
siRNA
Design
by Ali Afkar

Type of RNA in genetic engineering and gene manipulation

- 1. **mRNA (**آرانای پیامرسان**)
- 2. **rRNA (**آرانای ریبوزومی**)
- 3. **tRNA (**آرانای انتقالی**)
- 4. **siRNA (**آرانای کوچک مداخلهگر**)
- 5. **miRNA (**ریزآرانای**)
- 6. **tsRNA (آرانای کوچک مشتقشده از tRNA)**
- 7. **ysRNA (آرانای کوچک مشتقشده از Y RNA)**
- 8. **circRNA (**آرانای حلقوی**)
- 9. **lncRNA (**آرانای بلند غیرکدکننده**)
- 10. **piRNA (آرانای تعاملکننده با پیوی**)
- 11. **snRNA (**آرانای کوچک هستهای**)
- 12. **snoRNA (**آرانای کوچک هستکی**)
- 13. **ribozyme (**ریبوزیم**)
- 14. **exRNA (**آرانای خارج سلولی**)
- 15. **tasiRNA (آرانای کوچک مشتقشده از tasiRNA)**
- 16. **rasiRNA (آرانای کوچک مرتبط با تکرار)**
- 17. **scaRNA (**آرانای کوچک کایال**)
- 18. **srRNA (آرانای کوچک ریبوزومی)**
- 19. **gRNA (**آرانای راهنما**)
- 20. **crRNA (آرانای مرتبط با CRISPR)**

کاربردی ترین ..

- ۱. **siRNA (small interfering RNA)**: خاموشی ژن‌ها از طریق تجزیه mRNA. (۱۹-۲۱ باز)
- ۲. **miRNA (microRNA)**: تنظیم بیان ژن‌ها از طریق تعامل با mRNA. (۱۹-۲۱)
- ۳. **dsRNA (double-stranded RNA)**: شروع مسیر RNAi. (۱۰۰-۱۰۰۰ باز)
- ۴. **Antisense RNA**: خاموشی ژن‌ها از طریق اتصال به mRNA.
- (۲۰۰ تا ۲۰۰۰)

- ۵. **RNA: shRNA (short hairpin RNA)**های کوتاه با ساختار حلقه‌ای که برای خاموشی ژن‌ها استفاده می‌شوند.(۱۹-۲۰ بازی)
- ۶. **piRNA (Piwi-interacting RNA): **حفاظت از سلول‌های جنسی و مقابله با جابجایی عناصر ژنتیکی متحرک.(۲۴ تا ۳۱ باز)
- ۷. **CRISPR-Cas9: **یک سیستم اصلاح ژن که می‌تواند به طور خاص ژن‌ها را برش دهد و اصلاح کند.
- ۸. **ASOs (Antisense Oligonucleotides): **قطعات کوتاه DNA یا RNA که به mRNA متصل شده و تشخیص ...
- ۹. **RNA: Ribozymes**های کاتالیتیک که می‌توانند mRNA هدف را تجزیه کنند.
- ۱۰. **RNA: aptamer**یا DNA تک‌رشته‌ای که به مولکول‌های هدف مشخص متصل شده و فعالیت‌های آنها را مهار می‌کند.

- **circRNAیا ** RNA** حلقوی**** نوعی RNAغیرکدکننده است که به شکل حلقه‌ای تشکیل می‌شود. این نوع RNAبه دلیل ساختار حلقوی خود، در برابر تخریب توسط اگزونوکلئازها مقاوم‌تر است و نقش‌های مختلفی در **تنظیم بیان ژن، اسپانج کردن miRNAها و تعامل با پروتئین‌ها** دارد. circRNAها در بسیاری از فرآیندهای بیولوژیکی و بیماری‌ها نقش دارند و به عنوان بیومارکرهای بالقوه برای تشخیص و درمان بیماری‌ها مورد توجه قرار گرفته‌اند.

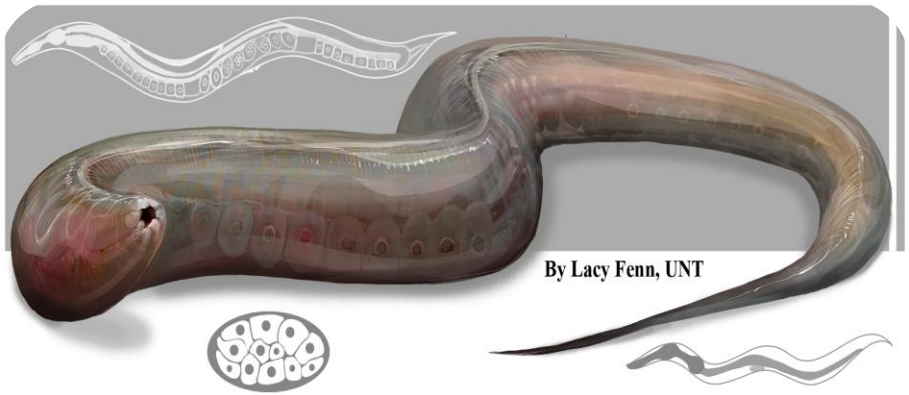
- **lncRNAیا ** RNA** بلند غیرکدکننده**** نوعی RNAاست که بیش از ۲۰۰ نوکلئوتید طول دارد و برخلاف RNAهای پیام‌رسان (mRNA) کد پروتئینی تولید نمی‌کند. این نوع RNAها نقش‌های مهمی در تنظیم بیان ژن، کنترل فرآیندهای سلولی، تنظیم ساختار کروماتین، و تعامل با سایر مولکول‌های زیستی دارند.
- lncRNAها در بسیاری از فرآیندهای بیولوژیکی و بیماری‌ها نقش دارند و تحقیقات زیادی روی آن‌ها در حال انجام است تا بیشتر درک شود که چگونه می‌توان از آن‌ها برای تشخیص و درمان بیماری‌ها استفاده کرد.

- **rasiRNAیا **راسی **RNA**نوعی از RNA** کوتاه مهارکننده تک‌رشته‌ای ** (siRNA)است که در سیستم‌های بیولوژیکی مختلف، از **جمله گیاهان، دانه‌های مخمار، و سلول‌های جنسی در موش‌ها،** شناسایی شده استrasiRNA .ها نقش مهمی در **مهار کردن عناصر جابجایی ژنتیکی** و **تغییرات هسته‌ای** دارند و معمولاً با پروتئین‌های زیرگروه Piwi از خانواده Argonaute تعامل دارند.
- .....

RNA interference

- **RNA interference (RNAi)** : a gene silencing mechanism where short interfering RNA (**siRNAs) and microRNA** (miRNAs) molecules inhibit the transcription and translation of target genes in a sequence-specific manner

- Firstly: *Caenorhabditis elegans:* degradation of par-1 mRNA followed by introducing double-stranded RNA to this nematode worm



- Practical tool for **new drug target discovery** and **RNAi drug development in mammalian cells**

- An appealing technique for decreasing the **virulence of pathogens** such as bacteria????, fungi and viruses
- siRNAs (short interfering RNAs) are primarily involved in **post-transcriptional regulation**.
- This means they function by interfering with gene expression **after the transcription process** has occurred but before the translation process begins.
- They achieve this **by binding to complementary mRNA** sequences and leading to their **degradation**, effectively silencing the gene.

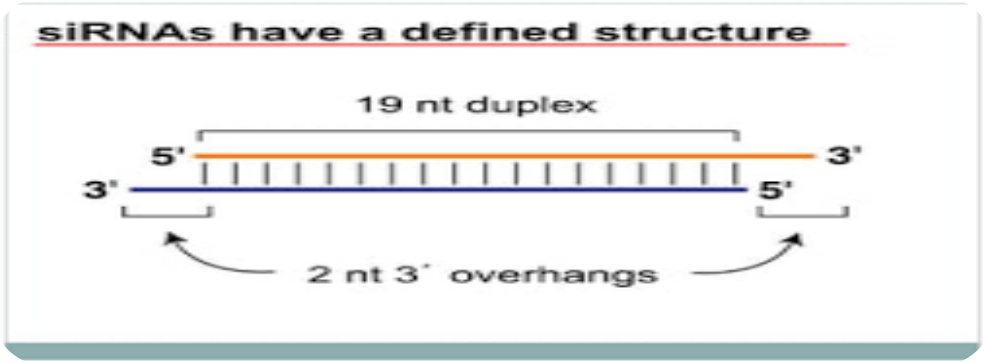
successes in siRNA :

- Drugs designed based on siRNA are in use and development.
- Three important drugs in this category, patisiran, givosiran, and lumasiran, have been approved by the U.S. Food and Drug Administration (FDA).
- **Patisiran**: For the treatment of hereditary transthyretin-mediated amyloidosis (hATTR).
- **Givosiran**: For the treatment of acute hepatic porphyria.
- **Lumasiran**: For the treatment of primary hyperoxaluria type 1.Additionally, 7 other siRNA-based drugs are in various stages of clinical evaluation.

What is 1-siRNA

2 pathway:

- 1-dsRNA is processed to **21-25 nt short interfering** RNA (siRNA) with 2 nt 3' overhangs by the RNAse III-like protein Dicer in the initiating step of RNAi
- 2-siRNAs are exogenously produced ~21 nucleotides **long double stranded RNA molecules**(**or synthetics**..) covering complementary with target sequence



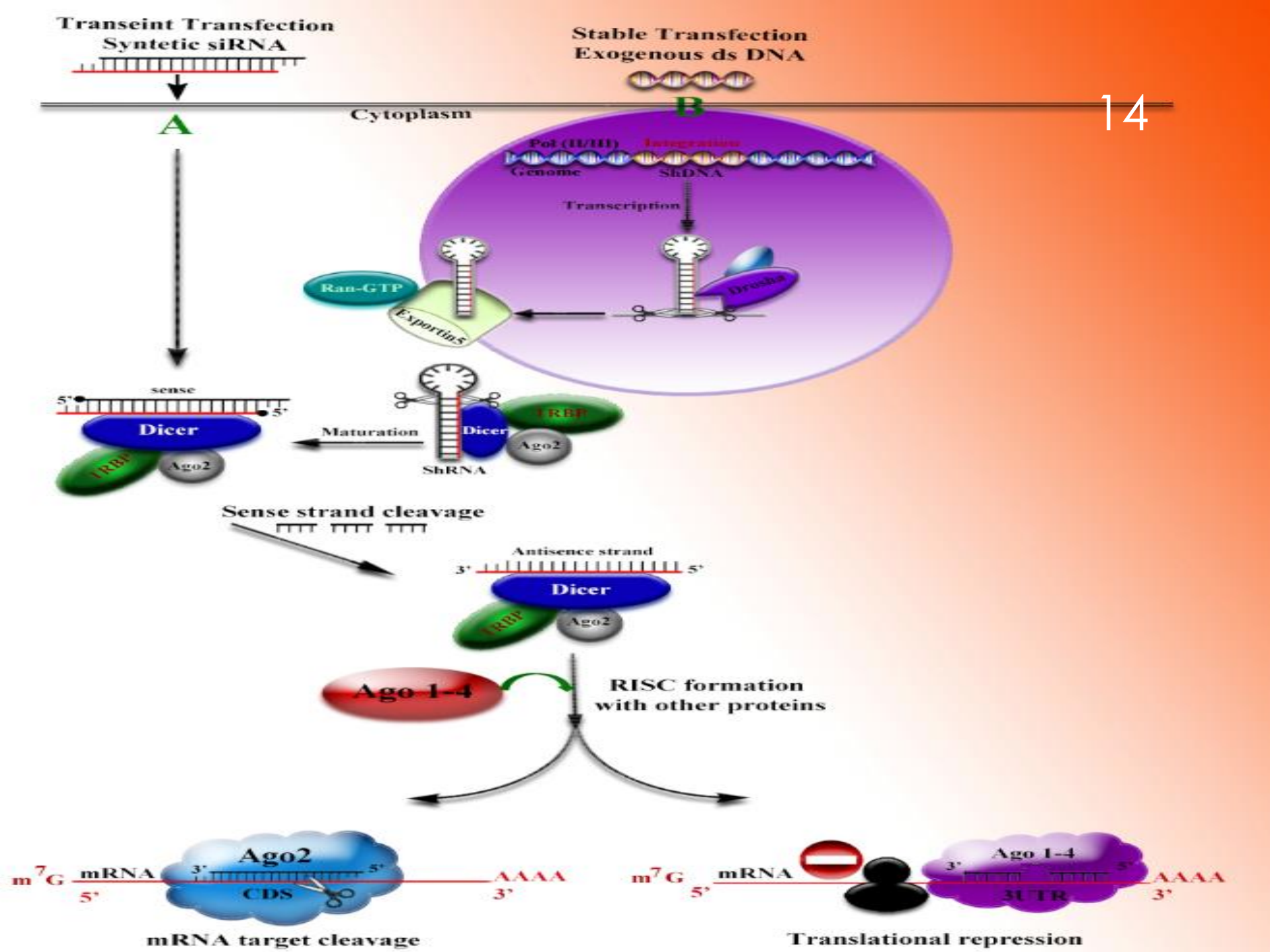
- In mammalian cells, **delivery of chemically synthesized short interfering RNA** specifically silences expression of the corresponding endogenous gene, **thus** bypassing the non-specific inhibitory mechanisms elicited by longer **ds RNA:**
- (like, Toll-like receptor 3 (TLR3), RNAse L, toxic effect on cell, Overall inhibition of translation..)

Mechanism of siRNA Interference:

- 1-During this process, long double-stranded RNA is broken down into smaller fragments **of 21-23 nucleotides** by the **ribonuclease enzyme Dicer** with the help of **ATP**. These small fragments are called siRNA.

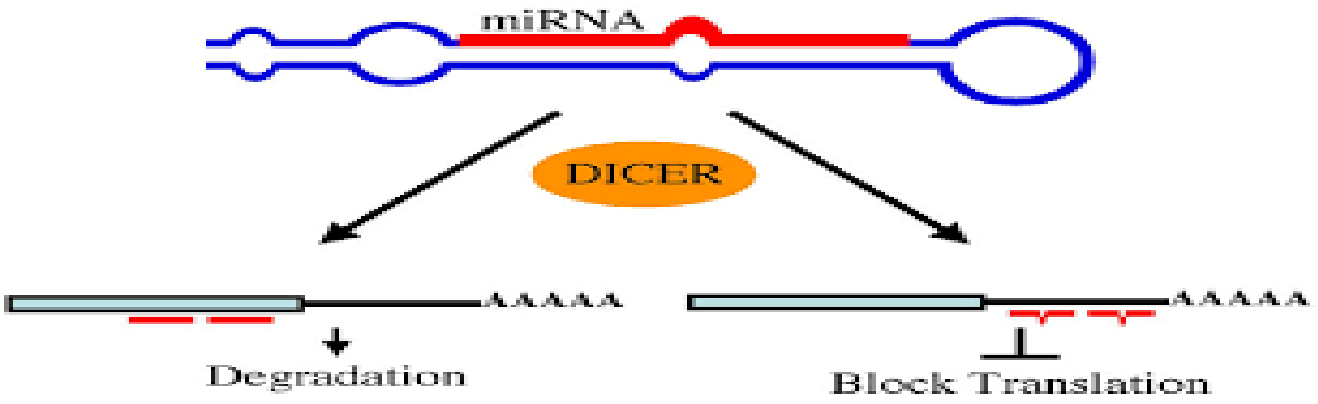
- 2-Then, the siRNA is incorporated into a multi-protein complex called **RISC** (RNA-Induced Silencing Complex), which includes the Argonaute 1-4 enzyme as part of the complex.

