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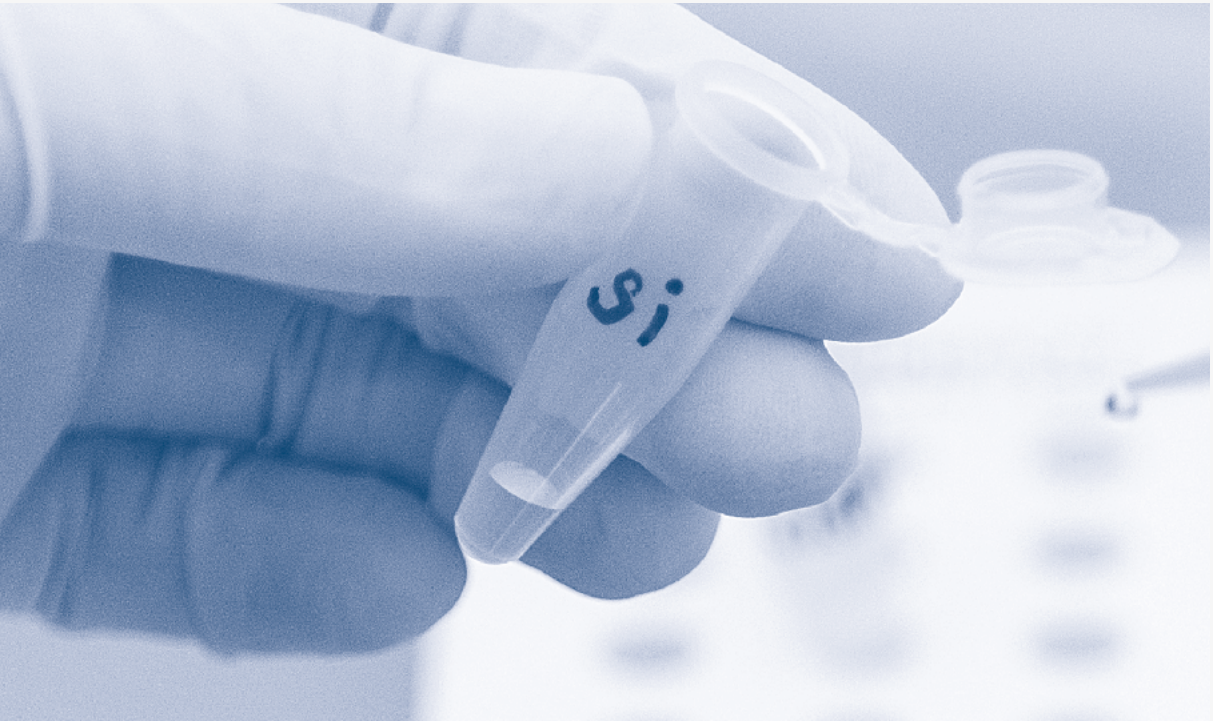
siRNA

Overview and Technical Tips



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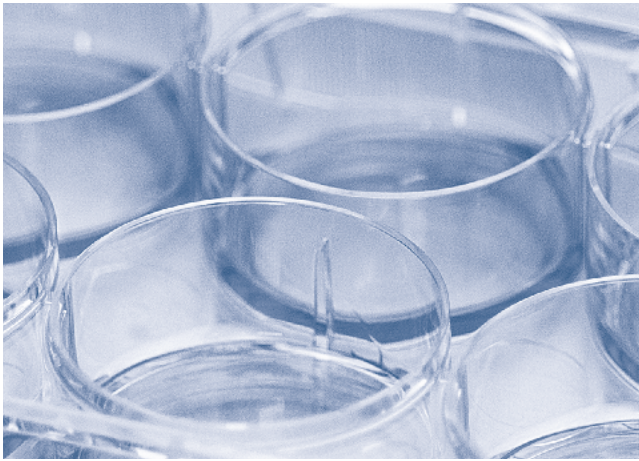

