Product Guide

RNA Interference, siRNA, shRNA, Dicer, RISC,
siRNA Design & Search, shRNA Design,
Chemical Synthesis of siRNA, shRNA DNA Oligos
RNAi Explorer™ Kit, siRNA Explorer™, shRNA Explorer™

RNAi Explorer™

Gene Link™

RNA Interference

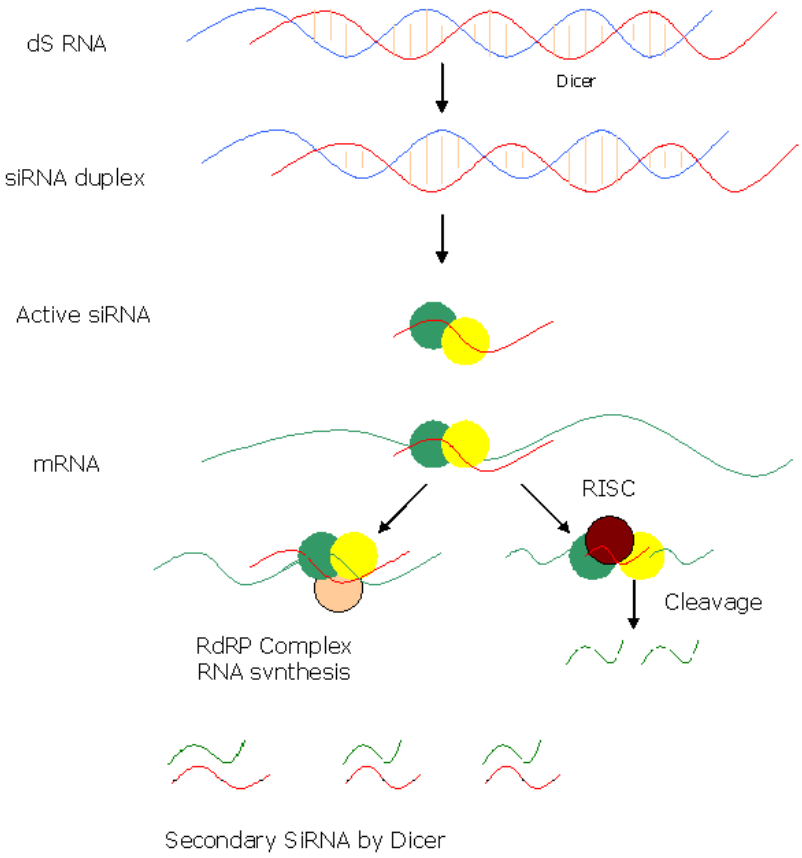
RNAi Explorer™

RNAi Explorer™ from Gene Link is a series of product and services to aid researchers in exploring RNA interference. The online search and design tools for siRNA or shRNA are developed based on current known guidelines. After selection of appropriate siRNA or shRNA, you can place an order. You are encouraged to try the robust search and design algorithm.

How does RNAi work?

