

CRISPR Gene Editing Tool for MicroRNA Cluster Network Analysis

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Materials

Name	Company	Catalog Number	Comments
0.2 mL PCR tubes, flat cap	Fisher	14-230-225	Plasticware
1.5 mL Microcentrifuge Tubes	Seal-Rite	1615-5500	Plasticware
24-well tissue culture plate	Corning Costar	09761146	Plasticware
5x Phusion HF Buffer	Thermo Scientific	F-518L	PCR reagent, genotyping
6-well tissue culture plate	Fisher	FB012927	Plasticware
96-well tissue culture plate	Falcon	08-772-2C	Plasticware
Anti-CRISPR-Cas9 antibody [7A9-3A3], Mouse monoclonal	AbCam	ab191468	Western blot reagent; 160 kDa; dilution 1:200
Antibiotic-Antimycotic (100x)	Gibco	15240062	Tissue culture reagent
BLAST nucleotide search engine	National Center for Biotechnology Information	NA	Freeware; website: blast.ncbi.nlm.nih.gov
Blasticidin S, Hydrochloride, Streptomyces griseochromogenes	Calbiochem	CAS 589205	CRISPR reagent; Chemical, Working stock = 1 mg/mL in water
Countess 3 Automated Cell Counter	Invitrogen	A50298	Equipment; Cell counter
Cryogenic tubes	Thermo Scientific	50001012	Plasticware
Dharmacon CRISPR Design Tool	Horizon Discovery Ltd.	NA	Freeware; website: horizondiscovery.com/en/ordering-and-calculation-tools/crispr-dna-region-designer
DharmaFECT siRNA Transfection Reagent #2	Dharmacon, Inc.	T-2002-02	Transfection reagent; LNCaP and PC3-ML cell lines
Dimethyl sulfoxide (DMSO)	Fisher	BP231100	Tissue culture reagent
DMEM Medium with L-Glutamine, 4.5g/L Glucose and Sodium Pyruvate	Corning	MT10013CV	Tissue culture reagent; Media for PC3-ML cells
Doxycycline Hydrochloride, Ready Made Solution	Sigma-Aldrich	D3072-1ML	CRISPR reagent; Chemical, Working stock = 1 mg/mL stock in water
DPBS, no calcium, no magnesium	Gibco	14190144	Tissue culture reagent
Edit-R CRISPR-Cas9 Synthetic tracrRNA, 20 nmol, designed	Dharmacon, Inc.	U-002005-20	CRISPR reagent; Universal tracrRNA oligonucleotides
Edit-R Modified Synthetic crRNA, desalted/deprotected, 2 nmol	Dharmacon, Inc.	crRNA-460XXX	CRISPR reagent; Designed crRNA oligonucleotides

EditR Inducible Lentiviral hEF1aBlastCas9 Nuclease Particles, 50 μ L, 10^7 TU/mL	Dharmacon, Inc.	VCAS11227	CRISPR reagent; Lenti-iCas9; Doxycycline-inducible lentiviral Streptococcus pyogenes Cas9 vector system
Ensembl genomic viewer	Ensembl	NA	Freeware; website: [ensembl.org] Use: Genome browser to identify a nucleotide sequences containing the miRNA cluster and surrounding gene/regulatory sequences.
GAPDH Antibody (FL-335), rabbit polyclonal	Santa Cruz Biotechnology	sc25778	Western blot reagent; 37 kDa; dilution 1:500
Gel/PCR DNA Fragment Extraction Kit	IBI Scientific	IB47010	Nucleic acid gel electrophoresis reagent
Gibco Fetal Bovine Serum, certified	Gibco	16000044	Tissue culture reagent
Hexadimethrine bromide	MilliporeSigma	H9268	CRISPR reagent; Chemical, Working stock = 0.8 mg/mL
Immobilon-FL PVDF Membrane	MilliporeSigma	IPFL10100	Western blot reagent; nitrocellulose
Intercept (TBS) Blocking Buffer	LI-COR	927-60001	Western blot reagent
IRDye 680RD Goat anti-Mouse IgG Secondary Antibody	LI-COR	926-68070	Western blot reagent
IRDye 800CW Goat anti-Rabbit IgG Secondary Antibody	LI-COR	926-32211	Western blot reagent
Microcentrifuge	Eppendorf	5425 R	Plasticware
miRBase microRNA viewer	miRBase	NA	Freeware; website: [mirbase.org] Use: Browser lists all annotated miRNA hairpins, mature miRNA sequences and associated clustered miRNAs mapping within 10 kb.
NuPAGE 4 to 12%, Bis-Tris, 1.0 mm, Mini Protein Gel, 10-well	Invitrogen	NP0321BOX	Western blot reagent
Odyssey CLx Imaging System	LI-COR	CLx	Equipment; Western blot imaging
Opti-MEM Reduced Serum Medium	Gibco	31985062	Transfection reagent
Owl D2 Wide-Gel Electrophoresis System	Owl	D2-BP	Equipment; Nucleic acid gel electrophoresis system
PCR Primers, designed (single-stranded DNA oligonucleotides)	Integrated DNA Technology	NA	PCR reagent, genotyping
Phusion High-Fidelity DNA Polymerase (2 U/ μ L)	Thermo Scientific	F530S	PCR reagent, genotyping
RIPA Lysis Buffer 10x	MilliporeSigma	20-188	Western blot reagent
Proteinase K Solution (20 mg/mL), RNA grade	Invitrogen	25530049	PCR reagent, genotyping
RNase A, DNase and protease-free (10 mg/mL)	Thermo Scientific	EN0531	PCR reagent, genotyping
RPMI 1640 Medium	Gibco	MT10041CV	Tissue culture reagent; Media for LNCaP cells
SnapGene Viewer	Snap Gene	NA	Freeware; website: [snapgene.com] Use: DNA sequence annotation software program to create a DNA file for gRNA design and which highlights the miRNA cluster locus (intergenic, intronic), each individual miRNA hairpin sequence belonging to the miRNA cluster, and other nearby coding and non-coding genes and/or regulatory features
TrypLE Select Enzyme (1x), no phenol red	Gibco	12563029	Tissue culture reagent; Recombinant trypsin

UltraPure Agarose	Invitrogen	16500100	Nucleic acid gel electrophoresis reagent
Veriti 96-Well Fast Thermal Cycler	ThermoFisher Scientific	4375305	Equipment
XCell SureLock Mini-Cell Electrophoresis System	Invitrogen	EI0001	Equipment; Protein gel electrophoresis system