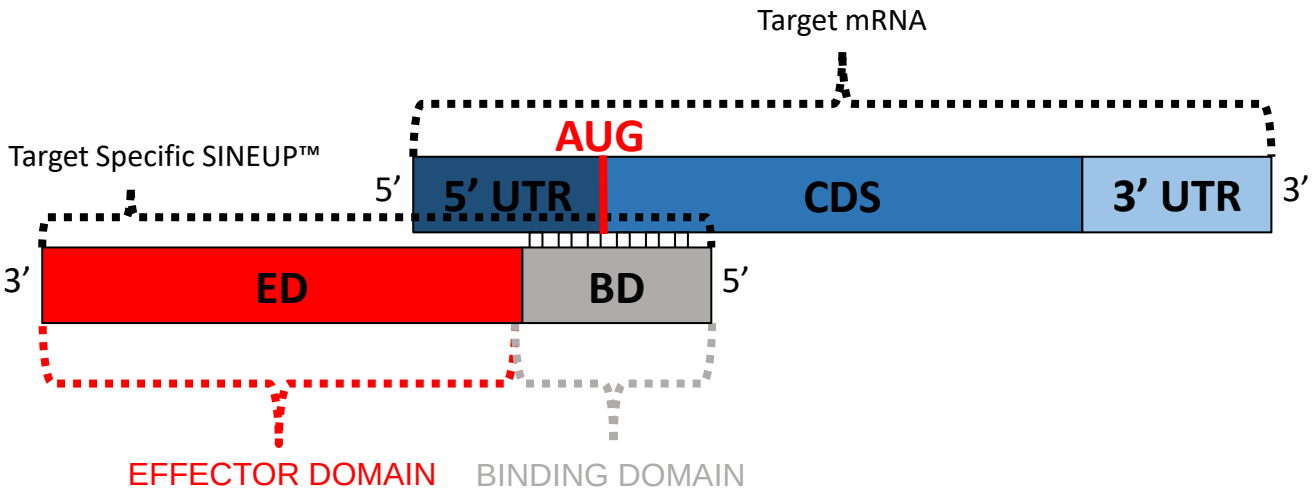
Technical Note for SINEUP™ Gene Knock-up System: Binding Domain Design

This technical note contains instructions for the design of a target gene-specific Binding Domain (BD).

To target a gene for knock-up of protein expression, a BD sequence complementary to the 5' untranslated region (UTR) and first several bases downstream of the initiation methionine of the target gene mRNA is required. This BD should be synthesized and used to replace the Stuffer Fragment in the pDUAL/ED plasmid. The BD should correspond to the antisense sequence immediately surrounding the initiation codon of the target gene. The nucleotide positions upstream of the AUG are referred to as negative and the nucleotide positions downstream of the AUG are referred to as positive. In this manner, + 1 is A in AUG. The Transcription Start Site should be identified prior to designing the BD. Below is a graphic illustration of the BD hybridized to the target mRNA.



An example of Binding Domain design for eGFP is shown in this technical note. In this case, the BD is obtained by annealing two partially complementary oligonucleotides which can be ordered from any DNA synthesis provider. The BD can also be obtained by dsDNA synthesis followed by digestion and purification of the digested product.
Upon digestion with XhoI and EcoRI the pDUAL/ED plasmid will release the 728 bp Stuffer Fragment. The resulting linearized and gel purified 4272 bp plasmid backbone is used to ligate with the BD of interest. This newly created plasmid represents the vector designed for up-regulating translation of the gene of interest.

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This particular BD is designed to target the eGFP mRNA by overlapping at 40 bases upstream of the initiation AUG (-40) and 32 bases downstream of the initiation AUG (+32).

1) Original sequence of the region of interest (-40/+32):

5′- TAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGCCACC**ATG**GTGAGCAAGGGCG AGGAGCTGTTCACCGG -3′

2) Generate reverse complement of the region of interest (sequence #1):

5′- CCGGTGAACAGCTCCTCGCCCTTGCTCAC**CAT**GGTGGCGACCGGTAGCGCTAGCGG ATCTGACGGTTCACTA -3′

The dsDNA for the BD is composed of two oligonucleotides: Forward (FWD) and Reverse (RVS). The oligonucleotides are designed and ordered from any DNA synthesis company. In order to generate both the FWD and RVS oligonucleotides, a few elements are added at each end of the sequences shown at step 1) and 2). These elements include restriction enzyme overhangs and a linker fragment. The latter is designed to introduce a linker between the BD and Effector Domain. The linker sequence can be any 10 nucleotides.

3) For the FWD oligonucleotide, add an **XhoI** restriction enzyme overhang at the 5′ end; add a **10 nucleotide linker** + an **EcoRI** restriction enzyme overhang at the 3′ end of sequence #1. For the RVS oligonucleotide, add a **10 nucleotide linker** + an **EcoRI** restriction enzyme overhang at the 5′ end; add an **XhoI** restriction enzyme overhang at the 3′ end of the original sequence.

FWD Oligonucleotide

5′- **TCGAG**CCGGTGAACAGCTCCTCGCCCTTGCTCAC**CAT**GGTGGCGACCGGTAGCGCT AGCGGATCTGACGGTTCACTA**ATCTGCATGCG** -3′

RVS Oligonucleotide

5′- **AATTCGCATGCAGAT**TAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGCCACC **ATG**GTGAGCAAGGGCGAGGAGCTGTTCACCGG**C** -3′

4) The final annealed product is prepared using the FWD oligonucleotide and RVS oligonucleotide:

5'- **TCGAG**CCGGTGAACAGCTCCTCGCCCTTGCTCAC**CAT**GGTGGCGACCGGTAGCGCTAGCGGATCTGACGGTTCACTA**ATCTGCATGCG** -3'
3'- **C**GGCCACTTGTCGAGGAGCGGGAACGAGTG**GTA**CCACCGCTGGCCATCGCGATCGCCTAGACTGCCAAGTGAT**TAGACGTACGCTTAA** -5'