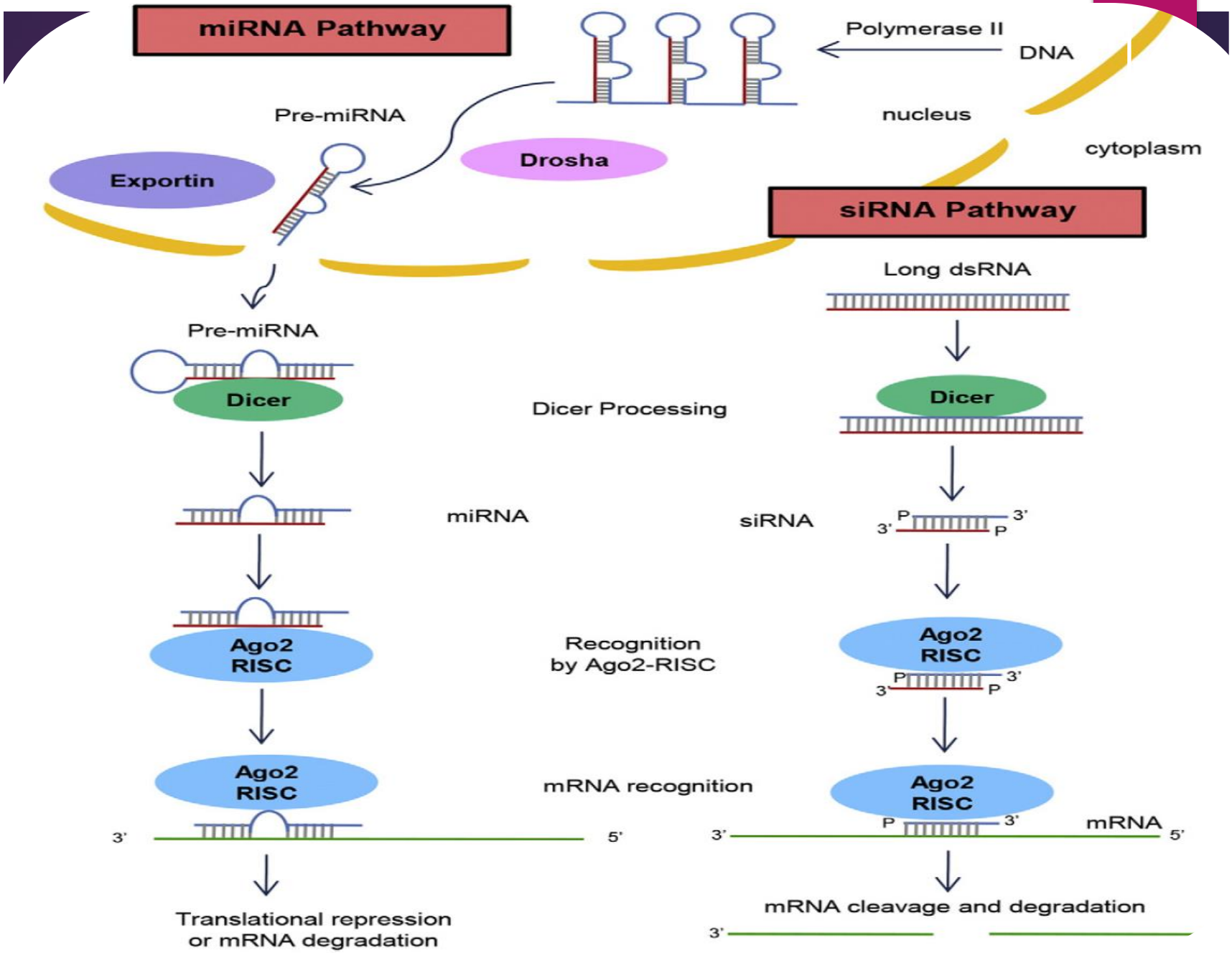
- 3-**Argonaute separates the sense** strand from the siRNA, and the **antisense strand remains** as the **guide** strand within the RISC complex.
- 4-After that, the **guide strand directs the activated RISC towards** the target mRNA.
- 5-Once the **guide strand fully pairs with the target mRNA**, the mRNA is cleaved by the **Argonaute enzyme.**
- 6-With the degradation of the mRNA, gene expression is halted, a state referred to as gene silencing.


Transeint Transfection
Syntetic siRNA
Stable Transfection
Exogenous ds DNA
Cytoplasm
A
B
Pol (II/III)
Integration
Genome
ShDNA
Transcription
Ran-GTP
Exportin5
Drosha
sense
5'
5'
Dicer
TRBP
Ago2
Maturation
Dicer
TRBP
Ago2
ShRNA
Sense strand cleavage
Antisence strand
3'
5'
Dicer
TRBP
Ago2
Ago 1-4
RISC formation
with other proteins
m7G
mRNA
5'
Ago2
3'
5'
CDS
AAAA
3'
mRNA target cleavage
m7G
mRNA
5'
Ago 1-4
3'
5'
3UTR
AAAA
3'
Translational repression

2-miRNA

- miRNAs are a family of **endogenously encoded** small noncoding RNAs, derived by processing of short RNA hairpins, that can inhibit the translation of mRNAs bearing **partially complementary** target sequences 3UTR ‘ 5UTR or CDs




miRNA Pathway
Polymerase II
DNA
nucleus
cytoplasm
Pre-miRNA
Drosha
Exportin
siRNA Pathway
Long dsRNA
Pre-miRNA
Dicer
Dicer Processing
Dicer
miRNA
siRNA
3'
P
P
3'
Ago2
RISC
Recognition
by Ago2-RISC
Ago2
RISC
Ago2
RISC
mRNA recognition
Ago2
RISC
3'
5'
3'
mRNA
5'
Translational repression
or mRNA degradation
mRNA cleavage and degradation
3'

basic criteria for designing the best siRNAs

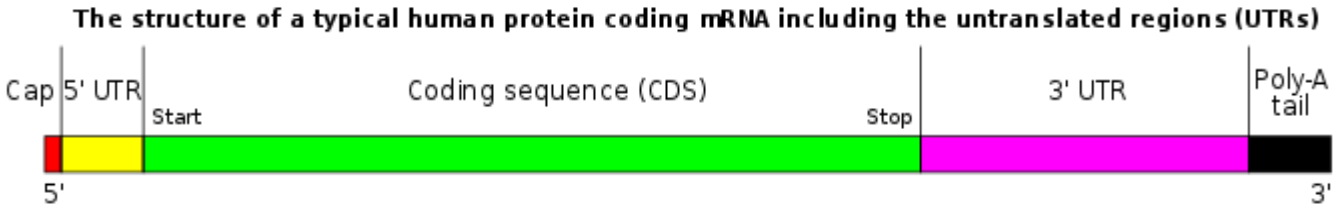
Different steps in designing a siRNA

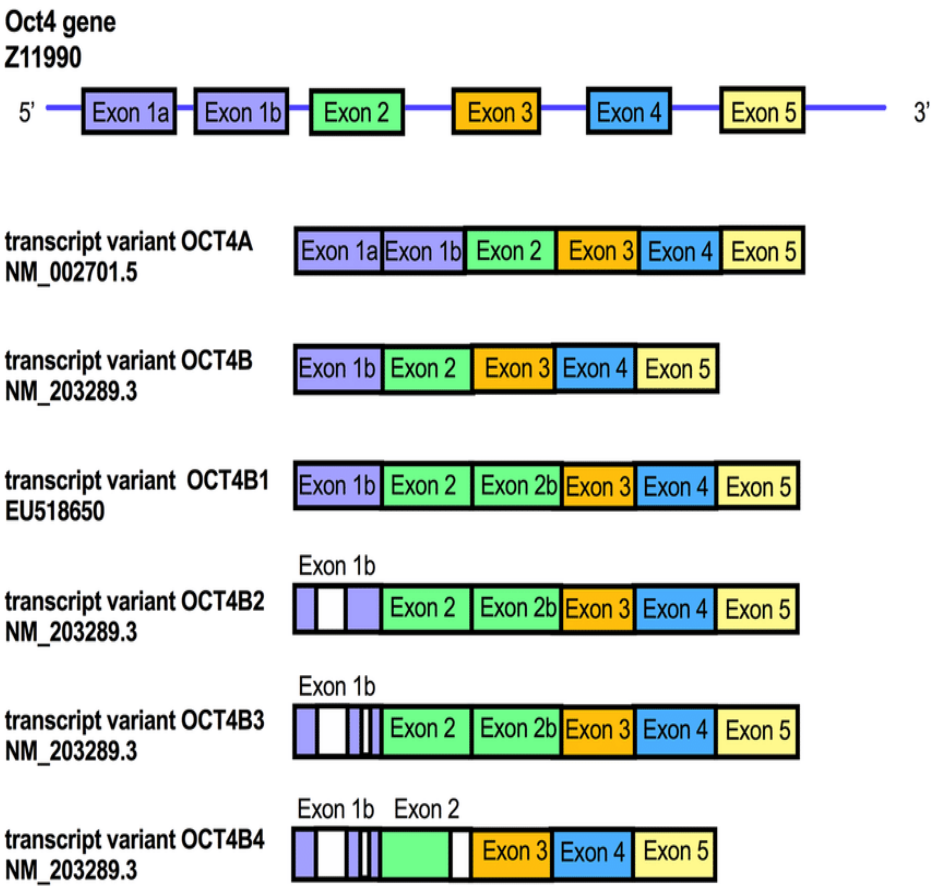
A. Finding the target gene
B. Selecting the best position for siRNA
C. Assessing its secondary structures(siRNA and mRNA target)
D. Assessing Its nucleotide content
E. Evaluating specificity of the designed siRNA

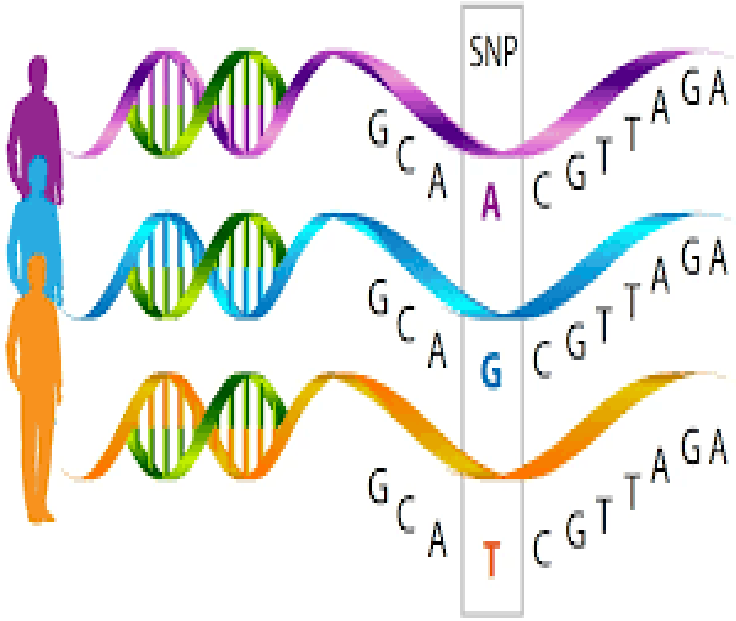
Target site
(best position
in the mRNA)

Target site

- Retrieving the gene from Common database(NCBI, genome browser or ensemble..)
- Assessment of Different Transcript variants ..
- Avoiding single-nucleotide polymorphism (SNP) locations(**300** to **500**); it might cause variation in the silencing efficiency in different situations(dbSNP..)




Oct4 gene
Z11990
5'
Exon 1a
Exon 1b
Exon 2
Exon 3
Exon 4
Exon 5
3'
transcript variant OCT4A
NM_002701.5
transcript variant OCT4B
NM_203289.3
transcript variant OCT4B1
EU518650
Exon 2b
transcript variant OCT4B2
NM_203289.3
transcript variant OCT4B3
NM_203289.3
transcript variant OCT4B4
NM_203289.3


SNP
G C A A C G T T A G A
G C A G C G T T A G A
G C A T C G T T A G A

Selecting the best positions

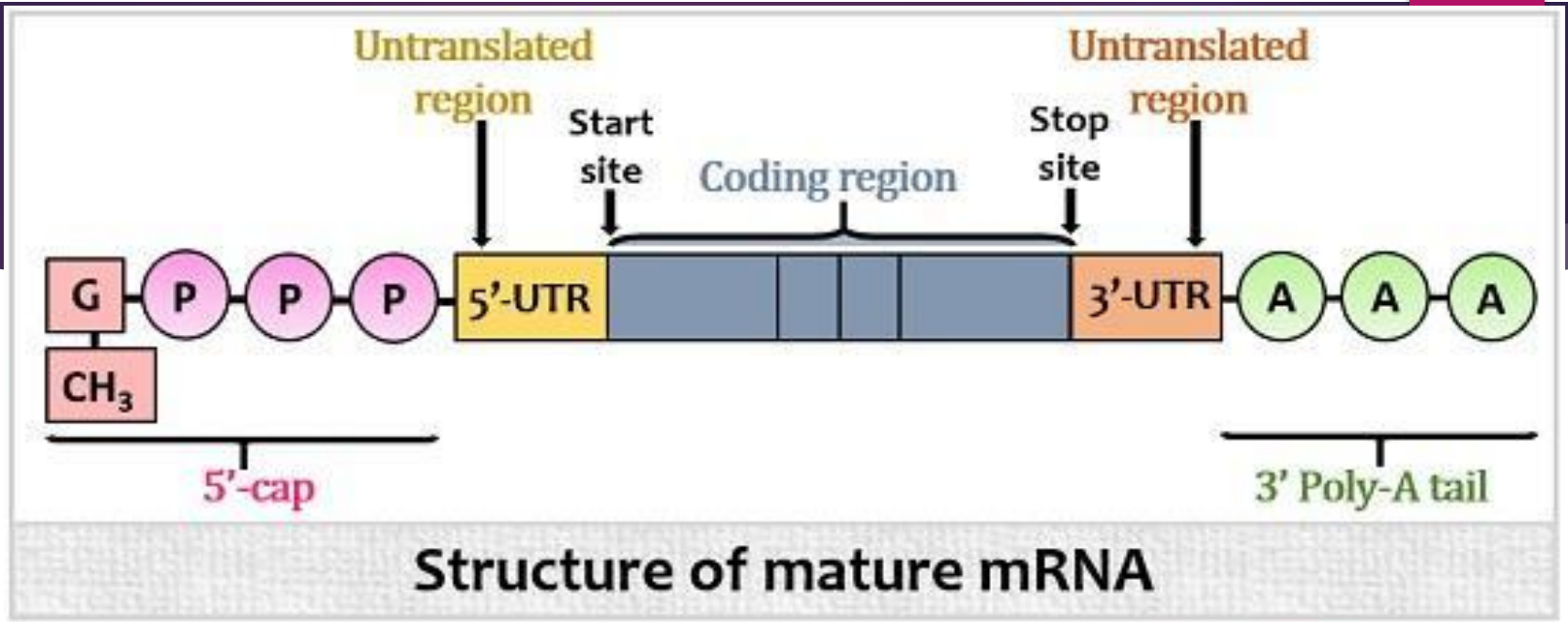
A. Avoid intronic parts and 5′UTR or 3′UTR.

