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Generation of pLKO.1 Plasmids Containing shRNA

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Shiyi Wang¹

¹Duke University



Shiyi Wang

Duke University

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We use this protocol and it's working

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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.