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Generation of pLKO.1 Plasmids Containing shRNA

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We use this protocol and it's working

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Disclaimer

Abstract

Generation of pLKO.1 Plasmids Containing shRNA

1 **Obtain shRNA Plasmids**

1.1 - Obtain pLKO.1 Puro plasmids containing shRNA sequences against mouse/rat Lrrk2 (shLrrk2: TRCN0000322193) and mouse/rat Atg7 (shAtg7: TRCN0000092163) from the RNAi Consortium (TRC) via Dharmacon.

2 **Synthesize Scrambled shRNA Sequences**

2.1 - Generate two scrambled shRNA sequences: GTTGCTGAATGGCGGATCTAT and GTTGCGGTTATGAATAGTACT.

3 **Cloning into pLKO.1 Vector**

3.1 - Clone the scrambled shRNA sequences into the pLKO.1 TRC cloning vector254 following the protocols provided by Addgene (https://www.addgene.org/protocols/plko/).

4 **pLKO.1-shRNA-EGFP Plasmid Construction**

4.1 - Remove CAG-EGFP from pLenLox-shNL1-CAG-EGFP255.

4.2 - Insert the CAG-EGFP sequence between the KpnI and SpeI sites in the pLKO.1 Puro vector, replacing the puromycin resistance gene to generate pLKO.1-shRNA-EGFP.

5 **pLKO.1-shRNA-mCherry Plasmid Construction**

5.1 - Replace EGFP with mCherry between the KpnI and NheI sites in the pLKO.1 Puro vector to generate pLKO.1-shRNA-mCherry plasmids.

6 Notes:

6.1 - Validate the integrity and orientation of cloned sequences through restriction digestion and sequencing.

6.2 - Use sterile techniques and appropriate safety measures when working with plasmid DNA.