Guidelines to designing genetic constructs:

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| --- | --- | --- | --- |
| **Name of part** | **Sequence (if applicable)** | **Location** | **Reason** |
| Universal Sequences | | | |
| BioBrick Prefix | gaattcgcggccgcttctagag | Before your part | Standard for iGEM |
| BioBrick Suffix | tactagtagcggccgctgcag | After your part | Standard for iGEM |
| VFR Primer Binding Site | ccacctgacgtctaagaaac | Before the BioBrick Prefix | To PCR amplify your g-blocks |
| VR Primer Binding Site | tcactcaaaggcggtaatac | After the BioBrick Suffix | To PCR amplify your g-blocks |
| For Transcription and Translation | | | |
| Promoter | Find one from registry (or other databases) | After prefix | For transcription initiation |
| RBS1 | Find one from registry (or other databases)  Make sure to locate the Shine-Dalgarno and annotate it (consensus sequence= aaggagg)  \*NOTE: the entire consensus sequence likely won’t be present | After promoter | For translation initiation |
| Coding Sequence | Make sure it’s codon optimized | After RBS and RBS Spacer (see below) | Your gene! |
| Bi-directional Terminator | Find one from registry (or other databases) | After coding sequence | You want bidirectional to prevent run-off transcription from abr gene |
| Spacer Sequences | | | |
| RBS Spacer | Lots of A nts and some T nts  7-9nts total | Between Shine- Dalgarno and start codon | Gives space for ribosome to bind properly |
| Terminator Spacer | ggatcc  \*If you’re using golden gate, see below | Between stop codon and terminator | Gives space for ribosome to finish translating and protects against exoribonucleases |
| VF2 primer binding site extra nts | cgaata | Before VF2 primer binding site | Gives room for primer to bind |
| VR primer binding site extra nts | ctatcg | After VR primer binding site | Gives room for primer to bind |
| ICARUS2 | GCTTCCGCGGGTGCCAGTGCGTCAGCGTCCGCATCAGCGAGTGCCTCACTGGTTCCGCGTGGTTCTGCAATTGCCATTGCGATTGATGATGACGATGACGATGCGTCTGCATCTGCGAGCGCCTCGGCAAGCGCCTCAGCCTCTGCGAGCGCCAGTcatcaccatcaccatcaTTTGattttaATCGCCGCGGCGGCAGCATAATGA  \*NOTE: Includes a 6-HIS tag and an end-spacer sequence | Between two sequences you want to link/separate | To separate the HIS-Tag from the rest of the protein (or any sequence) |
| Promoter Spacer3 | Ex. Atactaga  Ex. Tactagag | Between promoter and RBS | Gives room for Ribosome to bind and protects against exoribonucleases |
| Other sequences | | | |
| 6x-HIS tag4 | CACCATCACCATCACCAT | N-terminal or C-terminal of protein (i.e beginning or end of coding sequence) | For protein purification |
| Thrombin Protease Site5  (There are many other protease sites, just make sure you have the protease available) | CTGGTTCCGCGTGGTTCT  (Amino acids= LVPRGS)  Cuts between R and G | Before or after anything you want to cleave off (ex. To cleave of a HIS tag) | To cut off any sequences that are interacting with the function of the protein |
| OmpA Signal6 Peptide | atgaaaaaaaccgcgatcgcgatcgcggttgcgctggcgggtttcgcgaccgttgcgcaggcg | Beginning of coding sequence | To secrete protein to periplasm |
| MalE Signal6 Peptide | atgaagatcaagaccggcgccaggatcctggccctgagcgccctgaccaccatgatgttcagcgccagcgccctggcc | Beginning of coding sequence | To secrete protein to periplasm |
| DsbA Signal6 Peptide | atgaaaaagatttggctggcgctggctggtttagttttagcgtttagcgcatcggcg | Beginning of coding sequence | To secrete protein to periplasm |
| PhoA Signal6 Peptide | atggacaaattcgacgctaatcgccgcaaattgctggcgcttggtggcgttgcactcggtgccgccatcctgccgacccctgcgtttgca | Beginning of coding sequence | To secrete protein to periplasm |
| TorA Signal7 Peptide | atgaacaacaacgacctgttccaggcgtctcgtcgtcgtttcctggcgcagctgggtggtctgaccgttgcgggtatgctgggtccgtctctgctgaccccgcgtcgtgcgaccgcg | Beginning of coding sequence | To secrete protein to periplasm |
| YcbK Signal Peptide7 (Tat signal peptide) | atggacaaattcgacgctaatcgccgcaaattgctggcgcttggtggcgttgcactcggtgccgccatcctgccgacccctgcgtttgca | Beginning of coding sequence | To secrete protein to periplasm |