Nature Reviews Genetics 2001 Animation Nature Reviews Genetics 2004 Animation

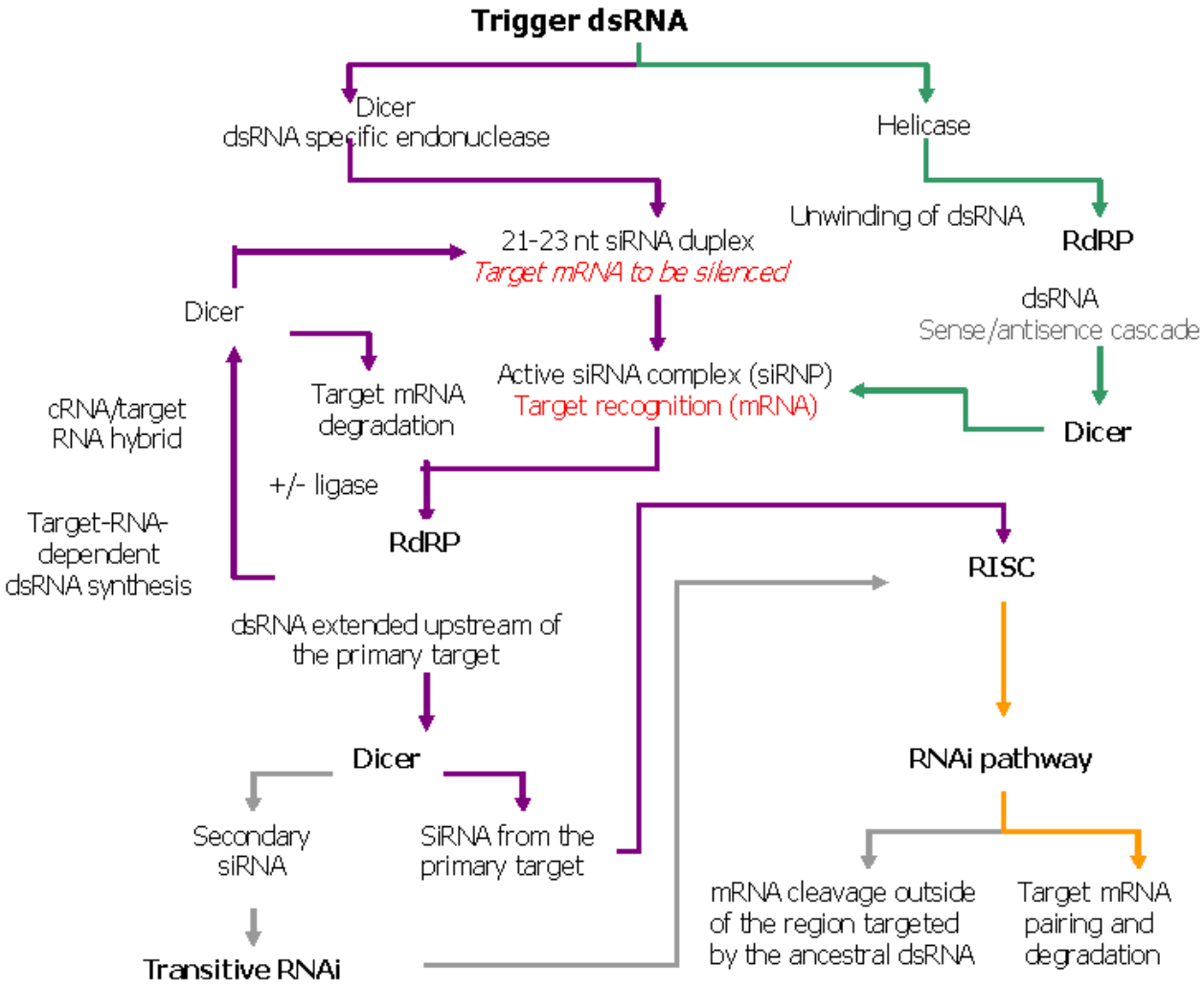
Recent advances in molecular biology have shown that gene expression can be effectively silenced in a highly specific manner through the addition of double stranded RNA (dsRNA) (1-3). The term RNA interference (RNAi) was coined to describe this phenomenon and, while the mechanism was originally observed in plants and later in the worm Caenorhabditis elegans, subsequent studies have shown that RNAi is present in a wide variety of eukaryotic organisms including mammals (4-6). For the most part, it is believed that RNAi serves as an antiviral defense mechanism although there is preliminary evidence that it also plays a role in the formation and maintenance of heterochromatin during mitosis and meiosis (7,8).



Once dsRNA enters the cell, it is cleaved by an RNase III –like enzyme, Dicer, into double stranded small interfering RNAs (siRNA) 21-23 nucleotides in length that contain 2 nucleotide overhangs on the 3' ends (9-11). In an ATP dependent step, the siRNAs become integrated into a multi-subunit

protein complex, commonly known as the RNAi induced silencing complex (RISC), which guides the siRNAs to the target RNA sequence (12). At some point the siRNA duplex unwinds, and it appears that the antisense strand remains bound to RISC and directs degradation of the complementary mRNA sequence by a combination of endo and exonucleases (13).

