Materials Required but not Supplied:
- Tissue culture reagents and media
- Mammalian cell line(s)
- Transfection reagents

Storage
- pSNAP Vector is supplied in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) at a concentration of 0.5 µg/µl. Plasmid solutions can be stored at 4°C for up to one week. For long-term storage –20°C is recommended.

Detailed Description
- A plasmid map and the sequence of the cloning region can be obtained by visiting the website at www.neb.com. This plasmid encodes the gene for the SNAP domain, which is a mutant form of the human gene for O-,alkylguanine-DNA-alkyltransferase (hAGT). The codon usage of the gene is optimized for expression in mammalian cells. In the plasmid sequence, the SNAP gene is encoded from 969 bp to 1514 bp.

This plasmid is intended for the cloning and stable or transient expression of SNAP-tag protein fusions in mammalian cells. It is suitable for the efficient production of stable cell lines expressing SNAP gene fusions. The plasmid contains the CMV promoter followed by the genes for SNAP, and neomycin resistance separated by the IRES of the encephalomyocarditis virus (ECMV), which permits the translation of two open reading frames from one messenger RNA; therefore after selection of stable mammalian cells for neomycin resistance, nearly all surviving colonies should stably express the SNAP protein.

Use your expression experiments require a pure population of cells, you can simplify use the pool of resistant cells, otherwise cell clones can be isolated and characterized using standard procedures.

The plasmid contains the β-lactamase (Ampicillin resistance) gene for maintenance in bacteria. The gene of interest can be cloned upstream or downstream of the SNAP, coding sequence, as a fusion to the N- or C-terminus of the SNAP-tag.

pSNAP, Vector can also be used as an expression control plasmid, expressing SNAP, alone, in which case the SNAP-tag protein is distributed throughout the cell. The SNAP gene can be isolated from the plasmid using PCR or direct cloning in order to subclone it into a different vector of choice.

Cloning of SNAP-tag Fusions in pSNAP
- Cloning by PCR
  - To subclone the gene of interest into pSNAP, fused to the N-terminus of SNAP, use the available restriction sites: NheI, EcoRV (blunt), Ascl, SwaI (blunt), BsrGI, AgeI or EcoRI which are located upstream of the SNAP-tag.
  - To subclone the gene of interest into pSNAP, fused to the C-terminus of SNAP, use the available restriction sites downstream of the SNAP-tag: SbfI, BamHI, Pmel (blunt), XhoI, PacI or NotI.

- Note: When fusing the gene of interest to the C-terminus of SNAP, note that there is a stop codon between the PacI and NotI sites, so SbfI, BamHI, Pmel, XhoI or PacI must be used as the 5’ cloning site for the insert.

- Note: Pmel and XhoI cannot be used together for cloning because they share a cytosine as part of their recognition sequences.

Expression of SNAP-tag Fusions
- Transient Expression
  - Expression of the fusion protein cloned in pSNAP, can be achieved by transiently transfecting cells in culture with standard transfection protocols. The appropriate reagent and time to permit adequate expression must be empirically determined. We recommend using pSNAP-H2B (NEB #N9186) as an expression control plasmid. H2B-SNAP fusion protein gives a nuclear localized signal when labeled with SNAP-Cell substrates. If the empty pSNAP, plasmid is used as a control vector for transfection, an even distribution of the SNAP-tag in nucleus and cytoplasm should be seen. Both pSNAP, and the localization control plasmid have performed well in stable and transient transfection of CHO-K1, COS-7, U-2 OS and NIH 3T3 cells. Note that the intensity of the fluorescence may vary, depending on the cell line and labeling substrate used.

Stable Expression
- pSNAP, and the localization control plasmids can be transfected by standard transfection methods. Twenty-four to 48 hours after transfection, begin selecting mammalian cultures in 600–1,200 µg/ml G418 (geneticin) depending on the cell line. It is recommended that a kill curve for each cell line be established to determine optimal selection conditions. After 8–12 days of continuous selection, stable colonies will become visible. It is possible to use pools of stable cell populations for initial cell labeling to test for the presence of SNAP-tag expression. In addition, monoclonal cell lines can be isolated and characterized, if desired.
Troubleshooting

Cloning of the Gene of Interest

If subcloning of the gene of interest with the SNAP-tag does not work, reconfirm all the cloning steps (primer design, choice of restriction site, DNA isolation, ligation and transformation, etc.). If all steps are confirmed as being correct, then try the cloning using different restriction sites. Be sure to include a positive and negative control for the ligation reaction. Alternatively, try to subclone the SNAP gene into a mammalian expression vector already containing the gene of interest.

Expression

In general, we have not experienced problems expressing SNAP-tag protein fusions. However, if the fusion protein does not appear to be expressed, try expressing the H2B-SNAP protein as a control using cells transiently transfected with pSNAP-H2B. Labeling of such cells with a fluorescent SNAP-Cell substrate should show strong nuclear localized fluorescence. The empty pSNAP plasmid can also be used as a control (cytosolic and nuclear fluorescence). Note that the intensity of this fluorescence may vary depending on cell line and substrate used. If the localization control is expressed but the fusion protein is not, then there are a variety of possible causes. It is possible that the gene of interest was not expressed or that the fusion protein is toxic for the cell line. It may also be that the fusion protein is not correctly expressed and the ligation reaction.

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Cloning Region of pSNAP

Unique restriction sites in the regions flanking the SNAP gene are displayed above the coding strand. The complete sequence for pSNAP, and the control plasmids can be downloaded at www.neb.com.

5' MCS

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3' MCS

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References:

Companion Products:
- pSNAP-ADR92 Control Plasmid #N9184S 20 µg
- pSNAP-H2B Control Plasmid #N9186S 20 µg