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Labeling of *Escherichia coli* Expressed SNAP-tag Fusion Proteins

Introduction

Escherichia coli (E. coli) is the leading host for expression of proteins, primarily due to its ease of use. SNAP-tag technology provides biologists with a versatile tool for expression and labeling of proteins in order to characterize their functions and interactions (1–3). New England Biolabs has introduced the pSNAP-tag (T7)-2 vector which allows a protein of interest to be fused to the N-terminus or C-terminus of an *E. coli* codon optimized SNAP-tag (Figure 1). The expression of SNAP-tagged fusion proteins are under the control of the IPTG-inducible T7/*lac* promoter and can be efficiently expressed using an *E. coli* T7 expression strain of your choice (4). Using this highly flexible system, a single gene construct creates a fusion protein that can be covalently labeled with a fluorophore of your choice, biotin or beads for fluorescent imaging, pull-down or other biochemical analysis (5,6).

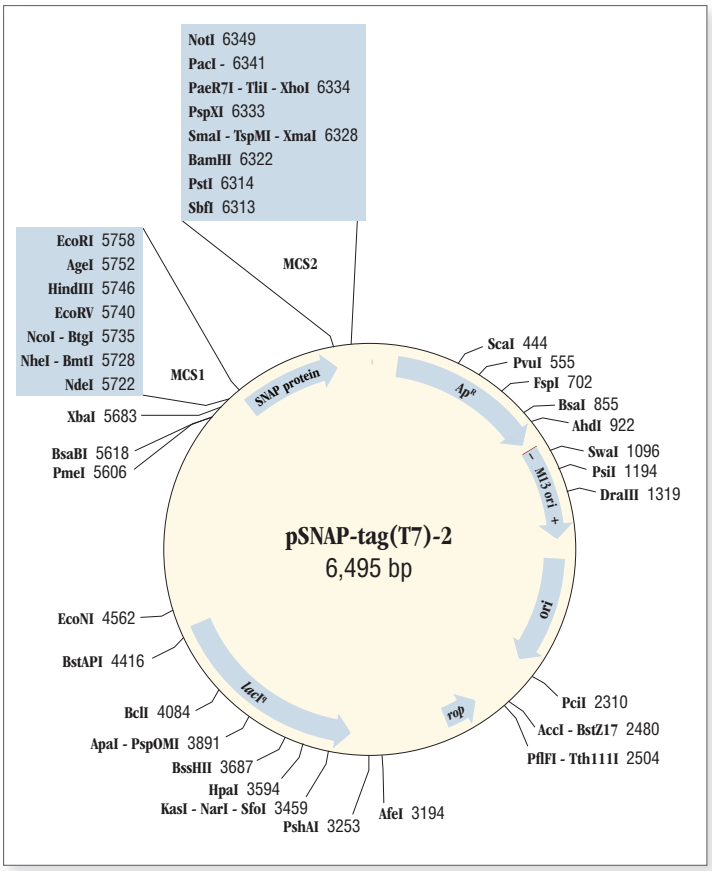

Figure 1: *pSNAP-tag(T7)-2 vector map*

Materials

- pSNAP-tag(T7)-2 vector (#N9181)
- SNAP-Vista Green (#S9174)
- SNAP-Cell TMR-Star (#S9105)
- *E. coli* T7 Express (#C2566)

Additional Materials

- Growth medium
- Ampicillin
- IPTG
- Phophate Buffered Saline (PBS)
- Dithiothreitol (DTT)
- 3X SDS Sample buffer (#B7709 or #B7703)

General Protocol

Cloning: The protein of interest can be expressed with the SNAP-tag (20 kDa) as either a N- or C-terminal in-frame fusion.

1. For fusion to the C-terminus of the SNAP-tag, subclone your gene of interest into the 3´ multiple cloning site (MCS) of the pSNAP-tag(T7)-2 vector and include a stop codon in the insert at the C-terminus of the fusion gene.

(see other side)

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2. For expression at the N-terminus of the SNAP-tag, the target gene should be cloned in-frame to the SNAP-tag and no stop codon should be included in the insert.