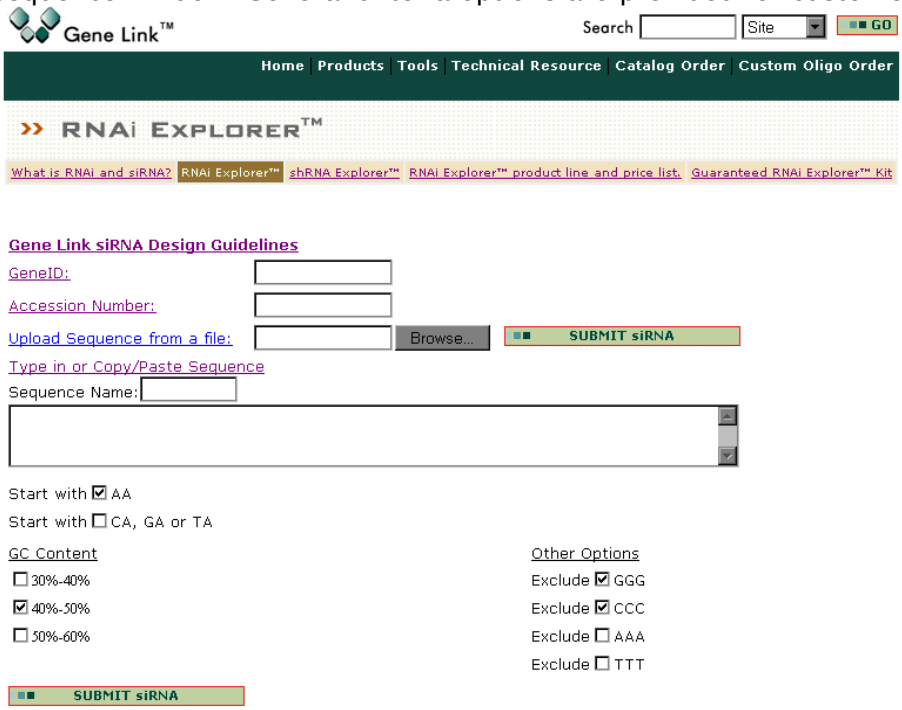
Preliminary studies in mammalian systems using long dsRNAs to initate the RNAi response failed because they led to the induction of a non-specific Type I interferon response that produced extensive changes in protein expression and eventually resulted in cell death (14,15). Subsequent studies, however, using synthetic, short double-stranded RNAs that mimic the siRNAs produced by the enzyme dicer, sequence specific gene silencing could be achieved in mammalian cells without inducing the interferon response (6,16). siRNA technology is now extensively recognized as a powerful tool for the specific suppression of gene expression and is presently being used by researchers in a wide range of disciplines for the assessment of gene function.



RNAi: Dicer and RNA directed RNA Polymerase (RdRP) Action

siRNA Explorer™

siRNA Explorer™ is an online tool for automated search and design of siRNAs. The search can be initiated by either entering the accession number, gene ID, uploading a sequence file or by pasting a sequence in the sequence window. Several criteria options are provided for customer optimization.



How are effective siRNA designed?

siRNA Design Strategies:
Default parameters: AA(19mer)

The default criteria selects target sequences of 21 nucleotides that begin with AA and are located within a region of the coding sequence that is within 50-100 nucleotides of the AUG start codon and within 50-100 nucleotides from the termination codon. The presence of AA at the start of the sequence allows for the use of dTdT at the 3'-end of the antisense sequence. The sense strand can also be synthesized with dTdT at the 3' end, because only the antisense strand is involved in target recognition. The use of dTdT reduces the cost of synthesis and also makes the siRNA duplex more resistant to exonuclease activity. Because a number of reports have demonstrated that the presence of AA at the beginning of the target sequence is not an absolute requirement, the selection program includes the option to search for sequences that begin with other nucleotide pairs.

GC Content

The G-C content of the sequence is also used as a condition for selecting target sequences. Ideally the GC content will be less than 50%, although successful gene silencing has been reported with siRNAs that have G-C contents between 50 and 60%. The default parameter selects for a G-C content in the 40-50% range, however, options are available that allow for selection over wider ranges.

