Cloning Strategy and Primer Design for C-terminal Rho-1D4 Fusion Proteins

The following diagrams lay out primer design to generate a construct for the addition of rho-1D4 to the C-terminus of a protein of interest (Fig.1), that can be inserted into expression vectors used with _E. coli_ expression systems. Adding a C-terminal rho-1D4 tag to a protein of interest is preferable when the protein has an intracellular C-terminus. Generally, the tag position should be chosen to minimize interference with binding sites of the native protein. **Note:** Primers should be purified by HPLC.

3' rho-1D4 primer without linker (TETSQVAPA)

Copy-paste sequence: GGGCTCGAGTCTCAAGCTGCGCCACCTGGAAGTCTCGGT

3' rho-1D4 primer with linker (TETSQVAPAGSSG)

Copy-paste sequence: GGGCTCGAGTCTCAAGCTGCGCCACCTGGAAGTCTCGGTGCGGAGGAGCC

5' primer

Copy-paste sequence: GGGCATATG

Fig. 1: The primers are designed to generate an expression construct with the rho-1D4 sequence at the 3' end of the gene of interest. As a result, the rho-1D4 tag appends to the C-terminus of the protein.