Guidelines for lentivirus production using gRNA and Cas9 vectors.

Considerations before packaging the pCLIP gRNA and Cas9 lentiviral vectors:

1. Non-Targeting Control DNA (provided) should be used to determine the packaging and transduction efficiency of the target cell used in the screen prior to packaging the DNA. This ensures optimal conditions and sufficient viral particle production for the screen. If required, additional control virus can be purchased from Transomic.
2. All plasmids should be purified using endotoxin-free purification.
3. All plasmids should be resuspended in dH_2O.
4. OMNIfect Transfection Reagent (0.3ml, Transomic Cat. # OTR1001) or FuGENE6 (Promega, Cat. # E2691) are two recommended transfection reagents.
5. All Transomic **constitutive** pCLIP lentiviral vectors are Tat independent, meaning that they can be packaged using 2nd, 3rd, or 4th generation packaging systems. Transomic's **pTOL-hCMV-TET3G-Hygro Transactivator vector** (used with the inducible Cas9 lentiviral vector) is the only exception since it contains a wildtype LTR, and thus can only be packaged using 2nd or 4th generation systems. In-house we use a combination of pCMV-dR8.2 - (Addgene) and pCMV-VSV-G - (Addgene). However, many researchers have achieved success with other systems including Clontech's Lenti-X single shots (4th generation).
6. A filter can be used to remove cellular debris from the generated virus, but the filter should not be nitrocellulose. Nitrocellulose binds proteins present in the membrane of lentivirus and destroys the viral particles.
7. Freeze/thaw events lower the viral titer and need to be kept to a minimum. Once the virus has been generated it needs to be aliquoted into cryovials and stored at -80°C prior to titering. Thaw only one of the aliquots for calculating the titer. This titer should accurately reflect the titer of the other frozen aliquots once they have been thawed for use. The relative transduction efficiency needs to be calculated on the same aliquot used for titering.

pCLIP vectors

Single and Dual CRISPR/Cas9 Vectors

- pCLIP-*dual*-SFFV-ZsGreen
- pCLIP-gRNA-EFS-Puro
- pCLIP-gRNA-EFS-Blast
- pCLIP-gRNA-EFS-ZsGreen
- pCLIP-gRNA-EFS-RFP
- pCLIP-All-EFS-Puro
- pCLIP-All-EFS-Blast
- pCLIP-All-EFS-ZsGreen

- pCLIP-All-EFS-RFP
- pCLIP-All-hCMV-Puro
- pCLIP-All-hCMV-Blast
- pCLIP-All-hCMV-ZsGreen
- pCLIP-All-hCMV-RFP

Cas9 Nuclease Vectors

- pCLIP-Cas9-Nuclease-EFS-Puro
- pCLIP-Cas9-Nuclease-EFS-Blast
- pCLIP-Cas9-Nuclease-EFS-ZsGreen
- pCLIP-Cas9-Nuclease-EFS-RFP
- pCLIP-Cas9-Nuclease-hCMV-Puro
- pCLIP-Cas9-Nuclease-hCMV-Blast
- pCLIP-Cas9-Nuclease-hCMV-ZsGreen
- pCLIP-Cas9-Nuclease-hCMV-RFP
- pCLIP-Cas9-Nuclease-TRE3G-Puro
- pCLIP-Cas9-Nuclease-TRE3G-Blast
- pCLIP-Cas9-Nuclease-TRE3G-ZsGreen
- pCLIP-Cas9-Nuclease-TRE3G-RFP

Cas9 Nickase Vectors

- pCLIP-Cas9-Nickase-EFS-Puro
- pCLIP-Cas9-Nickase-EFS-Blast
- pCLIP-Cas9-Nickase-EFS-ZsGreen
- pCLIP-Cas9-Nickase-EFS-RFP
- pCLIP-Cas9-Nickase-TRE3G-Puro
- pCLIP-Cas9-Nickase-TRE3G-Blast
- pCLIP-Cas9-Nickase-TRE3G-ZsGreen
- pCLIP-Cas9-Nickase-TRE3G-RFP

Related Vectors

- pTOL-hCMV-TET3G-Hygro Transactivator – requires 2nd or 4th generation packaging plasmids