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

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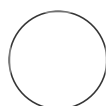
## shRNA knockdown

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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
pIKO.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 pIKO.1-Hygroplasmids-containing shRNA targeting DNAJC5 (ccggGCAACCTCAGATGACATTAACTCGAGTTTAATGTCATCTGAGGTTGCTTTTTG) 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.