B. UTRs and sequences close to start codon are more prone to regulatory proteins that can interfere with RISC complex .

C. Regions about **50–100 nucleotides downstream** of start codon in the open reading frame of the target gene are the **best target sites to be silenced.**

D. Worthwhile as **guanidine–cytosine (GC)** content of these **regions is less 50%; also**, they facilitate the function of RISC complex.

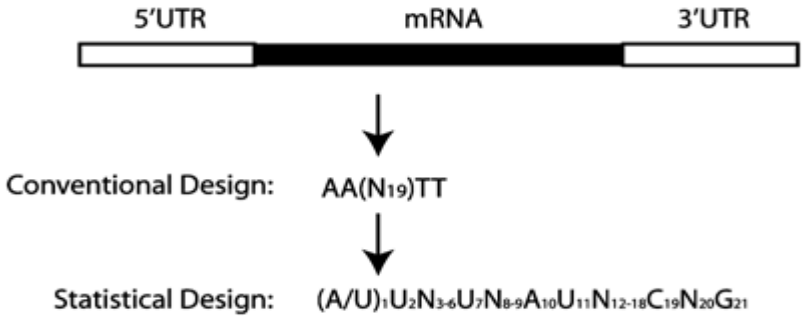
E. Accessibility of the target site owing to the **secondary structures of the mRNA is** another determinant of siRNA's **functionality** and any variations in **partial base pairing of the target site** will influence the effectiveness of siRNA.



Structure of mature mRNA

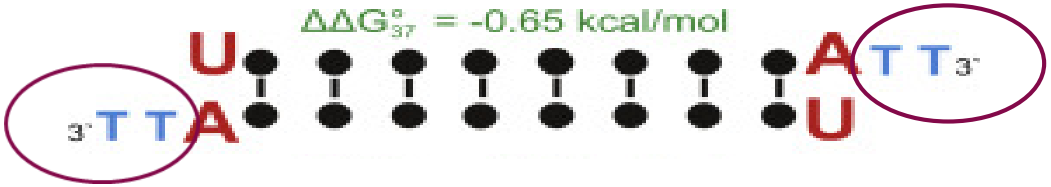
BIOLOGY READER

Selecting the best position



- Searching for :
- 5'-AA(N19)TT,
- 5'-AA(N21) or
- 5'-NA(N21) on the target sequence
- This helping the entry of **mRNA into RISC complex**
- Lowering the GC content of target site(*high GC Lead to the formation of stronger secondary structures with the target mRNA*)

- Native mRNAs have intrinsic **secondary structures** that can be predicted by the software such **as Mfold** (http://mfold.rna. albany.edu/?q=mfold) or **generunner** ..
- Target sites, including **2 consecutive unpaired bases** (within mRNA) at their 5′ -or 3′-ends are **more prone to the silencing effect of siRNAs than target sites** comprising unpaired regions **in their central region.**



- ***(1-These free bases act as initial binding points and facilitate proper recognition.***

- ***2-Free bases can reduce secondary and unstable structures at the ends of the siRNA, which can increase the overall stability of the siRNA during the gene silencing process.***
- ***This can help increase rapid degradation and decrease instability of the siRNA.***

- As the accessibility of the target site affects the siRNA efficiency, Tafer et al. constructed a software called **RNAxs** (http://rna.tbi.univie.ac.at/cgi-bin/RNAxs), which is a siRNA designing tool mainly based on **mRNA target site accessibility**.

Length of siRNA

- There is a **controversy** over the best length of siRNAs. Although some groups, including have worked with
- **A-19 nucleotide** ones and obtained very good results,
- **B- others** have used longer siRNAs ranging from **21 to 29** nucleotides.
- Although shorter siRNAs may lead to an unspecific binding, siRNAs **from 19 to 25** nucleotides have shown the same efficiency in silencing.

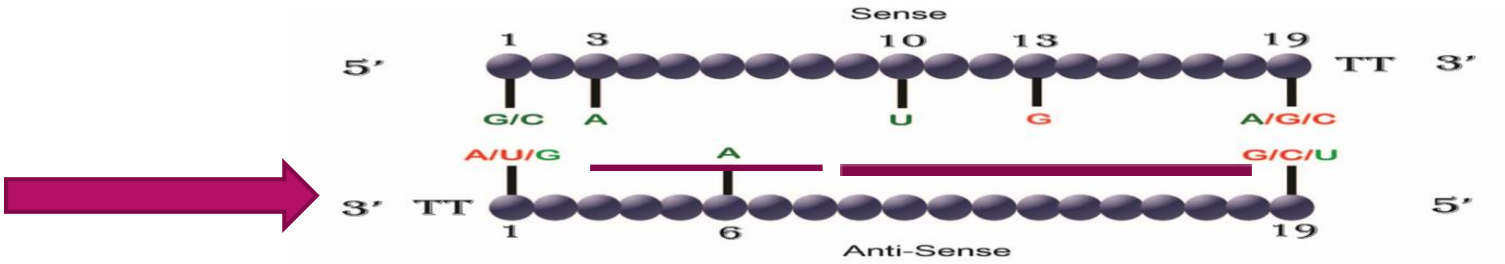
- However, **smaller siRNAs** are better to use for mammalian cells as **longer siRNAs** can immune rinduce mammalian response

Specificity checking

- After designing siRNAs with different methods, both sense and antisense strands should be checked **via blast with reference** sequence database (**Refseq-RNA and EST database**) of the desired organism to reduce the risk of silencing unintended genes.
- As their alignment may not be the result of chance, blast's results with smaller **E-values should not** be overlooked.
- Less than 78% query coverage with other **genes, ⩽15** nucleotides **out of 19** matching with the respective siRNA, is believed to be **tolerable**.
- Yet, it is worthwhile to mention that there is always a **probability of unpredictable off-target effects** for siRNAs.

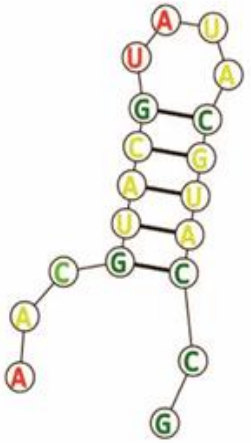
Nucleotide content of siRNA

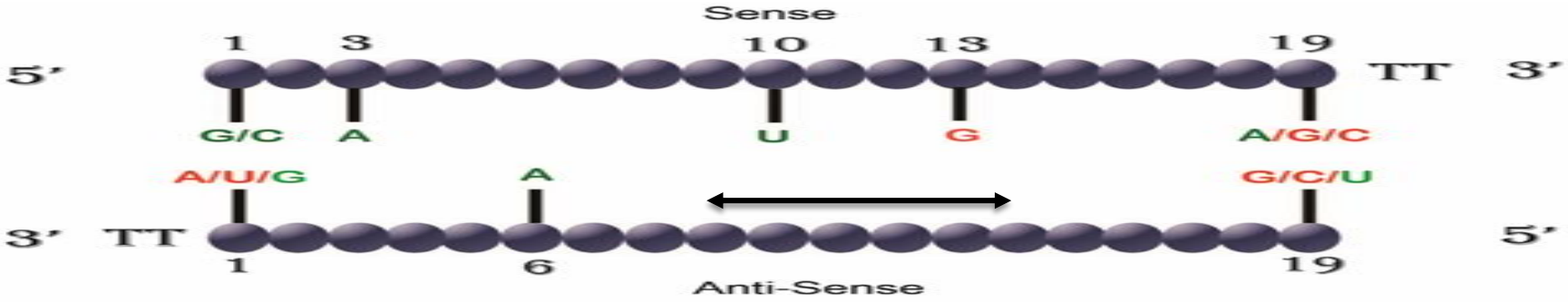
- ***Some of the criteria that are introduced by different algorithms overlap with each other.***
- 1-**GC** content is an obvious and **basic parameter** in the efficiency of siRNAs.
- This is due to the fact that **low GC content leads** to **unspecific and weak binding**, **while high GC content** may hinder unwinding the siRNA duplex by helicase and RISC complex.
- A- Suggested that the GC content should range from **31.6% to 57.9%.**
- B-Found the most functional siRNAs, which means up to **95%**, having GC content of **36–52%.**
- 2-In the **antisense strand**, GC percentages between the **2 to the 7** and the **8 to the 18** nucleotides were desirable to be 19% and 52%, respectively.



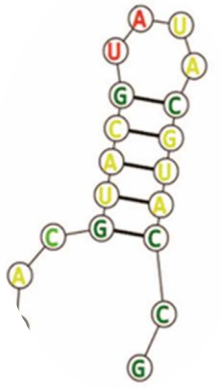
Nucleotide content of siRNA

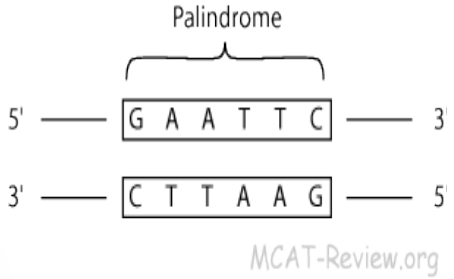
- 3- functional siRNAs have an unstable region **(lower GC content than others)** between the **9 to the 14** nucleotides, called **energy valley**, which is a **vital criterion for the** selection of siRNAs.
- 4-This **internal instability** increases the **RISC** complex functionality by **inducing** the most desirable conformation **during mRNA cleavage.**
- 5-Sense and antisense strands should have the ability to form the duplex properly and avoid any secondary structures that can be prohibitive.

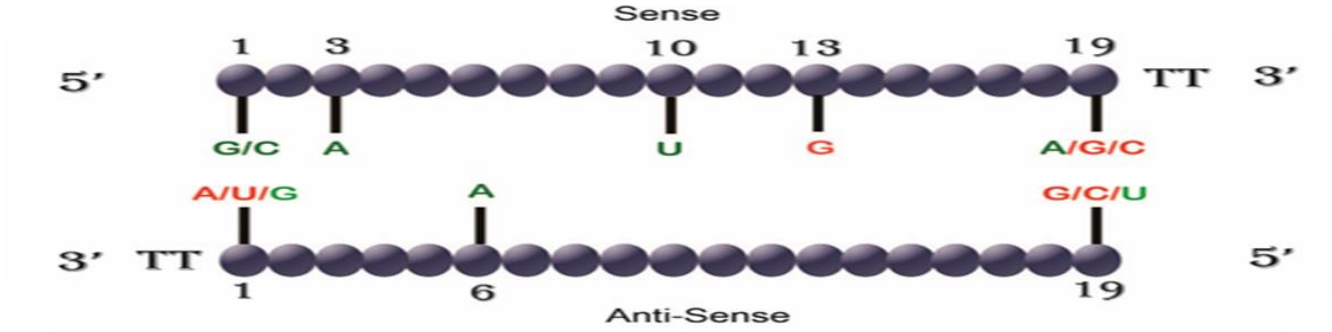




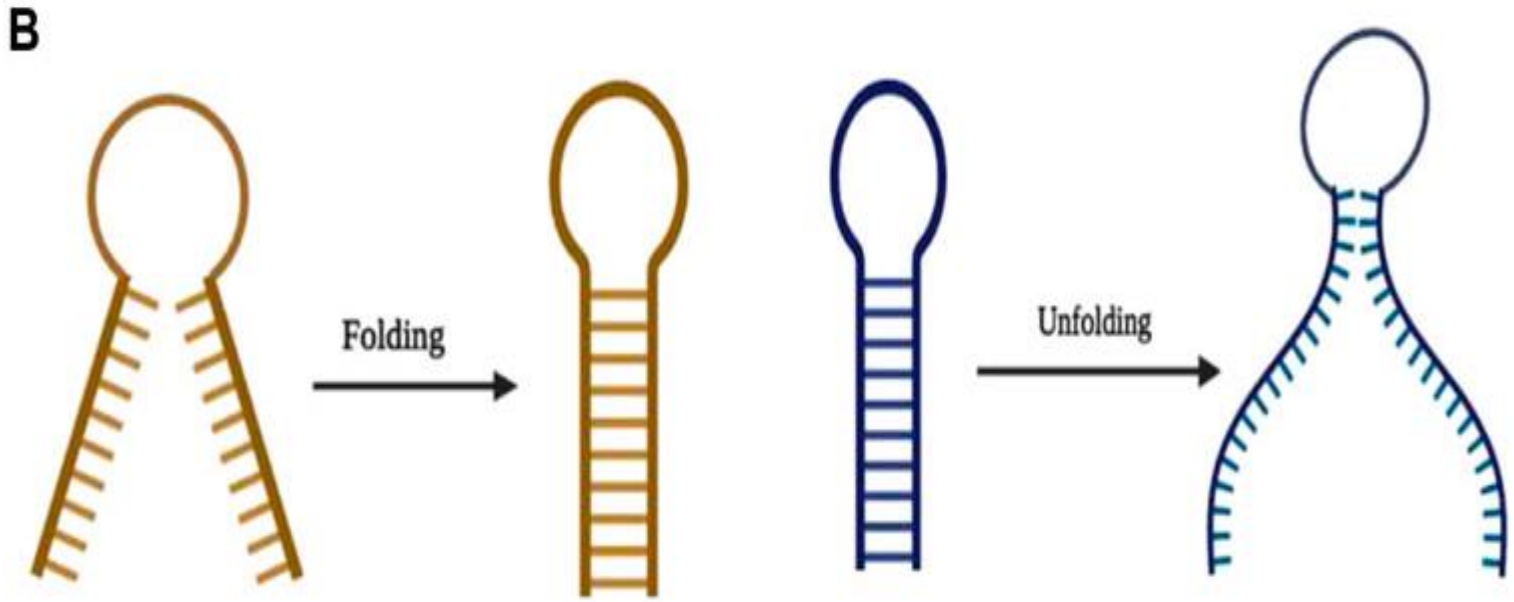
- 6- SiRNA sequences must be screened for internal repeats and palindromes.
- 7-However, siRNAs in which the internal secondary structures have Tm (melting temperature) values **less 20°C**, can be tolerated when the body temperature **reaches to 37°C**, which, in turn, unwinds the respective secondary structures.
- *(In general, secondary structures with **higher Tm are more stable**.*
- *The Tm of a molecule is usually between **50 and 90 °C**, but this depends on the **sequence and environmental** conditions.*



