Transomic Cloning Vectors

Vectors: CRISPR, shRNA, and LentiORF Select-Vectors™ cloning vectors
Format: Glycerol Stock

Vector Cloning Information

The information contained in this guide is to provide researchers with instructions necessary and specific to Transomic vectors. Details for methods of commonly used techniques (e.g. annealing oligonucleotides, restriction digests, PCR, Gibson assembly) are not included and should be developed by the individual user.

LentiORF Select-Vectors™

All LentiORF Select-Vectors™ select vectors contain a multiple cloning sequence (MCS) containing numerous unique restrictions sites for convenient cloning. Please refer to the plasmid map and sequence corresponding to the specific cloning vector configuration to design your cloning strategy. Please note that if you are using a vector that contains a P2A linker for expressing two proteins, it is necessary to remove the stop codon from the inserted expression gene.

CRISPR Cloning Vectors (includes CRISPR, CRISPRa, CRISPRi, single gRNA and All-In-One Vectors)

CRISPR cloning vectors contain a stuffer sequence that needs to be removed and replaced with the desired gRNA sequence. For all CRISPR cloning vectors, the appropriate restriction enzyme to use will be BsmBI.

1. **Design and synthesize appropriate gRNA oligonucleotides**

You may use on-line tools to design your gRNAs or contact Transomic directly for assistance in designing your gRNAs.

For cloning into the BsmBI site of the CRISPR cloning vector, the sequence of the insert (including the 20 bp gRNAs) should be:

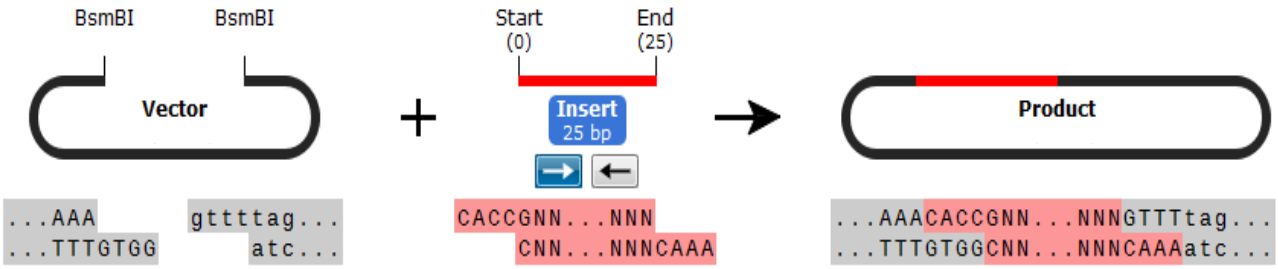
5’ caccgNNNNNNNNNNNNNNNNNNNN 3’

Reverse complement

5’aaacNNNNNNNNNNNNNNNNNNNNc 3’

This design provides the proper overhanging sequences for ligation into the BsmBI digested vector.

PhileKorea
Specialized in Medical & Bio Science since 2000



2. **Digest CRISPR cloning vector with BsmBI**

3. **Anneal the two oligonucleotides**

4. **Ligate annealed oligonucleotides into the digested vector**

5. **Transform into chemically competent *E. coli* cells such as Stbl2™ from ThermoFisher Scientific**

shRNA Cloning Vectors

A multistep cloning strategy is utilized to insert shRNA constructs in an UltramiR scaffold into Transomic's shRNA cloning vectors. You may use on-line tools to design your shRNAs or contact Transomic directly for assistance in designing your shRNAs.

1. **Generate the desired UltramiR clones using the following format (n=21 bp shRNA sequence). Notes:**
   a. **Oligo 1 and Oligo 2 sequences contain the reverse complement siRNA sequences.**
   b. **Oligo 1' and Oligo 2' siRNA sequences are the reverse compliment of Oligo 1 and Oligo 2 siRNA sequences respectively**
   c. **Included in the invariable sequences is a required 1 bp substitution (Green Font).**

Example 1 (n= 21bp siRNA sequences):

Oligo 1

Start (0) ***END (54)***

5' TCGAGAAGGTATATTGCTGTTGACAGTGAGCGAnnnnnnnnnnnnnnnnnnnn 3'

Oligo 1'

Start (0) ***END (60)***

5' GGCTTCACTAnnnnnnnnnnnnnnnnnnnnTCGCTCACTGTCAACAGCAATATACCTTC 3'

