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Generation of ATG3 KO Hela cells stably expressing HaloTag-LC3B

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol details generation of ATG3 KO Hela cells stably expressing HaloTag-LC3B.

Attachments

PDF

736-1852.pdf

129KB

Materials

Buffers and reagents:

- Growth broth: LB broth
- Stbl3 competent *E. coli* (MacroLab, UC Berkeley)
- DMEM medium with GlutaMAX containing 10% FBS and 10% Pen-Strep.

pVSV-G **addgene Catalog #**#138479

pBS-CMV-gagpol **addgene Catalog #**35614

pMRX-IP-HaloTag7-LC3 **addgene Catalog #**184899

Qiagen Hi-Speed MidiPrep kit **Qiagen Catalog #**12643

DMEM, high glucose, GlutaMAX™ Supplement **Thermo Fisher Catalog #**10566016

Gibco™ Fetal Bovine Serum value heat inactivated (formerly USDA-approved in North America or quali **Fisher Scientific Catalog #**A5256801

Penicillin-Streptomycin (10,000 U/mL) **Thermo Fisher Scientific Catalog #**15140122

Opti-MEM™ I Reduced Serum Medium **Thermo Fisher Catalog #**31985070

TransIT®-LT1 Transfection Reagent **Mirus Bio Catalog #**MIR 2300

Lenti-X™ Concentrator **Takara Bio Inc. Catalog #**631231

15-Dimethyl-15-diazaundecamethylene polymethobromide Polybrene **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**H9268

Puromycin Dihydrochloride **Gold Biotechnology Catalog #**P-600

Troubleshooting

Procedures

15m

1 Transform each plasmid into Stbl3 competent cells for the propagation. Next day, pick up one colony to inoculate Overnight culture in LB medium with ampicillin.

2 Midi prep the cultures to purify plasmids (Qiagen).

3 Plate 5 × 10^6 HEK 293T cells on a 10 cm plate in DMEM medium.

4 HEK293T transfection:

4.1 Add retroviral packaging plasmids (pVSV-G, pBS-CMV-gagpol) and pMRX-IP-HaloTag7-LC3, 5 µg each in 1.5 mL warm Opti-MEM medium.

4.2 Add 45 µL of TransIT-LT1 transfection reagent (Mirus) and swirl.

4.3 Incubate at Room temperature for 00:15:00 . 15m

4.4 Add 1.5 mL dropwise into 10 cm HEK293T plate.

4.5 At 72 hours post-transfection, collect retroviral supernatant into a falcon tube.

5 Concentrate retroviral supernatant to 1 mL using Lenti-X concentrator (Takara) with the manufacturer instruction.

6 Retroviral transduction:

6.1 Plate 1 × 10^5 ATG3 KO HeLa cells into 12-well plate one day before.

6.2 Next day, titrate 100 µL , 200 µL , 400 µL of concentrated retroviral solution with 8 µg/mL Polybrene (Sigma) into target HeLa cells.

6.3 At 24 hours post-transduction, remove retroviral supernatant and replace with fresh DMEM complete medium with 2 µg/mL puromycin (GoldBio).

6.4 Perform Puromycin selection for two weeks. Confirm Halo-LC3B positive cells by FACS sorting.