

Section 2C

Addition of an Epitope Tag Sequence to a Target Gene via an Adaptor-Duplex

An epitope tag DNA sequence may be fused to a target protein sequence on an expression vector via an adaptor-duplex.

Note: The guidelines and procedures in Section 2C assume you have already cloned the target sequence into a suitable expression vector.

This section discusses the design, construction, and insertion of a custom adaptor duplex (containing the tag sequence) into a vector restriction site at one end of the target protein sequence.

Critical factors for success: Designing an adaptor-duplex using the epitope tag sequence

Use the following general guidelines for designing an adaptor-duplex that will add the epitope tag to a target protein sequence on an expression vector:

Note: Details regarding the construction and insertion of specific examples of an adaptor-duplex are given in “Getting started: Procedures for adding a c-myc, HA, or VSV-G adaptor-duplex to the N-terminal coding region of a target gene on a prokaryotic expression vector” later in this section. Refer to those procedures for more information on the implementation of these guidelines.

- 1 From the restriction map of the vector containing the target protein, choose two restriction sites (RE 1 and RE 2) that are:
 - ▶ Unique (that is, occur only once in the vector)
 - ▶ Located (ideally) just before the N-terminus (for amino-terminal tagging) or just after the C-terminus (for carboxy-terminal tagging) of the target protein coding sequence.

Note: Location of the tag at the very end of a target protein will maximize the chances that the epitope sequence will be accessible to the tag-specific antibody. If necessary, however, you may choose unique restriction sites that are

located within the protein coding sequence, but (again, ideally) near either the 5' or 3' end of that sequence.

Note: If you are trying to answer a question about the function or cellular location of a certain domain within the target protein, you may choose RE 1 and RE 2 within the coding sequence of the domain. In that case, make sure that neither the removal of bases between RE 1 and RE 2 nor the insertion of a short foreign (tag) sequence will inactivate the target protein. Consult the literature (for instance, from Table 1B.1, Section 1B of this manual) for details of similar experiments.

- 2 Design two single-stranded adaptor oligonucleotides such that:

Note: For examples of oligonucleotides that meet these criteria, see Table 2C.1 in the procedures below.

- ▶ One oligonucleotide contains the complete coding sequence for the epitope tag; the other oligonucleotide contains nucleotides that will hybridize to that coding sequence

Caution: For the coding sequence, choose codons that are most likely to be used in the organism where the target protein will be expressed. See Table 2B.1 in Section 2B of this manual for more information on codon usage in various organisms.

- ▶ The core sequences of the two adaptors (containing the epitope tag DNA sequence) will hybridize to form a duplex DNA with no base mismatches
- ▶ The hybrid duplex will contain two appropriate single-stranded (“sticky”) 5' ends, which can hybridize to and be ligated into the RE 1 and RE 2 sites of the expression vector (chosen in guideline 1 above)
- ▶ The hybrid duplex, when fused with the target sequence, will encode the epitope tag in the same translational reading frame as the protein to be tagged