



pSmart VI

1. Product Description

The Twin Strep-tag II, while retaining the high specificity and mild purification conditions of the Strep-tag II, boasts an even higher affinity. Consequently, it can be utilized for direct purification of the target protein from culture supernatants. Furthermore, its ability to withstand various buffer conditions and additives, such as high salt concentrations, surfactants, reductants, metal ions, and chelating agents, renders it a versatile tag for a wide range of proteins. The pSmart VI vector is a modification of the pET-28a vector, primarily achieved by the insertion of the SUMO protein's nucleic acid sequence downstream of the tag to enhance protein solubility. Multiple cloning sites have been preserved to facilitate the insertion of gene fragments. Additionally, the inclusion of the RFP tag's nucleic acid sequence serves as a positive control for protein expression.

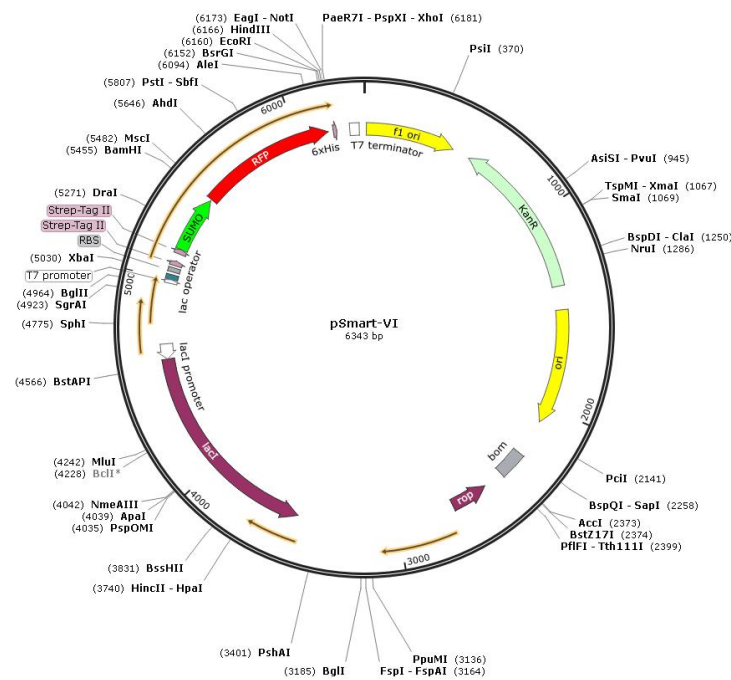


Fig.1. Schematic diagram

2. Multiple Cloning Site (MCS)

...CCATGGGTTGGAGCCATCCGCAGTTTGAAAAAGGTGGTGGCTCAGGTGGCGGCTCTGGCGTTCTAGTGCATGGAGTCATCCGC
AGTTTGAAAAATCAGATAGCGAAGTTAATCAGGAAGCCAAACCGGAAGTTAAACCGGAAGTTAAACCGGAACCCATATTAATCTGAA
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GGCAAAGAAATGGATAGCTTACGCTTTCTGTATGATGGCATTTCGATTACAGGCCGATCAGACCCCGGAAGATTTAGATATGGAAGATAA
TGATATTATTGAAGCACATCGTGAACAGATTGGCGGC **GGATCC** AAGGGCGAGGAGGACAACATGGCCATCATCAAGGAGTTCATGCG
GTTCAAGGTGCACATGGAGGAAGCGTGAACGGCCACGAGTTCGAGATCGAGGGAGAGGGAGAGGGAAGACCTTACGAGGGAAC
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GCTAAGAAGCCTGTGCAGCTGCCAGGAGCCTACAACGTGAACATCAAGCTGGACATCACCAGCCACAACGAGGACTACACCATCGT
GGAGCAGTACGAGAGGGCCGAAGGAAGACACAGCAGGAGGCATGGACGAGCTGTACAAG **GAATTC** **AAGCTT** **GCGGCCGC** **ACT**
CGAGCACCACCACCACCACCACTG...

The restriction enzymes corresponding to the above different background colors are **BamHI**, **EcoRI**, **HindIII**, **NotI**, **XhoI**, respectively. The green font is a Twin Strep-tag II tag, the orange font is a SUMO tag, the red font is an RFP tag, and the blue font is a 6×His tag.



Clone Method

It is recommended that the cloning method should be seamless cloning, and the design of primers should refer to the following:

Forward primer: ATCGTGAACAGATTGGCGGC + gene5' sequence

Reverse primer: CAGTGGTGGTGGTGGTGGT (TCA stop codon) + gene3' reverse complement sequence

Polyclonal sites were inserted, BamHI cleavage sites were selected upstream, and one of EcoRI/HindIII/NotI/XhoI was selected downstream.

3. Protease cleavage site

WSHPQFEKGGGSGGGSSAWSH**QFEK**SDSEVNQEAKPEVKPEVKPETHINKVSDGSSEIFFKIKKTTPLRRLMEAFKRQ GKEM
DSLRFLYDGIRIQADQTPEDLDMEDNDIEAHREQIGG↓GS

The arrow is the final cut point of the SUMO protease. After the protease has removed the label, there will be two more redundant amino acid residues Gly-Ser at the N-end of the target protein. If the target protein without redundant residues needs to be obtained, seamless cloning is recommended.

4. Sequencing Primer

The use of any general primers with pET-28a vectors is acceptable, but the sequencing primers T7-Pro or T7-Ter that are far away from the target protein are recommended here to obtain more reliable sequencing information.

5. Complete vector sequence

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6. Related Products

Product	Cat. No.	Size
pSmart VI	SLP028	100 ng/μl, 10 μl

