white paper

iRNAchek™

A complete high throughput siRNA designer

Insight and discovery are functionally separable. One precedes the other. Insight can happen every day. Discovery does not. Insight takes more intelligence, but it is discovery that is rewarded...

Francis Crick

A Revolution in Functional Genomics

The "Genomic Epoch" was ushered in by rapid advances in nucleic acid sequencing, making it possible to sequence complete genomes relatively quickly. The Human Genome Project accelerated development of the various techniques and technologies. The post-genomics era is characterized by a holistic approach to the study of biological systems and its use in the area of drug discovery, demanding many new analytical tools.

Functional Genomics prefaced a more holistic approach to the study of biological system of rules and its use in the field of therapeutics, involving many new analytical tools.

As scientists have overcome technical vaults and learned to harness RNA versatility, its promise as both a pharmaceutical agent as well as target has grown substantially. More recently, small interfering RNA (siRNA) and RNA interference (RNAi) have demonstrated the potency of double-stranded molecules to modulate gene expression. Collectively, these developments have spurred interest in RNA in therapeutics.

RNAi is one of the most interesting breakthroughs of functional genomics in the past decade and is rapidly becoming a significant method for analyzing gene functions in eukaryotes. It holds promise for the development of remedial gene silencing.

The Ultimate Gene Silencing Technology- RNAi

RNAi is a weighty new approach for attaining aimed gene silencing of disease-associated genes, using double stranded RNA (dsRNA) as the triggering agent. RNAi technology has been shown to work in a wide range of animal models and was voted the top scientific achievement of 2002 (Science Magazine 2002).

RNAi is a naturally occurring, highly catalytic gene regulation system thought to have evolved primarily as a defense mechanism against molecular pathogens. The pathway mounts a powerful cellular response to the presence of dsRNA molecules wherein complementary messenger RNA, whether of foreign or endogenous origin, is completely degraded, thereby silencing expression of the gene. Thus, with introduction of desired sequences containing dsRNAs into cells or organisms, the RNAi pathway can silence any target gene with maximal and tightly controlled specificity.

Used as a research tool with even therapeutipotential, RNAi offers an novel combination of:

- high potency, specificity and scalability
- wide cross species applicability
- excellent experimental reproducibility

As a result, RNAi is identified as the method of choice with a potential for a wide range of biomedical applications, replacing conventional antisense and ribozyme technologies. Apart from their role in gene silencing, siRNAs have been ascertained to play diverse biological functions like antiviral defense, transposon silencing, and genomic rearrangements. This functional heterogeneity has illustrated the importance of siRNAs within cells and has also aroused interest in their detection across species and tissues.

The Future of Drug Therapy

Recognition of gene function leads to the possibility of RNAi based therapeutics. This is no longer a distant dream, given RNA's inherent ability for selective gene silencing, fluxed with advances in nucleic acid chemistry and delivery technology made through crusades in oligonucleotide-based therapeutics.

RNAi has emerged from impressive findings as a mighty tool in reverse genetics. With RNAi libraries becoming available, much more will be accomplished. Although the technical challenges are not trivial, they are tractable and the quest for RNAi-based therapeutics looks bright with the ripening of nucleic acid delivery technology. RNAi technology has arisen at the right point in the long journey of oligonucleotide-based therapeutics. Small RNA's big quest is likely to be breathtaking.

The Science Behind RNAi

RNA interference (RNAi) is a phenomenon in which

- The insertion of double-stranded RNA (dsRNA) into a diverse range of organisms and cell types causes degradation of the complementary mRNA.
- In the cell, dsRNAs are cleaved into short 21-25 nucleotide siRNAs, by a ribonuclease known as Dicer.

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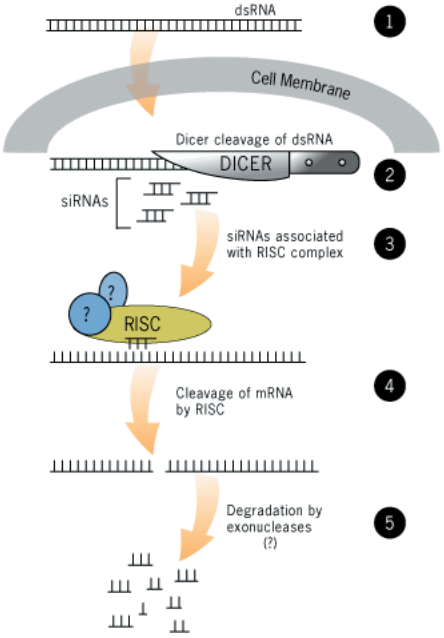
Arrest the Expression, Arrest the Disease. Small RNA's big quest is in all likelihood to be breathtaking.

If you do not want a protein, KILL THE MESSENGER!!!

