

Materials List for

Defining Gene Functions in Tumorigenesis by *Ex vivo* Ablation of Floxed Alleles in Malignant Peripheral Nerve Sheath Tumor Cells

Jody Fromm Longo¹, Stephanie N. Brosius^{2,3}, Steven L. Carroll¹¹Department of Pathology and Laboratory Medicine, Medical University of South Carolina ²Department of Pediatrics and Neurology, Perelman School of Medicine of the University of Pennsylvania ³Division of Child Neurology, Children's Hospital of Philadelphia

Corresponding Author

Steven L. Carroll

carrolst@musc.edu

Citation

Longo, J.F., Brosius, S.N., Carroll, S.L. Defining Gene Functions in Tumorigenesis by *Ex vivo* Ablation of Floxed Alleles in Malignant Peripheral Nerve Sheath Tumor Cells. *J. Vis. Exp.* (174), e62740, doi:10.3791/62740 (2021).

Date Published

August 25, 2021

DOI

10.3791/62740

URL

jove.com/video/62740

Materials

Name	Company	Catalog Number	Comments
Ad5CMV-eGFP	Gene Transfer Vector Core, Univ of Iowa	VVC-U of Iowa-4	
Ad5CMVCre-eGFP	Gene Transfer Vector Core, Univ of Iowa	VVC-U of Iowa-1174	
alexa 568 secondary antibody	Thermo/Fisher	GaR A11036	
Bioconductor Open Source Software for Bioinformatics	Bioconductor	http://www.bioconductor.org	alternative statistical analysis tool used for step 4.4
CD31	Abcam	ab28364	
Celigo Image Cytometer	Nexcelom Bioscience	N/A	
Cell Stripper	Corning	25-056-CI	mixture of chelators
DAB staining kit	Vector Labs	MP-7800	
DAVID (Database for Annotation, Visualization, and Integrated Discovery)	DAVID	https://david.ncifcrf.gov	functional enrichment analysis software used for step 4.5
DMEM	Corning	15-013-CI	
DreamTaq and Buffer (Genotyping PCR)	Thermo/Fisher	EP0701 and K1072	
erbB4 antibodies	Santa Cruz	sc-284	
erbB4 antibodies	Abcam	ab35374	
erbB4 antibodies	Millipore	HFR1: 05-1133	
FACS Sorter	BD Biosciences	Aria II	
Forskolin	Sigma	F6886	
GenomeSpace Tools and Data Sources	GenomeSpace	https://genomespace.org/support/tools/	general resource for several types of open source bioinformatic tools for step 4.5
Glutamine	Corning	25-005-CI	
Gorilla Gene Ontology enRichment analYsis and visualizAtion tool	Gorilla	N/A, http://cbl-gorilla.cs.technion.ac.il	functional enrichment analysis software used for step 4.5

GSEA Gene Set Enrichment Analysis	Broad Institute	N/A, https://www.gsea-msigdb.org/gsea/index.jsp	functional enrichment analysis software used for step 4.5
HSD: Athymic Nude-FOxn1nu mice	Envigo (Previously Harlan Labs)	69	
Illumina HiSeq2500 (next generation DNA sequencer)	Illumina	Hi Seq 2500	DNA sequencer used for step 4.2
Lasergene: ArrayStar Gene expression and variant analysis	DNAStar LaserGene software	N/A	software statistical and normalization analysis used for step 4.4
Lasergene: SeqMan NGen sequence alignment assembly	software alignment used for step 4.3	N/A	software alignment used for step 4.3
Matrigel, low growth factor basement membrane matrix	Corning	354230	
NRG1-beta	In house	Generated by SLC, also commercially available from R & D Systems(396-HB-050/CF).	
Nuclear Stain Hoeschst 33342	Thermo	62249	
Panther Gene Ontology Classification System	Panther	http://pantherdb.org	functional enrichment analysis software used for step 4.5
Partek (BWA aligner and analyzer)	Partek, Ver 7	N/A	software alignment and statistical/normalization used for step 4.3
Pen/Strep	Corning	30-002-CI	
Primer 1: 5'-CAAATGCTCTCTGTTCTTGT GTCTG- 3'	Eurofins Genomics	Primer 1 + 2: 250 bp ErbB4 null product and a 350 bp Floxed ErbB4 product;	
Primer 2: 5'-TTTGCCAAGTTCTAATTCCATC AGAACG-3'	Eurofins Genomics	Primer 1 + 2: 250 bp ErbB4 null product and a 350 bp Floxed ErbB4 product;	
Primer 3: 5'-TATTGTGTTCATCTATCATTGCA ACCCAG-3'	Eurofins Genomics	Primer 1 + 3: 350 bp wild-type ErbB4 product.	
Propidium Iodine	Fisher	51-351-0	
Proteom Profiler Phospho-Kinase Arrays	R&D Systems	ARY003B	
Real time glo	Promega	G9712	bioluminescent cell viability assay
ToppGene Suite	ToppGene	https://toppgene.cchmc.org	functional enrichment analysis software used for step 4.5
Trizol (acid-quanidinium-phenol and choloroform based reagent)	Invitrogen	15596026	
Tyramide Signal Amplification Kit	Perkin Elmer	NEL721001EA	