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Generating Ezrin Plasmids

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Abstract

Generating Ezrin Plasmids

1 1. pZac2.1-GfaABC1D-BioID2-HA Construction

1.1 - **Obtain Materials**

1.2 - pZac2.1-GfaABC1D-Lck-GCaMP6f plasmid from Dr. Baljit Khakh (Addgene plasmid #52924).

1.3 - pAAV-hSyn-BioID2-Linker-Synapsin1a-HA plasmid for BioID2 sequence.

1.4 - **PCR Amplification of BioID2**

1.5 - PCR amplify BioID2 sequence from pAAV-hSyn-BioID2-Linker-Synapsin1a-HA using the following primers:

1.6 - Forward Primer: 5’-ctagcctcgagaattcaccatgttcaaaaatcttatttg-3’

1.7 - Reverse Primer: 5’-ccgggtcgactctagatgcgtaatccggtacatcg-3’

1.8 - **Insertion into pZac2.1-GfaABC1D-Lck-GCaMP6f**

1.9 - Use In-Fusion cloning (TaKaRa) to insert the PCR-amplified BioID2 sequence into the EcoRI and XbaI restriction sites of pZac2.1-GfaABC1D-Lck-GCaMP6f.

2 2. pZac2.1-GfaABC1D-Ezrin WT-BioID2-HA and pZac2.1-GfaABC1D-Ezrin T567D-BioID2-HA Construction

2.1 - **Obtain Materials**

2.2 - pHJ421 (pEGFP-Ezrin WT) and pHJ423 (pEGFP-Ezrin T567D) plasmids from Stephen Shaw (Addgene plasmid #20680 and #20681).

2.3 - **PCR Amplification of Ezrin**

2.4 - PCR amplify Ezrin sequence from pHJ421 or pHJ423 using the following primers:

2.5 - Forward Primer: 5’-ctagcctcgagaattcaccatgccgaaaccaatca-3’

2.6 - Reverse Primer: 5’-tgaacatggtgaattccgacagggcctcgaactcg-3’

2.7 - **Insertion into pZac2.1-GfaABC1D-BioID2**

2.8 - Insert the PCR-amplified Ezrin sequence into the EcoRI restriction sites of pZac2.1-GfaABC1D-BioID2 for both Ezrin WT and Ezrin T567D variants.

3 3. pZac2.1-GfaABC1D-Ezrin T567A-BioID2-HA Construction

3.1 - **Mutagenesis**

3.2 - Use the Q5® Site-Directed Mutagenesis Kit (NEB) to generate the Ezrin T567A mutation in pZac2.1-GfaABC1D-Ezrin T567D-BioID2-HA.

3.3 - Perform mutagenesis using the following mutagenesis primers:

3.4 - Forward Primer: CAAGTACAAGGCGCTGCGGCAGA

3.5 - Reverse Primer: TCCCGGCCTTGCCTCATG

3.6 - **Confirmation**

3.7 - Verify the presence of the Ezrin T567A mutation in the plasmid through sequencing or restriction digest analysis.

4 Notes:

4.1 - Ensure proper sterile technique and use of appropriate safety precautions during handling of plasmids and reagents.

4.2 - Perform all steps under sterile conditions to avoid contamination.

4.3 - Validate all constructs through sequencing to confirm the correct insertion or mutation.