Sigma-Aldrich®
Lab & Production Materials

Protocol

Pairing CRISPR-Mediated Genome Engineering with 10x Genomics Single-Cell Analysis

Lentiviral CRISPR Pools From a 10x Genomics Compatible Partner

Compatible Products 10x GENOMICS

Introduction

CRISPR screening is a powerful approach to interrogate mechanisms underlying development, disease, and therapeutic responses. Single cell CRISPR screens extend this approach by directly linking CRISPR perturbations and single cell gene expression readouts, cell by cell. After you have completed your whole genome screen, a follow up screen using Sigma-Aldrich® custom CRISPR pools with 10x Genomics compatibility are a powerful method to validate your candidate list at single cell resolution. Profile hundreds of different CRISPR perturbations and detect individual sgRNAs with directly linked gene expression phenotypes in hundreds to tens of thousands of cells, without prior knowledge of cell types or markers. Leverage Chromium Single Cell Gene Expression with Feature Barcode technology to simultaneously measure CRISPR perturbations and the resulting transcriptional signature. This technology greatly expands the accessibility, scalability, and resolution of high-throughput functional screens, for mechanistic insights into biology.

Ordering Information: SigmaAldrich.com/10xCRISPRpools

Protocol for Lentiviral Transduction

It is recommended to use cells at low passage number for transduction experiments. Prior to transduction and screening, a kill curve should be established for all required antibiotics in each target cell line and stock used. Lentiviral infection rate, or CFU-based titer, should also be determined for your specific experimental conditions. This preparatory work should take ~2 weeks to complete. The experimental work, or the library screen, should take roughly 2–4 weeks, depending on cell growth rates. It is generally recommended to use BSL2 safety guidelines when working with lentivirus. An overview of the complete experimental protocol is provided in the following sections.

For detailed lentiviral protocols, visit SigmaAldrich.com/LentiProtocols

Preparatory Work

1. Perform a 7-day kill curve in each target cell stock for each antibiotic used.
2. For CRISPRi experimentation, the sensitivities to puromycin (puro) and blasticidin (blast) must be determined.
3. Determine your functional viral titer (transforming units per mL, TU/mL) for each target cell type.

Generation of Stable KRAB-dCas9 Helper Cells

Day 1: Seeding Cells

1. We generally recommend seeding cells in smaller volumes such as a 6-well cell culture plate with enough cells for 10% confluency.
2. Incubate at 37 °C with 5% CO_2 for 24 hours.

Day 2: Transduction

1. Remove growth medium from each well of the plate(s) prepared on day 1.
2. Add 8 mg/mL polybrene-containing medium to each well. Gently swirl the plate to mix.
3. Transduce the cells with KRAB-dCas9 helper construct: using the equation below, determine and add the appropriate volume of lentiviral particles for each construct. The desired MOI is <0.2. Leave at least one well for selection of non-transduced cells, one well for cells with virus but without selection, and one well for cells without virus or selection agent.

$$\textit{Volumes of Viral Particles (µL)} = 1000\ \mu L \times \frac{\textit{Number of Cells x Desired MOI}}{\textit{Viral Titer (TU/mL)}}$$

4. Incubate at 37 °C with 5% CO_2 for 24 hours.

Day 3: Medium Change

1. Remove the virus-containing medium from each well of the plate(s). Sterilize discarded virus medium and contaminated supplies using a 10% bleach solution for 24 hours.
2. Add fresh growth medium (without polybrene) to each well.
3. Incubate at 37 °C with 5% CO_2 for 24 hours.

Day 4: Antibiotic Selection

1. Remove the medium from each well.
2. Feed cells with fresh growth medium containing the appropriate concentration of blasticidin as determined in the kill curve prior to transduction.

Days 5–10: Continued Antibiotic Selection

1. Continue to select for transduced cells using concentrations and timelines established in the kill curve prior to transduction.
2. Replace the antibiotic-containing medium as necessary during the selection process (usually every 2 to 3 days). The non-transduced control cells should die completely after 7-day selection with antibiotic.
3. Following selection and subsequent expansion, split selected cells; bank a frozen stock for future culture using CryoStor® cell cryopreservation medium and grow the rest for transduction with the sgRNAs. Selected cells may remain under blasticidin selection to ensure retention of the KRAB-dCas9 cassette.

Transduction of the gRNA controls

Day 1: Seeding Cells

1. Determine the number of cells to seed in each flask using the equation below. The target MOI should be <0.2 to ensure that each cell integrates no more than a single sgRNA cassette. The desired coverage is 500–5000 cells per transduced guide.

$$\textit{Number of Cells} = \frac{\textit{Number of Guides x Desired Coverage}}{\textit{Desired MOI}}$$

2. Seed the appropriate number of cells per flask in fresh growth medium.
3. Incubate at 37 °C with 5% CO_2 for 24 hours.

Day 2: Transduction

1. Remove medium from each flask prepared.
2. Add 8 mg/mL polybrene-containing medium to each flask. Gently rock the flask to mix.
3. Using the equation below, determine and add the appropriate volume of lentiviral particles. For example, transduction of 20,000 cells with 2 control gRNAs at a MOI of 0.5 would require 10 µL of virus with a CFU functional titer of 1E+06 TU/mL. Be sure to leave some wells as negative controls for selection.

$$\textit{Volumes of Viral Particles (µL)} = 1000\ \mu L \times \frac{\textit{Number of Cells x Desired MOI}}{\textit{Viral Titer (TU/mL)}}$$

4. Incubate at 37 °C with 5% CO_2 for 24 hours.

Day 3: Medium Change

1. Remove the virus-containing medium from each flask.
2. Add fresh medium (without polybrene).
3. Incubate at 37 °C with 5% CO_2 for 24 hours.

Day 4: Antibiotic Selection

1. Remove the medium.
2. Feed cells with fresh growth medium containing the appropriate concentration of puromycin, as determined in the kill curve prior to transduction.

Days 5+: Continued Antibiotic Selection

1. Continue to select for transduced cells using concentrations and timelines established in the kill curve prior to transduction.
2. Replace the puromycin-containing medium as necessary during the selection process (usually every 2 to 3 days). The non-transduced control cells should die completely after selection with antibiotic.
3. Following selection and subsequent expansion, we recommend splitting the cells to bank a frozen stock of selected, transduced cells for future culture using CryoStor® cell cryopreservation medium. We recommend doing this in case you need to restart the experiment. Selected cells may remain under blasticidin and puromycin selection to ensure retention of the KRAB-dCas9 and gRNA cassettes.

Preparing Cells for Single Cell Analysis

As a 10x Genomics compatible partner, Sigma-Aldrich® gRNAs are compatible with each of the downstream Chromium analysis workflows. For next steps, please visit 10x Genomics Single Cell CRISPR Screening.

Ordering Information: SigmaAldrich.com/10xCRISPRpools

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices. Though the lentiviral transduction particles produced are replication incompetent, it is recommended that they be treated as Risk Group Level 2 (RGL-2) organisms in laboratory handling. Follow all published RGL-2 guidelines for laboratory handling and waste decontamination.

Biosafety Features

Replication incompetence is an important safety feature of Sigma-Aldrich® CRISPR Lentiviral Particles. Users should consult and observe their own institutional guidelines when working with viral systems.

Storage and Stability

Upon receipt, immediately store the product at -80 °C. Avoid freeze/thaw cycles, as this will severely reduce transduction efficiency.

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