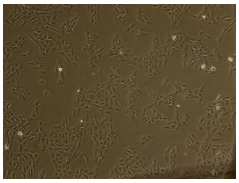
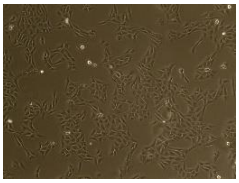
GENE KNOCKOUT: KIF5a

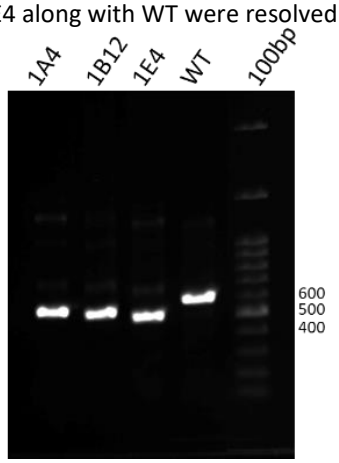
Institut et hôpital neurologiques de Montréal
Montreal Neurological Institute and Hospital

Product Information		
Cell Line	U2OS CRISPRn Kif5a Knockout	
Parental	U2OS-PDI-CRISPRn FH10a	
Product ID	U2OS PDI CRISPRn FH10a KIF5a KO 1A4	
Product Batch	U2On-Kif-1A4-190513	
Genotype	CRISPR/cas9-edited	
Passage	P7	
Date of Production	2019-May-13	
Properties		
Volume	1 ml/vial	
Storage Conditions	Liquid Nitrogen	
Cell Number/Vial	2.2 x 10^6 cells/ml	
Viability	85%	
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	Before Banking 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.	

GENE KNOCKOUT: KIF5a

Institut et hôpital neurologiques de Montréal
Montreal Neurological Institute and Hospital

Cell Line Characterization (DNA)	
Target Gene	Kif5a
Guide RNA Sequence	1. GAGGGUCGUGCAGCUUUCCC 2. GAACCUUGAGUUCCACAUCA
Genomic Location	Chromosome 12: 57,550,044-57,586,633
Gene	Kif5a ENSG00000155980
Target Protein	Kinesin heavy chain isoform 5A
Mutation	Clone 1A4 43bp deletion, 6bp insertion and 12 bp mismatch.
Forward Primer	**Reverse Primer**
TCTGGCTTTGGTGGTTGAGG	GGTAACCGGAAAGGGAGTCA

PCR Products	**Sequence**
Image description: PCR products of Kif5a KO clones 1A4, 1B12, and 1E4 along with WT were resolved on agarose gel 	TGACCATCTCCTAACTTAGGATGTTCTTCTTATTGACTC TTGCCTTGGTGTTCACCTGCATACATCTGAGTTGTCTCA TTCTCCCTGAGCCCCAGCTTCACTCTCAAATACCTTCAC TCGCCAGGGATCAAGGTGACCAGGGCACGACAGCTG GGCATTCAGATGGGGACTGGGAGGGGAAGATCTAAA ATCTTCCCACTGAAGAG

Sequence Alignment

Clone 1A4:

```
  1 tgaccatctcctaacttaggatgttcttcttattgactcttgccttggtg     50
    ||||||||||||||||||||||||||||||||||||||||||||||||||
  1 TGACCATCTCCTAACTTAGGATGTTCTTCTTATTGACTCTTGCCTTGGTG     50

 51 ttcacctgcatacaGGAAAGCTGCACGACCCTCAGCTGATGGGAATCATT    100
    ||||||||||||                    ||.||||...|..|||||
 51 TTCACCTGCATA--------------------CATCTGAGTTGTCTCATT     80

101 CCTC---GAATTGCCCGAGACATCTTCAACCACATCTACTCCATGGATGA    147
     |||   ||   ||||    ||.|||||       || |||.|   ||
 81 -CTCCCTGA---GCCC----CAGCTTCA-------CT-CTCAA---AT--    109

148 GAACCTTGAGT-TCCA--CATCAAGgtgaccagggcacgacagctgggca    194
      |||||.|.| .|||  .||||||||||||||||||||||||||||||
110 --ACCTTCACTCGCCAGGGATCAAGGTGACCAGGGCACGACAGCTGGGCA    157

195 ttcagatggggactgggaggggaagatctaaaatcttcccactgaagag    243
    ||||||||||||||||||||||||||||||||||||||||||||||||||
158 TTCAGATGGGGACTGGGAGGGGAAGATCTAAAATCTTCCCACTGAAGAG    206
```