shRNA knockdown

Nancy C. Hernandez
wusj[1], Villegas[2], schekman[1]

[1]Department of Molecular and Cell Biology, Howard Hughes Medical Institute, University of California, Berkeley, Berkeley, United States; [2]Helen Wills Neuroscience Institute, University of California, Berkeley, Berkeley, United States

Nancy C. Hernandez Villegas

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Protocol status: Working
We use this protocol and it's working

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ABSTRACT

This protocol describes a standard procedure to generate a stable cell line for a DNAJC5 knockdown using shRNA.

MATERIALS

Reagents

	Item	Catalog number	Manufacturer
	HEK 293T cells		Cell Culture Facility, UC Berkeley
	plKO.1-Hygroplasmids		
	DNAJC5-shRNA		
	pMD2.G plasmid		Addgene
	PsPAX2 plasmid		Addgene
	Lenti-X Concentrator	631231	Takara Bio
	SH-SY5Y cells		Cell Culture Facility, UC Berkeley
	Hygromycin B (50 mg/mL)	10687010	Thermo Scientific

shRNA knockdown

1 HEK293T cells were plated at a density such that on the day of transfection they are no more than 50% confluent.

2 plKO.1-Hygroplasmids-containing shRNA targeting DNAJC5 (ccggGCAACCTCAGATGACATTAAACTCGAGTTTAATGTCATCTGAGGTTGCTTTTTG) together with pMD2.G and PsPAX2 were transfected into HEK293T cells to produce lentiviral particles for 72 hr.

3 Lentivirus particles were concentrated with Lenti-X Concentrator follow manufacturer protocol (Takara Bio).

4 SH-SY5Y cells were transduced by lentivirus before differentiation.

5 Three days post transduction, cells were selected with 250 µg/ml hygromycin for 10 days.

6 The selected cells were differentiated, and the knockdown was verified with immunoblot.