

RJH Biosciences can Service your Transfection Needs at any R&D Stage



For each stage of the development pathway, a certain amount of transfection reagent is typically needed. The exact amounts will depend on the efficacy (or effective dose) of the nucleic acid formulation with the delivery agent. An increasingly higher amount of materials need to be available whose performance is reproducible at different manufacturing levels. At RJH Biosciences, we can upscale the preparation of transfection reagents at levels that support general research (GxP) and pre-clinical and IND-enabling studies (GLP). Capabilities for clinical studies (GMP) are under development and we look forward to working with partners on clinical development activities.

Performance of Leu-Fect Prepared at Different Scales



Testing the efficacy Leu-Fect reagent produced at different scales. Leu-Fect reagent from 0.5, 5 and 25 g synthesis 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.

RJH Biosciences Inc.
Suite 4000, 10230 Jasper Avenue,
Edmonton, Alberta, T5J 4P6
CANADA

www.rjhbiosciences.com
rjhbiosciences@gmail.com

V1.0