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shRNA plasmids

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Abstract

How to make shRNA plasmids

1. pLKO.1 Puro plasmids containing shRNA (pLKO.1-shRNA) against mouse/rat*Lrrk2*(sh*Lrrk2*: TRCN0000322193; GGCCGAGTTGTGGATCATATT), was obtained from the RNAi Consortium (TRC) via Dharmacon.

2. A scrambled shRNA sequence was generated (GTTGCTGAATGGCGGATCTAT) and cloned into the pLKO.1 TRC cloning vector according to Addgene protocols (**https://www.addgene.org/protocols/plko/**).

3. To generate pLKO.1 shRNA plasmids that express EGFP (pLKO.1-shRNA-EGFP), CAG-EGFP was removed from pLenLox-shNL1-CAG-EGFP and inserted between Kpn1 and SpeI sites in pLKO.1 Puro, replacing the puromycin resistance gene. pLKO.1 shRNA mCherry plasmids were generated by replacing EGFP with mCherry between KpnI and NheI sites.