

Jul 10, 2024

Generating Ezrin Plasmids

DOI

<https://dx.doi.org/10.17504/protocols.io.kqdg32361v25/v1>

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Protocol Citation: Shiyi Wang 2024. Generating Ezrin Plasmids. protocols.io

<https://dx.doi.org/10.17504/protocols.io.kqdg32361v25/v1>

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Protocol status: Working

We use this protocol and it's working

Created: July 10, 2024



Last Modified: July 10, 2024

Protocol Integer ID: 103192

Keywords: ASAPCRN

Funders Acknowledgements:

Aligning Science Across Parkinson's (ASAP) initiative

Grant ID: ASAP-020607

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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).

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- 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.