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PiggyBac plasmids

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Abstract

How to make PiggyBac plasmids

1. pPB-CAG-EGFP and pGLAST-PBase were a gift from Dr. Joseph Loturco.

2. To generate pPB-CAG-mCherry-CAAX, mCherry-CAAX was inserted between XmaI and NotI restrictions sites to replace EGFP.

3. To insert the hU6 promoter and shRNA in pPB-CAG-mCherry-CAAX, a DNA fragment containing hU6 and shRNA was amplified from pLKO.1-shRNA using Phusion High-Fidelity DNA Polymerase (NEB) with primers that introduced SpeI restriction sites (Forward Primer: GGACTAGTCAGGCCCGAAGGAATAGAAG; Reverse Primer: GGACTAGTGCCAAAGTGGATCTCTGCTG).

4. PCR products were purified, digested with SpeI, and ligated into pPBCAG-mCherry-CAAX at the SpeI restriction site.

5. An analytical digest with EcoRI followed by sequencing was used to confirm the orientation of the inserted DNA fragment.