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Supplementary Methods

The Knock-In Atlas: A web resource for targeted protein trap by CRISPR/Cas9 in human and mouse cell lines

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Materials & Reagents

- Cellmatrix Type I -C (Collagen, Type I, 3 mg/mL, pH 3.0) (Nitta Gelatin, #637-00773)
- HCl (pH 3.0)
- Porcine Gelatin (Sigma-Aldrich, #G1890)
- Puromycin Dihydrochloride, Animal-Free (Nacalai Tesque, # 19752-64)
- FuGENE® HD Transfection Reagent (Promega, #E2311)
- PEI MAX - Transfection Grade Linear Polyethylenimine Hydrochloride (MW 40,000) (PSI, #24765-2)
- Opti-MEM™ I Reduced Serum Medium (Termo Fisher Scientific, #31985070)
- Falcon® 5 mL Round Bottom Polystyrene Test Tube, with Cell Strainer Snap Cap, 25/Pack, 500/Case (Falcon, # 352235)

Protocols (Collagen coating or Gelatin coating)

1. Prepare 10-40% collagen (diluted collagen with HCl, pH 3.0) or 0.01-0.1% gelatin (dissolved gelatin with MilliQ) solutions.
2. Add diluted collagen or gelatin solutions into each well.
   1.0 mL of the solution/well in a 6 well plate
   0.5 mL of the solution/well in a 24 well plate
   50 µL of the solution/well in a 96 well plate
3. Incubate them at RT for 30 mins.
4. Wash the wells with 1×PBS three times.

Protocols (24 well plate-based transfection)

We prepared a variety of knock-in cell lines following to 24 well plate-based transfection for HEK293T, eHAP1, HeLa and Neuro2a. This protocol minimized DNA amounts and transfection reagents.

Day 0

1. Seed HEK293T (1.6×10^5 cells/well) of a 24 well plate with Cellmatrix Type I -C (Collagen, Type I, 3 mg/mL, pH 3.0) (Nitta Gelatin, #637-00773) (1:10-1:40 dilution), such that they will be 60-80% confluency.
   ※Total suspension should be around 400-500 µL to seed the cells at an appropriate density.
   ※eHAP1 (1.5×10^5 cells/well), HeLa (4.0×10^4 cells/well) and Neuro2a (1.6×10^5 cells/well) were used for transfection.
   ※Collagen coating is not necessary for eHAP1, HeLa and Neuro2a.

Day 1

2. Mix Plasmids or PCR products following to tables.
   Plasmid-based knock-in method

DNA	DNA (pmol)	DNA (µg)	PEI-Max or FuGene (µL)	OptiMEM(µL)
Intron gRNA expressio plasmid	0.038	0.225	1.8	30
Donor gRNA expression plasmid	0.038	0.225		
Plasmid-based knock-in donor	0.064	0.15		

   PCR-based knock-in method

DNA	DNA (pmol)	DNA (µg)	PEI-Max or FuGene (µL)	OptiMEM(µL)
Intron gRNA expressio plasmid	0.076	0.44	1.8	30
PCR-based knock-in donor	0.064	0.04		

3. Dispense pre-mixtures into each 1.5 mL tube.
4. Add Plasmid DNA (targeting an intron) into each 1.5 mL tube.
5. Add PEI-Max or FuGENE® HD Transfection Reagent to the mixture, incubate for 15 min at RT.
   ※We've already tried both transfection reagents for HEK293T. However, we have not tried PEI-Max for eHAP1, HeLa and Neuro2a.
6. Replace 0.25 mL of serum-free medium (DMEM) during Step5 incubation.
7. Apply the mixture to 24 well plate, and then incubate for 2 hours at 37℃.
8. 2 hours later, add 50 µL of serum to each 24 well plate incubate it for 22

hours at 37℃.