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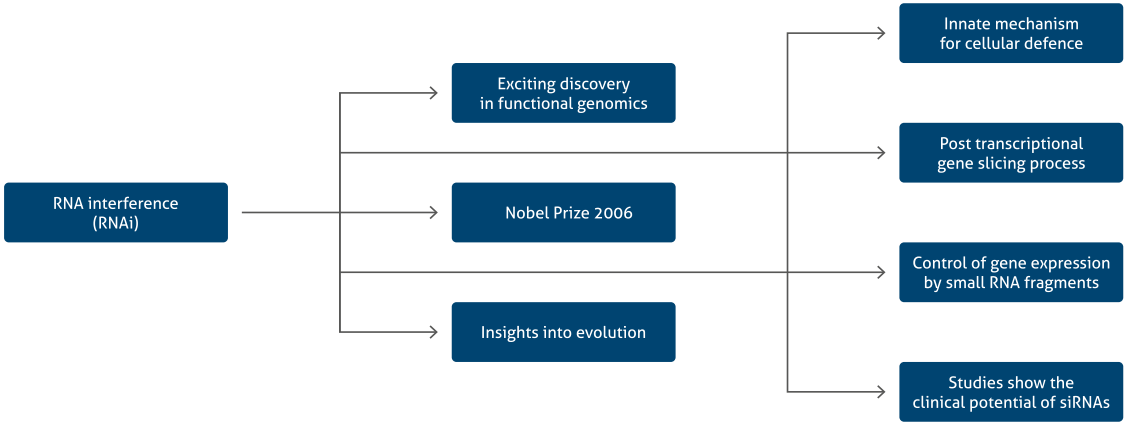
INTRODUCTION

At A Glance



In Conclusion

– **Applications as potential therapeutics and in drug development.**

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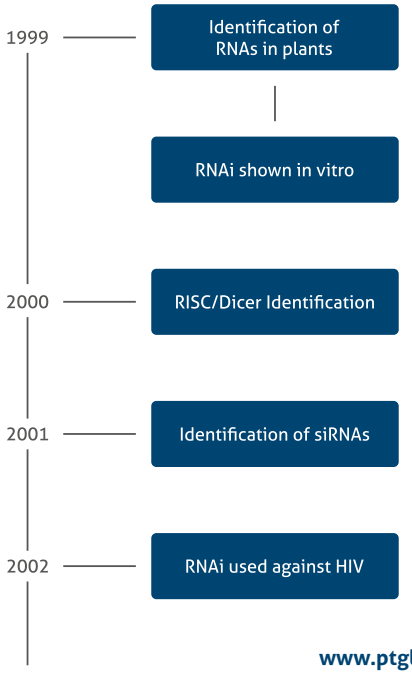
INTRODUCTION

At A Glance

RNAi interference (RNAi):

- RNAi is a post-transcriptional gene silencing process.
- RNAi leads to sequence specific degradation of mRNA.
- Highly specific process.
- Important genome regulation and defence mechanism.



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APPLICATIONS

General Ideas

siRNAs:

- Small interfering RNAs.
- 21–25 nt long fragments.
- Bind to complementary target mRNA.

Advantages of gene silencing:

- Cost effective method.
- Fast design of siRNA, mRNA sequence is needed.
- Highly specific method.
- Downregulation of target genes.
- Helpful tool for the analysis of genes and their functions.
- Powerful tool in gene therapy.

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APPLICATIONS

Big Potential From Small RNAs

Powerful tool for functional genomics:

- An accurate and potent gene silencing method.
- Knock-downs are quite easy.
- Study of functions.
- Variety of controls.
- A lot of published data exist of siRNA targeting specific genes.

"Billion Dollar Breakthrough"
- Fortune

"Technology Of The Year"
- Science (2002)

In Conclusion

- **Small RNAs have been identified to play multiple biological roles.**
- **Currently the most widely used gene-silencing technique.**

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APPLICATIONS

Oncology
- Analysis of signaling molecules.
- Defining oncogenes.

Infectious Diseases
- Virus targeting:
  HIV
  Hepatitis
  Respiratory infections

Stem Cells
- Observation of tumor phenotype.

Hematology
- Designing disease models (hematologic disorders are loss of function diseases).

Other Diseases
- Huntington, Macular Degeneration.

Some siRNA candiates entered clinical trials only 10 years after the discovery of the RNAi mechanism.

