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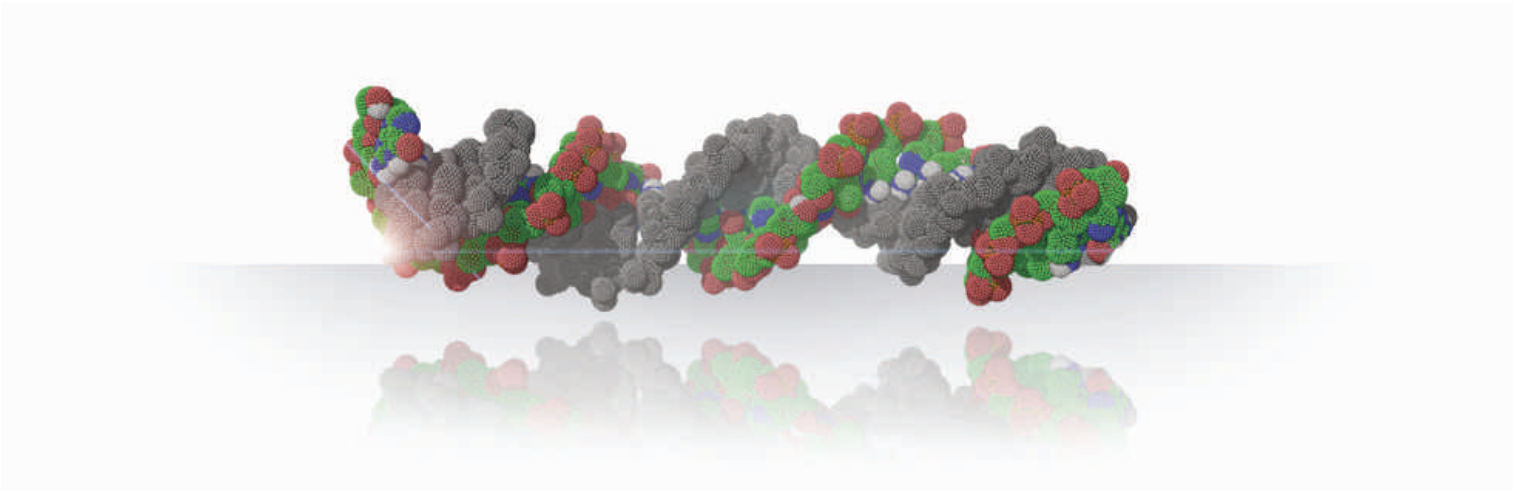


NOVEL TECHNOLOGY:

asymmetric siRNA Duplexes

[A-siRNA]

Novel **siRNA** with chemical and structural modifications to ensure asymmetric and potent RNAi activity.

ADVANTAGES OVER TYPICAL siRNA:

Greater Specificity of Suppression
Reduce Off-Target Suppression
Reliable for Drug Development
Degradation Resistant invitro/invivo
Superior Design and Chemistry

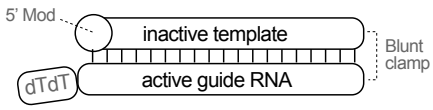
www.oligoengine.com

Asymmetric siRNA Duplex:

Right on target for Research and Therapeutics.

The A-siRNA Duplex was developed in 2003 by Oligoengine scientists as a more precise RNAi trigger than dsRNA based siRNA. The structure contributes to the asymmetric loading into RISC and the inactive sense strand prevents 'sense-strand' based off-target effects caused by canonical siRNA.

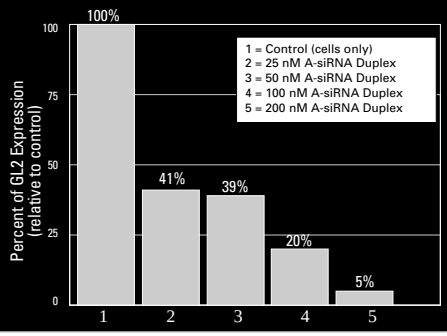
A-siRNA Duplexes have been used to specifically inhibit the expression of proteins in vitro, such as the GL2 luciferase protein. The A-siRNA Duplex is especially useful for in vivo studies and therapeutics development (see inset on opposite page).



