9 After you find a bacterial colony that contains the epitope-tagged gene, do one of the following:

If the tagged protein	Then
Is to be expressed in the bacteria	Optimize expression of the tagged protein by methods specific to the bacterial expression system used. Then prepare an extract containing the tagged protein.
Is to be expressed in eukaryotic cells	Purify microgram quantities of the plasmid containing the epitope-tagged gene by standard, published methods (for instance, Sambrook, Fritsch, and Maniatis, 1989; p. 17.1–17.44), then go to Procedure III.

III. Expressing the tagged protein in eukaryotic cells

After obtaining a plasmid containing the epitope-tagged protein sequence, you may want to get it into eukaryotic cells where it can be expressed. To do this, use one of many available transfection methods for eukaryotic cells (for instance, Sambrook, Fritsch, and Maniatis, 1989; p. 16.30–16.54).

Note: *Transfection conditions and amounts of plasmid needed depend upon the cells used and must be determined experimentally. For information on transfection, call your Boehringer Mannheim representative.*

After transfection, monitor expression of the tagged protein in the cells by methods specific to the protein (for instance, with a tag-specific antibody). Once the tagged protein is optimally expressed, prepare a cell lysate containing the tagged protein.

Note: *Any lysis method should effectively disrupt the cells, yet be gentle enough to preserve the epitope sequence of the tagged protein. See "Lysing different types of cells" in Chapter 3 of this manual for more information.*

Troubleshooting the PCR procedure

For a detailed discussion of the factors that influence PCR and how to troubleshoot the PCR procedure, see Erlich (1989), Innis *et al.* (1990), Kozak (1987), Thein and Wallace (1986), or the Boehringer Mannheim PCR Applications Guide.

Specific application of PCR procedures

Gill *et al.* (1996) used the above procedure to attach the HA tag to the amino terminus of the Green Fluorescence Protein (GFP) (Chalfie *et al.*, 1994). Briefly, modified versions of the upstream primer A (Figure 2B.3) and downstream primer B (Figure 2B.4) were produced, in which RE 1 = *Nco* I, RE 2 = *Xba* I, epitope tag sequence = HA tag sequence (from Table 2B.2), and primer A contained extra sequences for adding a polyhistidine (His_6) "tail" to the tagged protein. These primers were used to produce a His_6-HA-GFP fusion sequence and amplify it by PCR. The PCR product was cloned into a bacterial expression vector (pTrcHisB) and expressed in *E. coli* strain TOP10. The tagged protein was purified from a bacterial extract with the Xpress™ Purification System (Invitrogen). Finally, the purified, HA-tagged protein was analyzed on a Western blot with Anti-HA monoclonal antibodies, as described in Section 4A of this manual.

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