The application of RNAi to environmental health research will help address critical questions at a genome-wide level.

- An unwound strand of siRNA is subsequently housed in RNA-induced silencing complex (RISC) of protein molecules.
- Activated RISC then binds to complementary transcript by base pairing interactions between the siRNA antisense strand and it's target mRNA.
- The bound mRNA is cleaved and is made vulnerable to RNAses. Silencing thus achieved is as shown below.



In mammals, introducing dsRNA longer than about 30 nucleotides has not been successful due to invoking of nonspecific responses. However, transfection of synthetic 21 nucleotide siRNA duplexes into mammalian cells specifically suppresses endogenous genes. Challenges still persist as RNAi research moves from mice to man. While finding right genes to target is critical, of equal importance is the current focus on delivery of siRNAs to target cells. In addition, short and long-term side effects of RNAi therapy remain largely unknown.

The siRNA Design - A Challenge

Various methods for delivering siRNA include chemical synthesis, in vitro transcribed expression cassettes and siRNA expression vectors. Regardless of delivery mode, quality of specific silencing achieved is still a design-dependent constraint.

On selection of gene to silence, user needs to identify an ideal siRNA with numerous potential sites to bind on target mRNA. Effective silencing criteria identified to-date include factors such as GC% in range of 35 to 60 and lack of secondary structure that make target regions inaccessible.

On the same token, criteria that ensure specific silencing include sequence dependent factors such as avoiding siRNAs with strong homology to other gene sequences, carrying motifs recognized for occurrence within genome, and user-dependent annotation, to oligo-design based criteria such as absence of sequence patterns like G-tetrads.

Properties that characterize these effective sites have been analyzed. However, there is still ambiguity to warrant experimentation with at least three siRNAs to ensure silencing success. Accepting this has led to an an understanding that any software that designs siRNAs must provide user facility and flexibility to define emerging criteria that select valid siRNAs. Also appreciated was the need for a statistics module that could aid in identification of these probable success criteria, both from design and experimental data.

These were the key focus of design environment of iRNAchek™, as described further in the document.

Our Solution to the Challenge of siRNA Design

iRNAchek™ provides molecular biologists the essential tools and analytics necessary to design siRNAs. Ocimum Biosolutions has invested significant time to make iRNAchek™ powerful and user friendly, and believe that users will significantly speed up their research in the field of siRNA design.

Comprehensive and intuitive design environment of iRNAchek™ helps achieve the above through organization of sequence data, designing and filtering of siRNAs, and further tracking and analysis of successful templates. The tool provides user with great flexibility to define search templates; set regions of interest based on presence of Open Reading Frames (ORFs), untranslated regions (UTR), introns and exons, secondary structures, and motifs; apply mRNA stabilizing modifications; and more importantly carry out a post-experimentation analysis of successful siRNA for refining design criteria or constraints.

iRNAchek™ is the tool for those designing siRNAs in-house, be it a pharmaceutical industry or an individual researcher.

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Small RNA, big challenge…

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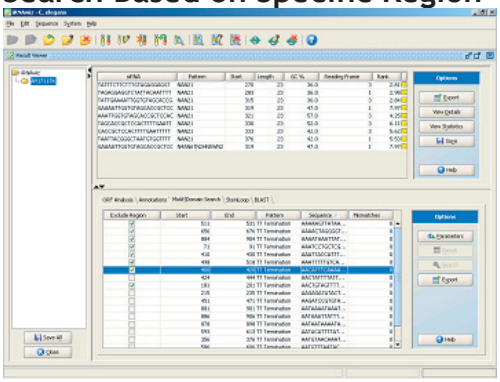
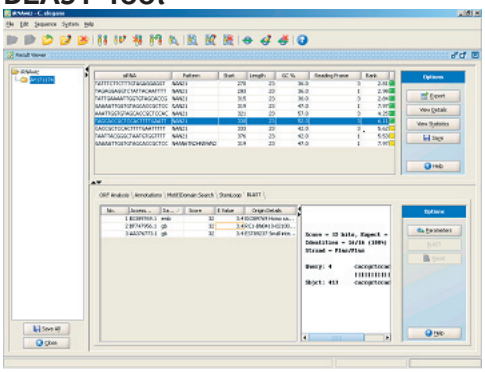
Important Features of iRNAchek™

Search Based on Specific Region



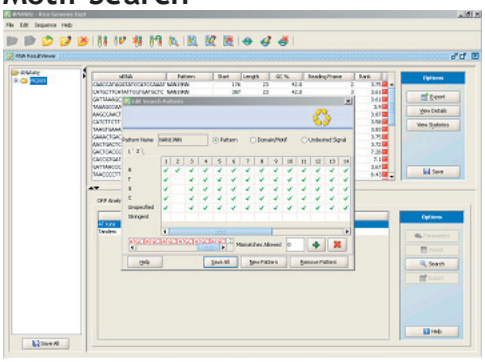
iRNAchek™ provides a facility to scan and filter siRNAs based on regions of interest defined by presence or absence of open reading frames, introns, exons, untranslated regions (UTR) and other user-defined or imported sequence annotations. There is also a provision to eliminate templates from specific regions.