Suppression of Firefly Luciferase Exppression

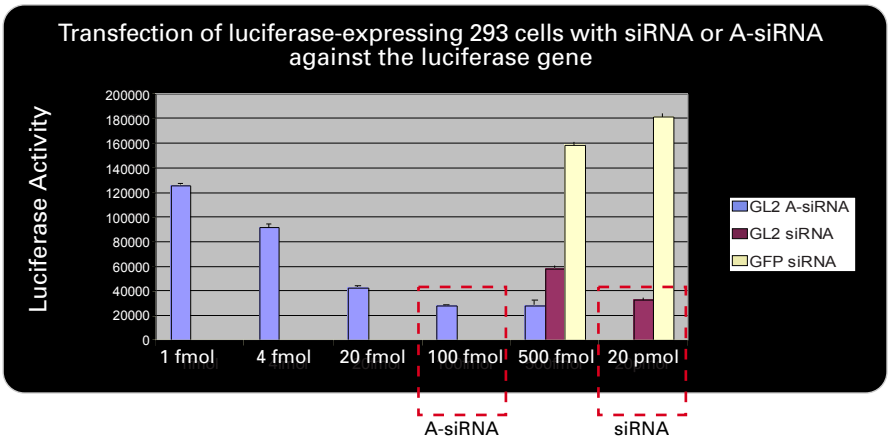
Effective Knockdown

To test the effectiveness of the A-siRNA Duplex in targeted gene suppression, an A-siRNA Duplex was designed against GL2 form of the firefly luciferase and tested in 3T3-Lux cells. As shown at right, GL2 expression was suppressed over 90% with the A-siRNA Duplex compared to a control lacking siRNA. Multiple runs of this same experiment demonstrated that RNAi directed by A-siRNA Duplexes are effectively repeatable and titratable.



Based on fast, proven, and scalable synthesis techniques, the A-siRNA technology provides an ideal platform for commercial applications. The dependability and cost-effectiveness of A-siRNA synthesis for target discovery and genome-wide RNAi applications supports the use of "just-in-time" arrays to vastly improve the drug development process.

Invitro Comparison



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A-siRNA Asymmetric siRNA Duplex

Removing off-target effects from RNAi

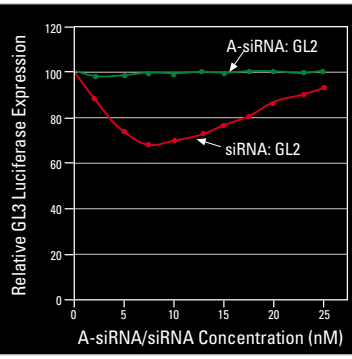
To prove the negative effect of typical siRNA, Oligoengine first designed an siRNA that shared GL2/GL3 complimentarity in both the sense and antisense strands. The ability of an siRNA to function in either direction would result in an undesirable suppression of GL3.

The same construct was tested using the Asymmetric siRNA composition which illustrated the benefit of de-activating the sense strand (template) of siRNA and prevention of sense-strand based off-target suppression.

Comparison of Specificity: A-siRNA Duplex vs. siRNA

Eliminates off-target Suppression

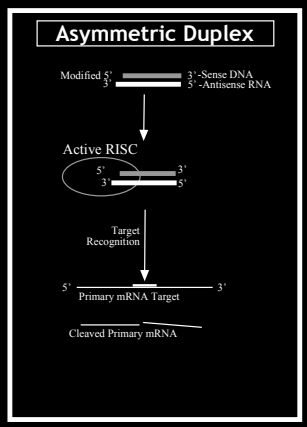
To demonstrate the specificity of the targeted A-siRNA Duplex response, NIH3T3 fibroblast cells that stably express the GL3 form of the firefly luciferase were transfected with varying concentrations of A-siRNA Duplex and chemically synthesized siRNA – both targeting the same region of the GL2 luciferase mRNA. In contrast to the A-siRNA Duplex, which showed no significant knockdown of GL3 luciferase expression, the siRNA demonstrated an "off-target" suppression of the GL3 protein, as shown in the chart at right.



