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VAMPseq Protocol

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We use this collection and it's working

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Abstract

This protocol describes the materials and method to conduct an experiment of Variant Abundance by Massively Parallel sequencing (VAMP-seq) assay. There are two designation plasmids available for a gene of interest (GOI) to fuse with a EGFP reporter system fused either N-terminally (N-terminal; EGFP-GOI) or C-terminally (C-terminal; GOI-EGFP).

Guidelines

Pre-barcoded Vector Design:

The current (as of 2023) working system (VAMP-seq v2) is modified based on the published version (Matreyek et al, 2018). There are two major modifications made for a two-step “plug and play” system compatible with Golden Gate Assembly. First, a pair of BsmBi/ Esp3I recognition sequences were added before the T7 promoter sequence in both of the N- & C- terminus systems. This is used to add barcode sequences to the vector. Secondly, a pair of SapI recognition sequences were added to assist cloning of the GOI variant library. In the N-terminal vector, the SapI sites are located between the linker sequences and IRES. For the C-terminal vector, the SapI sites are located between the Kozake site and the linker sequence of EGFP.

Barcodes and GOI Design

Barcode sequence design:

Below is the final amplicon sequence for adding barcodes to either N-& C- terminal plasmids.

5’-
GGCTACCGTCTCCaggc*TTAAGTTGGGTAACGCCAG*GACAACCGGTTAGAGCTCGTTTATGATACTA
GTATCGGCTAGCAGGACAGTTCTGCAATTGCGTGAGTAGGCAAGAACCGCTAGAAG***CGTCGCTGTA***
***CAAATAG*TTNNNNNNNNNNNNNNNNacgaTGAGACGGTAGCC**-3’

The Italicized template sequence is a double-stranded DNA fragment. BsmBi/EspsI sequences (in red) are added to both ends of the template by a primer set (in bold) using PCR amplification. The lowercase indicates fragment-specific overhangs for backbone assembly. In particular, a degenerative reverse primer has designed a 18nt bases (N) barcode. The lowercase indicates fragment-specific overhangs for backbone assembly. The underlined sequence is important for downstream genomic sequencing.

Gene of Interest flanking design:

VAMP-seq v2 vectors have two pre-existing SapI sites and pre-selected fragment specific overhangs ready to receive GOI sequence with complimentary flasking ends. The specific flasking sequences are as below:

In the N-terminus system, 5’-atg and 5’-CTA (reverse complement of 5’-tag”) are the pre-selected fragment-specific overhangs in the backbone. The insert fragment must contain the SapI recognition sequences (in red) and overhangs (indicated as lowercase) as the following:

5’-AAGACCGCTCTTCCatgNNNNNNNNNNNNNNNtagGGAAGAGCGGTCTT-3’

In the C-terminus system: 5’-atg and 5’-CGT (reverse complement of 5’-acg) are the pre-selected fragment-specific overhangs in the backbone to receive a compatible insert fragment. The insert fragment must contain the SapI recognition sequences (in red) and overhangs (indicated as lowercase) as the following:

AAGACCGCTCTTCCatgNNNNNNNNNNNNNNNacgGGAAGAGCGGTCTT-3’

*A stop codon should not be placed at the end of GOI sequence when assembling to the C-terminus backbone. It is already placed at the end of the EGFP. Albeit, in the N-terminus system Albeit, the stop codon is necessary for the GOI insert. It is a part of the SapI overhang.
Both Start and Stop codons cannot be mutated.

Wild-type and destabilizing variants design:

Wild-type of Gene of Interest:

Wild type sequences for gene of interest(s) can be obtained from PCR amplification of existing template, or synthesized as double-stranded DNA fragments. In both cases, the interior sequence should be free of SapI recognition sequences and flanked by the above sequences suitable for the desired designation vectors.

Destabilizing Variants Selection:

Variant Library design:

NGS primer/region design

Files

SEARCH

Protocol

NAME

Cloning with Golden Gate

VERSION 1

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Protocol

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Barcoded vector cloning

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Library cloning

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Protocol

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Tissue culture- Purity sorting HEK293T LLP iCasp9 cells

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Library transfection

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Genomic DNA Extraction from Sorted Cells

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PCR amplification of the barcode region

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