PROTOCOL

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INSPIRED CELL SOLUTIONS

Reverse Transfection (RTF) siRNA Libraries

Introduction

Dharmacon Reverse Transfection format (RTF) siRNA Libraries are designed for effective and convenient siRNA screens in human and mouse cells to study entire gene families or specific regulatory pathways using a reverse transfection format. The libraries are comprised of preselected groups of Dharmacon™ SMARTpool™ siRNA reagents targeting genes known to be relevant to a particular pathway or to be phylogenetically related to the indicated gene family.

The SMARTpool siRNA reagents and experimental controls are pre-dispensed at 6.25 pmol per well in replicate cell culture-ready 96-well plates for a one-time transfection into the cells of interest as described in Figure 1. This unique format eliminates the requirement for daughter plate creation, saving time and minimizing handling steps that might otherwise require robotics or high-throughput capabilities.

Reverse transfection format workflow

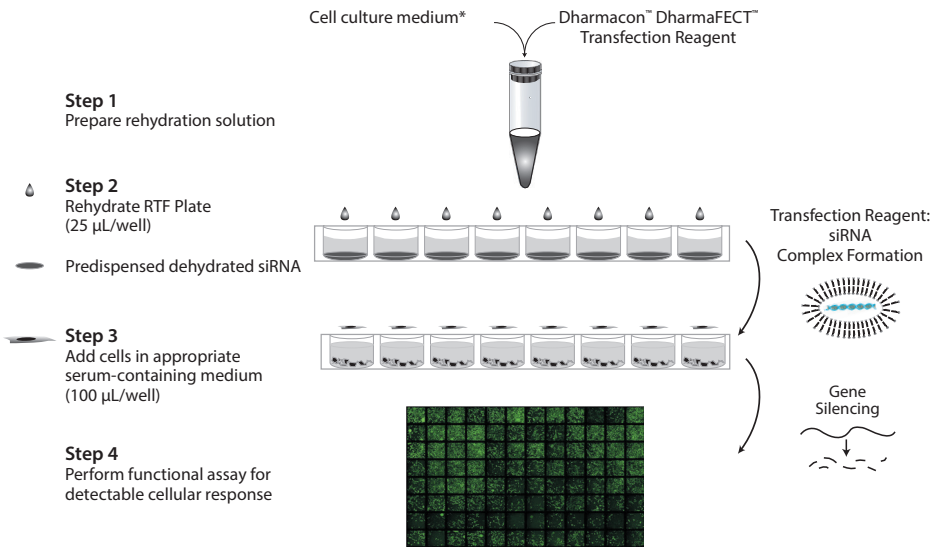

Figure 1. RTF siRNA Library Protocol Overview. **Step 1** – Combine DharmaFECT siRNA transfection reagent (or other optimized transfection reagent of choice) and cell culture medium. **Step 2** – Add mixture from Step 1 to RTF siRNA Library plates to rehydrate the SMARTpool siRNA and form transfection reagent: siRNA complexes. **Step 3** – Add cells to RTF siRNA Library plates and incubate to permit gene silencing **Step 4** – Carry out an appropriate screening assay to assess the cellular changes due to target gene knockdown. Shown is an example of data collected from a screen of targeted cell cycle genes using a Green Fluorescent Protein reporter as a measure of cell cycle status.

*Serum-free and antibiotic-free cell culture medium is recommended for preparation of transfection mix (for example, MEM-RS, HyClone Cat # SH30564).

Product description

Table 1. RTF siRNA Library Plate Format.

Wells	Contents	Description
A1 – F1	Negative and positive control siRNA reagents	Contains siRNA control reagents for human or mouse cells(as described in Table 2)
G1	Empty	Recommended for mock transfection control (transfection reagent only)
H1	Empty	Recommended for untreated cell control
A2 – H11	SMARTpool™ reagents	Number of SMARTpool reagents and plates are determined by size of specific siRNA library collection
A12-H12	Empty	Available for the user's specific screening assay controls, if desired

Table 2. Pre-plated siRNA controls for Dharmacon™ ON-TARGET*plus*™ and siGENOME™ RTF Human and Mouse Libraries.