Oligo 2

Start (0) ***END (56)***

5' TAGTGAAGCCACAGATGTAnnnnnnnnnnnnnnnnnnnnGTGCCTACTGCCTCGG 3'

Oligo 2'

Start (0) ***END (50)***

5' AATTCCGAGGCAGTAGGCACnnnnnnnnnnnnnnnnnnnnTACATCTGT 3'

Example 2 (Specific example of a 21bp siRNA sequence (Red font):

Oligo 1

Start (0) ***END (54)***

5' TCGAGAAGGTATATTGCTGTTGACAGTGAGCGAGGGACATCCATGTCCAGCAAT 3'

Oligo 1'

Start (0) ***END (60)***

5' GGCTTCACTAATTGCTGGACATGGATGTCCCTCGCTCACTGTCAACAGCAATATACCTTC 3'

Oligo 2

Start (0) ***END (56)***

5' TAGTGAAGCCACAGATGTAATTGCTGGACATGGATGTCCCGTGCCTACTGCCTCGG 3'

Oligo 2'

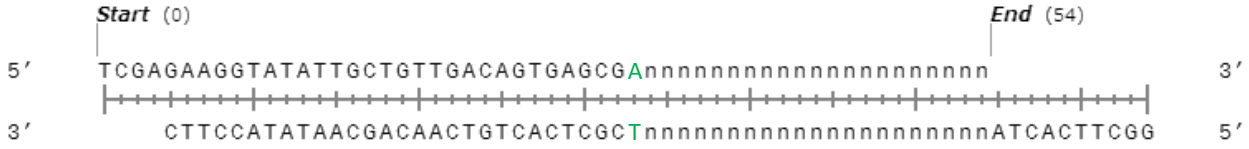
Start (0) ***END (50)***

5' AATTCCGAGGCAGTAGGCACGGGACATCCATGTCCAGCAATTACATCTGT 3'

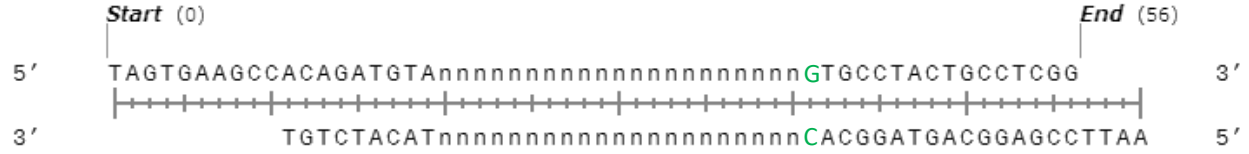
2. Anneal Oligo1 to Oligo1' and Oligo2 to Oligo2'

Oligo1 + Oligo1'

XhoI overhang Loop



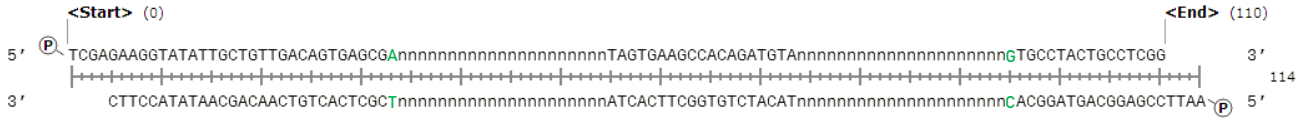
Oligo2 + Oligo2'



Loop EcoRI overhang

3. **Ligate the annealed oligos**

Following ligation, you will have the complete miR30 clone that has XhoI and EcoRI sites located at the 5’ and 3’ ends respectively.



4. **PCR UltramiR shRNA construct using “Hairpin-Hpa” primers that have the following sequence**

Hairpin HpaF (Forward)

5’CTGGGATTACTTCTTCAGGTTAACCCAACAGAAGGCTAAAGAAGGTATATTGCTGTT GACAGTGAGCG 3’

Hairpin HpaR (Reverse)

5’AGAGATAGCAAGGTATTCAGTTTTAGTAAACAAGATAATTGCTCCTAAAGTAGCCCC TTGAAGTCCGAGGCAGTAGGC 3’

5. **Digest the shRNA cloning vector with HpaI**

6. **Perform Gibson assembly reaction using the digested vector and PCR products**

7. **Transform into chemically competent *E. coli* cells such as Stbl2™ from ThermoFisher Scientific**