BLAST Tool



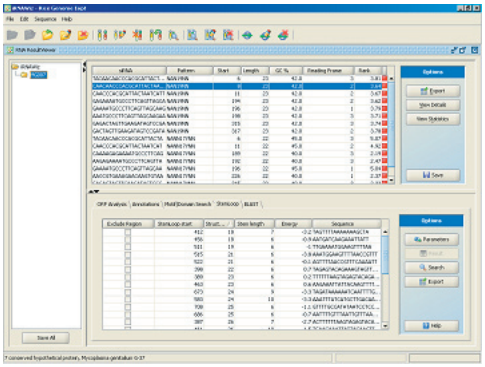
iRNAchek™ BLAST utility aids researcher to automatically filter siRNAs based on degree of non-target homology. Using this tool, users can search for potential matches from both NCBI databases or proprietary local databases. Further, there is a provision for setting one's own filter criteria.

Motif Search



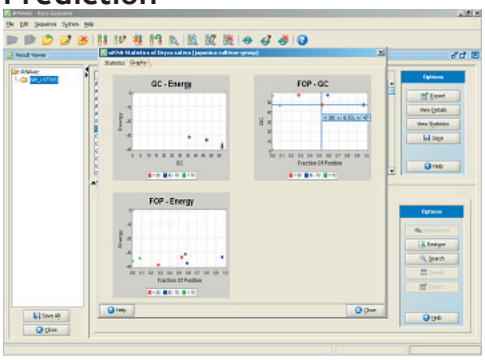
It is essential that siRNA do not target functionally significant regions across genome. iRNAchek™ efficiently eliminates for such possibilities by Motif-based filtering. Users are allowed to define motifs of interest from the tool.

StemLoopSearch



Efficacy of siRNA could be to limited by occurrence of strong local secondary structures making on regions on target mRNA inaccessible. iRNAchek™ identifies and lists all possible hairpins with facility to sort and filter by destabilization energies calculated from values in Turner tables, based on the nearest neighbor algorithm.

Statistical Analysis for Knockout Prediction



iRNAchek™ maintains and imports libraries of selected siRNAs. These templates may further be analyzed for identifying user-defined or design-time success criteria. A feature essential to design siRNA with better efficacy in targeted system. More important is the facility to predict success of imported siRNAs based on such identified parameters.

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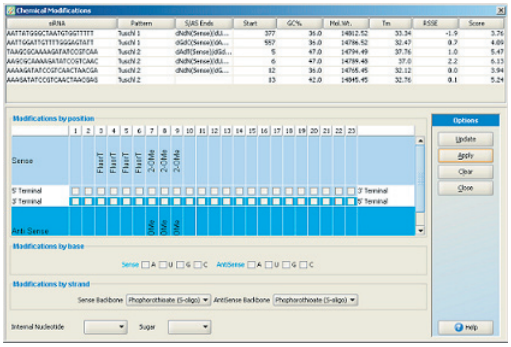
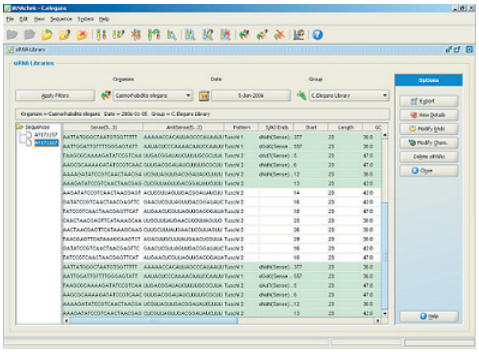
Chemical Modifications



iRNAchek™ allows users to enhance siRNAs using user-defined modification rules. This module facilitates changes in overhang length,varying bases of overhangs to include deoxy bases, applying modifications at backbone, sugar, and nucleotide levels and further, addition of terminal bases. These modifications may be applied by position, by base, and by strand.

siRNA library

iRNAchek™ catalogs unmodified and modified siRNAs saved as libraries. Users may apply various filters to select siRNAs for performance analysis - both by design and user-defined experimental criteria.



Technology Used to Make All This Possible

iRNAchek™ has been developed using advanced Java Technology and is platform independent. It can be used on varied operating systems like Windows, Linux, Unix or Macintosh. iRNAchek™ comes with an integral database that supports any of the several database systems such as Oracle, MySQL etc. with a friendly Graphical User Interface.

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