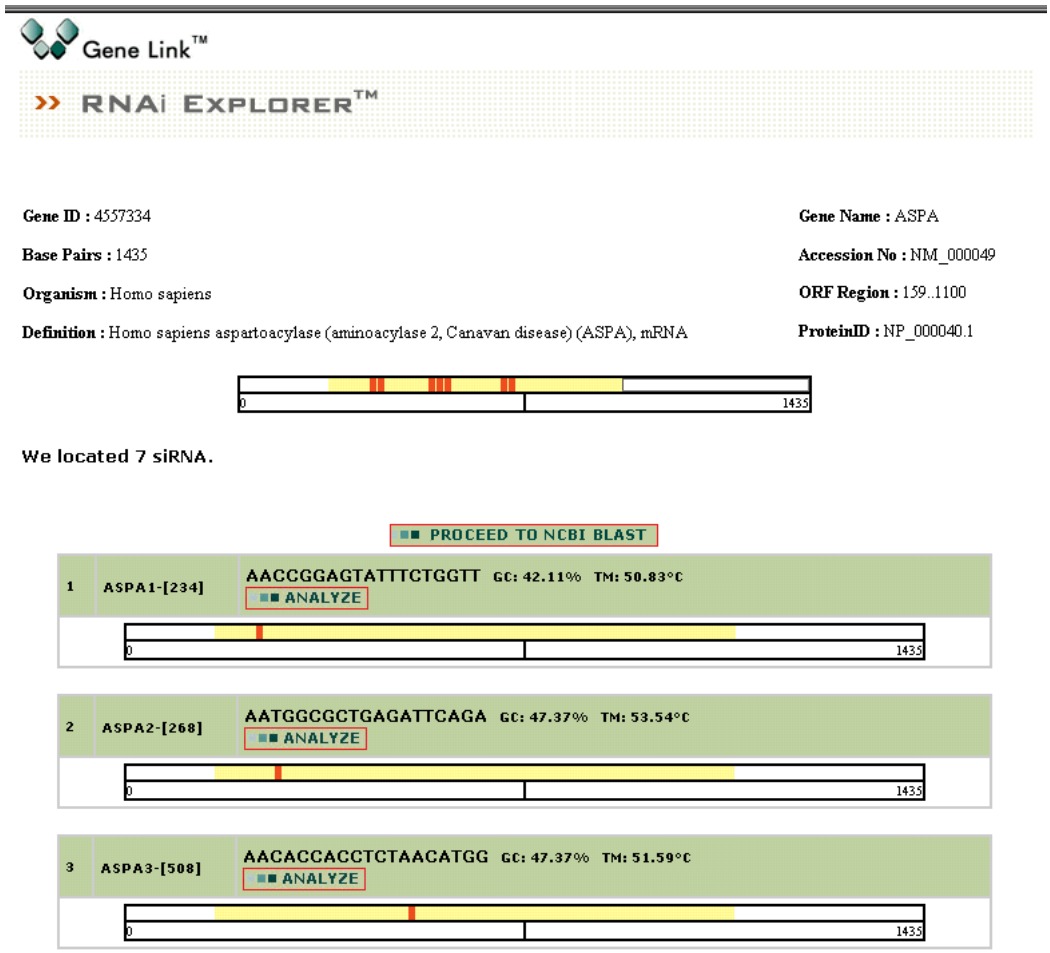
Stretches of Nucleotide Repeats

The default mode avoids sequences with repeats of three or more G's or C's, as their presence initiates intra-molecular secondary structures preventing effective siRNA silencing hybridization. As

an option, repeat stretches of A's and T's can also be eliminated, as they tend to reduce the specificity of the target sequence. If possible, this option is highly recommended.

Blast Search

Once a target sequence has been chosen, a BLAST search is initiated to ensure that your target sequence is not homologous to other gene sequences. Target sequences that have more than 15 contiguous bases pairs of homology to other genes in the NCBI database are eliminated.



Why use fluorescent and modified siRNA's?

It is not essential to monitor the subcellular localization of siRNA after transfection. When desired, the siRNA can be labeled with fluorescent dyes to track the delivery and uptake of siRNA. Usually after uptake the siRNA are present free in the cytoplasm and in complex formation with proteins in the endosomes.

The siRNA can be modified with various other modifications like 2'O methyl RNA, biotin or digoxigenin based on the researchers need. All these modifications are available from Gene Link. Please click here to see the list of modifications.

What are my siRNA delivery options?

Delivery of siRNA directly in cells can be achieved by using microinjection or electroporation. Another popular option is the use of transfection reagent. Several companies offer specialized siRNA-delivery reagents. Please consult the transfection reagent vendor's protocol for detailed information for the exact requirements and procedure. Careful optimization of variable factors should be ensured for all initial transfection experiment. It is based on this and further optimization that reproducible gene knock out results will be obtained. Usually RNAi effect is seen within 4 hours and the maximum down regulation observed in 24-48 hrs. The effect lasts several cell generations and from 4-10 days depending on cell culture type.

How does purity of synthetic siRNA's affect RNAi?
RNAi is a sequence specific chain of events. Chemical synthesis of siRNA's is based on coupling of bases to yield a particular sequence. The yield and purity depends on the coupling efficiency. Gene Link siRNA's can be used without further purification, but Gene Link recommends purified siRNA's for use in transfection.