Day 2

9. Add 0.25 mL of serum-free medium (DMEM). Incubate it at 37℃ for 24 hours.

Day 3

10. Passage cells from 24 well plate to 6 well plate with collagen coating.
11. Incubate cells at 37℃ for 24 hours.

Day 4

1. Collects the cells by using trypsinization.
2. Spin it at 4℃ 1,200 rpm for 3 min.
3. Remove the supernatant.
4. Resuspend 0.8-1.0 mL with FACS Sorting Buffer.
5. Filter the suspension with FACS tube.
6. Store them on ice before analysis.
7. Sort 500 fluorescent positive cells into a well of a collagen coated 24 well plate by using Cell Sorter MA900 (SONY) (Supplementary Methods Figure1).
   ※Please add 1.0 mL of fresh medium into each well of a 24 well plate before sorting.
   ※We recommend that tube sorting for rare knock-in events (e.g. ~0.1% of fluorescent knock-in efficiency) (Supplementary Methods Figure1). We alternatively sorted 10,000-60,000 cells into a collection tube (Falcon, #352063)
8. Incubate sorted cells at 37℃ for 2-3 weeks.

Protocols (6 well plate-based transfection)

We prepared a variety of knock-in cell lines following to 6 well plate-based transfection for mESC.

Day 0

1. Seed mESC (4.0×10^5 cells/well) of a 6 well plate with 0.01-0.1% gelatin coating.

Day 1

2. Mix Plasmids following to tables.

   Plasmid-based knock-in method

DNA	DNA (pmol)	DNA (μg)	PEI-Max or FuGene (μL)	OptiMEM(μL)
Intron gRNA expressio plasmid	0.114	0.68	5.43	90.5
Donor gRNA expression plasmid	0.114	0.68		
Plasmid-based knock-in donor	0.192	0.45		

   ※We recommend that Plasmid-based knock-in donor is feasible for mESC.
3. Dispense the mixtures into each 1.5 mL tube.
4. Add PEI-Max or FuGENE® HD Transfection Reagent to the mixture, incubate for 15 min at RT.

   ※We've already tried both transfection reagents for mESC.
5. Apply the mixture to the 6 well plate, and then incubate for 24 hours at 37℃.

   ※Please do not replace the medium with serum-free medium.

Day 2-4

6. Replace fresh medium containing puromycin (1 μg/mL), incubate them at 37℃ for 3 days.

   ※Puromycin selection was used for mESC Because we utilized gRNA expression plasmids derived from pSpCas9(BB)-2A-Puro (PX459) V2.0 (Addgene, #62988).

Day 5

7. Collects the cells by using trypsinization.
8. Spin it at 4℃ 1,200 rpm for 3 min.
9. Remove the supernatant.
10. Resuspend 0.5-1.0 mL with FACS Sorting Buffer.

11. Filter the suspension with FACS tube.
12. Store them on ice before analysis.
13. Sort more than 10,000 fluorescent positive cells into a well of a gelatin coated 6 well plate by using Cell Sorter MA900 (SONY) (Supplementary Methods Figure1).

    ※Please add 2.0 mL of fresh medium into each well of a 6 well plate before sorting.

    ※We recommend that second sorting for rare knock-in events (e.g. ~0.1% of fluorescent knock-in efficiency). We additionally sorted 10,000-60,000 cells into a 6 well plate after first sorting for some knock-in targets.
14. Incubate sorted cells at 37℃ for 1-3 weeks.

Protocols (electroporation)

We prepared knock-in cell lines following to electroporation for MEF and THP-1.

Day 1

1. Mix Plasmids following to tables.

   Plasmid-based knock-in method

DNA	DNA (pmol)	DNA (μg)
Intron gRNA expressio plasmid	0.171	1
Donor gRNA expression plasmid	0.171	1
Plasmid-based knock-in donor	0.429	1

   ※We recommend that Plasmid-based knock-in donor is feasible for MEF and THP-1.

2. Electroporate the cells with plasmid DNAs by using Neon Transfection System (Life Technologies).

   ※MEF (1.0×10^6 cells/reaction) and THP-1 (2.0×10^6 cells/reaction) were used for the electroporation.

3. Culture the cells for 1-2 weeks.

After 1-2 weeks

4. Detect fluorescent positive cells by using CytoFLEX S.