CRISPR glycerol stock clone receipt, storage, propagation, and verification

Depending on the size of the order, glycerol stocks of clones may be received either in individual tubes or rearrayed into micro-titer plates. E. coli (recombination resistant) stocks containing these clones are provided in LB broth with 8% glycerol. Note: Viral stocks will be shipped frozen on dry ice, to be placed at -80°C or liquid nitrogen immediately upon receipt.

Propagate culture for storage

Single gRNA vectors and All-in-One vectors

Cultures should be propagated in LB broth with **ampicillin or carbenicillin** (100 µg/ml) at 30°C for 30 hours or until the culture appears turbid. A 4 ml starter culture can be inoculated using 5 µl of the glycerol stock provided. Once turbid, place 920 µl of culture into a polypropylene tube and add 80 µl sterile glycerol (8% glycerol). Mix well and store at –80˚C. Glycerol stocks kept at -80°C are stable indefinitely as long as freeze/thaw cycles are minimized.

Dual gRNA vectors

Cultures should be propagated in LB broth with **ampicillin or carbenicillin (100 µg/ml) and zeocin (25ug/ml)** at 30°C for 30 hours or until the culture appears turbid. A 4 ml starter culture can be inoculated using 5 µl of the glycerol stock provided. Once turbid, place 920 µl of culture into a polypropylene tube and add 80 µl sterile glycerol (8% glycerol). Mix well and store at –80˚C. Glycerol stocks kept at -80°C are stable indefinitely as long as freeze/thaw cycles are minimized.

Plasmid Preparation

For transfection and transduction experiments, the plasmid DNA will first have to be extracted from the bacterial cells. Cultures should be grown in LB broth with ampicillin or carbenicillin (100 µg/ml) and zeocin (25ug/ml) at 37°C* overnight or until the culture appears turbid. A 4 ml starter culture can be inoculated using 5 µl of the glycerol stock provided. Either a standard plasmid mini-preparation or one that yields endotoxin free DNA can be used. When isolating plasmid DNA for virus production using endotoxin free kit will generally yield higher viral titers.

***Note: The temperature for propagation is 30°C while the temperature for plasmid preparation is 37°C**

Verification

While transEDIT CRISPR constructs are sequence-verified at Transomic as part of our stringent quality control, some researchers wish to verify the clones again upon delivery. This can be done using the primers specific to each vector as listed below. For vector maps and sequences for each CRISPR vector, please refer to the following website: http://www.transomic.com/Products/Vector-Maps-and-Sequences.aspx

Single gRNA vectors and All-in-One vector verification

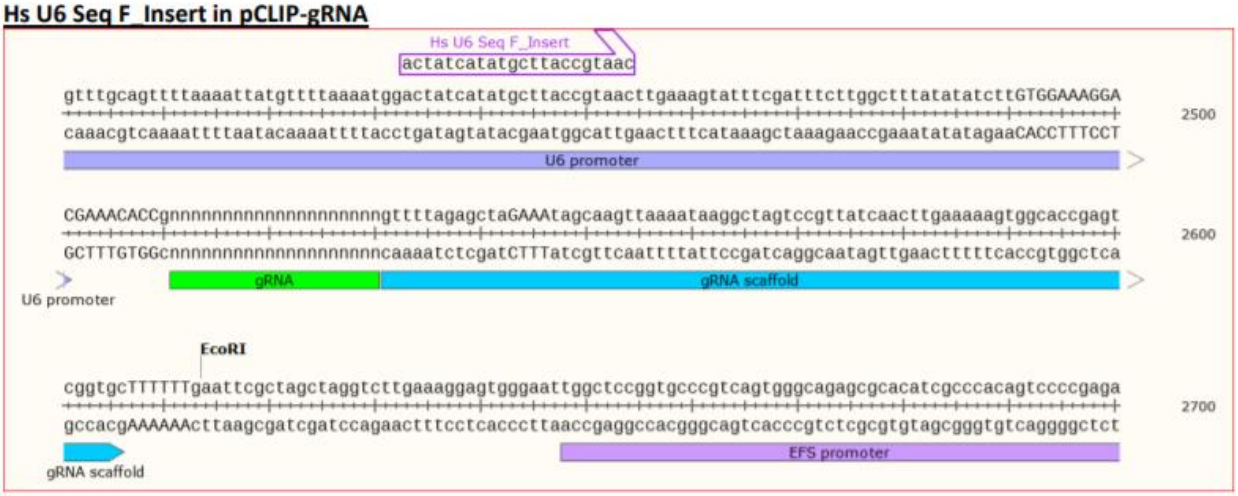
Use the following sequencing primer on both single gRNA and All-in-One (gRNA+Cas9) vectors in a Sanger sequencing reaction:

- Hs U6 Seq F_Insert – 5' ACTATCATATGCTTACCGTAAC 3'

This primer is placed to read through the gRNA of both single gRNA and All-in-One (gRNA+Cas9) vectors. More specifically, Hs U6 Seq F_Insert primer binds in the U6 promoter and sequences through gRNA. (Figure 1 and Figure 2)

Figure 1

Figure 2



Dual gRNA vector verification

Use the following two sequencing primers in two Sanger sequencing reactions:

- sgRNA1_5_F 5’ gggtacagtgcaggggaa 3’
- sgRNA_BC_5F 5’ gaacggcactggtcaact 3’

These primers are placed to read through the two gRNAs and the barcode. More specifically, sgRNA1_5_F binds in the cPPT and sequences through the more 5’ gRNA (which is transcribed off the human U6 promoter). sgRNA_BC_5F binds in the zeoR gene and sequences through the barcode and the more 3’ gRNA (which is transcribed off the chicken U6 promoter). (Figures 3 and 4)

Figure 3

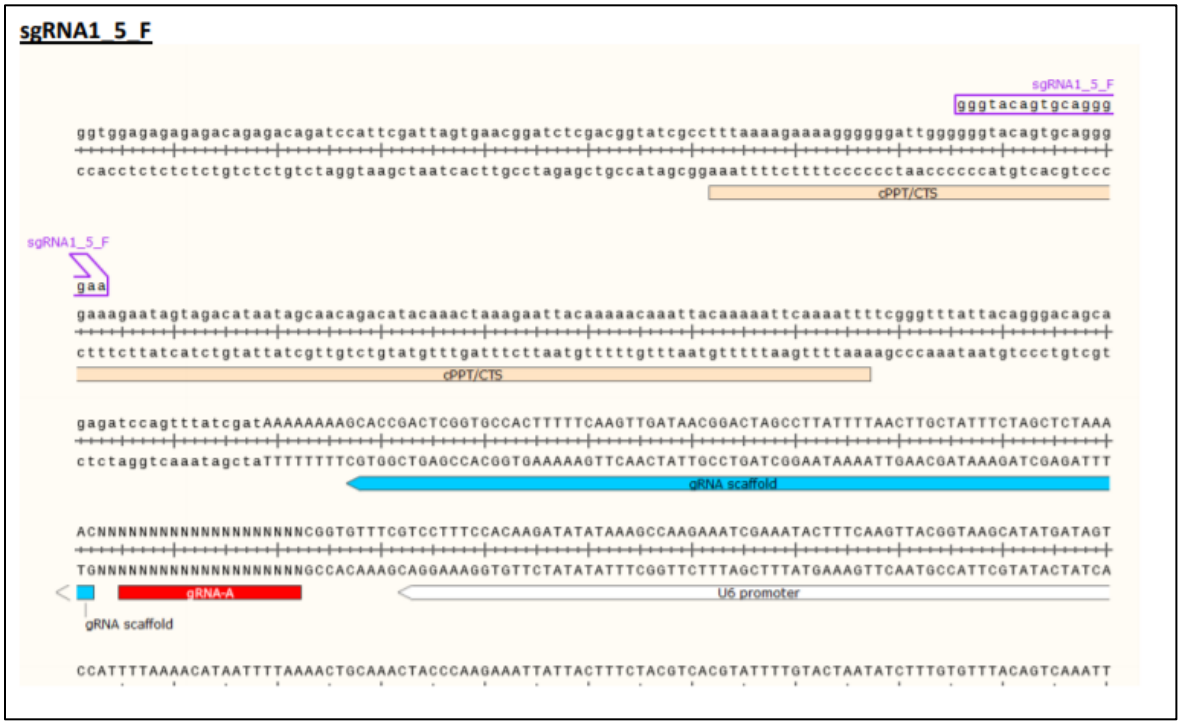
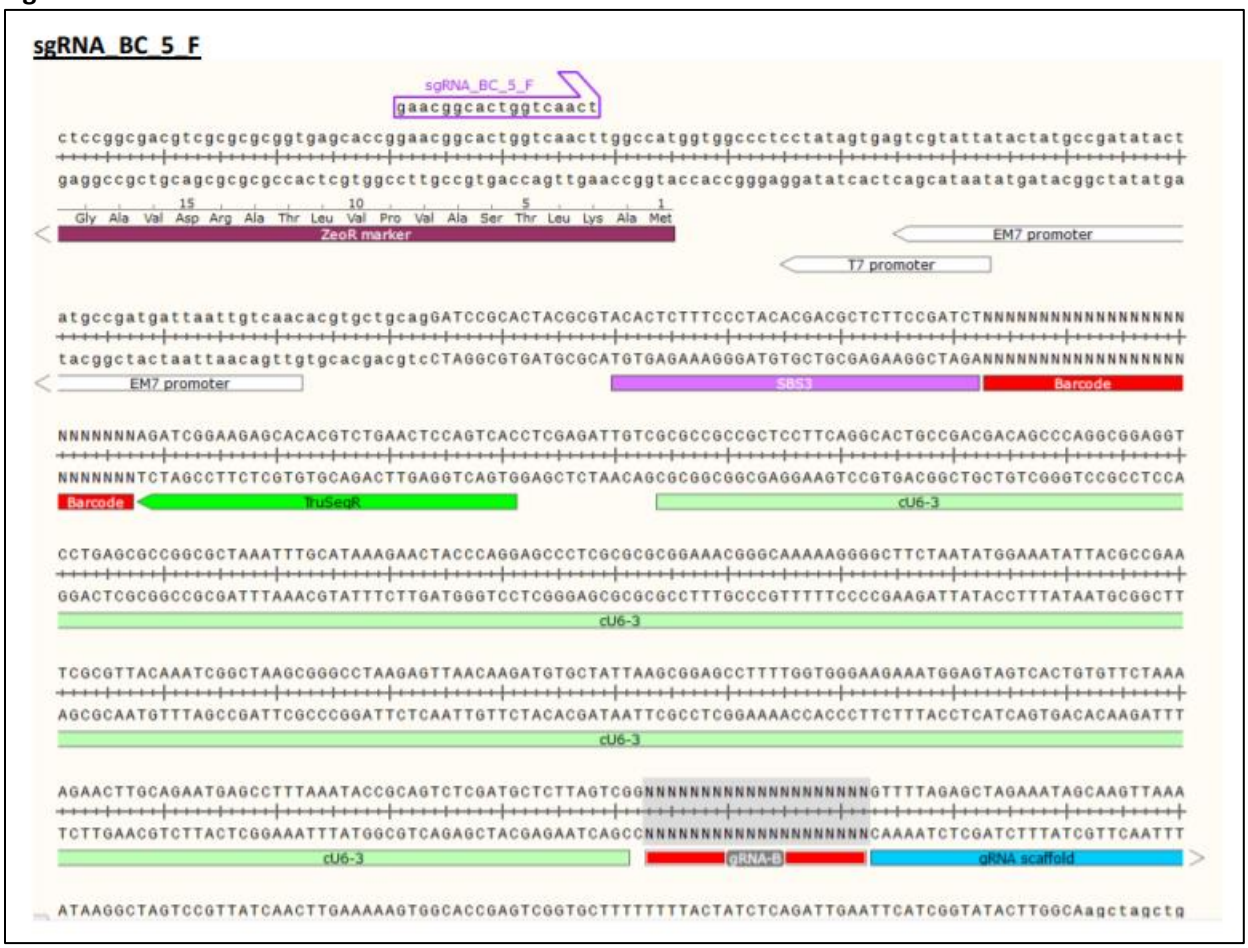


Figure 4


sgRNA_BC_5_F
sgRNA_BC_5_F
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gaggccgctgcagcgcgcgccactcgtggccttgccgtgaccagttgaaccggtaccaccgggaggatatcactcagcataatatgatacggctatatga
15 10 5 1
Gly Ala Val Asp Arg Ala Thr Leu Val Pro Val Ala Ser Thr Leu Lys Ala Met
ZeoR marker
EM7 promoter
T7 promoter
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tacggctactaattaacagttgtgcacgacgtcCTAGGCGTGATGCGCATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGANNNNNNNNNNNNNNNNNN
EM7 promoter
SBS3
Barcode
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NNNNNNNTCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTGGAGCTCTAACAGCGCGGCGGCGAGGAAGTCCGTGACGGCTGCTGTCGGGTCCGCCTCCA
Barcode
TruSeqR
cU6-3
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cU6-3
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cU6-3
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cU6-3
gRNA-B
gRNA scaffold
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