What concentration of siRNA is most effective?
As low as 1 nM concentration of siRNA have been shown to be effective in exhibiting RNAi. Initial experiments should be done at varying concentrations from 1-10 nM. Some reports have used as high as 25nM concentrations. High quality siRNA's should be used.

What is the optimal cell density for transfection?
This is another variable that has to be optimized and then maintained. A good starting point is 60-70% confluent cells. Time points should be taken after transfection to determine the maximum inhibition. Start at 4hrs and end at 72hrs initially.

How do I quantify down regulation?

RNAi down regulates a gene function without actually interacting with the gene. The subtle action is by mRNA degradation. Thus the degree of RNA interference achieved is directly proportional to the level of mature mRNA and the translated proteins. The options are:

1. Measurements of target protein(enzyme) activity. This option is suitable if a robust assay is available or has been in prior use. The assay would vary by the nature of the protein product.
2. Measurement of target mRNA level. This is the preferred method as it directly quantifies the level of mRNA. Quantitative PCR is very effective in measuring relative amount of target sequence. This can be achieved simply by SYBR green or by the use of TaqMan or Molecular Beacons.

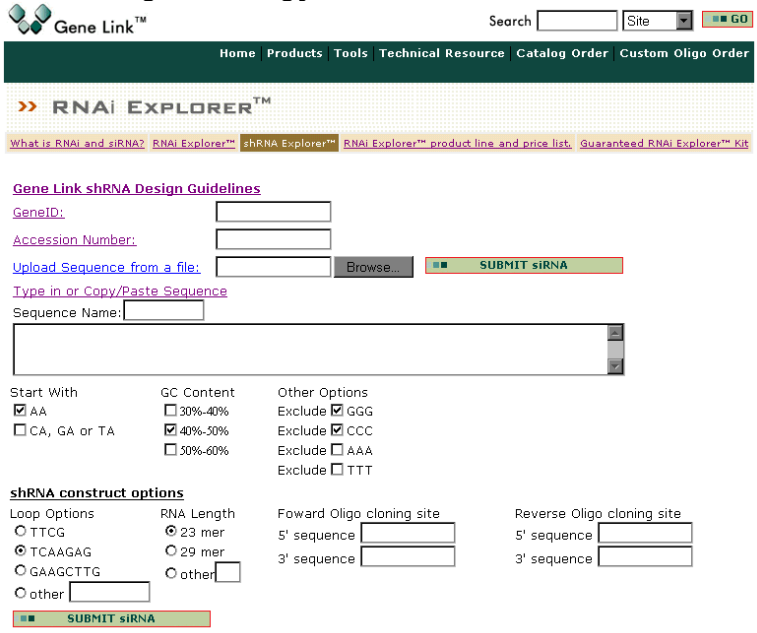
What are shRNA?

An alternate to individual chemical synthesis of siRNA is to construct a sequence for insertion in an expression vector. Several companies offer RNAi vectors for the transcription of inserts. Some use an RNA polymerase III (Pol III) promoter to drive expression of both the sense and antisense strands separately, which then hybridize in vivo to make the siRNA. Other vectors are based on the use of Pol III to drive expression of short "hairpin" RNAs (shRNA), individual transcripts that adopt stem-loop structures, which are processed into siRNAs by the RNAi machinery.

shRNA Explorer™

Designing Oligonucleotides for RNAi Expression Vectors

shRNA Explorer™ Design Strategy



1. Search for candidate siRNA sequences based on the following default parameters*. shRNA size parameters: AA>23-29. Default is 23 nt.

The default criteria selects target sequences of 23 nucleotides that begins **after** the AA and are located within a region of the coding sequence that is within 50-100 nucleotides of the AUG start codon and within 50-100 nucleotides from the termination codon. The AA sequence is NOT included in the shRNA sequence. The selection program includes the option to search for sequences that begin with other nucleotide pairs.

