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Ezrin plasmids

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Abstract

How Ezrin plasmids were made.

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

2. pZac2.1-GfaABC1D-BioID2-HA was generated by PCR of BioID2 from pAAV-hSyn-BioID2-Linker-Synapsin1a-HA (primers Fw: 5′-ctagcctcgagaattcaccatgttcaaaaatcttatttg-3′, Rv: 5′-ccgggtcgactctagatgcgtaatccggtacatcg-3′) and insertion into the EcoRI and XbaI restriction sites of pZac2.1-GfaABC1D-Lck-GCaMP6f using In-Fusion cloning (TaKaRa).

3. pHJ421(pEGFP-Ezrin WT) and pHJ423 (pEGFP-Ezrin T567D) were a gift from Stephen Shaw (Addgene plasmid # 20680 and # 20681).

4. pZac2.1-GfaABC1D-Ezrin WT-BioID2-HA and pZac2.1-GfaABC1D-Ezrin T567D-BioID2-HA were generated by PCR of Ezrin from pHJ421 or pHJ423 (primers Fw: 5′-ctagcctcgagaattcaccatgccgaaaccaatca-3′, Rv: 5′-tgaacatggtgaattccgacagggcctcgaactcg-3′) and insertion into the EcoRI restriction sites of pZac2.1-GfaABC1D-BioID2, respectively.

5. To generate pZac2.1-GfaABC1D-Ezrin T567A-BioID2-HA plasmid, Q5® Site-Directed Mutagenesis Kit (NEB) was used with mutagenesis primers (Fwd: CAAGTACAAGGCGCTGCGGCAGA; Rev: TCCCGGCCTTGCCTCATG)