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Generating pPB-CAG-mCherry-CAAX Plasmid

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Protocol status: Working

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: GGACTAGTCAGGCCCCGAAGGAATAGAAG - 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.