

Materials List for:

# Efficient Generation of hiPSC Neural Lineage Specific Knockin Reporters Using the CRISPR/Cas9 and Cas9 Double Nickase System

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URL: <https://www.jove.com/video/52539>

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## Materials

Name	Company	Catalog Number	Comments
pStart-K	Addgene	20346	
pWS-TK6	Addgene	20350	
pKD3	Addgene	45604	
pKD46	The Coli Genetic Stock Center, CGSC	7634	
PGK-neo-bpA sequence	Addgene	13442	
Human BAC clones of target genes	<a href="https://bacpac.chori.org">https://bacpac.chori.org</a>		
JDS246 (Cas9-003), Mammalian codon-optimized streptococcus pyogenes Cas9-3X Flag	Addgene	43861	
MLM3636, Human-gRNA-ExpressionVector with U6 promoter	Addgene	43860	
pX335-U6-Chimeric_BB-CBh-hSpCas9n(D10A)	Addgene	42335	
sgRNA design, ZIFit	<a href="http://zifit.partners.org/ZIFit/">http://zifit.partners.org/ZIFit/</a>		
Off target prediction tool CasOT	<a href="http://eendb.zfgenetics.org/casot/download.php">http://eendb.zfgenetics.org/casot/download.php</a>		
Perl	<a href="http://www.perl.org/get.html">http://www.perl.org/get.html</a>		
NCBI	<a href="http://www.ncbi.nlm.nih.gov/">http://www.ncbi.nlm.nih.gov/</a>		
BLAT	<a href="https://genome.ucsc.edu/cgi-bin/hgBlat?command=start">https://genome.ucsc.edu/cgi-bin/hgBlat?command=start</a>		
sgRNA Primer synthesis	Sigma		
One shot Top10 Electrocomp E. Coli Competent cells	Life Technologies	C4040-50	
AccuPrime Pfx SupperMix	Life Technologies	12344-040	
Restriction enzymes	NEB and Life Technologies		
Zymoclean Gel DNA Recovery Kit	Zymo Researech	D4007	
DNA quick extraction buffer	Epicentre	QE0905T	

Herculase II Fusion DNA Polymerases	Agilent Technologies	600675	
Digestion buffer 2	New England Biolab		
6X DNA Loading Dye	Thermo Scientific	R0611	
TeSR-E8 Kit for hESC/hiPSC Maintenance	Stem Cell Technologies	05940	
UltraPure Agarose	Life Technologies	16500-100	
50xTAE	Life Technologies	B49	
Essential 8 medium	Life Technologies	A1517001	
Dulbecco's Phosphate Buffered Saline without Calcium and Magnesium	Life Technologies	A12856-01	
D-MEM/F12 with Glutamax	Life Technologies	10565018	
D-MEM with Glutamax	Life Technologies	10566040	
Fetal Bovine Serum-ES cell qualified	Life Technologies	10439	
Knockout serum replacement	Life Technologies	10828010	
2-mercaptoethanol 1000X	Life Technologies	21985023	
Non Essential Amino Acid	Life Technologies	11140050	
Stempro Accutase	Life Technologies	A1110501	
Dispase	Life Technologies	17105-041	
0.25% Trypsin- EDTA solution	Life Technologies	25200-056	
Geltrex	Life Technologies	12760-013	
ROCK inhibitor Y-27632	Millipore	SCM075	
SMC4 reagent	BD	354357	
Neomycin resistant MEF	Millipore	PMEF-NL	
Hygromycin resistant MEF	Millipore	PMEF-HL	
G418 (Geneticin)	Life Technologies	11811	
Hygromycin B	Life Technologies	10687010	
FIAU (Fialuridine, 1-2-Deoxy-2-fluoro- $\beta$ -D-arabinofuranosyl-5-iodouracil)	Moravek Biochemicals and Radiochemicals	M251	
Electroporator: Gene Pulser Xcell	Bio-Rad		
0.4 cm electroporation cuvette	Bio-Rad	165-2088	
DIG-High prime DNA labeling and detection starter kit II	Roche	11585614910	
Hybridization denature solution	VWR	82021-478	
PCR DIG probe synthesis kit	Roche	11636090910	
DIG wash set	Roche	11585762001	
Anti-Digoxigenin (DIG-AP)	Roche	11093274910	
CSPD chemiluminescence system	Roche	11755633001	
DIG wash and block buffer set	Roche	11585762001	
50X TAE buffer	Life Technologies	24710030	
Blotting buffer (25 mM Tris pH 7.4, 0.15 M NaCl, 0.1% Tween20)			
Hoefer Ultraviolet Crosslinker	Fisher Scientific	03-500-308	
Spermidine	Fisher	AC13274-0010	
Tris HCl 2M (pH 7.5)	VWR	200064-506	

Denville Scientific blue bio film 8x10	Fisher	nc9550782	
DNA molecular weight marker II ( DIG-labeled )	Roche	11218590910	
Amersham Blotting membrane Hybond-N+	Roche	95038-400	
Pyrex glass drying tray	Fisher	15-242A	
Kimberly-Clark C-fold paper towels	Fisher	06-666-32B	
Whatman 3MM paper (26X41)	Fisher	05-713-336	
Hybridization bag	Roche	11666649001	
Hybridization tubes	Fisher	13-247-300	
Hybridization oven rotisserie Shake 'n' Stack	Fisher	HBMSOV14110	