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LABEL-seq Protocol V.1

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Atlas of Variant Effects ...

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We are still developing and optimizing this collection

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Abstract

Pre-barcoded Vector Design:

This protocol outlines the materials and methods for conducting a LABEL-seq (Labeling with Barcodes and Enrichment for Biochemical Analysis by Sequencing) assay. The assay utilizes designated plasmids containing Bxb1-attB sites and MS2 phage coat protein (MCP) binding elements that link with small MS2 RNA hairpins. A gene of interest (GOI) can be fused to MCP either at the N-terminus (N-term) or C-terminus (C-term), enabling the expression of stable circular RNAs (circRNAs) that incorporate MS2 hairpins along with a unique 18-nucleotide barcode. Each protein variant is tagged with a specific barcode, allowing quantitative measurement of gene activity and protein abundance. The current (as of 2025) working system (LABEL-seq v2) is modified based on the published version (Simon et al. 2024). There are two major modifications made for a two-step cloning system compatible with Golden Gate Assembly. First, a pair of BsmBi/ Esp3I recognition sequences, located on the circRNA architecture, 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.

Guidelines

Barcodes and GOI Design

Barcode sequence design:

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

5'-GTATAGTTTGTGCGGTGGTCCGTCTCAagatGTACANNNNNNNNNNNNNNNNTGTACAAcgtcTGAGA
CGCGAAGGTGTAGGGGATTGAT-3'

The template sequence is a double-stranded DNA fragment with a 18nt bases (N) barcode.The lowercase indicates fragment-specific overhangs for backbone assembly using BsmBi/EspsI enzyme. This double-stranded DNA is created by a Klenow fill-in process from a single stranded template with primer 5′-ATCAATCCCCTACACCTTCGCG-3′

Gene of Interest flanking design:

LABEL-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 MCP. Albeit, in the N-terminus system, 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.

Variant Library design:

A protein variant library can be designed and synthesized as a site-saturation mutagenesis library, introducing 22 possible amino acid substitutions (20 common codons along with one deletion and Stop) and at each codon position, excluding the start and stop codons of a gene.

Files

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Protocol

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Cloning with Golden Gate

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Barcoded vector cloning

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Library cloning

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Tissue culture: Library Cell Culturing and Transfection

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Abundance Assay with Flag & HaloTag Enrichments

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Activity Assay with E40 & pEM1 Enrichments

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