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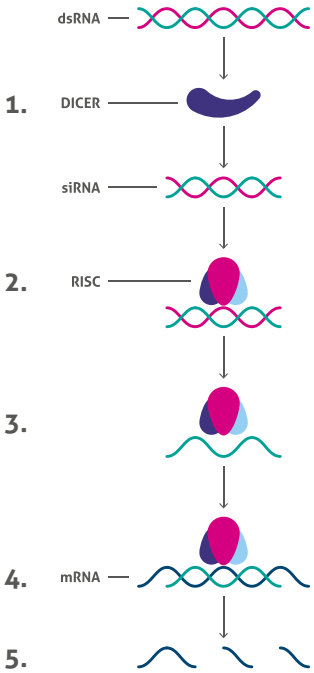
HOW DOES IT WORK?



1. dsRNA is processed into sense/antisence RNA by Dicer.
2. siRNA associates with RISC.
3. Antisense siRNA/RISC complex associates with the target mRNA.
4. RISC cuts the target mRNA.
5. mRNA degradation.

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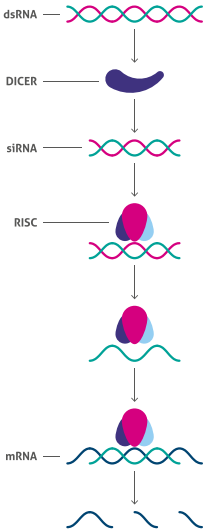
HOW DOES IT WORK?

Inspired by siRNA



Normal gene pool

↓

Mutant gene

↓

Mutant mRNA → Synthetic siRNA

↓

Altered protein → KD of mutant gene

↓

Disease/disorder → Alleviation of disease/disorder

siRNA sequences can be tailored to block disease-causing genes.

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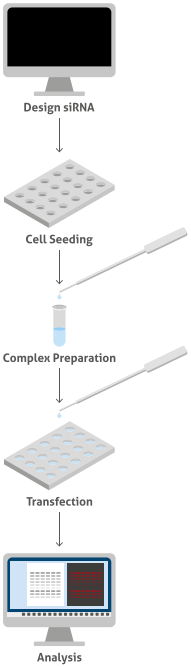
HOW DOES IT WORK?

Protocol Overview

Important factors influencing siRNA experiments
Cell type
Cell growth rate
Cell density
Cell viability
Transfection method
Quality/quantity of siRNA
Time of transfection



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HANDY TIPS

RNase-free Environment

- Use RNase-free tips.
- Use pipettes only for RNA work.
- Use gloves.
- Changes gloves after touching any surface.
- Clean your work surface with a RNase-decontaminating solution or wipes.
- Working in a RNase free zone, no air vents.

Working With A New Target/siRNA/ Cell Type

- Requires multiple test transfections to optimize the best conditions.
- Transfection efficiency should be as high as possible.
- Fluorescently labeled siRNA simplifies targeting of the knock-down effect.

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HANDY TIPS

Validation Of siRNA Data

- Use positive/negative controls.
- Use a second siRNA against the same target.
- Titrate the siRNA concentration.
- Monitor RNA and protein level.

Optimizing siRNA Delivery Into Cultured Cells

- Keep culture conditions consistent.
- Healthy cells.
- Optimal cell density.
- Optimize culture conditions (media, etc).
- Optimize transfection reagent.

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HANDY TIPS

Designing siRNA

- Around 21–23nt long.
- G/C content: 30–50%.
- No basepair mismatch.
- siRNA should not bind to introns.
- No sequence that shows homology other coding sequences (BLAST).
- Work with two or three different siRNA constructs to get reliable results.

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TROUBLESHOOTING

FAQs

FAQs	Recommendations
Serum or serum-free medium?	Transfection reagents require serum-free medium for dilution of the siRNA complex. Serum quality/lot might affect the experiment.
Antibiotics or antibiotics-free medium?	Dependent on combination of cell type and transfection reagent: Cell permeability is very sensitive during transfection. Antibiotics can cause cell death.
Replacement of transfection medium?	In general it can be replaced after 6h as at this time the transfection will be completed. Not required step, depends on tranfection reagent.
Storage of siRNA reagents?	siRNA: frozen (clean tube before use), reagents: 4°C.