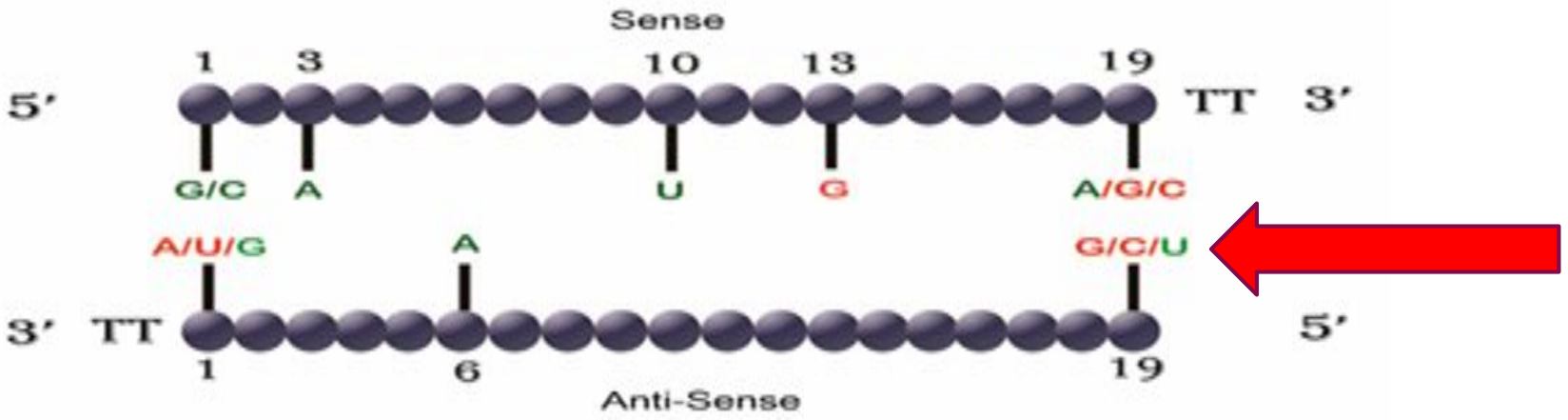




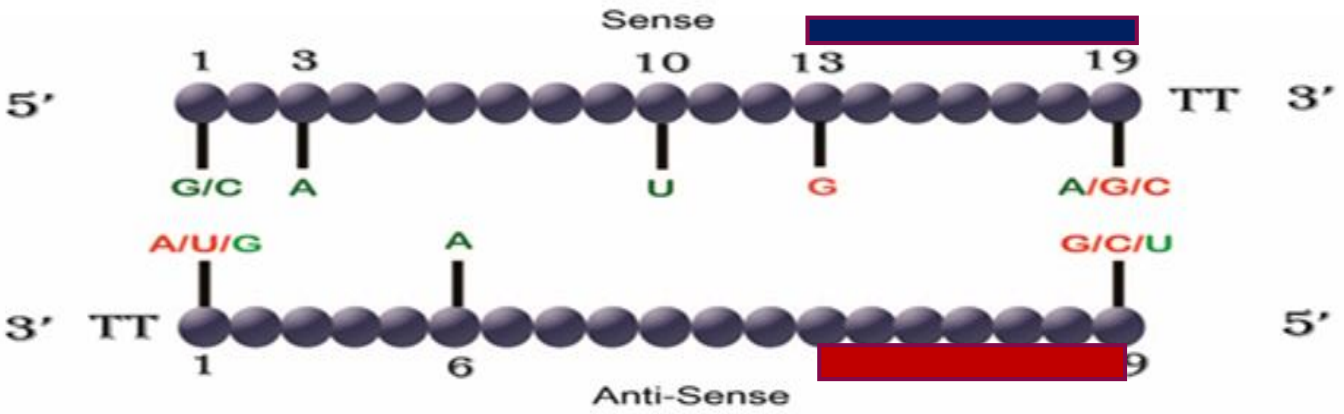
- ***Therefore, at temperatures(37) above Tm(20..), double-stranded or secondary structures become unstable and unfold to the single-stranded state.***

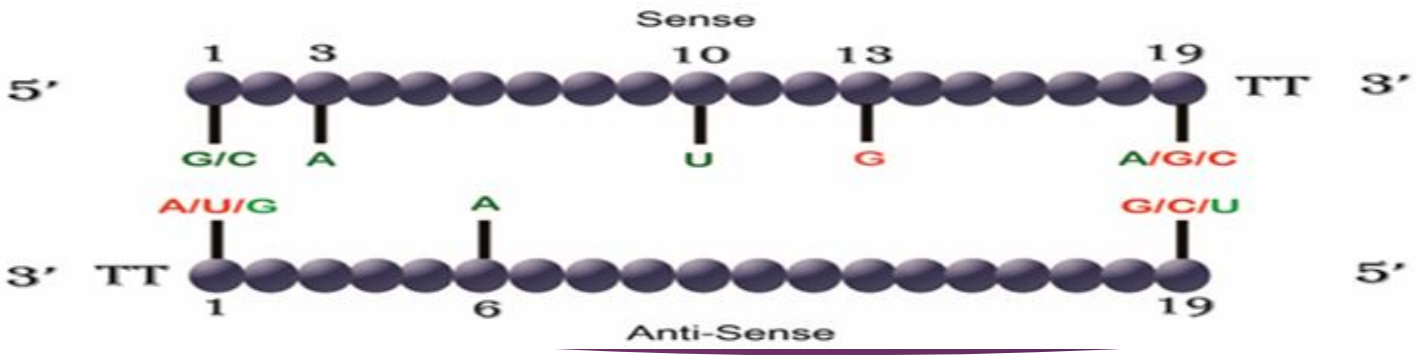


- This Evaluation of secondary structure can be carried out **by Mfold or oligoanalyzer 3.1 or gene runner..**
- 8-The other most substantial criterion for siRNA selection is ***low internal stability at 5'-end of the antisense strand***, which is probably an **important factor** for proper **unwinding of siRNA duplex and entering into the RISC complex**

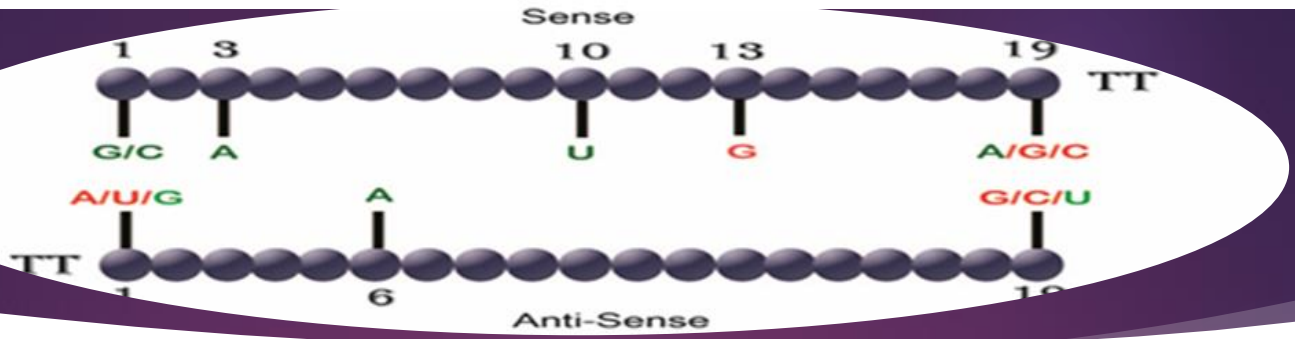


- 9-This instability can be easily assessed by the number of A/U nucleotides.
- A- for having an effective siRNA, **at least 4 out** of the **7 nucleotides** in the 5'-end of **antisense strand** should be **A or U.**
- 10- Or the others suggested at least 3 from **A/U bases** between the **13 and the 19 nucleotides** of **the sense strand** were needed.
- 11-Any way indicated that **A/U duplex differential between two strands**, especially in the last **three base pairs**, correlates with functional **siRNAs(sencse)**.

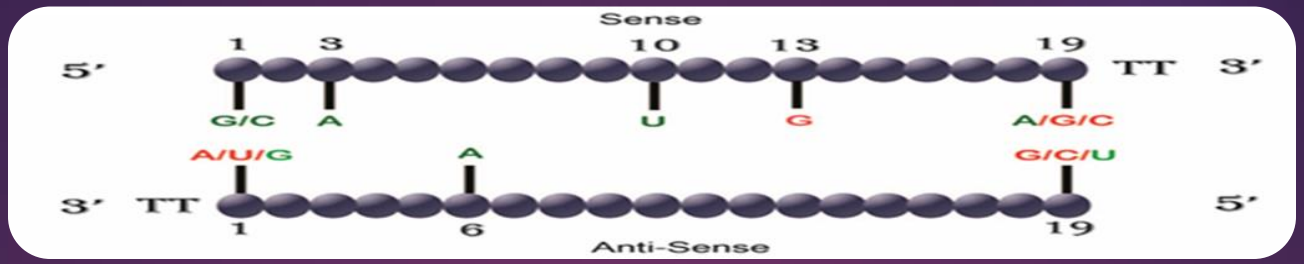




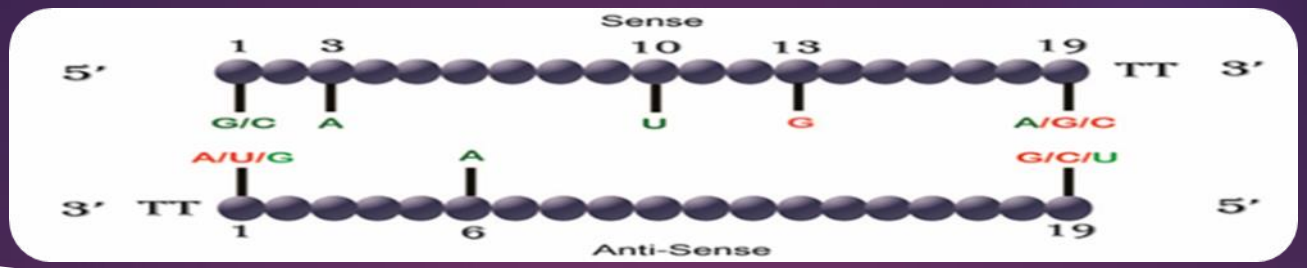
- 12-Also confirmed that the presence of **more 3 A/Us** at the **13 to 19** position is important for **siRNA's functionality**.
- 13-One other hand **high internal stability** or **high G/C** content **at 3'-end of the antisense** strand is influential in the efficiency of siRNA.
- 14-Sequences such as **GGGG or CCCC** should be avoided as they increase the risk of **hairpin structures.**
- Point: **Off course**; some topics, more than **three A or U nucleotides** can also be detrimental because RNA **polymerase III tends** to **end the transcription** at poly U site.
- The presence **of purine at the 5'-end of the sense** strand makes the initiation of transcription, by RNA polymerase III, more efficient.(for expression in vector.. **Contrary to what has been said so far.)**



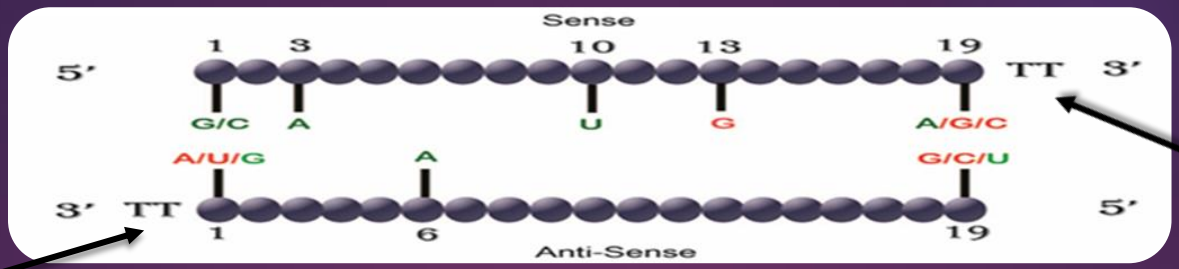
- 16- Nucleotide 6 of the antisense strand is preferred to be A.
- Conversely, U1 and G19 in the antisense strand decrease the efficiency of siRNA.
- 17-The presence of **A at the 19 position**, **A at the 3** position **and U at the 10 position**, besides the absence **of G or C at the 19** and **G at the 13** nucleotide of **sense strand are influential**.
- 18- **U10 showed the strongest correlation** with the efficiency of siRNA owing to the fact **that RISC cleaves target mRNA between nucleotide 10 and 11 and**, similar to **most of endonucleases**, prefers to cut 3′ of U rather than other bases.(power base of G, C, T and U important)



- **Jagla** and his research team **analyzed 600 chemically** synthesized siRNAs and revealed four sets of rules based on the base composition of **the sense strand**.
- According to their findings, the **first rule** is the best one that increases the chance of functional siRNA prediction up **to 90%**. Here are the rules:
- **Rule 1:** Presence of A/U at position 19, GC at the first position, A/U at position 10, more A/Us at position 13–19.
- **Rule 2**: Presence of A/U at position 19, GC at the first position, GC at position 10, more A/Us at position 13–19.
- **Rule 3**: Presence of G/C at position 19, GC at position 1, GC at position 11, morec A/Us at position **5–19**.
- **Rule 4:** Presence of A/U at position 19, A/U at position 1, more 3 A/Us at position 13–19.



- برای نتیجه گیری خلاصه شده در مورد طراحی siRNAبر اساس قوانینی که ارائه کردید، معیارهای نهایی را می توان به صورت زیر ترکیب کرد:
- معیارهای کلیدی برای طراحی siRNA:
- موقعیت 19: حضور A/U.
- .موقعیت اول: به طور معمول GC، اگرچه A/Uنیز در برخی قوانین قابل قبول است.
- موقعیت 10/11: بسته به قانون، وجود A/Uیا GC (البته A یا U)
- موقعیت های 13-19: A/Uهای بیشتر در این محدوده ترجیح داده می شوند.
- موقعیت های 5-19: در قانون 3، A/Uهای بیشتری در این محدوده وسیع تر ترجیح داده می شوند.



- **Two nucleotides** can be added chemically to the 3′-end of sense and antisense strands and these siRNA duplexes tend to be more efficient than duplexes with ⩾3 **3' overhanging nuclotides.**

- **Benefits of TT Overhangs:**
- **Enter ti RISC complex**
- **Stability siRNA structure**
- **Cost Reduction**: TT overhangs help reduce the expense of RNA synthesis.
- **Increased Persistence**: These overhangs can increase the persistence of siRNA duplexes by enhancing their resistance to RNAase
- Decrease off-target , improve connection to the target mRNA….

