transEDIT-*dual* 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.

Propagation

pCLIP-dual-SFFV-ZsGreen cultures should be propagated in LB broth with ampicillin or carbenicillin (100µg/ml) and zeocin (25ug/ml) at 30°C for 30 hours or until the culture appears turbid. Two-10ml starter cultures can be inoculated using 2 to 10µl of the glycerol stock provided. Once turbid, place 920µl of culture into a polypropylene tube and add 80µl 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 pCLIP-dual-SFFV-ZsGreen plasmid DNA will first have to be extracted from the bacterial cells. Cultures should be grown in LB broth with ampicillin or carbenicillin (100µg/ml) and zeocin (25ug/ml) at 30°C for 30 hours or until the culture appears turbid. Two-10ml starter cultures can be inoculated using 2 to 10µl 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.

Verification

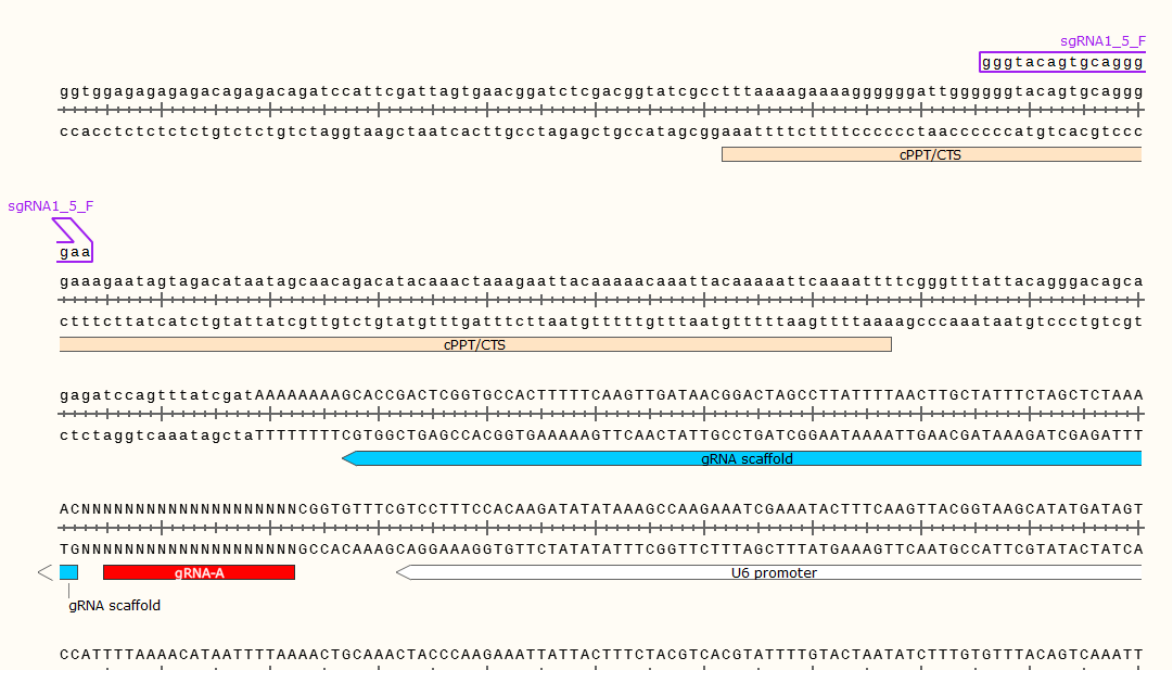
While pCLIP-dual-SFFV-ZsGreen constructs are sequence-verified at Transomic to ensure the presence of the correct two gRNAs and the barcode, some researchers wish to verify the clones again upon delivery.
This can be done by using the following two sequencing primers in two Sanger sequencing reactions:

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

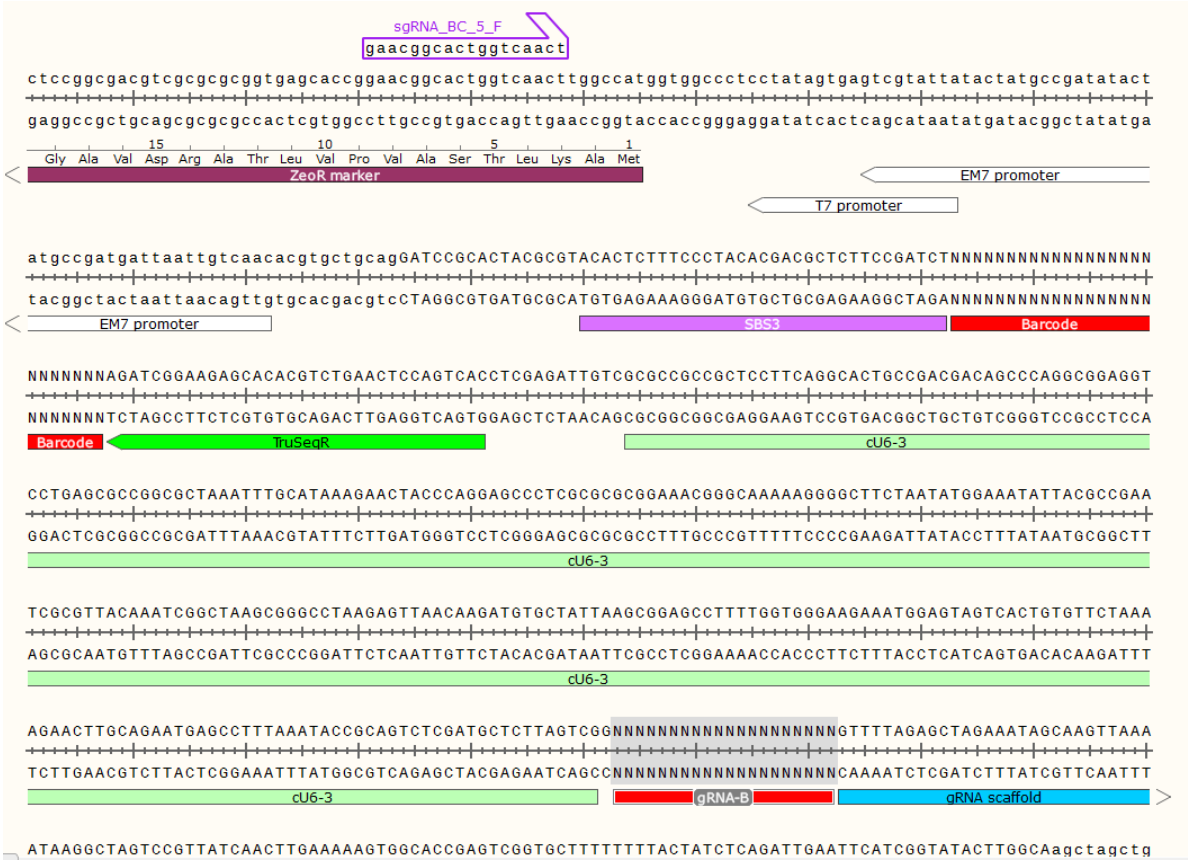
These primers are placed to read through the two gRNAs and the barcode. More specifically, sgRNA1_5_F binds in the cPPT and sequences through the more 5’ gRNA (which is transcribed off the human U6 promoter). sgRNA_BC_5F binds in the zeoR gene and sequences through the barcode and the more 3’ gRNA (which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

For the vector map and sequence for pCLIP-dual-SFFV-ZsGreen, please refer to the following website:
http://www.transomic.com/Products/Vector-Maps-and-Sequences.aspx

sgRNA1_5_F



sgRNA_BC_5_F


sgRNA_BC_5_F
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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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tacggctactaattaacagttgtgcacgacgtcCTAGGCGTGATGCGCATGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGANNNNNNNNNNNNNNNNN
EM7 promoter
SBS3
Barcode
NNNNNNNAGATCGGAAGAGCACACGTCTGAACTCCAGTCACCTCGAGATTGTCGCGCCGCCGCTCCTTCAGGCACTGCCGACGACAGCCCAGGCGGAGGT
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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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