Considerations for *E. coli* amplification of Tet-pLKO-puro (Addgene #21915) with strong shRNA inserts

- When it contains a shRNA insert, Tet-pLKO-puro vector backbone is prone to recombination; this is especially pronounced with highly efficient shRNAs that form strong secondary structures. When recombination occurs, the shRNA insert is often lost, but the ampicillin resistance gene is retained, allowing propagation in bacteria.

- To minimize recombination, we recommend using NEB Stable competent cells (NEB C3040I) grown at 30°C. However, even under these conditions we still observe some recombination at a rate of approximately 10-15%; isolating additional colonies should allow for identification of a correct clone.

- To confirm that a correct clone has been isolated we recommend:

    - Plasmid DNA Sanger sequencing to confirm presence of the insert (using Tet-pLKO-puro 5' sequencing primer GGCAGGGATATTCACCATTATCGTTTCAGA);

    - Plasmid DNA diagnostic restriction digest using either EcoRI-HF (not destroyed), or EcoRI-HF (not destroyed) / AgeI-HF (destroyed) double digest – either should result in a single linearized band of about 8kb. Presence of additional shorter or faster migrating bands is indicative of recombined products (please see examples below).