transEDIT pCLIP-ALL and transEDIT pCLIP-gRNA 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-ALL and pCLIP-gRNA cultures should be propagated in LB broth with ampicillin or carbenicillin (100µg/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 pCLIP-ALL and pCLIP-gRNA 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) 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.

Note: The pCLIP-ALL plasmid is large and may yield lower DNA amounts than expected.

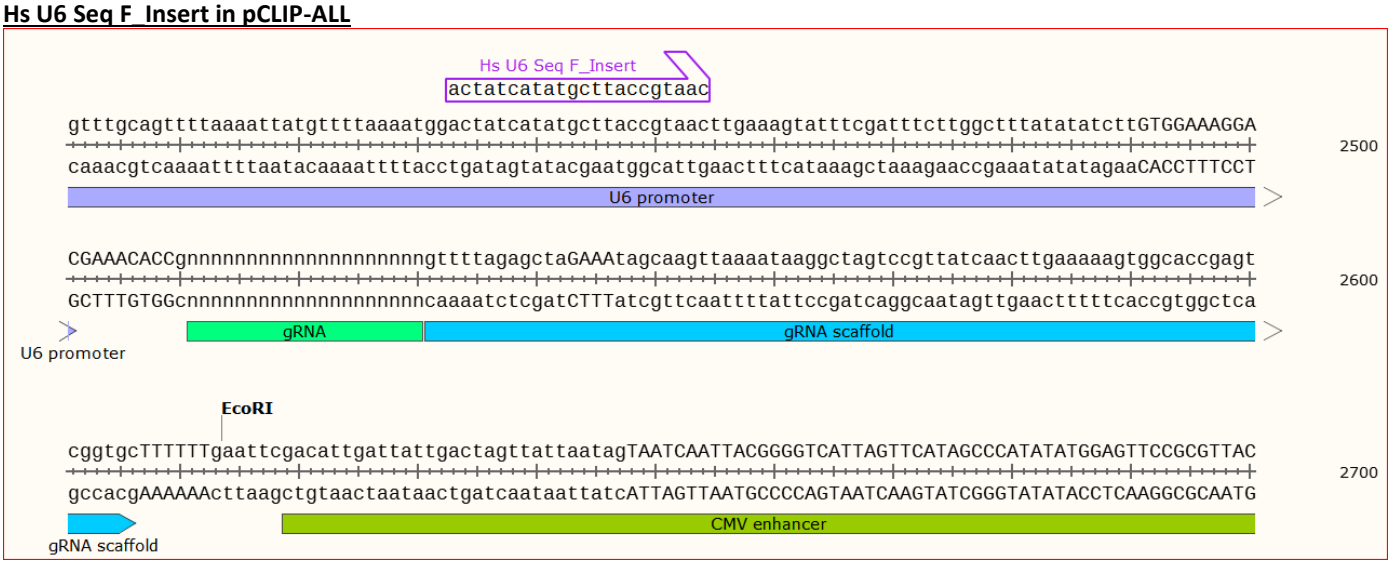
Verification

While pCLIP-ALL and pCLIP-gRNA constructs are sequence-verified at Transomic to ensure the presence of the correct gRNAs, some researchers wish to verify the clones again upon delivery.
This can be done by using the following sequencing primer on both pCLIP-ALL and pCLIP-gRNA in a Sanger sequencing reaction:

> Hs U6 Seq F_Insert – 5’ ACTATCATATGCTTACCGTAAC 3’
>
> *This primer is placed to read through the gRNA of both pCLIP-ALL and pCLIP-gRNA. More specifically, Hs U6 Seq F_Insert primer binds in the U6 promoter and sequences through gRNA.*

For the vector maps and sequences for pCLIP-ALL and pCLIP-gRNA, please refer to the following website:
http://www.transomic.com/Products/Vector-Maps-and-Sequences.aspx

Hs U6 Seq F_Insert in pCLIP-ALL



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Hs U6 Seq F_Insert in pCLIP-gRNA