CRISPR Arrayed Multi-Guide Library Lipofection using Cas9-Expressing Cells

Developed by EditCo

Introduction

EditCo's Screening Libraries are easy-to-use kits for loss-of-function arrayed screening. Knockouts are achieved through a multi-guide approach, in which up to three chemically modified synthetic sgRNAs are used to concurrently target each gene. Each well of the plate contains 150 pmol of multi-guide sgRNA. This protocol describes a high-throughput library lipofection method using Cas9-expressing cells.

This protocol is written with a particular sgRNA concentration and Lipofectamine™ volume (i.e., 0.2 µM sgRNA and 0.08 µl Lipofectamine™/reaction) for HEK293 cells. However, we highly recommend optimizing your transfection to your particular cell type prior to embarking on a screen using an Arrayed Multi-guide Library. Once you have completed the optimization in your cell type, you can apply the appropriate sgRNA concentration and Lipofectamine™ volume to this protocol. Upon request, EditCo will provide a positive control multiguide sgRNA (TRAC) to customers for use in the optimization.

Abbreviations:

CRISPR: clustered regularly interspaced short palindromic repeats
Cas9: CRISPR associated protein 9
sgRNA: single guide RNA
RNP: ribonucleoprotein
PCR: polymerase chain reaction
ICE: inference of CRISPR edits
TE: Tris EDTA
PBS: phosphate-buffered saline
KO Score: Knockout Score

Materials Required

Material	Ordering Information
Arrayed multi-guide library	EditCo (included)
Nuclease-free water	Multiple vendors (e.g., Thermo Fisher Scientific)
Lipofectamine™ RNAiMAX Transfection Reagent	Thermo Fisher Scientific, Catalog #13778030
Opti-MEM™ I Reduced Serum Medium	Thermo Fisher Scientific, Catalog #31985062
Positive control (optional)	Recommended: *TRAC* multi-guide sgRNA (available upon request, EditCo)
Nuclease-free 1X TE buffer	Multiple vendors (e.g., Thermo Fisher Scientific, Catalog #AM9849)
Multiwell plate adhesive sealing film	Thermo Fisher Scientific, Catalog #CMAX00001
384-well V-bottom plates (145 µl)	E&K Scientific, Catalog #EK-30281
Cell-culture treated 384-well clear polystyrene plates	Multiple vendors (e.g, Thermo Fisher Scientific)
Normal growth medium	Multiple vendors, cell-type dependent
Trypsin or preferred cell dissociation reagent	Multiple vendors (e.g., TrypLE, Thermo Fisher Scientific)
PBS buffer	Multiple vendors (e.g., Thermo Fisher Scientific)
Fetal bovine serum (FBS)	Multiple vendors (e.g., Thermo Fisher Scientific, Millipore Sigma)
Cell counter	Multiple vendors (e.g., Thermo Fisher Scientific)
PCR primers (sequence provided)	Multiple vendors
Sequencing primer (sequence provided)	Multiple vendors
Microcentrifuge tubes	Multiple vendors (e.g., Eppendorf)

Important Considerations

- All EditCo reagents should be stored according to the manufacturer's recommendations.
- Synthetic sgRNA should be dissolved in TE buffer and diluted to a working concentration using nuclease-free water.
- Cell seeding is based on the rate of cell growth. For fast-growing cells, seed fewer cells.
- For adherent cells, be sure to include trypsinization (step 2.5a-b)
- Use cells at lowest passage number possible.
- Wearing gloves and using nuclease-free tubes and reagents are recommended in order to avoid RNase contamination.
- Always maintain sterile technique and use sterile filter pipette tips.

Recommended Optimization

To optimize the transfection conditions to your cell type, we suggest that customers optimize the concentration of sgRNA and volume of Lipofectamine™ used using a positive control multi-guide sgRNA (e.g., TRAC). Once optimized, the values can replace the current sgRNA concentration and Lipofectamine™ volume in this protocol.

The volume of Lipofectamine™ is the most critical parameter to optimize, as it may greatly impact the efficiency of your transfection. The concentration of sgRNA is the next most critical component. Additionally, optimizing cell number may improve results.

Suggested Controls

Control	Description	Purpose
Negative	non-targeting sgRNA or no sgRNA	Ensure that there are no false positives due to contamination (no effect expected=wild type).
Positive	sgRNA with high editing efficiency (e.g., TRAC)	Ensure all reagents, protocol, and equipment are functioning (effect expected). Optimize transfection conditions.
Transfection	pMAX GFP (Lonza), GFP mRNA (SBI)	Assess transfection efficiency of sgRNA

Timeline

Pre–Lipofection	Setup & Lipofection	Post–Lipofection
Day 1 Seed cells Incubate (2 days)	**Day 3** Rehydrate Multi-guide sgRNAs Dilute Multi-guide sgRNAs to Working Stock Prepare Lipofectamine™ Master Mix Form Lipofectamine RNAiMax-sgRNA Complexes Prepare Cells Reverse Transfection	**Days 4-7** Analysis

Protocol

1. Pre-Lipofection

1.1. Seed Cells

a. Seed cells in an appropriately-sized vessel at least 2 days before lipofection so that they are 60-80% confluent on the day of transfection.

