Oct 22, 2025

Molecular cloning of SHIP164 plasmids for expression in mammalian cells

DOI
dx.doi.org/10.17504/protocols.io.8epv5z5kjv1b/v1

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Protocol Citation: michael.hanna , Michael G Hanna, Pietri De Camilli 2025. Molecular cloning of SHIP164 plasmids for expression in mammalian cells. **protocols.io https://dx.doi.org/10.17504/protocols.io.8epv5z5kjv1b/v1**

Protocol status: Working

Created: July 18, 2021

Last Modified: October 22, 2025

Protocol Integer ID: 51653

Keywords: Molecular cloning of SHIP164 plasmids, expression in mammalian cells, ASAPCRN, molecular cloning of ship164 plasmid, molecular cloning of ship164, ship164 plasmid, molecular cloning, expression in mammalian cell, ship164, mammalian cell, sequences of other protein, other protein, protein, cell

Abstract

This protocol is to help with the molecular cloning of SHIP164 and the sequences of other proteins.

Attachments

Molecular cloning of...

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Troubleshooting

Safety warnings

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Before start

We experienced difficulty in amplifying SHIP164 from a cDNA library, therefore we opted to generate a codon optimized SHIP164 open reading frame synthesized. The codon optimized cDNA was cloned into pUC57 and was subsequently subcloned into multiple other mammalian expression vectors.

The general cloning strategy is described below.

Cloning strategy

1 Linearize destination vector using restriction enzyme digest following manufacturers protocol (New England Biolabs).

2 Amplify desired insert by PCR making sure to include 15-20 bp overhang homology with destination vector.

3 Run both cut vector and PCR product on an agarose gel (e.g., 1% supplemented with Midori Green or Ethidium Bromide for visualization) and purify the DNA from gel using a NucleoSpin Gel and PCR Clean-up kit (Takara).

4 Ligate PCR insert into vector using NEB HiFi assembly mixture following manufacturers protocol (https://www.neb.com/protocols/2014/11/26/nebuilder-hifi-dna-assembly-reaction-protocol).