CELLECTA

RNAi and shRNA Design. Simplified.

Principles of RNAi and shRNA Design

shRNA versus siRNA

RNA interference (RNAi) is a biological process where RNA molecules are used to inhibit gene expression. Typically, short RNA molecules are created that are complementary to endogenous mRNA and when introduced into cells, bind to the target mRNA. Binding of the short RNA molecule to the target mRNA functionally inactivates the target mRNA and sometimes leads to degradation of the target mRNA.

Historically, two types of short RNA molecules have been used in RNAi applications. Small interfering RNA (siRNA) are typically double-stranded RNA molecules, 20-25 nucleotides in length. When transfected into cells, siRNA inhibit the target mRNA transiently until they are also degraded within the cell. Small hairpin RNAs (shRNA) are sequences of RNA, typically about 80 base pairs in length, that include a region of internal hybridization that creates a hairpin structure. shRNA molecules are processed within the cell to form siRNA which in turn knock down gene expression. The benefit of shRNA is that they can be incorporated into plasmid vectors and integrated into genomic DNA for longer-term or stable expression, and thus longer knockdown of the target mRNA (Figure 1).

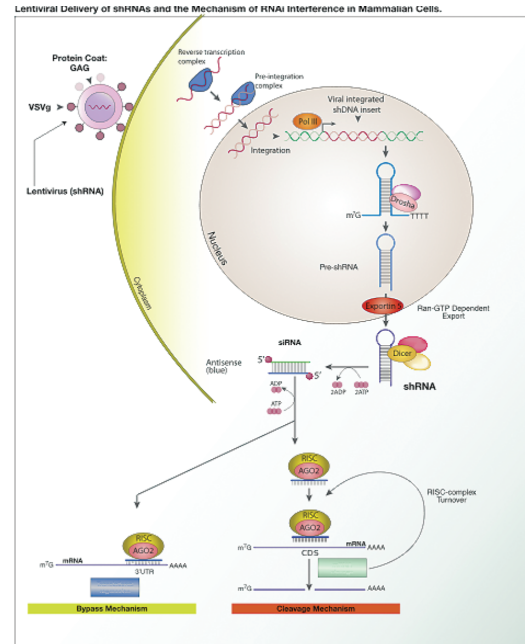

Figure 1

shRNA is processed in the cell by Exportin 5, Dicer, and the RISC/AGO2 complex. Lentiviral delivery of the shRNA enables stable expression and permanent knockdown of the target gene. (Image by Dan Cojocari - Wikimedia Commons.)

Effective Expression of shRNAs From RNA Polymerase III Promoters

Small RNAs including shRNAs are transcribed endogenously with RNA Polymerase III (Pol III). This is different from cDNAs which are transcribed using RNA polymerase II. Therefore, to guarantee high expression of shRNAs, Cellecta uses promoters that specifically bind Pol III, the U6 and H1 promoters. Both the U6 and H1 promoters work well to express high levels of shRNA across many cell types, as compared to Pol II promoters such as CMV and EF1, which do not express shRNAs as effectively due to their small size. In addition, Pol II promoters used for expressing shmiRs may not work in most cell types, where U6 and H1 work well.

Lentiviral Vectors Enable Permanent Knockdown of Targets

The shRNA becomes stably integrated into the host cell genome. As cells divide, the shRNA is passed on to daughter cells. Using lentiviral vectors for expression of shRNAs provides permanent knockdown without needing to transfect the cells multiple times (Figure 2).

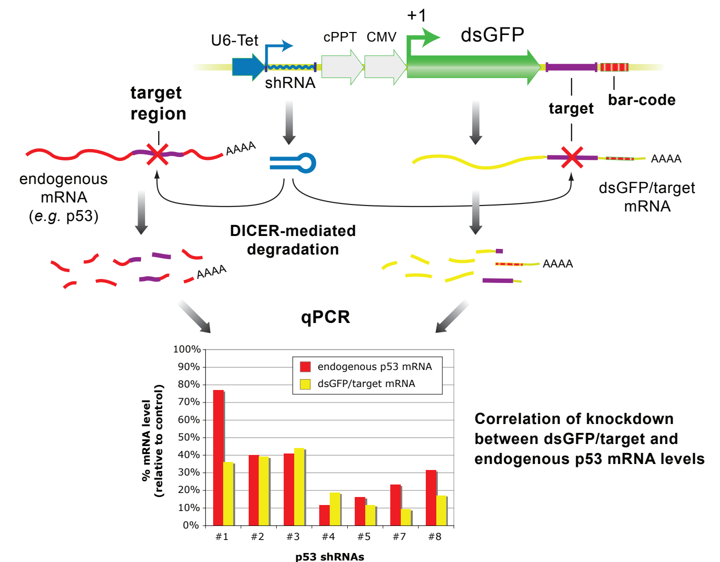

Figure 2

Map of shRNA-target lentiviral reporter construct integrated in genomic DNA and mechanism of knockdown of GFP-target reporter. The cells transduced with functional shRNA constructs will have low GFP mRNA levels, while non-functional shRNAs permit a high level of GFP mRNA expression.