2. Setup & Lipofection

2.1 Rehydrate Multi-guide sgRNA

Note: EditCo RNA oligos ship dry at ambient temperature and remain stable for several weeks at room temperature. Please store dried RNA oligos at -20ºC for long-term storage (up to 6 months).

a. Briefly centrifuge the 384-well plate to make sure that all of the RNA is collected at the bottom of each well.
b. Carefully rehydrate the 150 pmol of the multi-guide sgRNA in each well of the plate using 30 µl of nucleasefree 1X TE buffer to obtain 30 µl of 5 µM (5 pmol/µl) sgRNA.
c. Seal the plate with sealing film and then shake or vortex the plate to resuspend completely or use a liquid handler to pipette 5 times.
d. Allow about 30 minutes for resuspension. This will be your concentrated rehydrated sgRNA stock.

Note: Rehydrated sgRNA stock can be stored at -80ºC.

2.2. Dilute Multi-guide sgRNA to Working Stock

a. In each well of a 384-well V-bottom plate (volume of 145 µl), add 4 µl multi-guide sgRNA (5 µM, 5 pmol/µl) and 96 µl of nuclease-free water.
b. Mix gently. This makes a working stock of 0.2 µM (0.2 pmol/µl).
c. To reduce the number of freeze/thaw cycles, the working stock can be aliquoted into single-use volumes and stored at -20ºC. Under these conditions, the provided sgRNA is stable for up to 6 months.

Note: The concentration of multi-guide sgRNA working stock should be optimized for every cell line. This protocol uses a 0.2 µM (0.2 pmol/µl) sgRNA concentration (Step 2.2b), as this concentration has been effective for HEK293 cells.

2.3. Prepare Lipofectamine™ Master Mix

a. Determine the number of wells/conditions to be transfected in the experiment (denoted below as “X wells”)

Calculation for Lipofectamine™ Master Mix (16 µl per reaction)

b. To a suitable tube, add Opti-MEM™ according to the formula below:
(X wells) x (15.92 µl Opti-MEM™) x (1.3*)
c. To the same tube, add Lipofectamine™ RNAiMax Reagent according to the formula below:
(X wells) x (0.08 µl RNAiMax Reagent) x (1.3*)

Note: The amount of Lipofectamine™ RNAiMax Reagent used should be optimized for every cell line. This protocol uses 0.08 µl/well Lipofectamine™ RNAiMax Reagent (Step 2.3c) as this volume has been effective for HEK293 cells.

d. Pipette 16 µl of the Lipofectamine™ master mix into each well of a 384-well cell culture plate.

2.4. Form Lipofectamine RNAiMax-sgRNA Complexes

a. Transfer 4 µl of multi-guide sgRNA (0.2 µM, 0.2 pmol/µl) from the working concentration plate to the 16 µl of Lipofectamine™ RNAiMax Reagent in Opti-MEM™ in each well of the 384-well cell culture plate.
b. Incubate at room temperature for 5 minutes.

2.5. Prepare Cells

Note: For suspension cells, skip step 2.5a-b.

a. Wash cells with 1X PBS (enough to cover the bottom of each well), then aspirate PBS.
b. To dissociate cells, add trypsin or preferred dissociation agent (enough to cover the bottom of each well), incubate for 5 minutes in a humidified 37°C/5% CO2 incubator. Resuspend cells in an equivalent volume of growth medium to stop the trypsin reaction.
c. Count cells to determine cell density.
d. Transfer the appropriate number of cells according to the formula below:
(1.5 X 103 cells) x (X wells) x (1.3*)
e. Centrifuge cells at 100 g for 10 minutes. Aspirate medium.
f. Resuspend the cell pellet in Opti-MEM™+ 10% FBS according to the formula below:
(X wells) x (20 µl FBS/Opti-MEM™) x (1.3*)

2.6. Reverse Transfection

Note: EditCo highly recommends reverse transfection (i.e., multi-guide sgRNA is added to wells first and cells in suspension are added second), as this method has resulted in high editing efficiencies.

a. Add 20 µl of adequately mixed cell suspension in FBS/Opti-MEM™ to the 20 µl of Lipofectamine™ RNAiMaxsgRNA complexes in each well of the cell culture plate (40 µl total reaction volume). Incubate cells for 1–3 days in a humidified 37°C/5% CO_2 incubator. (X wells) x (20 µl FBS/Opti-MEM™) x (1.3*)
b. Incubate at room temperature for 5 minutes.

3. Post-Lipofection

3.1. Analysis

a. Extract DNA from cells 1-3 days post-transfection.
b. PCR-amplify and Sanger-sequence the target region of the DNA.
c. Conduct Inference of CRISPR Edits (ICE) analysis.
ICE is a free online tool that provides an easy quantitative assessment of genome editing using Sanger sequencing data. The software compares the sequence traces of amplicons generated from genomic DNA isolated from both the edited and unedited pools of cells. The results of an ICE analysis include ICE Score (indel frequency) and Knockout (KO) Score (the percentage of sequences that lead to a putative knockout). The ICE analysis tool is available at ice.editco.com.

Additional Information

For an up-to-date list of all protocols and other resources, please visit this link.

For technical assistance, contact our Scientific Support Team at technicalsupport@editco.bio.

For common FAQs, please visit this link.