Thermodynamic features

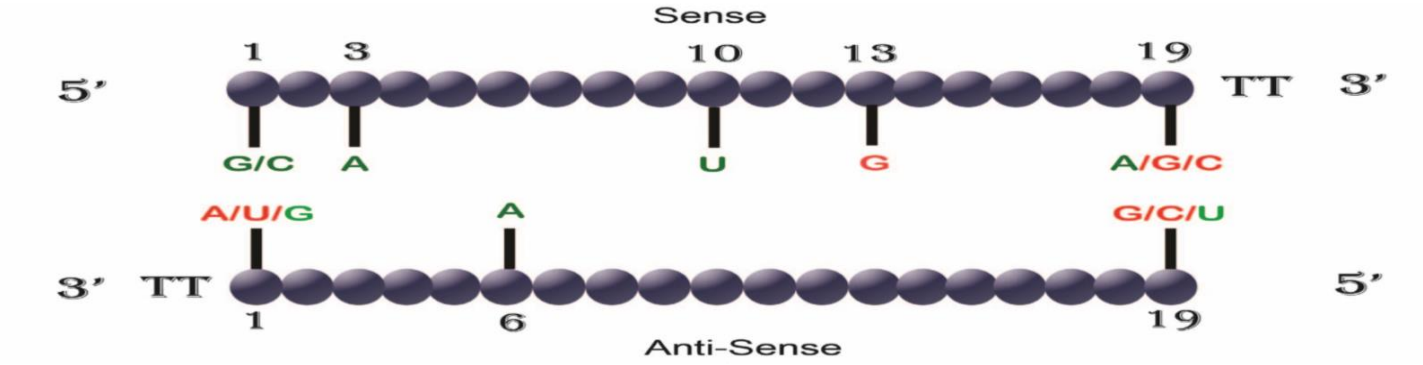
Thermodynamic features:

- Thermodynamic characters of functional siRNA molecules are guiding siRNA design…

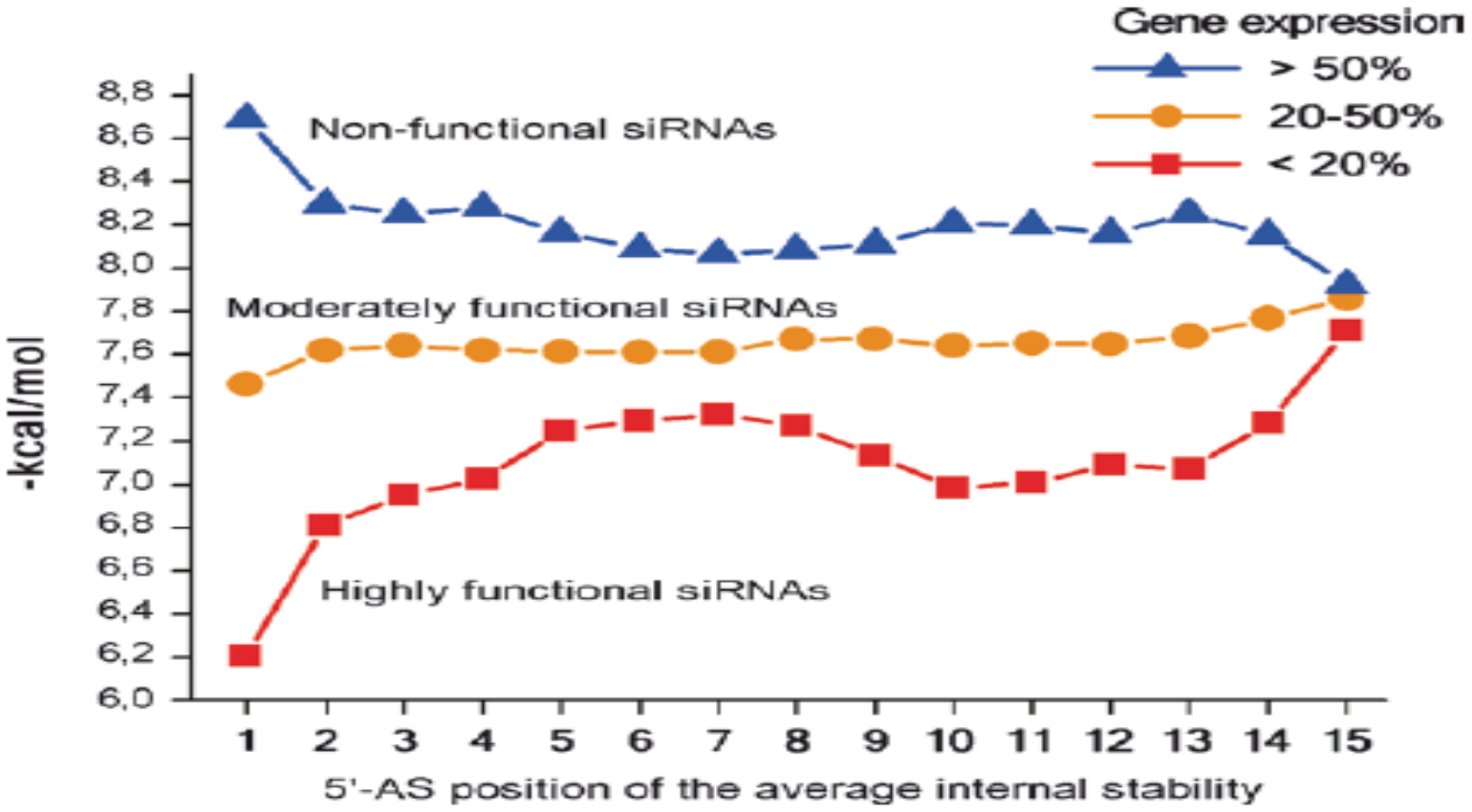
- Thermodynamic differences in the base-pairing stabilities **of the 5´-ends** of both **siRNA and miRNA** molecules play a **critical role** in determining **which strand** initiates RNA induced silencing complex (**RISC)** activation (**base pair; like A/U..)**

Thermodynamic features

- 1-Low stability in 5′ end of AS
- 2-Higher stability in 3′ of AS
- 3-Also; Energy valley in 9-14 nt
- Then ; A/U richness in 3′ end, GC richness in 5′ end **is important**



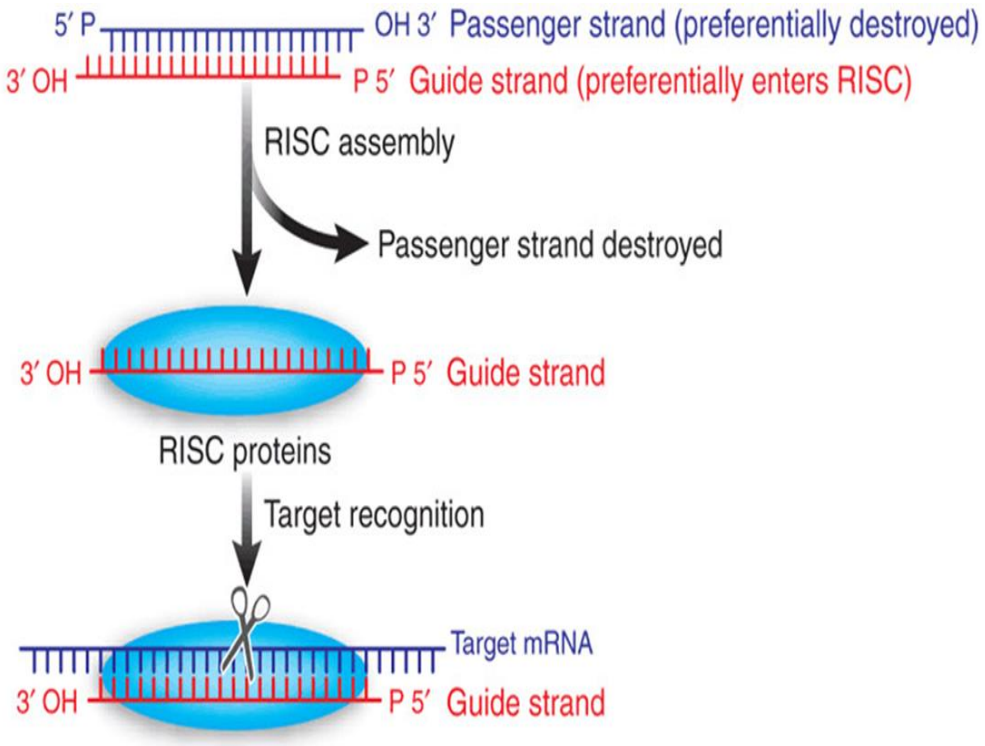
The best result



- Suggest an energy balance of **5 to 8 kilocalories** per mole is suitable for the **arms of siRNA**:

- **1-Correct Strand Selection**: **Lower energy in the 5' arm AS (SO LOW STABILITY)** ensures that this arm is selected as the guide strand by the RNA-induced silencing complex (RISC) instead of the 3' arm. This ensures **that the correct strand** is chosen by RISC and the RNAi process is executed correctly.
- **2-Greater Stability**: Arms with energy levels between **5 to 8** kilocalories per mole may contribute to **better and more stable activity siRNA**. This can increase the duration of siRNA activity in cells, ultimately **improving their efficiency**.

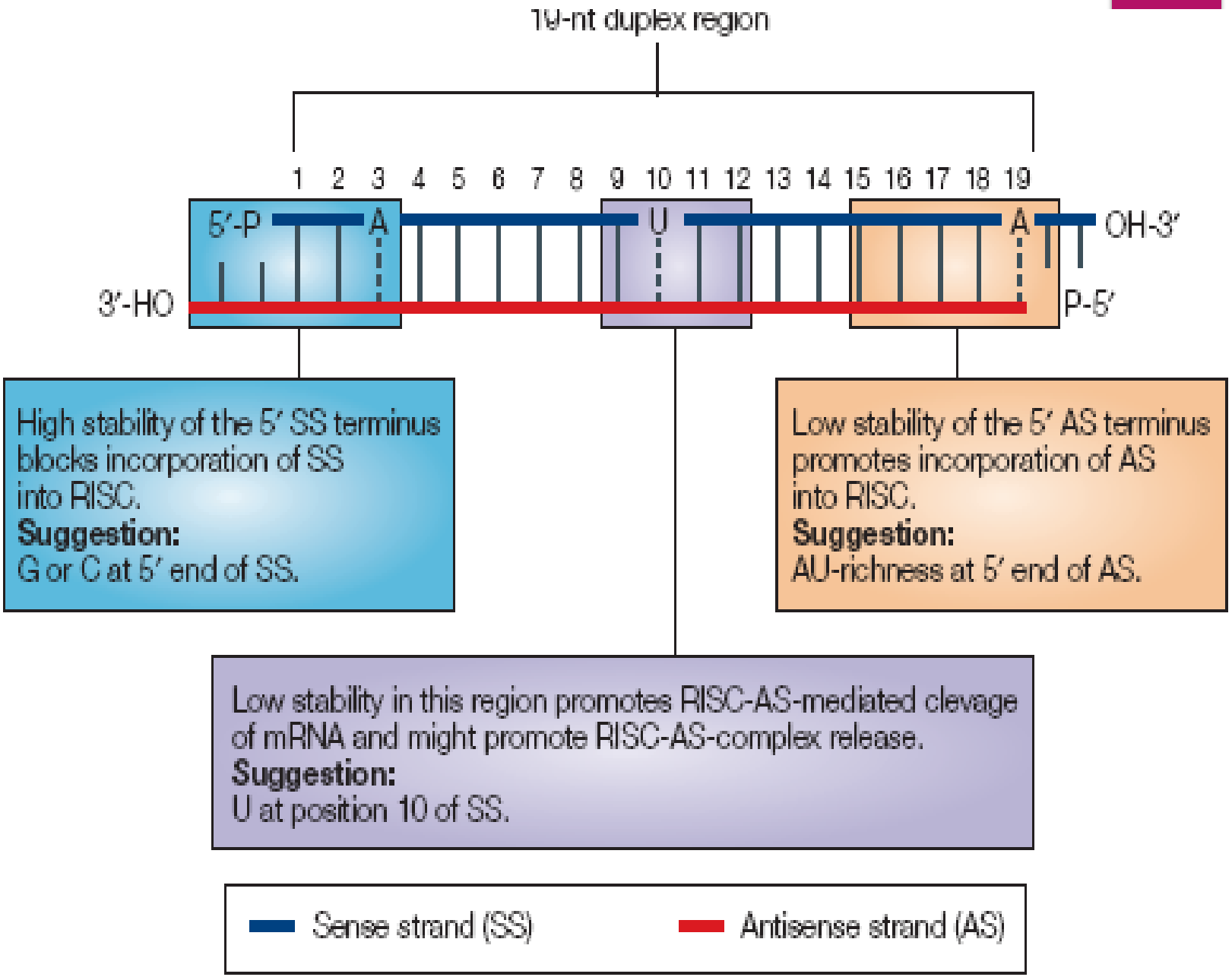
- ### Summary:-
- ****Sense strand**:** Separated and has no specific role in the RNAi process.-
- ****Antisense strand**:** Binds to the target mRNA and causes it to be degraded or inhibited.



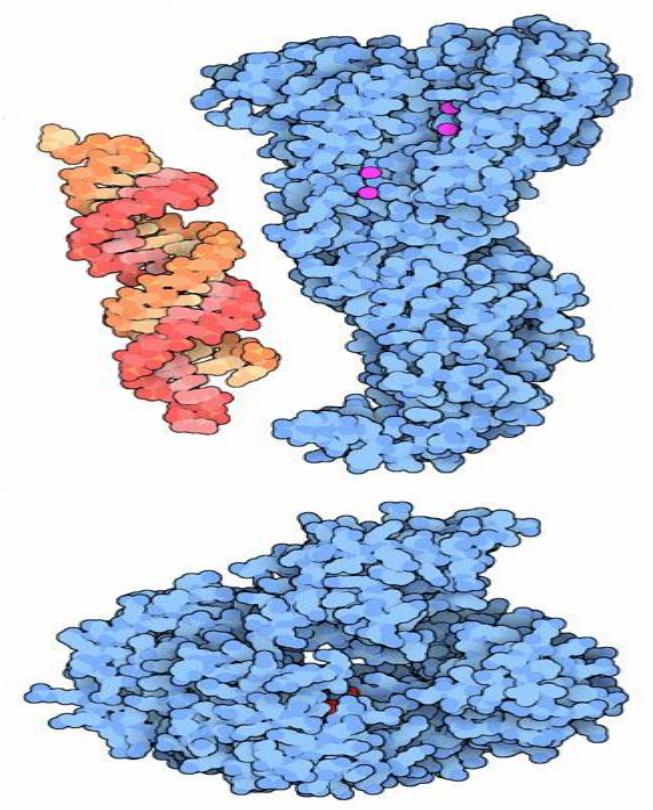
Transfection

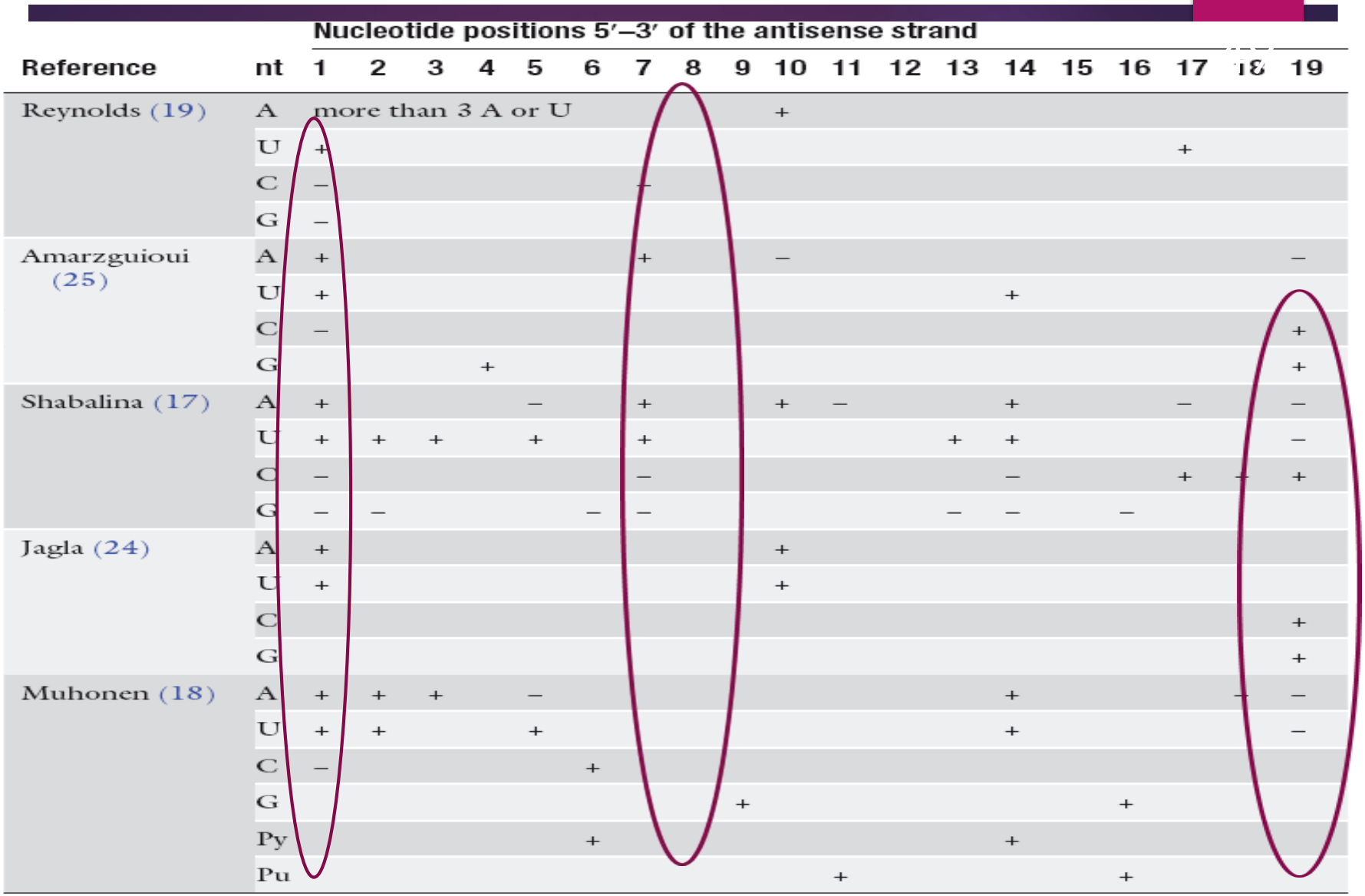
- Transient or Stable of (naked or in vector)
- In transient one: the double-stranded RNA(19-21 base) is introduced to the target cells by different methods…