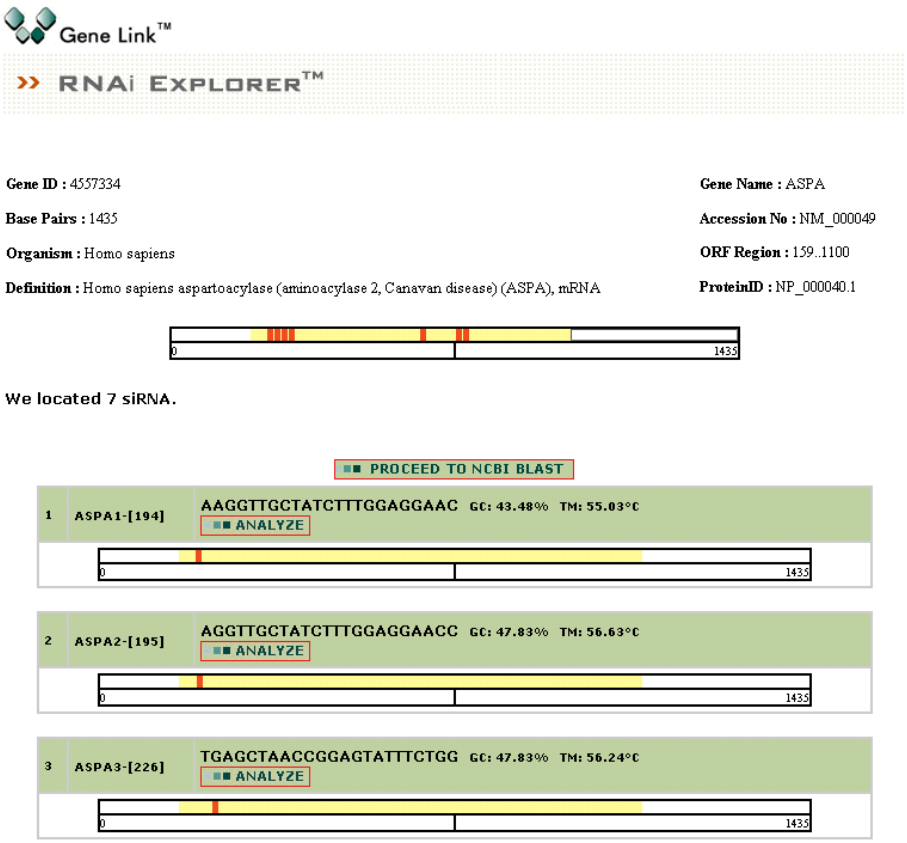
The upper strand of the target sequence should start with a G or an A, as RNA polymerase III prefers to initiate transcription with a purine. If a G or A is not present, then it must be inserted immediately upstream of the target sequence. A 'G" is added to the sequence at the 5' end if it is not present. A terminator sequence consisting of 6 dTs is added immediately downstream of the target sequence.

GC Content

The G-C content of the sequence is also used as a condition for selecting target sequences. Ideally the GC content will be less than 50%, although successful gene silencing has been reported with siRNAs that have G-C contents between 50 and 60%. The default parameter selects for a G-C content in the 40-50% range, however, options are available that allow for selection over wider ranges.

Stretches of Nucleotide Repeats

The default mode avoids sequences with repeats of three or more G's or C's, as their presence initiates intra-molecular secondary structures preventing effective siRNA silencing hybridization. As an option, repeat stretches of A's and T's can also be eliminated, as they tend to reduce the specificity of the target sequence. If possible, this option is highly recommended.



Select Loop Sequence

A loop sequence is necessary for construction of shRNA. Based on reported findings the default sequence for the loop is TCAAGAG, other options are TTCG and GAAGCTTG (HindIII site) and an option to enter your own sequence.**Enter Cloning Site Sequence**

Option is provided to enter cloning site sequence for 'forward' and 'reverse' oligo.

3. Submit for siRNA search

After selecting all the options, select 'Submit siRNA Search". This will initiate the program to download the sequence based on the accession number, Gene ID or to upload sequence you provided. Initially the ORF is determined and on the basis of the criteria suitable sequence is selected and a NCBI BLAST search is initiated. A list of siRNA sequences are presented for selection. The selected siRNA sequences are then converted for shRNA constructs.

