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Cell line construction and maintenance for Lyso-IP with or without genes linked with lysosomal storage disease

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DISCLAIMER

ABSTRACT

Lyso-IP is a method that allows for the isolation of lysosomes for proteomics and metabolomics using HA-tagged TMEM192 (dx.doi.org/10.17504/protocols.io.bybjpskn; dx.doi.org/10.17504/protocols.io.bx9hpr36). Here, we describe methods for cell line construction and maintenance of HeLa cells with TMEM192-3xHA with or without deletion of genes linked with lysosomal storage diseases.

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MATERIALS TEXT

A	B	C
REAGENT or RESOURCE	**SOURCE**	**IDENTIFIER**
Chemicals, peptides, and recombinant proteins		
Puromycin	Sigma-Aldrich	P9620
G418 (Geneticin)	Invivogen	ant-gn-2
Dulbecco's MEM (DMEM), high glucose, pyruvate	GIBCO / Invitrogen	11995
Experimental models: Cell lines		
HeLa cells	ATCC	CCL-2
HeLa: TMEM192-3xHA	This study	
Recombinant DNA		
pSMART TMEM192-3xHA (targeting vector for genomic tagging)	35	Addgene #175777
pX459-gRNA-APP (for making APP deletion by CRISPR/Cas9)	This study	Addgene #176487

DISCLAIMER:

Cell line maintenance

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

Endogenous tagging of TMEM192 with 3xHA

2 For endogenous tagging of TMEM192 with 3xHA, co-transfect HeLa cells with pX459 containing a gRNA (5'-AGTAGAACGTGAGAGGCTCA) targeting adjacent to the translational termination sequence in TMEM192 and pSMART containing 5' and 3' homology arms for TMEM192 in which the termination codon is replaced by a 3xHA epitope sequence followed by a TAA stop codon (Addgene #175777).

3 Identify homozygously targeted clones by immunoblotting cell extracts with α-HA and α-TMEM192. These are referred to as HeLa-TMEM192-HA cells for Lyso-IP.

Targeted knock-out specific genes including GRN, HEXA, NPC1 and NPC2

4 For GRN knock-out, phosphorylate and anneal oligonucleotides (Top: 5'-ATCGACCATAACACAGCACG, Bottom: 5'-CGTGCTGTGTTATGGTCGAT), and clone into a pX459 vector. For HEXA knock-out, phosphorylate and anneal oligonucleotides (Top: 5'-CGGCCGAGCTGACATCGTAC, Bottom: 5'-GTAGCATGTCAGCTCGGCCG), and clone into a pX459 vector. For NPC1 knock-out, phosphorylate and anneal oligonucleotides (Top: 5'-TACCTGGACAGAAACTGTAG, Bottom: 5'-CTACAGTTTCTGTCCAGGTA), and clone into a pX459 vector. For NPC2 knock-out, phosphorylate and anneal oligonucleotides (Top: 5'-AGCTGCCAGGAAACGCATCG, Bottom: 5'-CGATGCGTTTCCTGGCAGCT), and clone into a pX459 vector.

5 Transfect HELA-TMEM192-HA cells with the pX459-gRNA-APP plasmid (Addgene #176487) with Lipofectamine 3000, and select with 1.2 µg/mL of puromycin. Select monoclonal cells, and confirm target gene deletion by Western blotting, and/or Sanger sequencing of the edited alleles.

Rescue of GRN expression

6 The entry vector pDONR223 containing full-length GRN open reading frame (1179 base pairs) is recombined with a pHAGE lentivirus destination vector using Gateway cloning technology (Thermo Fisher).

7 Make lentivirus for transduction of pHAGE-GRN by transfecing 293T cells along with psPAX2, pMD2.G (Addgene Cat#12260 Cat#12259) and pHAGE-GRN in a 4:2:1 ratio using polyethyleneimine. Virus-containing supernatant was harvested 2 days after transfection and filtered through a 0.45-micron syringe filter. Polybrene was added to a final concentration of 8 mg/ml to the viral supernatant.

8 HeLa Tmem192-3xHA GRN KO cells were infected with 50 mL of viral supernatant, and stable cell lines were selected 48 h post-infection using hygromycin at a concentration of 100

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mg/mL.

8.1 Maintain HeLa cells in Dulbecco' Modifies Eagles Medium (DMEM) with 10% fetal bovine serum and optional 1% penicillin-streptomycin with hygromycin at a concentration of 100 mg/mL.

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