Validation of siRNAs — from conceptual design to process

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Introduction

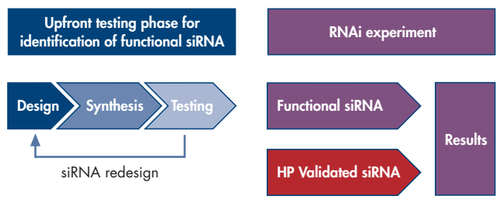
The success of RNAi experiments depends on a number of factors including functionality of the siRNA, characteristics of the cell type, and functionality of tools for downstream analysis

To increase the chance of successful RNAi, we have designed and functionally tested a large number of siRNAs targeting important human genes and shown that they provide high knockdown efficiency. These HP Validated siRNAs allow researchers to avoid upfront design and testing experiments and to start experiments immediately.

A standardized and robust procedure for the validation of siRNAs using quantitative, real-time RT-PCR has been established. Only siRNAs that result in at least 70% knockdown satisfy internal specifications and are called HP Validated siRNAs.

siRNAs were designed using the innovative HiPerformance siRNA Design Algorithm licenced from Novartis AG. A stringent, in-house homology analysis tool was used to ensure siRNA specificity. Validation was performed using QuantiTect® Primer Assays, which are validated primer sets for SYBR® Green based real-time RT-PCR.

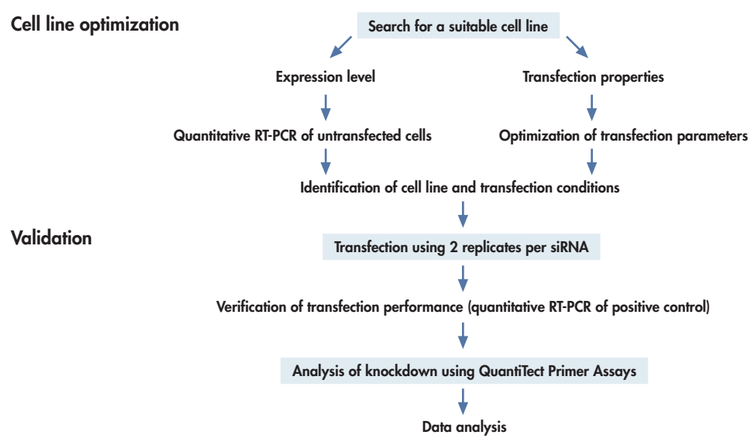
Use of Validated siRNAs Saves Time in Upfront Testing



The validation workflow includes optimization of the cell system and functional testing

- A cell type with appropriate gene expression and transfection properties is identified. Transfection parameters are optimized.
- siRNAs are transfected and knockdown is assessed by quantitative, real-time RT-PCR using QuantiTect Primer Assays and QuantiTect SYBR Green Kits.

Validation Workflow



Optimization of transfection is critical for successful RNAi

Gene knockdown of 70% is usually necessary for functional analysis. This means that transfection efficiencies of ≥70% are required. A reduction of transfection efficiency by 10–20 % will reduce knockdown and as a result will also reduce phenotypic effects. This reduction may make phenotypic effects undetectable and will reduce the reproducibility of the experiment. Data in Figure 1 show how suboptimal conditions that lower transfection efficiency also affect knockdown efficiency.

Important factors for optimal transfection include:

- **The transfection reagent**
  A highly effective reagent, such as HiPerFect Transfection Reagent, should be used.
- **The transfection method**
  Conventional transfection or reverse transfection procedures may be used.
- **Optimized parameters**
  The siRNA concentration and the amount of transfection reagent used must be optimized.

Optimal Transfection Leads to Efficient Knockdown

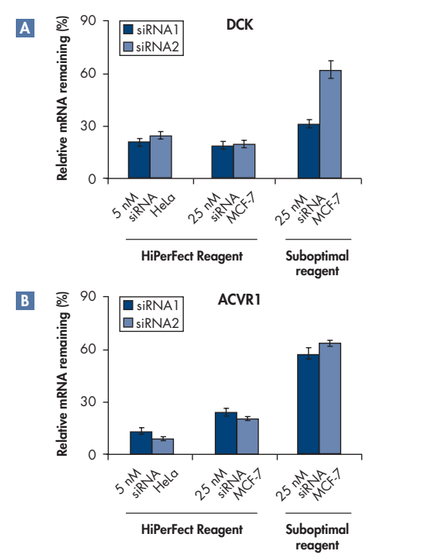


Figure 1 HeLa or MCF-7 cells were transfected with 25 nM or 5 nM of HP Validated siRNAs targeting A DCK or B ACVR1. Either HiPerFect Transfection Reagent or a suboptimal transfection reagent was used. Knockdown was assessed by quantitative RT-PCR. Suboptimal transfection conditions resulted in less efficient transfection and knockdown