Well	ON-TARGET*plus* RTF Libraries	Cat#	siGENOME RTF Libraries	Cat #
A1	ON-TARGET*plus* Non-targeting siRNA #1	D-001810-01	siGENOME Non-targeting siRNA #2	D-001210-02
B1	ON-TARGET*plus* Non-targeting siRNA #2	D-001810-02	siGENOME Non-targeting siRNA #3	D-001210-03
C1	ON-TARGET*plus* Non-targeting siRNA #3	D-001810-03	siGENOME Non-targeting siRNA #4	D-001210-04
D1	ON-TARGET*plus* Non-targeting siRNA #4	D-001810-04	siGENOME Non-targeting siRNA #5	D-001210-05
E1	ON-TARGET*plus* Non-targeting pool	D-001810-10	siGENOME Non-targeting pool #2	D-001206-14
F1	ON-TARGET*plus* Human or Mouse Cyclophilin B control pool	D-001820-10 or D-001820-20	siGENOME Cyclophilin B control pool (H, M, R) Control siRNA	D-001136-01

Each RTF siRNA Library plate is provided in the format described in Table 1 and Figure 2. Prior to using an RTF siRNA Library, we recommend optimizing transfection conditions for your cells of choice using Dharmacon RTF Optimization Plates described in Related Products section at the end of protocol.

RTF Libraries are available in clear, white, or black-walled culture plates (clear bottoms) for support of assays with fluorescent or luminescent detection.

- Clear: Thermo Scientific™ Nunc™ Cat# 152039; Fisher Scientific Cat# 12-565-901
- White: Thermo Scientific™ Nunc™ Cat# 152028; Fisher Scientific Cat# 12-565-903
- Black: Thermo Scientific™ Nunc™ Cat# 152037; Fisher Scientific Cat# 12-565-902

All plates are sealed with Thermo Scientific™ Easy Pierce™ Heat Seal Cat# AB 3738

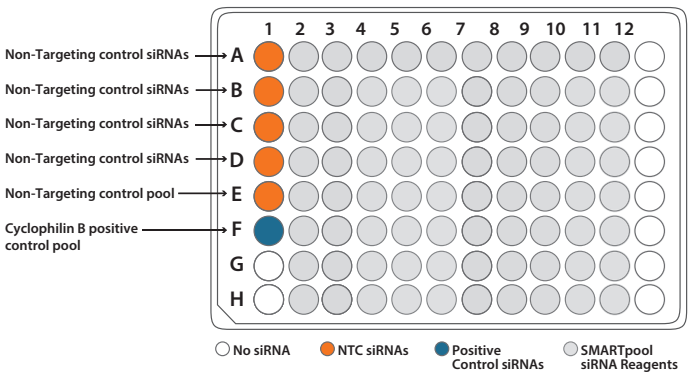

Figure 2. RTF siRNA Library Plate Format.

Table 3. Materials required for use of RTF siRNA Libraries.

96-well RTF siRNA Library plates, containing 6.25 pmol of SMARTpool siRNA reagent per well (triplicate experiment recommended)
DharmaFECT transfection reagent, or other optimized transfection reagent (sold separately)
Serum-free and antibiotic-free cell culture medium such as MEM-RS, Hyclone Cat# SH30564
Equipment, reagents, and supplies required for cell culture and screening assay of choice
USB containing plate map information for determination of total wells per replicate and use in downstream data analysis (provided with siRNA Library purchase)

Shipping and storage

- RTF siRNA Library reagents are shipped dried-down at ambient temperature in sealed 96-well plates. Under these conditions, the reagents are stable for at least 4 weeks.
- Upon receipt, RTF library plates should be stored at +4 °C. Freezing is not recommended.

Handling precautions

- Oligonucleotides are susceptible to enzymatic degradation by nucleases and to chemical degradation by extreme pH and temperatures. Wearing gloves and maintaining nuclease-free conditions when handling the oligonucleotides is strongly recommended.

Protocol

The following protocol should be performed in a laminar flow hood. All calculations are for a triplicate experiment on a single plate with a 10% pipetting allowance. See Table 3 for required materials.

