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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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## 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 vector<sup>254</sup> 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-EGFP<sup>255</sup>.
- 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.