**shRNA cloning**

**About shRNA**

Inducible shRNA is a good approach to knock down target gene.

**Best strategy**

How to prove the result is not due to off-target effect? cDNA rescue experiment.

3'UTR is considered as signature of a gene. so shRNAs targetting 3'UTR. so there will be minimum off targetting. 5'UTR of the gene is not considered as signature region of the gene. so shRNA targetting 5'UTR may target the other genes and other regions as well.

The clones that are against the 3' UTR of the target are included so one could rescue the knockdown phenotype with the introduction of a cDNA clone corresponding to the target. Transcripts from an exogenous cDNA clone would not be susceptible to silencing compared to the endogenous copy. Many publications/review committees require such an experiment to verify the observed phenotype. However, while most target sets have this added feature, not all target sets have a 3' UTR clone.

**Plasmid**

[Inducible TET-Cellecta vector](https://cellecta.com/collections/tet-inducible-systems-and-vectors?pf_t_category=shRNA+Constructs)

[pRSITEP-U6Tet-sh-noHTS-EF1-TetRep-2A-Puro](https://www.snapgene.com/plasmids/viral_expression_and_packaging_vectors/pRSITEP-U6Tet-sh-noHTS-EF1-TetRep-2A-Puro)

**Resources:**

[Design Hairpins for a Target Transcript Sequence (RNAi)](https://portals.broadinstitute.org/gpp/public/)

[ShRNA sequence for 5´UTR or 3´UTR?](https://www.researchgate.net/post/shRNA_sequence_for_5_UTR_or_3_UTR)

**Step 1: Digest plasmid/vector with bdsl enzyme in 1.5 ml tube (~1 day)**

* 20 ug cellecta plasmid (conc is 3031 ug/ml, so add 6.5 ul pRSITEP-U6Tet)
* 5ul bdsl enzyme (can be find in green enzyme box)
* 10 ul Rcutsmart buff (can be find in green enzyme box)
* Add up to 100ul (100-20-5-10=65 ul dw)
* 37 ℃ water bath overnight

**Step 2: Purify plasmid/vector using bead**

* Magnetic bead (XP in 4 ℃) 1:1 ratio (volume)
* Vortex bead, mix with digested vector
* Put onto magnetic base
* Remove liquid
* Wash with 80% EtOH
* Wash with 80% EtOH
* Open lip, let bead dry
* Elution with 50ul dw

**Step 3: Phosphorylation of 5’ end of oligo**

* 1 ul T4 DNA ligase 10X buff
* 0.5 ul PNK Enzyme (keep on ice)
* 6.5 ul dw
* 2 ul oligos mixture
* PCR machine (Phosphorylation profile)
* Dilute phosphorylated oligos 100x (1 ul product + 99 ul dw)

**Step 4: Ligation (connect digested plasmid with oligos) (no Gibson)**

* 1 ul T4 DNA ligase
* 1 ul T4 DNA ligase 10X buffer
* 50 ng digested vector (measure conc before use)
* 2 ul phosphorylated oligos mixture (diluted 100 times)
* Add up to 10 ul per Rxn