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TROUBLESHOOTING

FAQs

FAQs	Recommendations
siRNA concentration?	The lowest functional siRNA amount evaluated in test transfection should be used for the experiments.
Duration of siRNA silencing?	In general, the silencing effect can be observed earliest after 24h. It retains cell type dependent for 4–7 days.
Stock solution of siRNA.	Consult manufacturer's instructions, generally between 50–100 μM.
Working solution of siRNA?	Has to be optimized. Generally, a rage of 5–100 nM is used.

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TROUBLESHOOTING

Controls

Control type	Recommendations
Read-out	Analyse mRNA and protein level.
Use lowest effective siRNA concentration	Higher concentrations of siRNA lead to more off-target effects.
GAPDH siRNA control	GAPDH is high expressed in virtually all mammalian cells. It is a useful tool for evaluating transfection efficiency and cell viability.
Transfection of two or more different siRNA against the target of interest	Helps to identify sequence specific off-target effects.
New target/siRNA/cell type	Requires multiple test transfections to optimize the best conditions.
Toxic impact	Cell sample just treated with the transfection reagent.
Endogenous protein level	Non-treated/transfected cell sample.

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TROUBLESHOOTING

Controls

Control type	Recommendations
Titrate siRNA amount	Use different concentrations.
siRNA conjugated to a fluorescence label	Microscopic evaluation of transfection efficiency.
Include a cell line with a known high transfection efficiency.	If the cell line of interest is difficult to transfect, use a control cell line.
Scrambled siRNA	Helps to differentiate between sequence-specific silencing from non-specific silencing affects.
Time points	Check silencing affects at different post-transfection time points.
Recombinant protein	Re-introduce the protein by transfection with the recombinant protein.

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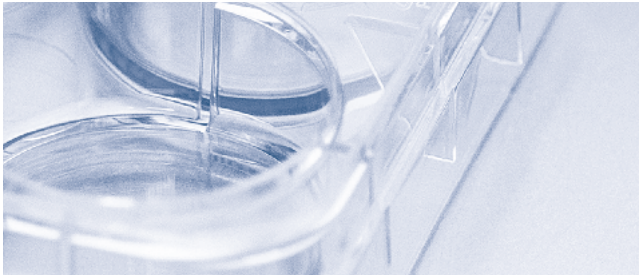
TROUBLESHOOTING

Unhealthy Cells

Recommendations
Passage cell lines frequently, do not allow cells to overgrown.
Passage fresh cells lines at least 1–2 times before the experiment.
Usually a high cell density is needed > 70 %*.
Transfect cells at the same passage numbers.

** Cell density and passage number depends on the type of cell line.*



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CONCLUSIONS

- RNAi is an important part of the **cellular machinery** that provides viral immunity and mechanism for the control of gene expression.
- siRNA triggers function in the RNAi mechanism result in **gene suppression.**
- Potential therapeutic application include **viruses and cancer.**
- Technical challenges:
  - Stability
  - Targeting
  - Off-target effects
  - Immune response
  - Delivery
- The field is relatively young and much remains to be discovered.

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FURTHER REFERENCES

- K. Huppi, S. Martin, et al., Defining and Assaying RNAi in Mammalian Cells. Mol. Cell. 17(1), 1-10 (2005).
- Whither RNAi? Nat. Cell. Biol. 5(6), 489-90 (2003).
- L.P. Lim, N.C. Lau, et al., Microarray analysis shows that some microRNAs down regulate large numbers of target mRNAs. Nature. 433(7027), 769-773 (2005).
- F. Barthel et al, Gene transfer optimization with lipospermine-coated DNA, DNA Cell Biol. 12, 553–60(1993).
- P.L. Felgner et al, Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. J. Biol. Chem. 269, 2550–61 (1994).
- D.H. Kim et al., Nature Reviews Genetics 8, 173–184 (2007).
- S. Loyter et al., Mechanisms of DNA uptake by mammalian cells: Fate of exogenously added DNA monitored by the use of fluorescent dyes. Proc. Natl. Acad. Sci. USA 79, 422–6. (1982).
- D.S . Anson et al., The use of retroviral vectors for gene therapy-what are the risks? A review of retroviral pathogenesis and its relevance to retroviral vector-mediated gene delivery. Genet. Vaccines Ther. 2, 9. (2004).

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