OCT 02, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.6qpvr3462vmk/v1

Protocol Citation: Harper JW, Kelsey Hickey, sharan_swarup 2023. Gene editing of YIPF3, YIPF4, CALCOCO1, FIP200, and ATG7 in HEK293 and HeLa cells V2. **protocols.io** https://dx.doi.org/10.17504/protocols.io.6qpvr3462vmk/v1

MANUSCRIPT CITATION:
https://www.biorxiv.org/content/10.1101/2022.12.06.519342v1

Gene editing of YIPF3, YIPF4, CALCOCO1, FIP200, and ATG7 in HEK293 and HeLa cells V2

Forked from Gene editing of YIPF3, YIPF4, CALCOCO1, FIP200, and ATG7 in HEK293 and HeLa cells

Harper JW[1], Kelsey Hickey[1], sharan_swarup[1]

[1]Harvard Medical School

ASAP Collaborative Research Network

Kelsey Hickey

ABSTRACT

This protocol describes the creation of YIPF3, YIPF4, FIP200, CALCOCO1, and ATG7 knockout cell lines in HEK293 and HeLa cells using CRISPR-Cas9.

MATERIALS

Puromycin (Gold Biotechnology, P-600-100)
pX459 (Addgene #62988)
HEK293 (ATCC CRL-1573, RRID: CVCL_0045)
anti-YIPF4 (Sino Biological 202844-T46)
anti-ATG7 (Cell Signaling Technology, 8558S)
anti-CALCOCO1 (Abclonal A7987)
Dulbecco's MEM (DMEM), high glucose, pyruvate (Gibco / Invitrogen, 11995)

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

Created: Aug 03, 2023

Last Modified: Oct 02, 2023

PROTOCOL integer ID:
85934

Keywords: ASAPCRN

Cell line maintenance

1 Maintain HEK293 and/or HeLa cells in Dulbecco' Modifies Eagles Medium (DMEM) with 10% fetal bovine serum and optional 1% penicillin-streptomycin.

Targeted knock-out specific genes including YIPF3, YIPF4, CA...

2 For YIPF4 knock-out, the following sgRNA sequences were designed and ordered (5' ATCTCGCGGCGACTCCCAAC 3' / 5' CGGCCTATGCCCCCACTAAC 3'), and cloned into a pX459 vector to create pX459-gRNA-YIPF4-KO.

For CALCOCO1 knock-out, the following sgRNA sequences were designed and ordered (5' AAGTTGACTCCACCACGGGA 3' / 5' CTAAGCCGGGCACCATCCCG 3'), and cloned into a pX459 vector to create pX459-gRNA-CALCOCO1-KO.

For ATG7 knock-out, the following sgRNA sequence was designed and ordered (5' ATCCAAGGCACTACTAAAAG 3'), and cloned into a pX459 vector to create pX459-gRNA-ATG7-KO.

For FIP200 knock-out, the following sgRNA sequence was designed and ordered (5' ACTACGATTGACACTAAAGA 3'), and cloned into a pX459 vector to create pX459-gRNA-FIP200-KO.

For YIPF3 knock-out, the following sgRNA sequences were designed and ordered (5' CCATTTCGGGCGCCGCCCGC 3' / 5' GGCGGCGCCCGAAATGGAGC 3'), and cloned into a pX459 vector to create pX459-gRNA-YIPF3-KO.

Sequence validate by Sanger sequencing.

CRISPR editing and confirmation

3 Transfect HEK293 and/or HeLa cells with the pX459-gRNA-YIPF4-KO, pX459-gRNA-CALCOCO1-KO, pX459-gRNA-ATG7-KO, gRNA-FIP200-KO, or gRNA-YIPF3-KO with Lipofectamine 3000, and select with 1.2 µg/mL of puromycin for 24-48 hours. Select monoclonal cells by limiting dilution or by cell sorting (SONY SH800S sorter) in 96 well plates.

3.1 Individual clones were subjected to immunoblotting with anti-YIPF4 (Sino Biological 202844-T46), anti-ATG7 (Cell Signaling Technology, 8558S), anti-FIP200 (Cell Signaling Technology, 12436), anti-YIPF3 (Invitrogen PA566621), or anti-CALCOCO1 Rabbit mAb antibody (Abclonal A7987) and clones lacking the relevant protein were selected for further analysis by Sanger sequencing of the edited alleles.