

MISSION[®] esiRNA for RNAi Screening



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Introduction

RNA interference (RNAi) is a powerful tool for loss of gene function studies through the knock-down of specific mRNA transcripts¹. RNAi is induced by long double stranded (ds)RNA that is homologous to the target gene. Introduced into cells, this dsRNA is chopped into shorter fragments by an endoribonuclease of the RNase III family (Dicer). These fragments of approximately 21 base pairs in length are bound by the RNA-induced silencing complex (RISC) and separated into single strands, where one strand remains bound by RISC. This strand serves as the template for the recognition of the corresponding mRNA. Once a target mRNA is recognized, the protein Argonaut, which is a component of the RISC, now cleaves the mRNA and initiates its further degradation. The path from gene to protein is abrogated at the mRNA level.

Shortly after its discovery RNAi became a widely used technology for loss-of-function studies in invertebrates like the nematode *Caenorhabditis elegans* or the fruit fly *Drosophila melanogaster*. In most mammalian cells, however, long dsRNA induces a potent and often detrimental interferon response. Hence the approach of delivering long dsRNA is not useful to study the specific function of individual genes in most mammalian cells.

This problem was solved in an elegant way in the year 2001²: Short double stranded RNAs – similar to the Dicer products – do not induce an interferon response but mediate gene-silencing in mammalian cells. Importantly, such short interfering RNAs (siRNAs) are easily accessible through chemical solid phase synthesis, making RNAi experiments in mammalian cells straightforward.

Design of siRNAs

A key step for good siRNA design is the determination of the best target region in the mRNA sequence. Since the target regions differ in their susceptibility for RNAi the corresponding siRNAs differ largely in their knock-down efficacies. A high quality siRNA should therefore efficiently destroy the intended target mRNA.

An equally important criterion for a high quality siRNA is its specificity. It is now well established that siRNAs are co-silencing other transcripts beside their target-genes. These so-called off-target effects arise mostly from partial homologies of the siRNA sequence to other mRNAs. Consequently, observed phenotypes can be due to the knock-down of the intended target, or due to other, unintended silenced transcripts. This explains why off-target effects are a central challenge in the RNAi field, especially in high-throughput screening.

Some improvements to increase the specificity of siRNAs have recently been made, e.g. by avoiding the 3'-untranslated regions, but a design algorithm that can faithfully predict and prevent off-target effects is presently not available.

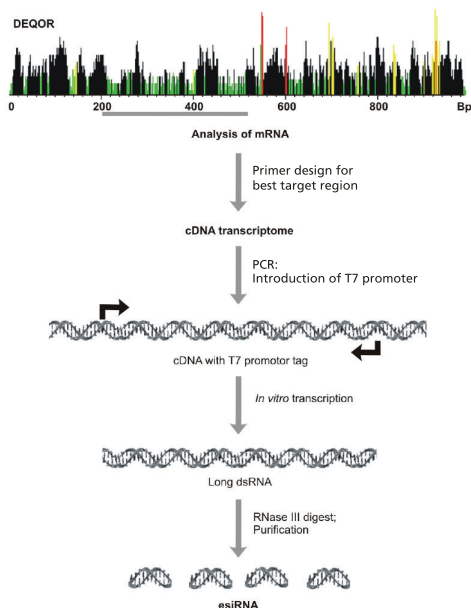


Figure 1: Flow chart of MISSION esiRNA production

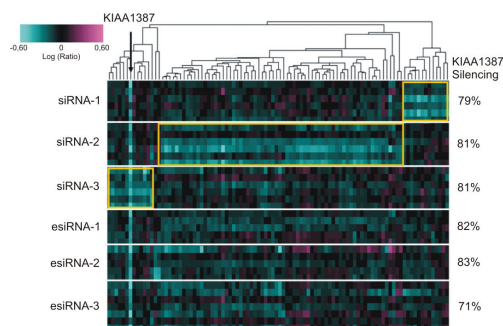


Figure 2a: Microarray analysis of the specificity of esiRNAs compared to siRNA

An alternative concept to the chemical synthesis of siRNAs is the enzymatic digestion of long double stranded RNAs *in vitro*³. In this case a cDNA template is amplified by PCR and tagged with two bacteriophage-promotor sequences. RNA polymerase is then used to generate long double stranded RNA that is homologous to the target-gene cDNA. This RNA is subsequently digested with RNase III from *Escherichia coli* to generate short overlapping fragments of siRNAs with a length between 18-25 base pairs. This complex mixture of short double stranded RNAs is similar to the mixture generated by Dicer cleavage *in vivo* and is therefore called endoribonuclease-prepared siRNA or short esiRNA.

Off-Target Effects

For the production of a high-quality esiRNA a 300-600 base pair long region in the target mRNA is selected that has a high susceptibility for RNAi without homology to other genes of the same species. For the automatic localization of the best target region we have developed the algorithm DEQOR. This algorithm (<http://cluster-1.mpi-cbg.de/Deqor/deqor.html>) initially determines every possible 21mer in a given mRNA. Based on the quality constraints for siRNA design each of these 21mer sequences is assigned an efficacy and specificity score. On the basis of this data the region with the highest percentage of good quality silencers is determined. An esiRNA synthesized from a cDNA representing the DEQOR-predicted region will therefore contain a high percentage of good quality siRNAs⁴ (Figure 2a). In order to evaluate the performance of esiRNAs, we compared the silencing efficacy and specificity of DEQOR-optimized esiRNAs with chemically synthesized siRNAs using microarray expression analysis and quantitative real-time PCR. These studies revealed a similar silencing efficacy of siRNAs and esiRNAs, making them equally suitable for efficient transcript silencing. In contrast, esiRNAs showed a more than 10 fold higher target specificity in comparison to individual siRNAs⁴ (Figure 2b), making esiRNAs the silencing trigger of choice for transcript silencing with minimal off-target effects.

A possible explanation for the increased specificity lays in the complexity of the esiRNA pool. The high numbers of different siRNAs in the mixture share the same on-target but differ in their sequence-dependent off-target signatures. As a consequence, many individual siRNAs add to the overall silencing of the target, while off-target effects are diluted out.

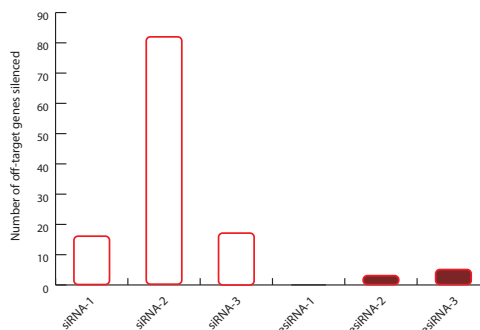


Figure 2b: Microarray analysis of the specificity of esiRNAs compared to siRNA

High-Throughput Production of MISSION® esiRNA

The generation of esiRNAs has been adapted to high-throughput protocols. A production pipeline has been established at the Max Planck Institute for Molecular Cell Biology and Genetics in Dresden. This pipeline allows for rapid generation of genome-wide libraries of esiRNAs at relatively low costs. Sigma-Aldrich® has entered into an exclusive agreement with MPI for manufacture of all MISSION esiRNA libraries.

A human genome-scale MISSION esiRNA library with more than 16,000 esiRNAs and a mouse genome library with over 14,000 esiRNAs are available⁴. Future plans include the building of additional MISSION esiRNA libraries for other model organisms. These libraries will likely represent one of the most specific mammalian RNAi resources available and hence will aid many research groups in carrying out high-quality, large-scale loss-of-function studies.

Literature

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