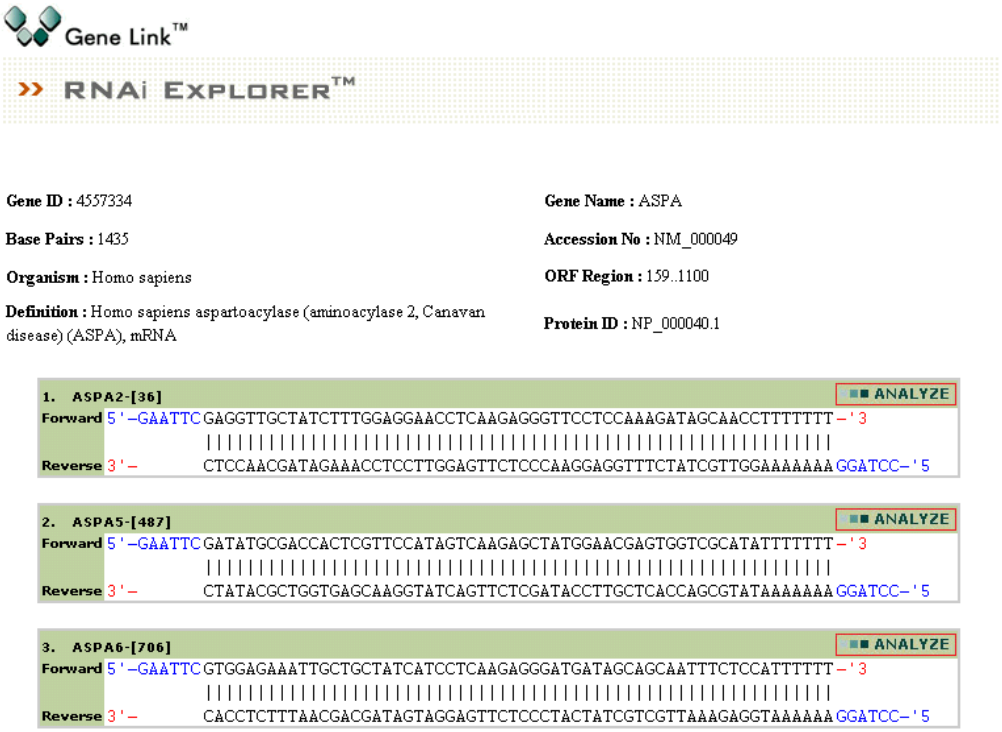
4. View shRNA Construct

Annealed View

All selected features are incorporated in the construct and is presented for viewing and editing if required. This is presented in 'annealed form' for visual inspection of the sequence.

shRNA view

Use this feature to view the forward oligo construct in a short hairpin view.



5. Final Reviewing and or Editing before placing order

Final reviewing of all oligo sequence is presented before actual submitting the order. Two 'view' formats are available; 'Simple View' and 'Classic View'.

6. Submit Order

Pressing 'Submit Order' places an order. An immediate email confirmation with an order number is emailed. Please call or email if you do not receive an email confirmation.

*RNAi and siRNA

RNA interference (RNAi) is a specific and sequence dependent targeted gene silencing activity. RNAi acts by post transcriptional degradation of mRNA by small interfering RNAs (siRNA's) of the same sequence. The silencing approaches 100% and has to be empirically determined and optimized. Not every siRNA can effectively down regulate a gene. The process of RNA interference varies by individual siRNA while some do not exhibit any interference at all.

References

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RNAi Explorer™ Product Price List

RNAi Explorer™ from Gene Link is a series of product and services to aid researchers in exploring RNA interference. The online search and design algorithms for siRNA or shRNA are developed based on current known guidelines. You can place an order for the Guaranteed RNAi Explorer™ kit by simply providing Gene Link the appropriate Gene ID or Accession Number of your gene of interest. Alternatively, you can design your own siRNA or shRNA at Gene Link's website.

siRNA Synthesis
The siRNA duplexes provided are synthesized as a single strand oligo and annealed after complete deprotection and purification steps are completed. Gene Link utilizes the most reliable standard RNA synthesis chemistry. The RNA duplex is supplied lyophilized and ready to use.

Guaranteed RNAi Explorer™ Kit

Please consult and save enclosed 'Order Specifications' sheet, this gives your gene specific siRNA information. This kit contains the following.

1. 3 siRNA duplexes 10 nmols each of 19+2 dT 3' overhangs. Supplied lyophilized, ready to dissolve and use. Please see order report for sequence information.
2. 1 negative control non-silencing siRNA duplex. 5 nmols supplied lyophilized, ready to dissolve and use.
3. Choice of detection probe or custom oligos if requested at time of order.