Validation requires a robust experimental setup

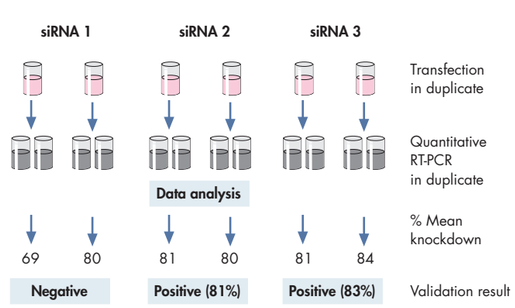
Control experiments include the following:

- **Cell line control for abundance of target** — quantitative RT-PCR of untransfected cells with the same primer pair as used for validation
- **Transfection control** — a positive control (MAPK1) tests transfection efficiency

Features of the experimental setup are as follows:

- At least 2 replicate transfections are performed for each siRNA.
- At least 2 quantitative RT-PCRs are performed for each transfection.
- The housekeeping gene GAPDH is used as an internal reference.
- All siRNAs must show ≥70% knockdown

Experimental Setup



Validation identifies highly effective siRNAs

- HP Validated siRNAs must show knockdown of ≥ 70%. The average knockdown achieved is 82% (calculated from a large group of randomly chosen HP Validated siRNAs, see Figure 2).
- HP Validated siRNAs resulted in efficient knockdown in cell lines with equivalent target gene expression. Conversely, siRNAs which failed validation specifications gave comparable results in 2 cell lines (Figure 3).
- For the majority of targets, 2 HP Validated siRNAs are available, allowing confirmation of phenotypic results using an additional siRNA.

HP Validated siRNAs Result in High Knockdown

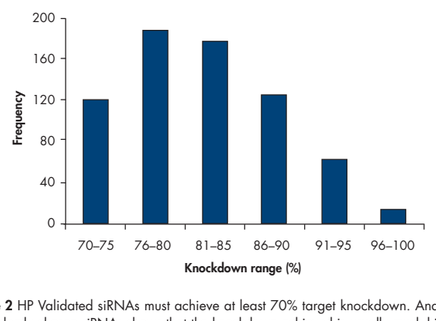


Figure 2 HP Validated siRNAs must achieve at least 70% target knockdown. Analysis of randomly chosen siRNAs shows that the knockdown achieved is usually much higher. The average knockdown efficiency was 82%.

Comparable Knockdown in a Range of Cell Lines

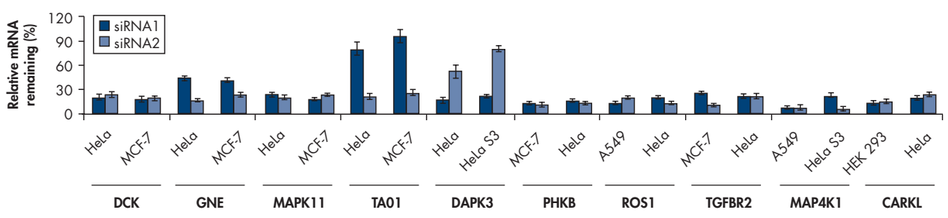


Figure 3 HP Validated siRNAs and siRNAs that failed to meet validation criteria were transfected into a variety of cell lines using optimized siRNA concentrations. After 48 hours, knockdown was assessed by quantitative RT-PCR. siRNAs resulted in comparable knockdown efficiencies in a variety of cell lines with equivalent gene expression and transfection efficiency.

Summary

- HP Validated siRNAs are analyzed in a standardized, stringent validation procedure that allows high-throughput and robust testing and provides reliable results. HP Validated siRNAs show ≥ 70% knockdown. In most cases, 2 siRNAs are provided for the gene, allowing confirmation of results. The range is continually expanding with siRNAs targeting genes from important gene families
- HP Validated siRNAs result in equivalent knockdown in various cell lines with similar gene expression levels and transfection properties.
- HP Validated siRNAs are provided with details about the experimental parameters used (e.g., cell line and transfection conditions). Together with QuantiTect Primer Assays, they provide a matching integrated solution for RNAi and expression analysis

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