1RBS Annotation example:

2Model the use of ICARUS first

3Promoter spacer example:

4Use modelling and information about the structure of the protein to determine where to place the HIS tag

5Thrombin protease site example:

6For the sec secretion system

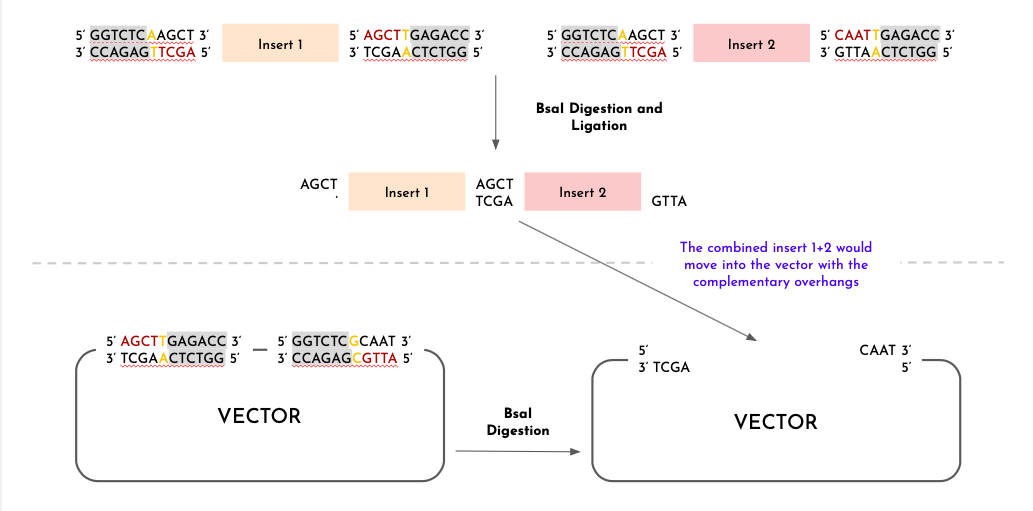
7For the tat secretion system

Golden Gate Design:

NOTE: Remember the BsaI recognition site is not palindromic, so you must use that property carefully.

BsaI Recognition site= 5’ GGTCTC 3’ or 5’ GAGACC 3’

3’ CCAGAG 5’ 3’ CTCTGG 5’



Your inserts should have the recognition site on the EXTERIOR

5’ GGTCTC 3’ before insert

5’ GAGACC 5’ after insert

Your vector should have the recognition site on the INTERIOR

5’ GAGACC 3’ on the left

3’ GGTCTC 3’ on the right

This allows for seamless cloning and the removal of the recognition sites in the final product (this is VERY beneficial)

NOTE: If you’re ligating two inserts that are a part of the coding sequence (ex. A signal peptide and the gene coding sequence) then make the overhang the same sequence as the normal coding sequence. Examples below:

**MoClo Standard:** This cloning standard outlines the overhang sequences that should be used for specific types of inserts:

1. Promoter+rbs
2. Signal peptide
3. Gene sequence
4. Terminator