Guaranteed RNAi Explorer™ Kit*		
Components	**Qty**	**Price**
3 Target siRNAs	10 nmol each	$995.00
1 negative control siRNA	5 nmol	
1 QPCR probe or 6 free oligos*	5 nmol	

*Select one choice from list below when placing order

27-6402-01	*Guaranteed RNAi Explorer kit with Fluorescein/Dabcyl or BHQ-1 Molecular Beacon
27-6402-02	*Guaranteed RNAi Explorer kit with Fluorescein/Tamra or BHQ-1 TaqMan
27-6402-06	*Guaranteed RNAi Explorer kit with 6 oligos up to 30mer at 50 nmol scale

Custom siRNA Duplex Synthesis

Custom siRNA's can be ordered using Gene Link's online search and design proprietary algorithms bound to silence the gene. Customers can also place an order for pre-designed siRNA's. Each siRNA duplex is supplied lyophilized and ready to use after appropriate reconstitution.
Please consult and save enclosed 'Order Specifications' sheet, this gives order specific data including the exact nmol quantity supplied of each duplex.

siRNA (duplex)				
Catalog Number	**27-6401-06**	**27-6401-05**	**27-6401-02**	**27-6401-10**
Purification	**20 nmol scale**	**50 nmol scale**	**200 nmol scale**	**1 µmol scale**
Crude	$195.00	$295.00	$350.00	$650.00
RPC	$210.00	$350.00	$450.00	$710.00
Gel	$295.00	$410.00	$510.00	$810.00

RNAi Explorer™ Control siRNA Duplexes

The negative and positive control siRNA duplexes serve as validation for your experiments including monitoring transfection and silencing efficiencies. These are supplied lyophilized.

Control siRNA Duplexes			
Product	**Size**	**Catalog Number**	**Price, $**
Negative Controls non-silencing siRNA for human, mouse and rat			
Negative Control unlabeled, non-silencing	5 nmols	27-6410-05	$110.00
Negative Control unlabeled, non-silencing	20 nmols	27-6410-20	$250.00
Negative Control FL labeled, non-silencing	2 nmols	27-6410-02FL	$110.00
Negative Control FL labeled, non-silencing	10 nmols	27-6410-10FL	$350.00
Negative Control FL labeled, non-silencing	20 nmols	27-6410-20FL	$495.00
Positive Controls, Unlabeled Verified high efficiency silencing of target gene			
Human Vimentin; NM_003380	5 nmols	27-6412-05	$110.00
Human Vimentin; NM_003380	20 nmols	27-6412-20	$250.00
Human Beta Actin; NM_001101	5 nmols	27-6413-05	$110.00
Human Beta Actin; NM_001101	20 nmols	27-6413-20	$250.00
Mouse Beta Tubulin; AF312873	5 nmols	27-6414-05	$110.00
Mouse Beta Tubulin; AF312873	20 nmols	27-6414-20	$250.00
Rat Chromogranin-A; NM_021655	5 nmols	27-6415-05	$110.00
Rat Chromogranin-A; NM_021655	20 nmols	27-6415-20	$250.00
Positive Controls, Fluorescein Labeled Verified high efficiency silencing of target gene			
Human Vimentin; NM_003380; FL labeled	2 nmols	27-6412-02FL	$110.00
Human Vimentin; NM_003380; FL labeled	10 nmols	27-6412-10FL	$350.00
Human Beta Actin; NM_001101; FL labeled	2 nmols	27-6413-02FL	$110.00
Human Beta Actin; NM_001101; FL labeled	10 nmols	27-6413-10FL	$350.00
Mouse Beta Tubulin; AF312873; FL labeled	2 nmols	27-6414-02FL	$110.00
Mouse Beta Tubulin; AF312873; FL labeled	10 nmols	27-6414-10FL	$350.00
Rat Chromogranin-A; NM_021655;FL labeled	2 nmols	27-6415-02FL	$110.00
Rat Chromogranin-A; NM_021655;FL labeled	10 nmols	27-6415-10FL	$350.00

DNA Oligo Synthesis for shRNA

shRNA (DNA oligonucleotides)		
Product	**200 nmol scale**	**1 µmol scale**
DNA Oligo Synthesis	$1.80/base	$3.25/base
Gel Purification	$75.00	$150.00

Handling & Storage

Follow established stringent RNase free handling conditions. The lyophilized siRNA duplex should be stored immediately at -20° C. The lyophilized siRNA is stable for ~6 months at -20° C.

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Prices subject to change without notice All Gene Link products are for research use only

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