Expression: pSNAP-tag(T7)-2 can be used for expression in all NEB T7 Express strains: T7 Express Competent *E. coli* (NEB #C2566), T7 Express I^q Competent *E. coli* (NEB #C3016), T7 Express *lysY* Competent *E. coli* (NEB #C3010), T7 Express *lysY/*I^q Competent *E. coli* (NEB #C3013) and BL21(DE3) (NEB #C2527).

1. Grow the transformed cells at 37°C until OD_{600}~0.5 (or 0.6–0.8 for 15°C induction).
2. Induce the cells with 0.4 mM IPTG at 30°C for 3–5 hours or 15°C for 16 hours.
3. Harvest the bacteria by centrifuging the culture for 15 min at 5000 x g.
4. The bacterial pellets may be stored frozen at -20°C prior to lysis and SDS-PAGE analysis of protein expression. (See Figure 2A)

Labeling in cell lysate:

1. Resuspend the cells in 1X PBS supplemented with 1 mM DTT and lyse the cells by sonication.
2. Sonicate to lyse the cells and add 10 µM of the substrate.
3. Incubate at 37°C for 30 minutes.
4. Add 3X SDS Sample buffer and conduct SDS-PAGE.
5. Perform in-gel fluorescent detection using a Typhoon 9400 Imager (Figure 2B and 2C).

Note: It is possible to label a SNAP-tag fusion protein at a wide range of temperatures (4°–65°C); however, optimization of various parameters (labeling time, substrate concentration etc.) should be conducted.. The expressed SNAP-tag fusion protein can also be purified and subsequently labeled in 1X PBS buffer supplemented with 1mM DTT.

Results

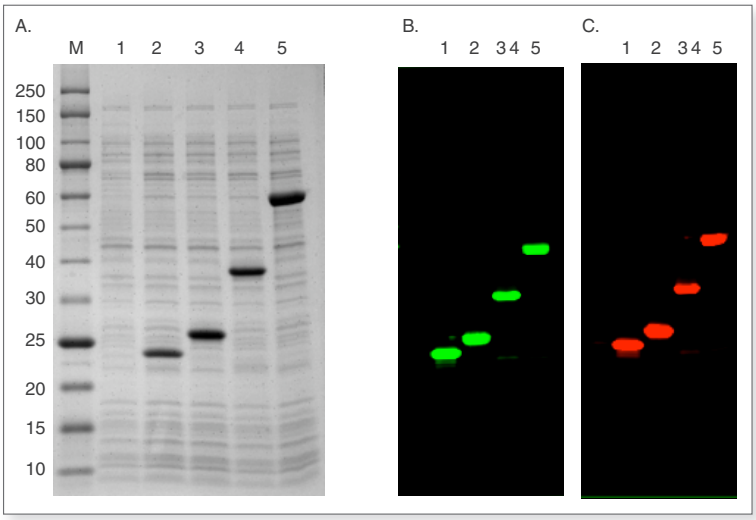

Figure 2. *Expression and labeling of SNAP-tag fusions in E. coli T7 Express host cells. SNAP-tag(T7)-2 plasmids expressing SNAP-tag (23 kD, lane 2), SNAP-tag-6XHis-tag–chitin binding domain (27 kD, lane 3), MCP-SNAP-tag-6XHis tag (31 kD, lane 4) and maltose binding protein-SNAP-tag (63 kD, lane 5) were transformed into E. coli T7 Express. Induction was carried out at 30°C for 4 hours. Uninduced cells are present in lane 1. The resulting cell lysates were subjected to SDS-PAGE and stained with Coomassie blue (A), labeled with SNAP-Vista Green (B) or labeled with SNAP-Cell TMR Star (C). The fluorescent signals were detected by Typhoon Imager 9400 with the excitation/emission filter sets of 488nm/520nm (B) and 532nm/580nm (C). M contains the Protein Ladder (NEB #P7703).*

Summary

This note demonstrates the high level expression of SNAP-tag and three SNAP-tag fusion proteins from the pSNAP-tag (T7)-2 vector in *E. coli* T7 Express host cells. Specific protein labeling was readily performed in cell lysates by addition of a fluorescent substrate, either SNAP-Vista Green or SNAP-Cell TMR Star. This bacterial SNAP-tag expression system provides a versatile tool in site-specific labeling of proteins for a variety of applications.

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