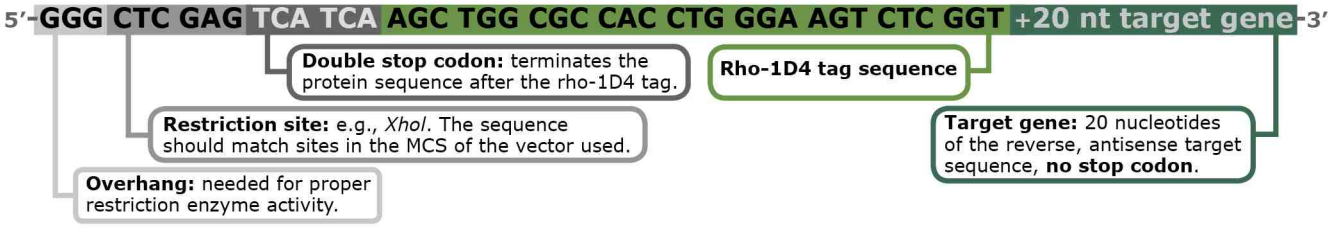
Cloning Strategy and Primer Design for C-terminal Rho-1D4 Fusion Proteins

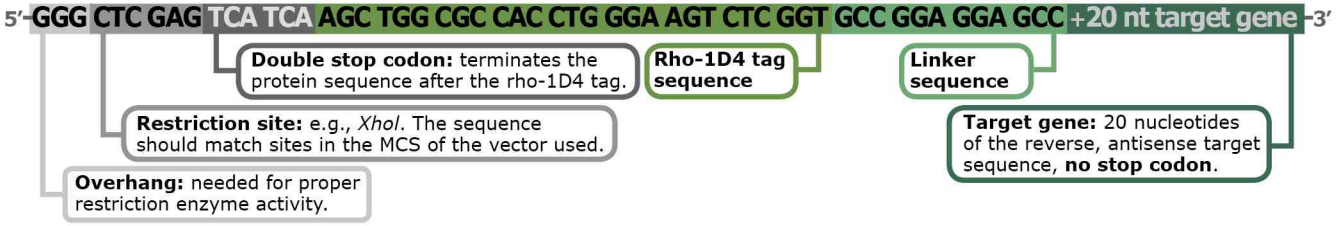
The following diagrams lay out primer design to generate a construct for the addition of rho-1D4 to the C-terminus of a protein of interest (Fig.1), that can be inserted into expression vectors used with *E. coli* expression systems. Adding a C-terminal rho-1D4 tag to a protein of interest is preferable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



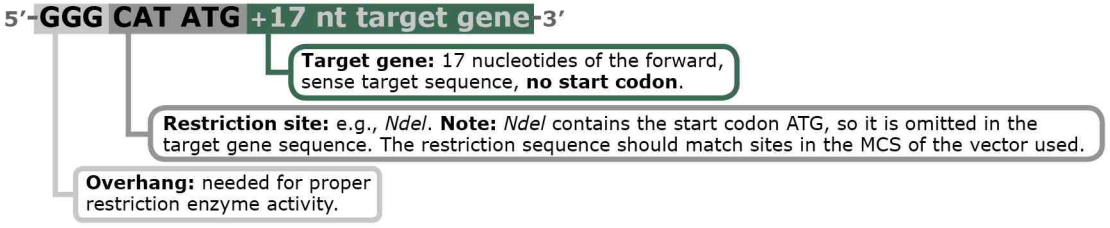
Copy-paste sequence: GGGCTCGAGTCATCAAGCTGGCGCCACCTGGGAAGTCTCGGT

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



Copy-paste sequence: GGGCTCGAGTCATCAAGCTGGCGCCACCTGGGAAGTCTCGGTGCCGGAGGAGCC

5' primer



Copy-paste sequence: GGGCATATG

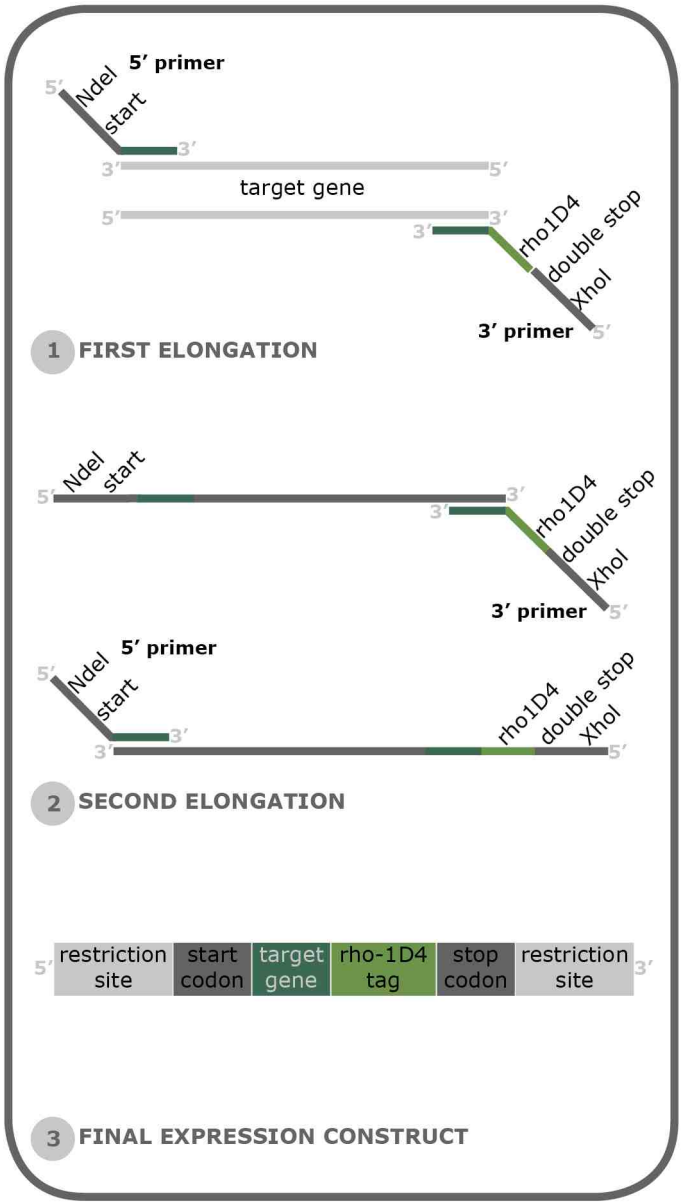


Fig. 1: The primers are designed to generate an expression construct with the rho-1D4 sequence at the 3′ end of the gene of interest. As a result, the rho-1D4 tag appends to the C-terminus of the protein.