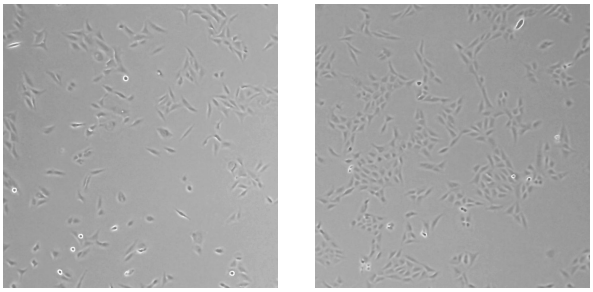
Institut-Hôpital neurologique de Montréal
Montreal Neurological Institute-Hospital

GENE KNOCKOUT: LIMK1

Product Information		
Cell Line	CRISPRn LIMK1 Knockout cell line	
Parental	U2OS PDI CRISPRn FH10a	
Product ID	U2OS CRISPRn PDI FH10a LIMK1 KO 2E2	
Product Batch	U2OSn –LIMK1-2E2-201022	
Genotype	CRISPR/cas9-edited	
Passage	P3	
Date of Production	2020-10-22	
Properties		
Volume	1 ml/vial	
Storage Conditions	Liquid Nitrogen	
Cell Number/ Vial	77%	
Viability	2.3 x 10^5 cells/mL	
Quality Control		
Test	**Test Method**	**Pass/Fail**
Viability	Post thawing culture	Pass
Mycoplasma	MycoAlert™ Mycoplasma Detection Kit (Lonza)	Pass
Cell Line Characterization	Sanger Sequencing (DNA)	Pass
Morphology Images	10x objective 24h Post-Thaw 48h Post-Thaw 	
Growth Conditions		
Culture Media	Dulbecco's Modified Eagle's Medium (DMEM) supplemented with tetracycline-free FBS 10%, L-glutamine 2mM, Penicillin-Streptomycin 100U/ml.	
Passage Method	Trypsin	
Freezing Media	Tetracycline-free FBS with 10% DMSO	
Recommended Subculture	Cells are cultured as a monolayer at 37°C in a humidified atmosphere with 5% CO_2. Cells should be passaged every 5-7 days. Split at 80-85% confluency, approximately 1:10-1:20.	
Cell Line Revival	Rapidly thaw cells in a 37°C water bath. Transfer contents into a tube containing 5 ml pre-warmed media. Centrifuge cells, remove supernatant wash cells with 10 ml PBS, centrifuge cells, remove PBS and seed into a 10 cm flask containing pre-warmed media.	

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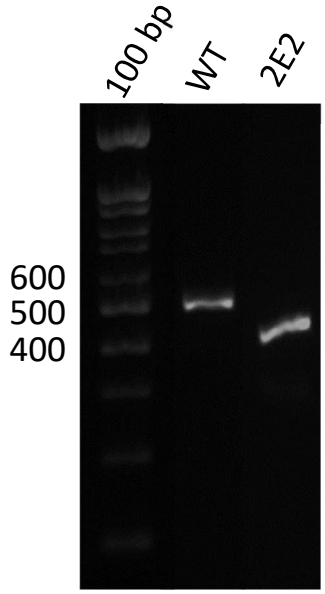
Cell Line Characterization (DNA)	
Target Gene	LIMK1
Guide RNA Sequence	1. AUAGUACUGGUGCGACAGGG 2. UGAUUUGCUCAGAGCACCCA
Genomic Location	Chromosome 7: 74,082,933-74,122,525 forward strand.
Gene	LIMK1 ENSG00000106683
Target Protein	LIMK1
Mutation	81 bp deletion in Exon 3
Forward Primer	**Reverse Primer**
TACAAAGGTGCCTGTAGCCG	ACACCCTTGGAAGAGGCCTA

PCR Products

Gel Image description

Image description: PCR products of LIMK1 KO clones 2E2 along with WT were resolved on 2% agarose gel



DNA Sequence of edited region:

AACTTGGCRGGGGAMCCGGGCTGGCCCAGCTGCTGAGCTC
CTGGACAGAGGCTGGCTCCATAGATGGGGGCGCTGAGGGA
TACAGACAGACAGTCAGGCTCAGAAAGCAGCTCCAAAGACC
AGCAAAAAGGAGACCAGGAGAGACAATGATGGGAGAGAG
ACCTGGAGCATGGGGTCTGCTTGGATACCCCCACCCCAGGT
GAGTGTGCAAGGCAGGGGGCGCTCACCATAACCAGTCCCTT
GGTGATTTGCTCAGAGCACAGGGAGGCACTGCAGTCACAAC
ACCTGCAGGAGAGGGGCCGGGCTGAGACGTCCTCTCCCG
CCCTCCTCCACCTCACCTGGGCCCCTGCCTCGGCTACAGGCA
CCTTTGTAAAGGGCGAATTCTGCAGATATCCATCACACTGGC
GGCCGCTCGAGCATGCATCTAGAGGGCCCAATTCGCCCTAT
AGTGAGTCGTATTACAATTCACTGGCCGTCG

Sequence Alignment

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  1 GTGTTGTGACTGCAGTGCCTCCCTGTCGCACCAGTACTATGAGAAGGATG     50
    ||||||||||||||||||||||||
  1 GTGTTGTGACTGCAGTGCCTCCCT-------------------------     24

 51 GGCAGCTCTTCTGCAAGAAGGACTACTGGGCCCGCTATGGCGAGTCCTGC    100

 25 -------------------------------------------------     24

101 CATGGGTGCTCTGAGCAAATCACCAAGGGACTGGTTATGGTGAGCGCCCC    150
        ||||||||||||||||||||||||||||||||||||||||||||||||
 25 -----GTGCTCTGAGCAAATCACCAAGGGACTGGTTATGGTGAGCGCCCC     69
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