

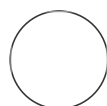


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Generation of ATG3 KO Hela cells stably expressing HaloTag-LC3B

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ABSTRACT

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

ATTACHMENTS

[736-1852.pdf](#)

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

OPEN ACCESS

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

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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

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.