1. Allow RTF siRNA Library plates and DCCR to equilibrate to room temperature.
   a. For inclusion of assay-specific controls (purchased separately), we suggest adding 6.25 pmol in 1 µL to appropriate empty wells.
2. Prepare the Rehydration Solution by mixing the DharmaFECT transfection reagent with cell culture medium.
   - For each set of triplicate plates, prepare 8 mL of Rehydration Solution in a 15 mL tube. Table 4 describes the volumes of DharmaFECT Transfection Reagent necessary to obtain the various DharmaFECT Transfection Reagent volumes (0.03 to 1.0 µL/well) in the final transfection medium.
   - For final volumes of DharmaFECT Transfection Reagent that are different than those described in Table 4, please adjust the volumes of stock DharmaFECT Transfection Reagent and cell culture medium accordingly.
   - The Rehydration Solution may be stored under sterile conditions at room temperature for up to 2 hours prior to use.
3. Rehydrate RTF plates with the Rehydration Solution.
   - Dispense 25 µL of cell culture medium to well H1 in all three plates (black wells, Figure 3). Next, dispense 25 µL of rehydration solution to all remaining wells (gray wells, Figure 3). Allow DharmaFECT Transfection Reagent to complex with siRNA by incubating the rehydrated RTF siRNA Library plates at room temperature for 15-90 minutes. Optimum incubation time is 30 minutes.
     Note: The Rehydration Solution may not coat the bottom of the wells but rather "ring" the bottom of the well. This is expected and does not interfere with rehydration of the siRNA.
4. Add cells to the rehydrated RTF siRNA Library plates.
   a. Prepare at least 35 mL of cell suspension in antibiotic-free complete medium. Complete medium is the medium in which the cells are normally cultured [for example DMEM plus 5-10% Fetal Bovine Serum (FBS)].
   b. Add 100 µL of cell suspension to each well in Columns 1-11. Do not add cells to Column 12 unless being used as untreated controls. The cell concentration in the cell suspension should be such that when 100 µL is dispensed into each well, the required cell number per well is achieved. For example, to add 1×10^3 cells per well, the cell suspension should have 1×10^4 cells per mL. The final volume in each well will be 125 µL and the concentration of siRNA in each well will be 50 nM.
5. Incubate the RTF siRNA Library plates under appropriate culture conditions for the cells being used (for example 37 °C, 5% CO_2).
   - For most consistent results, we recommend that the RTF plates be incubated for at least 2 days before performing your screening assay of choice.
6. Perform the screening assay of choice.

Table 4. Rehydration Solution Dilution Guide (assumes single plate in triplicate).

Final volume of DharmaFECT in transfection medium (µL/well)	Volume of DCCR (mL)	Volume of Stock DharmaFECT (µL)
1.0	7.7	320
0.5	7.8	160
0.25	7.9	80
0.12	8.0	38.4
0.06	8.0	19.2
0.03	8.0	9.6

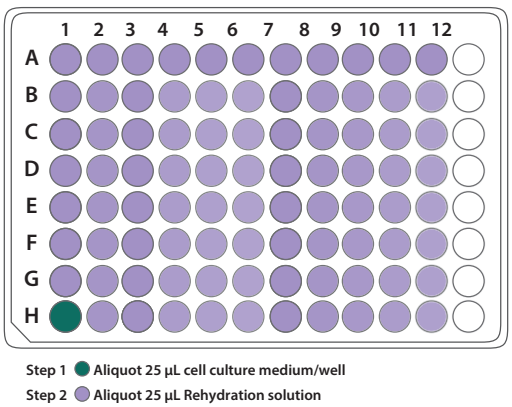

Figure 3. RTF siRNA Libraries Plate Rehydration Guide.

Related products

Product	Description
Dharmacon DharmaFECT siRNA transfection reagents	• siRNA transfection reagents available in four unique lipid-based formulations • Optimized for efficient delivery of siRNA into mammalian cells with minimal impact on cell viability. • Cat #T-2001, T-2002, T-2003, T-2004
RTF Optimization Plates	• Useful for establishing the best transfection conditions using and non-targeting control for each specific assay • Available with Dharmacon siGENOME or ON-TARGET*plus* standard controls for either human or mouse applications • Provided as triplicate in clear (standard), black, or white walled plates • Cat #H-102200, H-112200, H-002200

If you have any questions, contact

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