Additional genome-wide assays have shown the specific signature of A-siRNA.

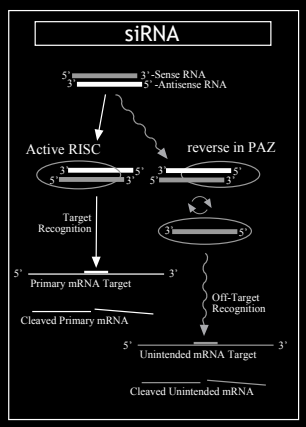
Efficient Processing: A-siRNA Duplex vs. siRNA

A-siRNA vs. siRNA processing



A-siRNA provides advantages of potency and specificity when compared to other RNAi methods.

vs.



- **A-siRNA's non-functional sense results in elimination of "Off-Target" suppression of genes complimentary to sense RNA.**
- **100% processing effiency to target mRNA due to asymmetric structure and end thermodynamics.**
- **More stable in Cytosol and Serum than dsRNA.**

- **siRNA results in "Off-Target" suppression of genes partial complimentary to sense RNA.**
- **Overall molecule is double-processed (forward/reverse) resulting in decreased efficiency.**

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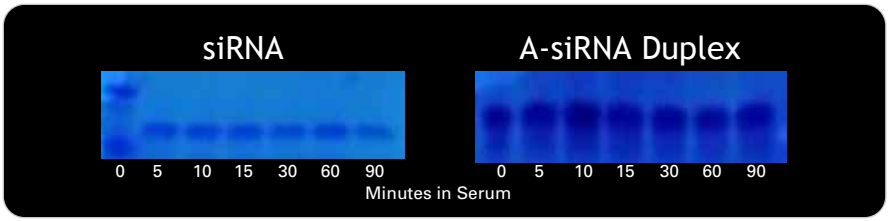
A-siRNA Asymmetric siRNA Duplex

Invivo use of Asymmetric siRNA Duplexes.

The A-siRNADuplex is more effective in-vivo than siRNA due to it's stability and processing efficiency. A direct comparison was performed against siRNA for serum stability and invivo efficacy by a third party.

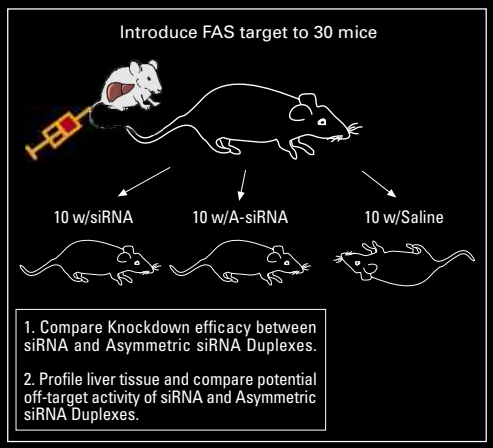
Serum Stability



Hydrodynamic tail vein injection of 10mg of siRNA or A-siRNA against the Fas receptor mRNA.

In-vivo against fas

Results from studies at an independent, third-party research institution indicate that intravenous injection of A-siRNA Duplexes targeting the Fas gene in mouse hepatocytes reduces Fas mRNA levels and expression of Fas protein in the liver. The goal is to demonstrate the potential therapeutic value of the A-siRNA Duplex; in this case, in preventing and treating acute and chronic liver injury induced by viral and autoimmune hepatitis, alcoholic liver disease, acute and chronic liver failure and rejection of liver transplants.



