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CRISPR-Cas9 Screening Protocol for Gene Perturbation Analysis

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We use this protocol and it's working

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Abstract

This protocol describes CRISPR-Cas9 Screening for gene perturbation analysis.

Materials

- 2 mg/ml puromycin
- High Pure PCR Template Preparation Kit (Roche # 11796828001)
- Phusion HF DNA polymerase (ThermoFisher Scientific #F-530XL)
- Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoFisher Scientific #Q33232)

High Pure PCR Template Preparation Kit **Roche Catalog #**11796828001

Phusion™ High-Fidelity DNA Polymerase (2 U/µL) **Thermo Fisher Catalog #**F-530XL

Quant-iT™ 1X dsDNA HS Assay **Thermo Fisher Catalog #**Q33232

Procedure

1w 5d 0h 5m

1 Plate 10 × 150 mm of cells at 8 × 10^6 cells per dish in complete growing medium.

2 Incubate Overnight .

3
- Next day, infect cells with human CRISPR Brunello lentiviral pooled library.

> **Note**
>
> Four unique sgRNAs (single guide RNAs) per gene, comprising a total of ~76,000 sgRNAs.

- Incubate Overnight .

> **Citation**
>
> Doench, J., Fusi, N., Sullender, M. et al. (2016)
> . Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9.
> Nature Biotechnology.
>
> https://doi.org/10.1038/nbt.3437 LINK

4 The following day, trypsinize cells from all 10 plates, pool and plate into 20 × 150 mm dishes (5 × 10^5 cells per dish) with growing medium containing 2 µL puromycin. Incubate for 72:00:00 .

3d

5 Change to regular medium and incubate for 168:00:00 to allow complete editing.

1w

6 Trypsinize all 20 plates, pool and determine cell number.

7 Plate into 100 × 150 mm dishes (5 × 10^5 cells per dish).

8 Infect 50 plates with Ad-rtTA and 50 plates with Ad-MAPL-flag adenovirus at 10 pfu/cell. Incubate for 48:00:00 . 2d

9 Change to regular medium and refresh every 2-3 days.

> Note
>
> For Ad-rtTA, cells reached confluency in 1.5 weeks and for Ad-MAPL-flag, ~3 weeks.

10 Harvest cells by trypsinization, pool together all 50 plates, disperse to single cell, and determine cell number.

11 Make aliquots of 20 × 10^6 cells and centrifuge for 1000 x g, Room temperature, 00:05:00 . 5m

12 Remove supernatant and store pellets at -80 °C .

13 Isolate genomic DNA using High Pure PCR Template Preparation Kit (Roche #11796828001) as described by the manufacturer.

14 Amplify sequences of the gRNA from genomic DNA by PCR using Phusion HF DNA polymerase (ThermoFisher Scientific #F-530XL) using a 2-step amplification adding a unique 6-bp index per sample and sequencing adapter sequences as described in S. Huang et al. (2012) Cell 151, 937-950.

15 Quantify using the Quant-iT™ PicoGreen™ dsDNA Assay Kit (ThermoFisher Scientific #Q33232).

16 Sequence amplified gDNA using HiSeq2500 System (Illumina).

17 Map sequence reads to the library using xcalibr and analyze counts with MAGeCK (version 0.5.8) as described in W. Li et al., (2014) Genome Biol 15, 554.

18 Use Robust Rank Aggregation (RRA) algorithm to identify genes whose perturbation (knockout or overexpression) primarily enhanced fitness in the MAPL overexpressing group but not the control group.

Protocol references

Doench, J.G. *et al.* Optimized sgRNA design to maximize activity and minimize off-target effects of CRISPR-Cas9. *Nature biotechnology* **34**, 184-191 (2016).

S. Huang et al. (2012) Cell 151, 937-950.

W. Li et al., (2014) Genome Biol 15, 554.