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Generating pPB-CAG-mCherry-CAAX Plasmid

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We use this protocol and it's working

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Abstract

Generating pPB-CAG-mCherry-CAAX Plasmid

1 **Obtain Plasmids** - Obtain pPB-CAG-EGFP and pGLAST-PBase plasmids from Dr. Joseph Loturco.

2 **Insert mCherry-CAAX into pPB-CAG-EGFP** - Insert mCherry-CAAX between XmaI and NotI restriction sites in pPB-CAG-EGFP to replace EGFP.

3 **Insert hU6 Promoter and shRNA into pPB-CAG-mCherry-CAAX** - Amplify a DNA fragment containing hU6 promoter and shRNA from pLKO.1-shRNA using Phusion High-Fidelity DNA Polymerase with primers introducing SpeI restriction sites: - Forward Primer: GGACTAGTCAGGCCCGAAGGAATAGAAG - Reverse Primer: GGACTAGTGCCAAAGTGGATCTCTGCTG - Purify the PCR products and digest them with SpeI.

4 **Ligation into pPB-CAG-mCherry-CAAX** - Ligase the purified and SpeI-digested DNA fragment containing hU6 promoter and shRNA into pPB-CAG-mCherry-CAAX at the SpeI restriction site.

5 **Confirmation by Analytical Digest and Sequencing** - Perform an analytical digest with EcoRI to confirm the correct orientation of the inserted DNA fragment. - Sequence the plasmid to confirm the accurate insertion of hU6 promoter and shRNA sequences.