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

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CRISPR nuclease (CRISPRn) genome editing

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ABSTRACT

CRISPR nuclease (CRISPRn) genome editing

1

LentiCRISPRv2 plasmid (RRID:Addgene_52961) was used for α-syn genome editing. All sgRNAs were designed using CRISPick (https://portals.broadinstitute.org/gppx/crispick/public) and examined for genome-wide sequence specificityusing the National Center for Biotechnology Information's (NCBI) Basic Local Alignment Search Tool NCBI BLAST (RRID:SCR_004870)(http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sgRNAs (Control 5' GACGGAGGCTAAGCGTCGCAA3' and α-syn CRISPRn 5' GGTTCATGAAAGGACTTTCAA3') were cloned as described previously

CITATION

Shalem, O., Sanjana, N.E., Hartenian, E., Shi, X., Scott, D.A., Mikkelsen, T.S., Heckl, D., Ebert, B.L., Root, D.E., Doench, J.G., and Zhang, F. (2014). (2013). Genome-scale CRISPR-Cas9 knockout screening in human cells. Science.
LINK
10.1126/science.1247005

2 To validate the sgRNAs constructs, DIV-3 hippocampal primary neurons were infected with lentiviruses per 6hrs (MOI=5). Transduced neurons were cultured to maturity (DIV17-DIV21) and then lysate for western blotting analysis and genomic analysis.