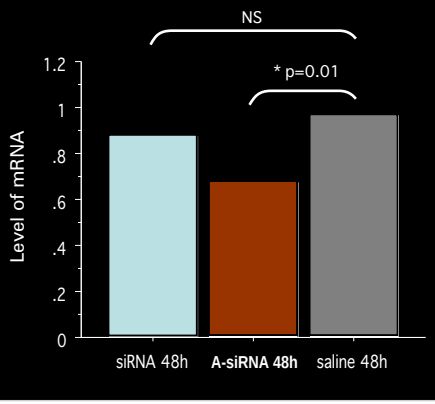
1. Compare Knockdown efficacy between siRNA and Asymmetric siRNA Duplexes.

2. Profile liver tissue and compare potential off-target activity of siRNA and Asymmetric siRNA Duplexes.

In-vivo results

Sacrifice at 48 hours.

Analysis by real time PCR of Fas mRNA.



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A-siRNA Asymmetric siRNA Duplex

Asymmetric siRNA Duplex Summary.

Summary

- More Potent
- More Specfic
- More Stable

Prices start at $399.00 per 40 nm duplex. Please visit http://www.oligoengine.com/oe3 to design and order your A-siRNA Duplex or call Customer Service.

Halo-Bio
RNAi THERAPEUTICS, INC.

Intellectual Property

The A-siRNA Duplex was developed and is supplied exclusively by OligoEngine's parent company, Halo-Bio RNAi Therapeutics.

Like small interfering RNA, the A-siRNA Duplex consists of two short polynucleotides, custom synthesized to target a specific gene sequence, and annealed in a duplex molecule. And like siRNA, the A-siRNA Duplex has been demonstrated as a potent RNAi trigger.

However, important differences in structure, chemistry and configuration exist between the A-siRNA Duplex and "standard" siRNA molecules. As such, the A-siRNA Duplex is the subject of a patent application filed by Oligoengine scientists with the United States Patent and Trademark Office (patent pending).

Licensing

Licensing of A-siRNA for Target Validation, Drug Discovery, and Therapeutic Applications

The A-siRNA Duplex has utility across all stages of the gene-to-drug pathway. Commercial parties can obtain a license of the A-siRNA technology for the purposes of performing target validation, drug discovery and development, or therapeutics applications. In addition, any products, including cell lines, transgenic animals and therapeutic compounds developed using the A-siRNA Duplex will be considered as licensed products, upon which Halo-Bio will seek to support such uses by licensing.

Terms of a commercial license are available for various uses and geographies. For specific licensing information, please contact an OligoEngine sales manager or Halo-Bio RNAi Therapeutics directly:

http://www.halo-bio.com

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EXCLUSIVE:

Asymmetric siRNA Duplex™

Advanced RNAi Kit

Order the **A-siRNA Duplex** to achieve greater suppression, specificity and stability over typical siRNA.

KIT CONTENTS:

- **40 nMole of A-siRNA Duplex**
- **Dilution Buffer**
- **Optional positive or negative controls**

starting at

$399.00

fax-back quote form

Fax it back, we'll take care of the rest!

Fax to 206.254.0300

IDENTITY:

NAME: _____

PHONE: _____

EMAIL: _____

GENE OR SEQUENCE:

No Additional Charge for Design. Results guaranteed to acheive greater than 70% suppression.

GENE(S): _____

_____

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N19 SEQUENCE(S): _____

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KIT OPTIONS:

- ☐ ADD CUSTOM NEGATIVE CONTROL $199
- ☐ 5' LABEL 20 nM of Si2 Duplex $199
  - ☐ 6-FAM
  - ☐ HEX
  - ☐ TET
  - ☐ TAMRA
- ☐ POSITIVE/NEGATIVE CONTROLS
  - ☐ 5 nmoles anti-GFP duplex $99
  - ☐ 5 nmoles Mamm-X Scramble duplex $99

A-si**RNA** Duplex

For more information and licensing terms:
1.800.516.5446

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