Custom shRNA Knockdown Constructs

CELLECTA

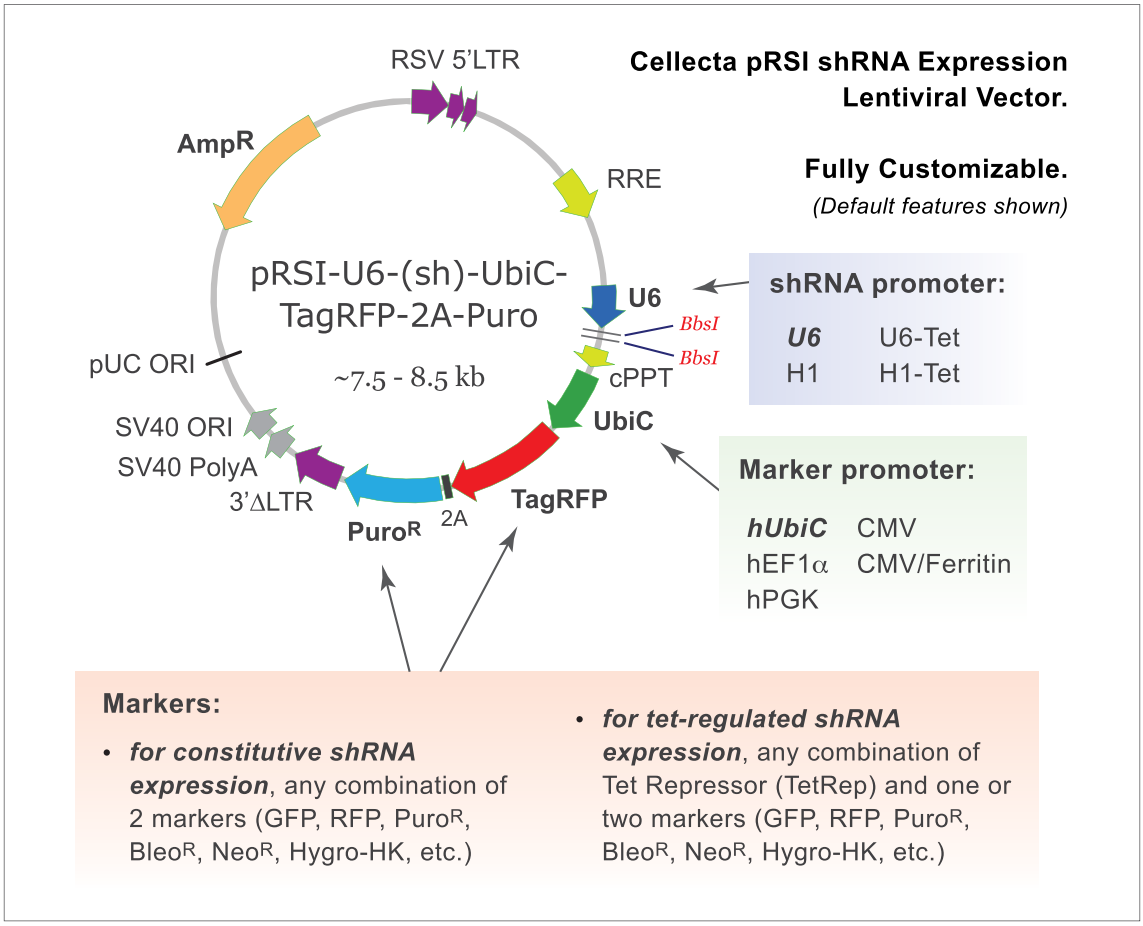
Cellecta provides complete services for construction of custom constructs expressing shRNA targeted to any transcript. Confirm hits from a library screen, access effects of specific gene knockdown on cells, or explore mechanism of action for a drug.

Construct Choices

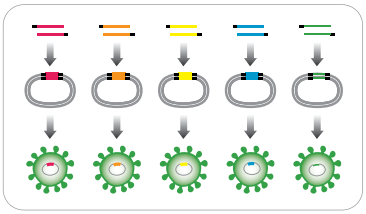
- Choose constitutive or inducible version of H1 or U6 shRNA Promoters
- Select GFP, RFP, PuroR, BleoR, NeoR or Hygro-HK markers
- Obtain constructs as plasmid or packaged lentiviral particles

Knock Down Targets with Lentiviral shRNA Constructs

- You provide the RefSeq number or gene ID
- We can design 3-5 shRNA constructs that target the transcript
- Our proprietary shRNA design delivers ~70% shRNAs that generate >70% transcript knockdown in standard cell lines (measured by qRT-PCR)



Simple shRNA Cloning



- We clone one shRNA sequence that you specify into a vector and verify the correct construction

Targeted Sets of shRNA Constructs

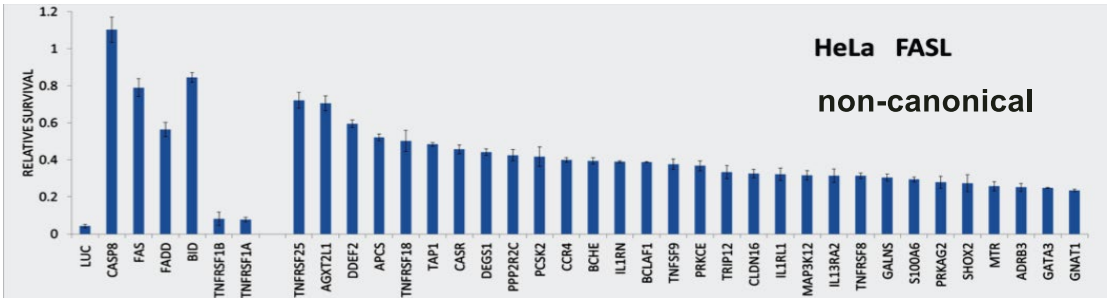
- We clone 3 or 5 shRNA sequences targeting a specific human or mouse gene
- Constructed based on design parameters used in creating our libraries
- Usually at least 2/3 or 3/5 of the constructs knock down at least 70% of target transcript, but this can vary between different targets and is not guaranteed

Validated shRNA Expression Constructs

- After cloning several shRNA sequences designed with our algorithm, we confirm expression of the target gene using qRT-PCR
- We use the cell line of your choice
- Target gene transcript expression is knocked down by at least 70%
- We provide the most effective construct to you in both plasmid and packaged virus form

Example shRNA Validation Data

Gene Hit Confirmation



To confirm isolated hits from a positive selection shRNA screen for FAS mediators, two shRNA constructs were made targeting each of the top 100 hits. 293 cells expressing each shRNA were tested for inhibition of apoptosis after treatment with FAS ligand. The specificity of knockdown of the shRNA targets was assessed by real-time qRT-PCR with gene-specific primers. This approach allowed us to come up with a set of verified FAS targets. The best responders are shown, with known FAS mediators on the left and the non-canonical hits, which were not expected to be mediators for FAS apoptosis, on the right.