- In the stable transfection: the exogenous double-stranded DNA expressing **short hairpin RNA** (shRNA) is exposed to the cells
- The stable procedure : **more effective in** mammalian cells. The double-stranded **DNA will be integrated** to the cell's genome and **transcribed to shRNA** by RNA **polymerase II/III**. (Dividing cell, Hela and non –dividing cells, neurons)
- Then the processed shRNA is exported from the nucleus to the cytoplasm


19-nt duplex region
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
5′-P
A
U
A
OH-3′
3′-HO
P-5′
High stability of the 5′ SS terminus blocks incorporation of SS into RISC.
Suggestion:
G or C at 5′ end of SS.
Low stability of the 5′ AS terminus promotes incorporation of AS into RISC.
Suggestion:
AU-richness at 5′ end of AS.
Low stability in this region promotes RISC-AS-mediated clevage of mRNA and might promote RISC-AS-complex release.
Suggestion:
U at position 10 of SS.
Sense strand (SS)
Antisense strand (AS)

A review of nucleotide content based on the most important studies



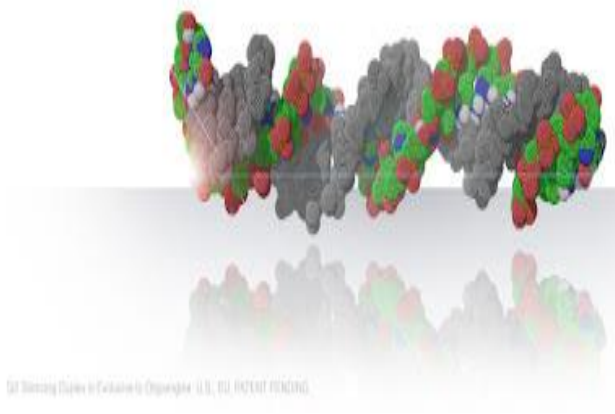


Reference	nt	Nucleotide positions 5′–3′ of the antisense strand																	
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Reynolds (19)	A	more than 3 A or U									+									
	U	+																+		
	C	–						–												
	G	–																		
Amarzguioui (25)	A	+						+			–									–
	U	+													+					
	C	–																		+
	G				+															+
Shabalina (17)	A	+				–		+			+	–			+			–		–
	U	+	+	+		+		+						+	+					–
	C	–						–							–			+	+	+
	G	–	–				–	–						–	–		–			
Jagla (24)	A	+									+									
	U	+									+									
	C																			+
	G																			+
Muhonen (18)	A	+	+	+		–									+				–	–
	U	+	+			+									+					–
	C	–					+													
	G									+							+			
	Py						+								+					
	Pu											+					+			

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
G1	Reynolds et al	effective			A							T			A C T						A T
G2	Ui-tei et al	effective	G C																		A T
		ineffective	A T																		G C
G3	Amarzguioui et al	effective	G C					A							T			C			A T
		ineffective	T									T									G

Essential Parameters	algorithms
Asymmetrical nucleotide content in the duplex (More A/U at 5' end of antisense sense strand and More G/C at 5' end of sense strand)	Amarzguioui et al Reynold et al Ui-tei et al
Weak base pairing at 5'end of antisense (presence of A/U)	Amarzguioui et al Ui-tei et al Jagla et al
Absence of internal repeats	Reynold et al Ui-tei et al
Presence of A at 6^{th} position of antisense strand	Amarzguioui et al
Presence of A at 3^{rd} and 19^{th} position of sense strand	Reynold et al
Absence of G at 13^{th} position and G/C at 19^{th} position of sense strand	Reynold et al Jagla et al
Presence of U at 10^{th} position of sense strand	Reynold et al Jagla et al

finally

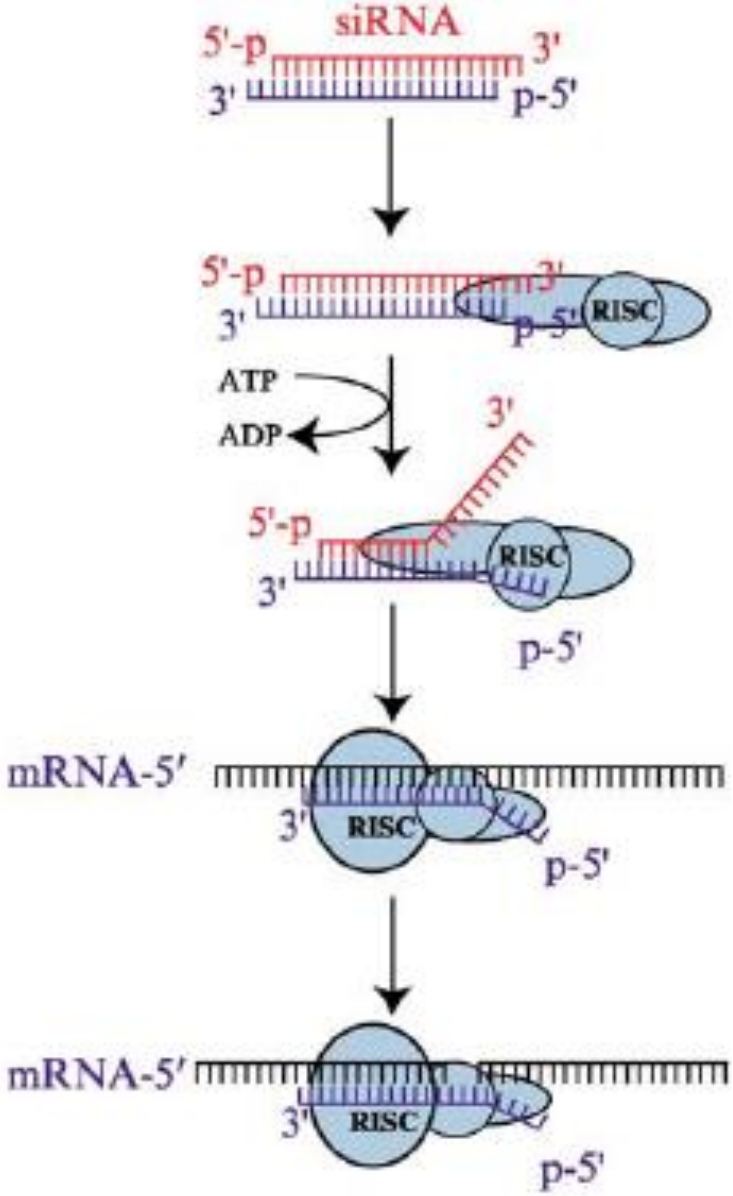


Advantages of siRNA

1. **Specificity**: siRNA offers highly **specific gene silencing** by targeting complementary mRNA sequences, reducing off-target effects.
2. **Reversible Effects**: The effects of siRNA are temporary and reversible, providing a controlled method of gene regulation.
3. **Versatility**: siRNA can be designed to target virtually any gene, making it a versatile tool for research and therapeutic purposes.
4. **Rapid Knockdown**: siRNA can achieve rapid knockdown of target genes,??! allowing for timely study of gene function

Disadvantage

- 1-Off-target effects.
- 2- Minimal Immune Response
- 3- Scalable Production (cost..)
- 4-Time Knockdown (24h; 48h..naked)



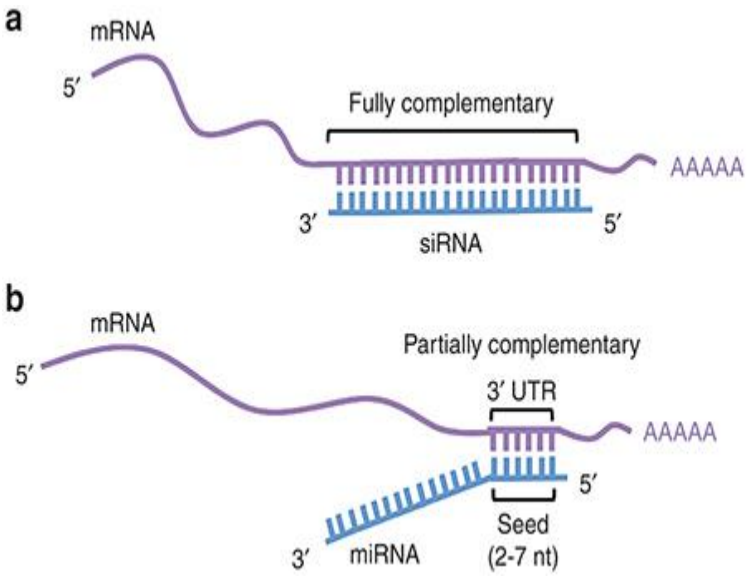
1. siRNA-preRISC complex formation
   - No internal repeats

2. siRNA unwinding; RISC ATP dependent activation
   - GC content < 50%
   - 5'AS end flexibility
   - A19
   - No C19

3. Target site recognition
   - GC content > 30%

4. mRNA cleavage
   - U10
   - A3
   - No G13

Target features?



mRNA features

- 1-Secondary structure of mRNA.
- 2-Target region incorporated in various **hairpin structures.**
- 3-Linear correlation between siRNA silencing of the target gene **and local free energy of the target region.**

- **Local Free Energy and Its Importance:**

- **Secondary Structure of mRNA**: mRNA can form various secondary structures, such as hairpins, which can affect the accessibility of the target region for siRNA binding.
- **Hairpin Structures**: These structures can be **stable or unstable**, depending on the **local free energy**. More stable hairpins (with lower free energy) are harder to unwind and target.
- **Linear Correlation**: There is a direct relationship between the efficiency of siRNA silencing and the local free energy of the target mRNA region. Regions with lower free energy or positive ΔG (less stable hairpins) are generally more accessible and thus more efficiently silenced by siRNA.

Free energy calculation: S fold, M fold, genebee

RNA Folding Form

M. Zuker

Mfold web server for nucleic acid folding and hybridization prediction.

Nucleic Acids Res. **31 (13)**, 3406-15, (2003)

[Abstract] [Full Text] [Supplementary Material] [Additional Information]

The folding temperature is fixed at 37°. You may still fold with the older *version 2.3* RNA parameters, which allow the temperature to be varied.

DNA mfold server. Quikfold. Fold many short RNA or DNA sequences at once.

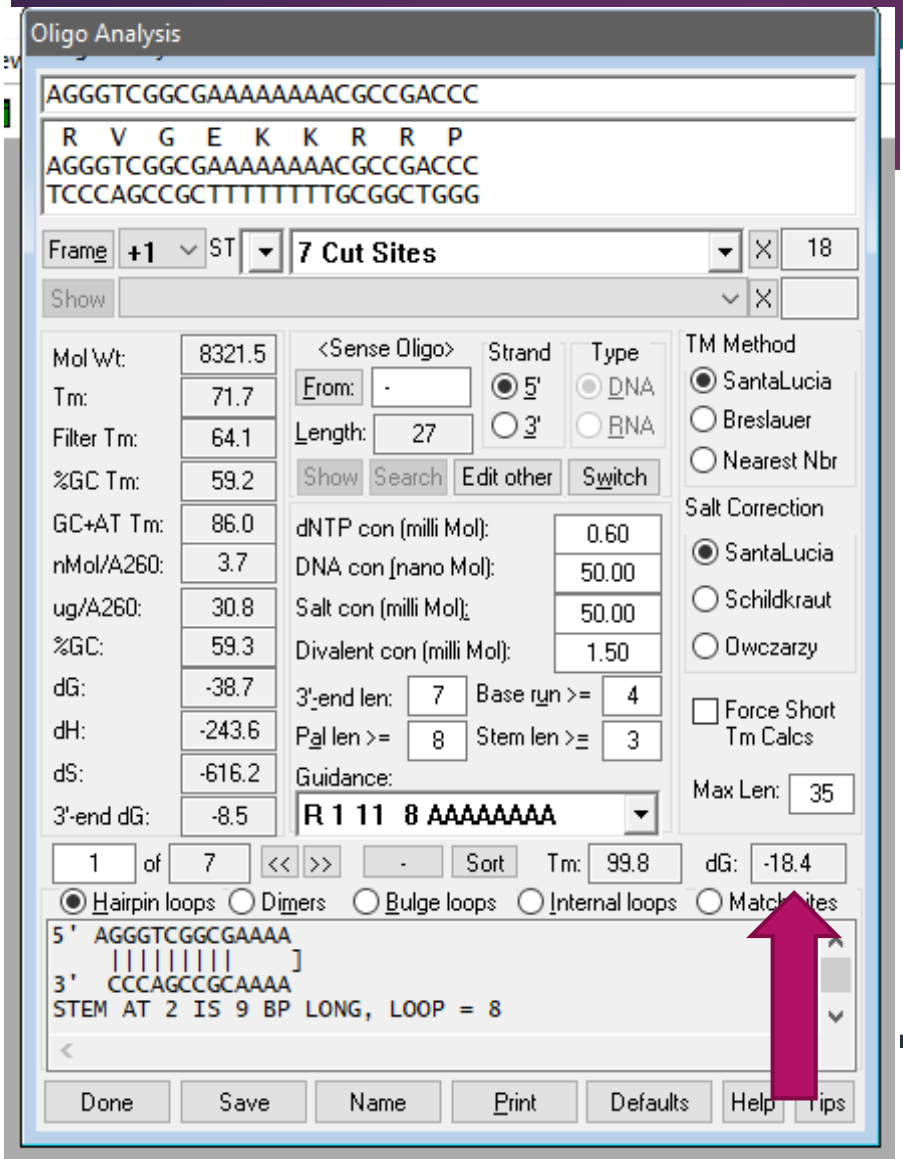
Enter sequence name:

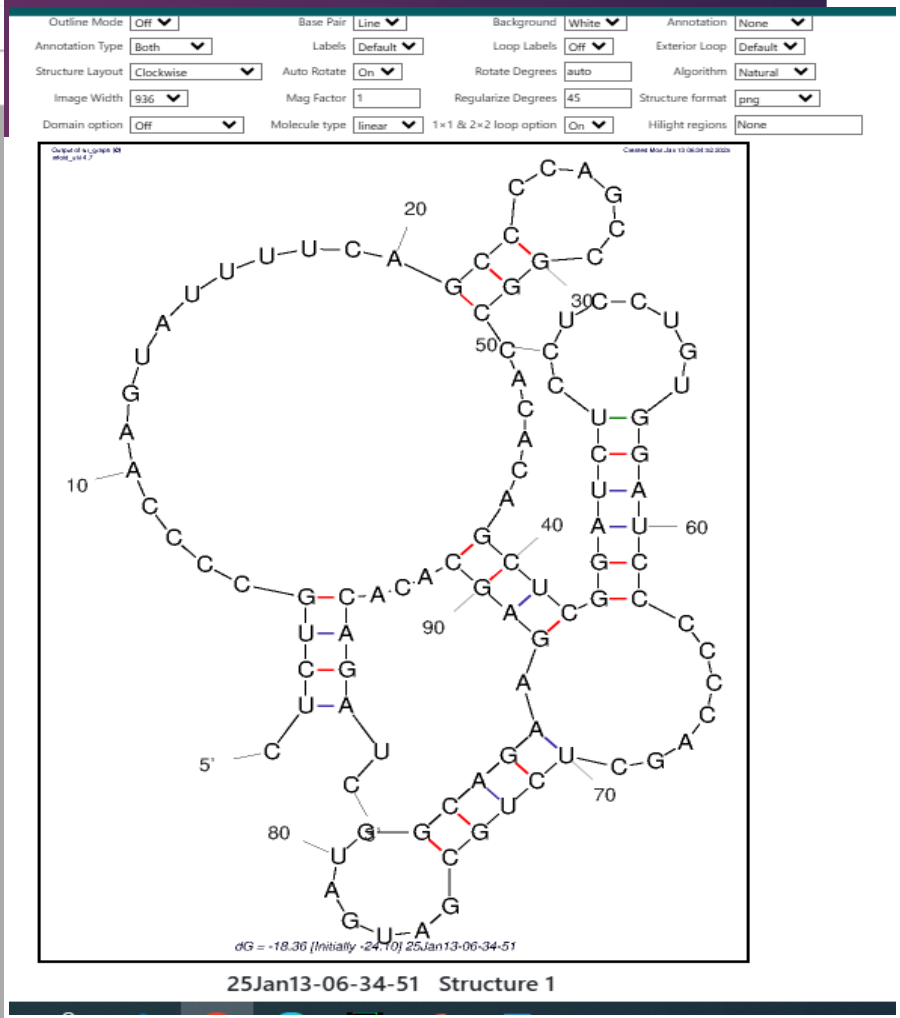
Enter the sequence to be folded in the box below. All non-alphabet characters will be removed.
FASTA format may be used.

```
seq1>
CTCTGCCCCAAGTATTTTCAGCCCCAGCCGGCCACACAGCTCGGATCTCCTCCTGTGGATCCCCCCAGCTCTGCGATGATGGCAGAAGAGCACACAGATCTCGAGGCCCAGATCGTCAAGGAT
ATCCACTGCAAGGAGATTGACCTGGTGAACCGAGACCCCAAGAACATTAACGAGGACATAGTCAAGGTGGATTTTGAAGACGTGATCGCAGAGCCTGTGGGCACCTACAGCTTTGACGGCGTG
TGGAAGGTGAGCTACACCACCTTCACTGTCTCCAAGTACTGGTGCTACCGTCTGTTGTCCACGCTGCTGGGCGTCCCACTGGCCCTGCTCTGGGGCTTCCTGTTCGCCTGCATCTCCTTCTGC
CACATCTGGGCGGTGGTGCCATGCATTAAGAGCTACCTGATCGAGATCCAGTGCATCAGCCACATCTACTCACTCTGCATCCGCACCTTCTGCAACCCACTCTTCGCGGCCCTGGGCCAGGTC
TGCAGCAGCATCAAGGTGGTGCTGCGGAAGGAGGTCTAAAGCCAGGGACTGCTCCATACCCCATGATGGAGCACACGGTGTAGGGAAGCCAGAAAGAAAAGACGGCCCAGCCACAGAAGCACA
ATGG
```

Format Sequence | Clear Constraints | Check Constraints

Secondary structures





Immune stimulatory effect

- 1-Double-stranded RNA **(dsRNA)** induces the production of **type I interferon** (IFN), which plays a crucial role in the antiviral immune response. This induced type I interferon **activates the enzymes PKR and OAS as follows**:
- **A-PKR**: **Binds to dsRNA** and becomes activated. This enzyme **phosphorylates various** proteins in the cell, halting protein translation and preventing viral replication
- **B-.OAS**: Also becomes activated by dsRNA. These enzymes produce 2',5'-oligoadenylates, which activate **RNAase L**. RNAase L degrades viral RNA and prevents viral replication.
- 3-5'-UGUGU-3' and 5'-GUCCUUCAA-3': high immune stimulatory
- 4-5'-UGU-3' motif is recognized by TLR7/8

Off target effect

- Meticulous blast search in 5' region of Anti sense strand , Searches in :
- 1- mRNA data bases
- 2- EST databases
- 3- nucleotides database...

Criterion in summary

Criteria	Probable reason
Biophysical, thermodynamic and structural considerations	
Overall low to medium G+C content (30–50%)	Facilitates interaction with RISC and unwinding
Low internal stability at the 5′ antisense strand	Promotes antisense-strand selection by RISC
High internal stability at the 5′ sense strand	Blocks sense-strand selection by RISC
Absence of internal repeats or palindromes	Increases the concentration of functional, stable hairpins
A-form helix between siRNAs and target mRNA	Enhances RNA–RNA interactions and promotes cleavage
Base preferences at specific positions in the sense strand	
Presence of an A at position 3 and 19* of sense strand	Promotes antisense-strand selection by RISC
Absence of a G or C at position 19 of sense strand	Promotes antisense-strand selection by RISC
Presence of a U at position 10 of sense strand	Promotes RISC mediated cleavage of mRNA and dissociation of the RISC–siRNA complex
Absence of a G at position 13 of sense strand	Promotes efficient unwinding
Enhancing specificity of siRNA-mediated gene silencing	
Perform stringent homology searches	Minimizes potential nonspecific gene silencing
Avoid low-stringency sequence interactions between siRNA and 3′UTR	Minimizes potential nonspecific gene silencing

Programs

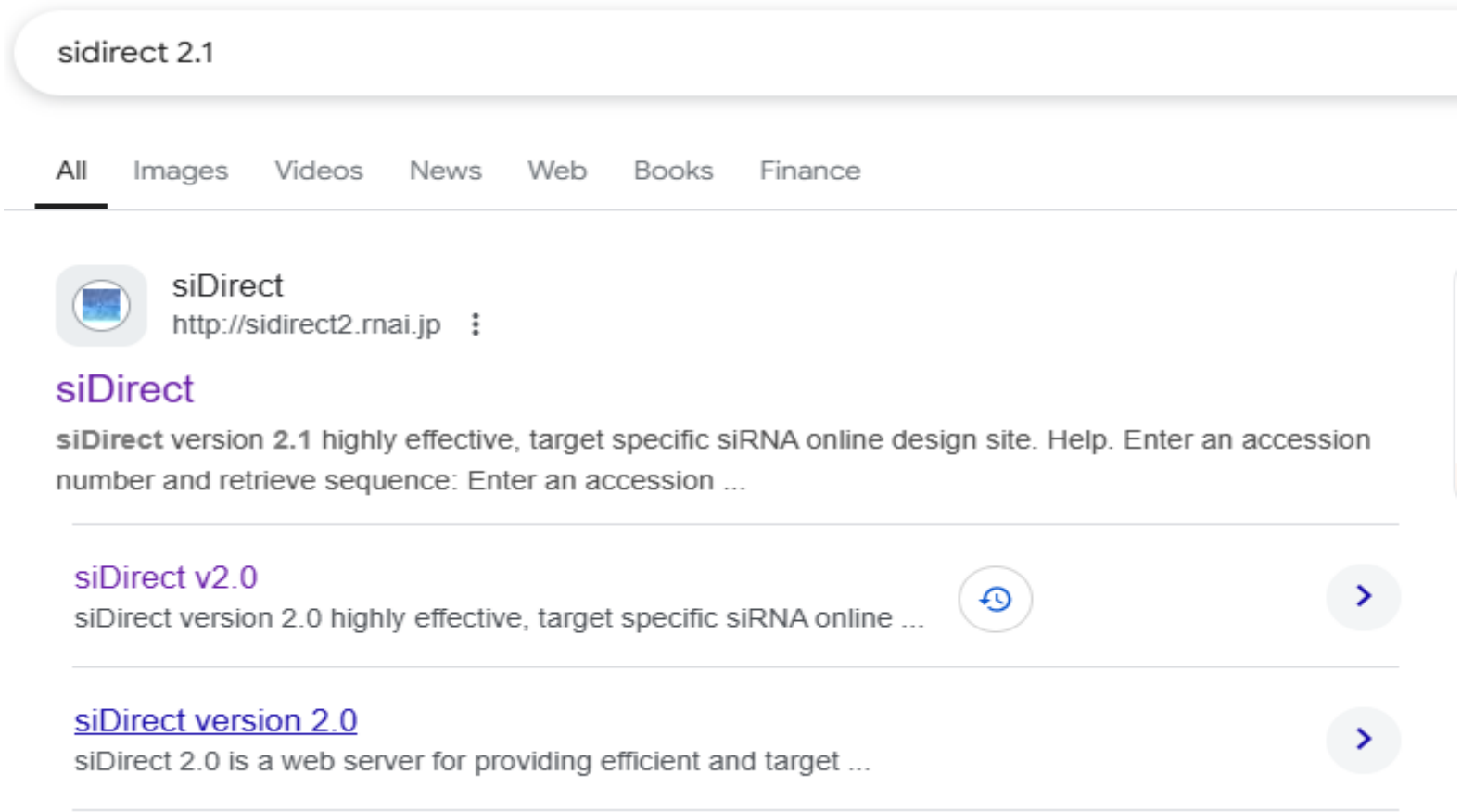
- Invitrogen
- Qiagen
- Dharmcon
- IDT
- Si Direct……

URLs

Source	Web site
Ambion	www.ambion.com
Dharmacon	www.dharmacon.com
Integrated DNA Technologies	Biotools.idtdna.com
Iris Genetics	www.irisgenetics.com
Qiagen	www.qiagen.com
Oligoengine	www.oligoengine.com
Open Biosystems	www.openbiosystems.com
Sfold	Sfold.wadsworth.org
The Whitehead Institute	jura.wi.mit.edu/pubint/ www.iona.wi.mit.edu/siRNAext

Tools	URLs	Comments
siDESIGN	http://www.dharmacon.com/	Scores and ranks candidate siRNAs based on thermodynamic and sequence-related criteria. BLAST search is conducted by default.
RNAi Designer	https://rnaidesigner.invitrogen.com/rnaiexpress/	Ranks candidate siRNAs using a primitive scoring system. BLAST search is automatic and the results are shown.
BIOPRED*si*	http://www.biopredsi.org	An artificial neural network–based tool, which was trained with ~2,500 experimentally assessed siRNAs. Analysis of genome-wide specificity is included.
Whitehead siRNA Selection server	http://jura.wi.mit.edu/bioc/siRNA	Offers flexibility in defining siRNA sequence patterns and selection of filter functions. Different properties of selected siRNAs are calculated, including thermodynamic values, polymorphisms are identified and the results of configurable BLAST search and filtering are shown. The user can sort the output in various ways and balance decisions.
siDE	http://side.bioinfo.ochoa.fib.es/	Developed for high-throughput applications of siRNAs using several published algorithms for efficacy prediction and a nonredundant database for specificity analysis.
siSearch	http://sisearch.cgb.ki.se/	The kernel algorithm focuses primarily on energy features of effective siRNAs. Alternative algorithms are also implemented and integrated in the tool. siSearch is expandable to include newly discovered rules.
Sirna	http://sfold.wadsworth.org/sirna.pl	Sequence selection tool, which incorporates the target accessibility in the evaluation. No specificity analysis.
siRNA design software	http://www.cs.hku.hk/~sirna	Candidate siRNAs proposed by various previously developed sequence selection tools are classified based on target accessibility.

Design web server



http://sidirect2.rnai.jp/

siDirect version 2.0 highly effective, target specific siRNA online design site. Help

Enter an accession number and retrieve sequence:

retrieve sequence

or Paste in a nucleotide sequence:

```
>sample sequence
ggctgccaag aacctgcagg aggcagaaga atggtacaaa tccaagtttg ctgacctctc
tgaggctgcc aaccggaaca atgacgccct gcgccaggca aagcaggagt ccactgagta
ccggagacag gtgcagtccc tcacctgtga agtggatgcc cttaaaggaa ccaatgagtc
cctggaacgc cagatgcgtg aaatggaaga gaactttgcc gttgaagctg ctaactacca
agacactatt ggccgcctgc aggatgagat tcagaatatg aaggaggaaa tggctcgtca
ccttcgtgaa taccaagacc tgctcaatgt taagatggcc cttgacattg agattgccac
ctacaggaag ctgctggaag gcgaggagag caggatttct ctgcctcttc caaacttttc
ctccctgaac ctgagggaaa ctaatctgga ttcactcccc ctggttgata cccactcaaa
aaggacactt ctgattaaga cggttgaaac tagagatgga caggttatca acgaaacttc
tcagcatcac gatgaccttg aataaaaatt gcacacactc agtgcagcaa tatattacca
```

design siRNA

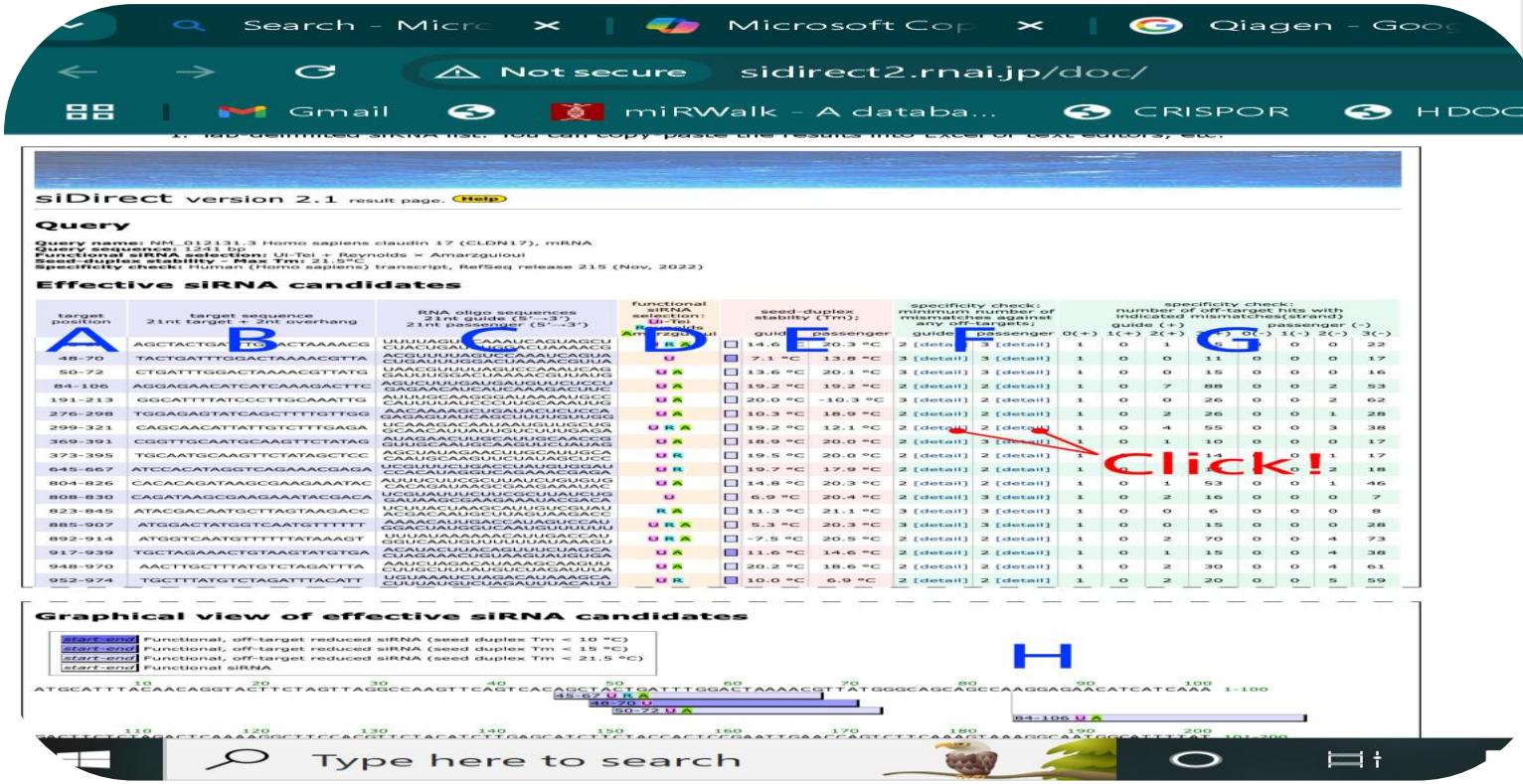
Options: click here

siDirect v.2.0 | Last modified on Dec 3, 2009.

