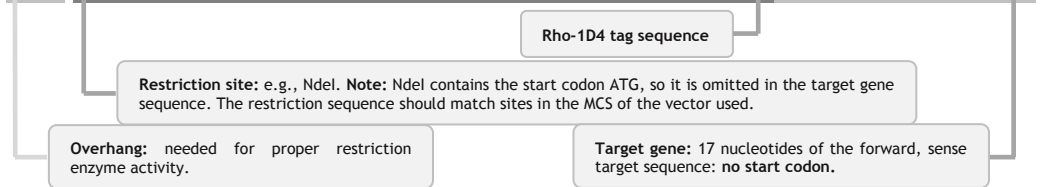


## Cloning Strategy and Primer Design for N-terminal Rho-1D4 Fusion Proteins

The following diagrams lay out primer design to generate a construct for the addition of rho-1D4 to the N-terminus of a protein of interest (Fig. 1), that can be inserted into expression vectors used with *E. coli* expression systems. Adding a N-terminal rho-1D4 tag to a protein of interest is preferable when the protein has an intracellular N-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

### 5' rho-1D4 primer without linker (TETSQVAPA)

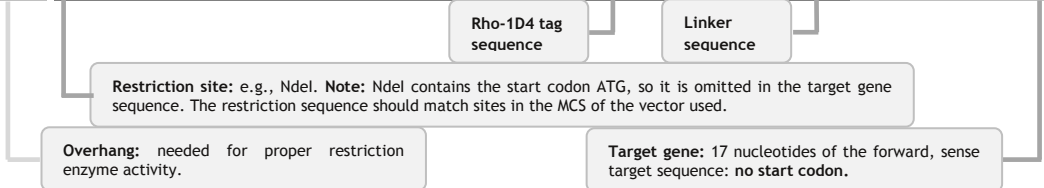
5'-GGG CAT ATG ACC GAG ACT TCC CAG GTG GCG CCA GCT + 17 nt target gene-3'



Copy-paste sequence: GGGCATATGACCGAGACTTCCCAGGTGGCGCCAGCT

### 5' rho-1D4 primer with linker (TETSQVAPAGSSG)

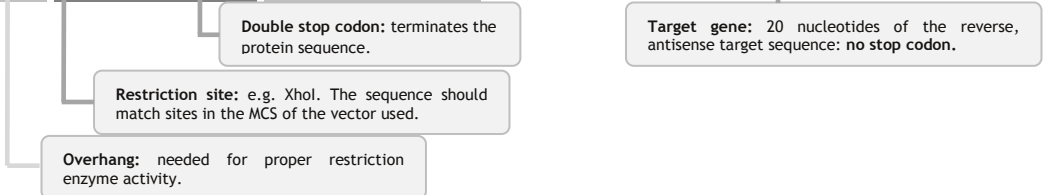
5'-GGG CAT ATG ACC GAG ACT TCC CAG GTG GCG CCA GCT GGA AGC AGC GGA + 17 nt target gene-3'



Copy-paste sequence: GGGCATATGACCGAGACTTCCCAGGTGGCGCCAGCTGGAAGCAGCGGA

### 3'-primer

5'-GGG CTC GAG TCA TCA + 20 nt target gene-3'



Copy-paste sequence: GGGCTCGAGTCATCA

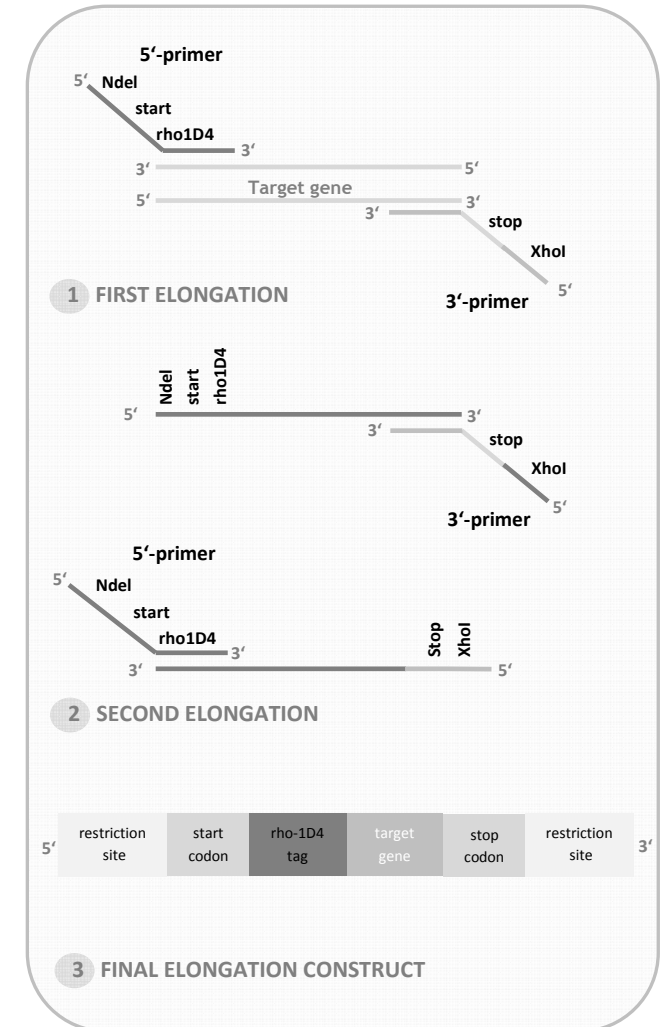


Fig. 1: The primers are designed to generate an expression construct with the rho-1D4 sequence at the 3'-end of the gene of interest. As a result, the rho-1D4 tag appends to the N-terminus of the protein.