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Optimization of shRNA Design

Designing effective shRNAs is essential for effective RNAi knockdown screening. In addition to choosing the optimal sequence, there are a number of structural factors that affect shRNA efficacy. In addition, the stem-loop hairpin structure of shRNA poses problems for library construction and amplification. To create representative quality shRNA libraries, we have focused significant effort to optimize effective of the shRNA inserts in our libraries. We have developed an in-house algorithm to predict the most effective shRNA sequences, and combine this with published data of validated sequences when constructing our libraries. We have also optimized the structure of shRNA for library construction.

To efficiently test shRNA structure variations on a large-scale, we developed a high-throughput shRNA efficacy testing technology based on a reporter assay. It was previously demonstrated that a reporter gene (such as GFP) with a 3' fusion to a cDNA fragment or short oligonucleotide from a target gene could be effectively used to monitor the efficacy of siRNA and shRNA constructs against this target gene. Based on these findings, we developed a lentiviral reporter vector for identification of functional shRNA constructs (Figure 3). Our proprietary shRNA testing reporter vector allows for cloning of both shRNA template downstream of the H1 promoter and target sequences at the 3' end of the GFP reporter. When this construct is transduced into cells, transcription from the H1 promoter produces shRNA, while transcription from the CMV promoter generates a GFP-sense target mRNA fusion transcript that can be used as a reporter for screening functional shRNAs. To optimize the structure of the shRNA, we constructed an shRNA-target library in the shRNA validation vector for 150 shRNAs, with each shRNA constructed in 40 different designs described in the literature.

We performed RNAi screens in CHO-T-REx cells transduced with this 150x40 barcoded shRNA-Target library and measured representation (in the library and transduced cells) and knockdown efficiency of each shRNA construct. Some representative data from this screening is shown in Figure 4

The ten best designs were selected based on those having the highest target knockdown activity, equal amplification efficiency of same-design pooled oligonucleotides, and equal representation of same-design shRNAs in the pooled RNAi libraries. The knockdown efficiency of three p53 shRNAs constructed with these 10 best designs in a lentiviral vector was further analyzed by RT-PCR after transduction into mouse fibroblast cells (Figure 5).

For more information, email info@cellecta.com or call +1-650-938-3910

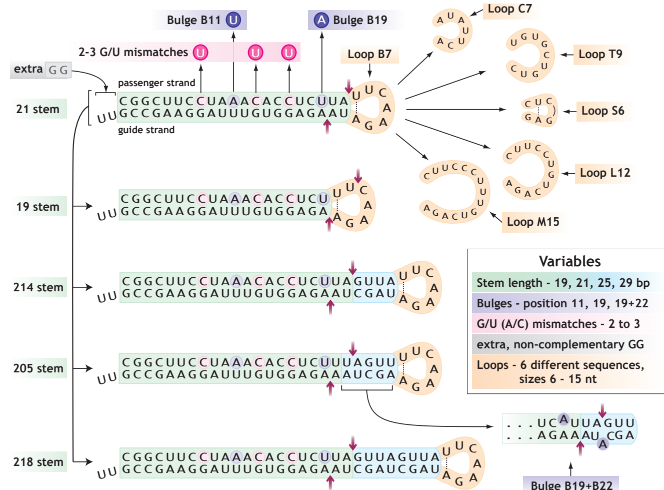

Figure 3

To optimize the structure of the shRNA, we constructed an shRNA-target library in the shRNA validation vector for 150 shRNAs, with each shRNA constructed in 40 different designs described in the literature.

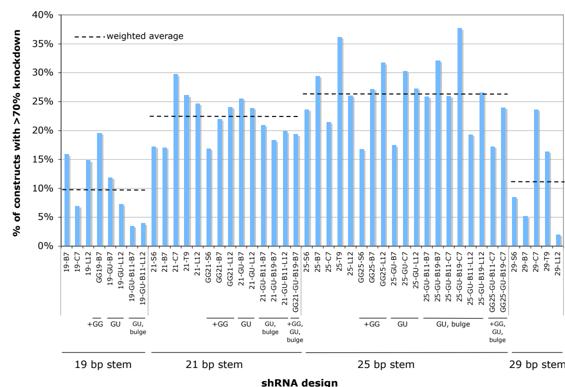

Figure 4

Representative data from RNAi screens in CHO-T-REx cells transduced with a 150x40 barcoded shRNA-Target library.

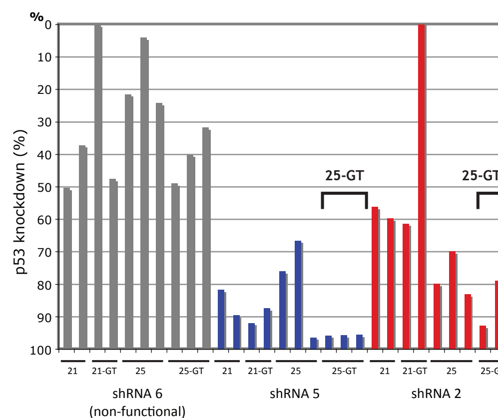

Figure 5

The 10 best shRNA designs were selected based on highest target knockdown activity, equal amplification efficiency, and equal representation of same-design shRNAs in the pooled RNAi libraries.

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