Examples

Example 1 | Designing siRNAs for human claudin 17 (CLDN17)

1. Enter the accession number for human claudin 17 (NM_012131).
2. Click 'retrieve sequence' to obtain the nucleotide sequence.
3. Alternatively, you can directly paste a nucleotide sequence.
   Accepted input types are FASTA or a plain nucleotide sequence up to 10 kbp.
4. Click 'design siRNA'.



Results:

A-siRNA target positions.

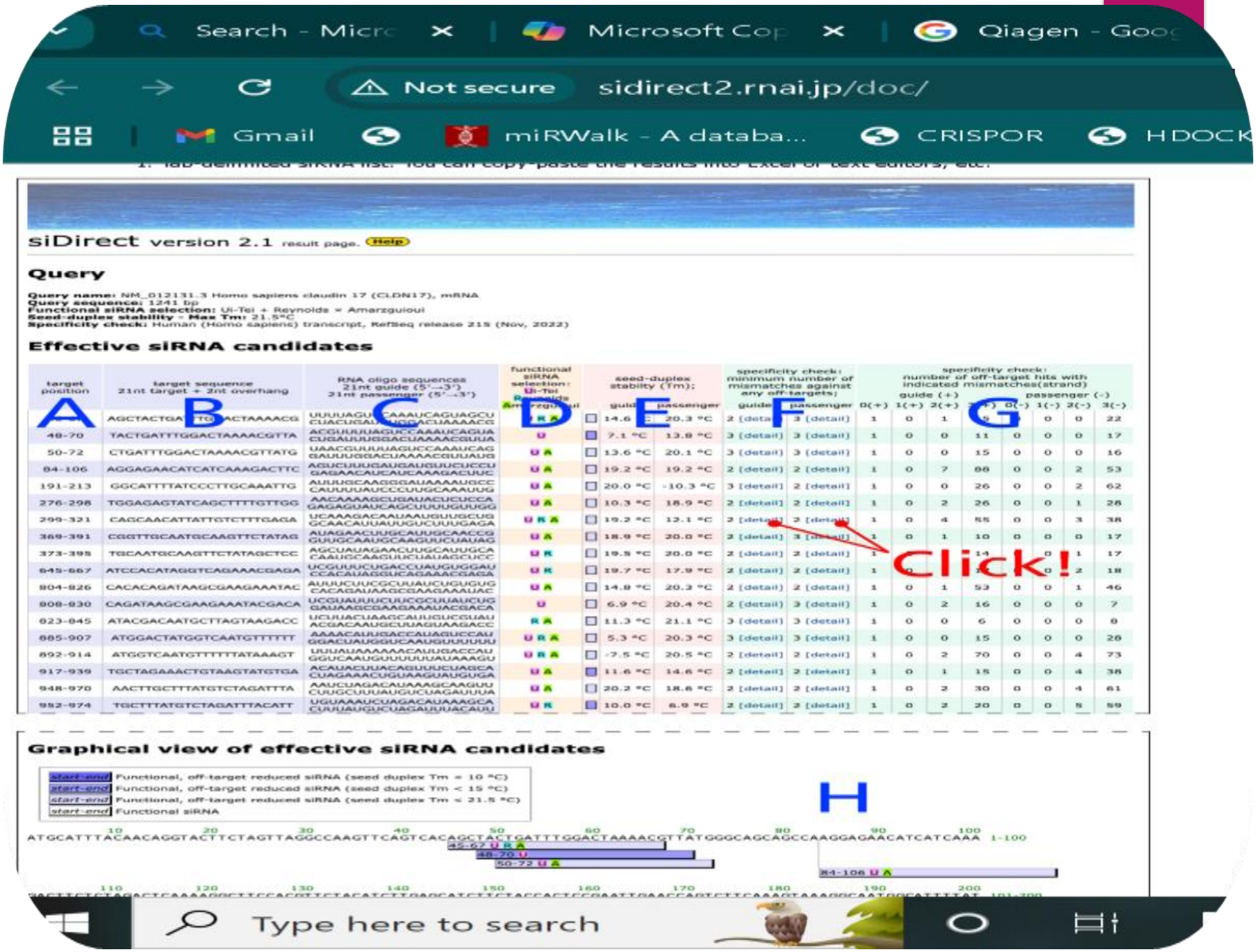
B-siRNA target sequences (21nt + 2nt overhang).

C-Oligonucleotide sequences (21nt guide strand and 21nt passenger strand).

D-siRNA efficacy predictions. siRNAs with U, R, A satisfy the functional siRNA design algorithms of Ui-Tei et al. (reference 3), Reynolds et al. (reference 4), and Amarzguioui et al. (reference 5), respectively.

Calculated Tm of the siRNA seed region.

E-Selecting an siRNA with a lower seed Tm reduces off-target effects. → Ui-Tei et al. (reference 6)


Search - Micr
Microsoft Cop
Qiagen - Goog
Not secure
sidirect2.rnai.jp/doc/
Gmail
miRWalk - A databa...
CRISPOR
HDOCK
siDirect version 2.1 result page. Help
Query
Query name: NM_012131.3 Homo sapiens claudin 17 (CLDN17), mRNA
Query sequence: 1241 bp
Functional siRNA selection: Ui-Tei + Reynolds × Amarzguioui
Seed-duplex stability - Max Tm: 21.5°C
Specificity check: Human (Homo sapiens) transcript, RefSeq release 215 (Nov, 2022)
Effective siRNA candidates
target position
target sequence 21nt target + 2nt overhang
RNA oligo sequences 21nt guide (5'→3') 21nt passenger (5'→3')
functional siRNA selection: Ui-Tei Reynolds Amarzguioui
seed-duplex stability (Tm): guide passenger
specificity check: minimum number of mismatches against any off-targets: guide passenger
specificity check: number of off-target hits with indicated mismatches(strand) guide (+) passenger (-)
0(+) 1(+) 2(+) 0(-) 1(-) 2(-) 3(-)
A
B
C
D
E
F
G
Click!
Graphical view of effective siRNA candidates
start-end Functional, off-target reduced siRNA (seed duplex Tm = 10 °C)
start-end Functional, off-target reduced siRNA (seed duplex Tm < 15 °C)
start-end Functional, off-target reduced siRNA (seed duplex Tm < 21.5 °C)
start-end Functional siRNA
H
45-67 U R A
48-70 U
50-72 U A
84-106 U A
Type here to search

- **متن :Fحداقل تعداد mismatchبا هر توالی غیرهدف**
- این بخش فقط کمترین تعداد mismatchبین siRNAو هر mRNAغیرهدف رو گزارش می‌ده.
- یعنی اگر یک توالی غیرهدف فقط ۱ mismatchبا siRNAداشته باشه، اون رو به عنوان خطرناک‌ترین off-targetدر نظر می‌گیره.
- این عدد به شما می‌گه که بدترین حالت چقدر نزدیک به تطابق کامل بوده.
- اما نمی‌گه چند تا توالی با اون تعداد mismatchوجود داره—فقط حداقل رو می‌گه.
- کاربردش: برای غربال اولیه—اگر حداقل mismatchبرابر 0 یا 1 باشه، اون siRNAممکنه خطرناک باشه و باید حذف بشه.
- ---
- **متن :Gتعداد تطابق‌ها با درجات مختلف mismatch**
- این بخش تعداد توالی‌هایی رو که با siRNAتطابق دارند، با درجات مختلف mismatchگزارش می‌ده:
  - 19/19: تطابق کامل
  - 18/19: یک mismatch
  - 17/19: دو mismatch
  - 16/19: سه mismatch
- ستون 0(+) معمولاً نشون‌دهنده‌ی تطابق کامل با ژن هدف اصلیه (مثلاً claudin 17).
- کاربردش: برای ارزیابی دقیق‌تر—مثلاً اگر یک siRNAبا ۵۰ توالی غیرهدف فقط یک mismatchداشته باشه، حتی اگر در متن Fعدد "1" بیاد، در متن G می‌فهمی که اون خطر جدی داره چون تعدادش زیاده.

siDirect version 2.1 result page. Help

Query

Query name: NM_012131.3 Homo sapiens claudin 17 (CLDN17), mRNA
Query sequence: 1241 bp
Functional siRNA selection: Ui-Tei + Reynolds + Amarzguioui
Seed-duplex stability - Max Tm: 21.5°C
Specificity check: Human (Homo sapiens) transcript, RefSeq release 215 (Nov, 2022)

Effective siRNA candidates

target position	target sequence 21nt target + 2nt overhang	RNA oligo sequences 21nt guide (5'→3') 21nt passenger (5'→3')	functional siRNA selection: Ui-Tei Reynolds Amarzguioui	seed-duplex stability (Tm); guide	passenger	specificity check: minimum number of mismatches against any off-targets; guide	passenger	specificity check: number of off-target hits with indicated mismatches(strand) guide (+) 0(+)	1(+)	2(+)	guide (+) passenger (-) 0(-)	1(-)	2(-)	3(-)
46-68	AGCTACTGATTTGGACTAAAACG	UUUUAGUCCAAAUCAGUAGCU CUACUGAUUUGGACUAAAACG	U R A	14.6 °C	20.3 °C	2 [detail]	3 [detail]	1	0	1	5	0	0	0	22
48-70	TACTGATTTGGACTAAAACGTTA	ACGUUUUAGUCCAAAUCAGUA CUGAUUUGGACUAAAACGUUA	U	7.1 °C	13.8 °C	3 [detail]	3 [detail]	1	0	0	11	0	0	0	17
50-72	CTGATTTGGACTAAAACGTTATG	UAACGUUUUAGUCCAAAUCAG GAUUUGGACUAAAACGUUAUG	U A	13.6 °C	20.1 °C	3 [detail]	3 [detail]	1	0	0	15	0	0	0	16
84-106	AGGAGAACATCATCAAAGACTTC	AGGUCUUUGAUGAUGUUCUCCU GAGAACAUCAUCAAAGACUUC	U A	19.2 °C	19.2 °C	2 [detail]	2 [detail]	1	0	7	88	0	0	2	53
191-213	GGCATTTTATCCCTTGCAAATTG	AUUUGCAAGGGAUAAAAAUGCC CAUUUGUUUCCCUUGCGAAAUUG	U A	20.0 °C	-10.3 °C	3 [detail]	2 [detail]	1	0	0	26	0	0	2	62
276-298	TGGAGAGTATCAGCTTTTGTTGG	AACAAAAGCUGAUACUCUCCA GAGAGUAUCAGCUUUUGUUGG	U A	10.3 °C	18.9 °C	2 [detail]	2 [detail]	1	0	2	26	0	0	1	28
299-321	CAGCAACATTATTGTCTTTGAGA	UCAAAGACAAUAAUGUUGCUG GCAACAUUAUUGUCUUUGAGA	U R A	19.2 °C	12.1 °C	2 [detail]	2 [detail]	1	0	4	55	0	0	3	38
369-391	CGGTTGCAATGCAAGTTCTATAG	AUAGAACUUGCAUUGCAACCG GUUGCAAUGCAAGUUCUAUAG	U A	18.9 °C	20.0 °C	2 [detail]	3 [detail]	1	0	1	10	0	0	0	17
373-395	TGCAATGCAAGTTCTATAGCTCC	AGCUAUAGAACUUGCAUUGCA CAAUGCAAGUUCUAUAGCUCC	U R	19.5 °C	20.0 °C	2 [detail]	2 [detail]	1	0	2	70	0	0	4	73
645-667	ATCCACATAGGTCAGAAACGAGA	UCGUUUCUGACCUAUGUGGAU CCACAUAGGUCAGAAACGAGA	U R	19.7 °C	17.9 °C	2 [detail]	2 [detail]	1	0	1	15	0	0	4	38
804-826	CACACAGATAAGCGAAGAAATAC	AUUUCUUCGCCUUAUCUGUGUG CACACAGAUAAGCGAAGAAAUAC	U A	14.8 °C	20.3 °C	2 [detail]	2 [detail]	1	0	2	30	0	0	4	61
808-830	CAGATAAGCGAAGAAATACGACA	UCGUAUUUCUUCGCUUAUCUG GAUAAGCGAAGAAAUACGACA	U	6.9 °C	20.4 °C	2 [detail]	3 [detail]	1	0	1	53	0	0	1	46
823-845	ATACGACAATGCTTAGTAAGACC	AAAACAUUGACCAUAAGUCCAU ACGACAAUGCUUAGUAAGACC	U R A	11.3 °C	21.1 °C	3 [detail]	3 [detail]	1	0	0	6	0	0	0	8
885-907	ATGGACTATGGTCAATGTTTTTT	AAAACAUUGACCAUAGUCCAU GGACUAUGGUCAAUGUUUUUU	U R A	5.3 °C	20.3 °C	3 [detail]	3 [detail]	1	0	0	15	0	0	0	28
892-914	ATGGTCAATGTTTTTTATAAAGT	UUUAUAAAAAACAUUGACCAU GGUCAAUGUUUUUUAUAAAGU	U R A	-7.5 °C	20.5 °C	2 [detail]	2 [detail]	1	0	2	70	0	0	4	73
917-939	TGCTAGAAACTGTAAGTATGTGA	UCACAUACUUACAGUUUCUAGCA CUAGAAACUGUAAGUAUGUGA	U A	11.6 °C	14.6 °C	2 [detail]	2 [detail]	1	0	1	15	0	0	4	38
948-970	AACTTGCTTTATGTCTAGATTTA	AAUCUAGACAUAAAGCAAGUU UAGAUCUGUAUUUCGUUCAAU	U A	20.2 °C	18.6 °C	2 [detail]	2 [detail]	1	0	2	30	0	0	4	61
952-974	TGCTTTATGTCTAGATTTACATT	UGUAAAUCUAGACAUAAAGCA CUUUAUGUCUAGAUUUACAUU	U R	10.0 °C	6.9 °C	2 [detail]	2 [detail]	1	0	2	20	0	0	5	59

A B C D E F G

Click!

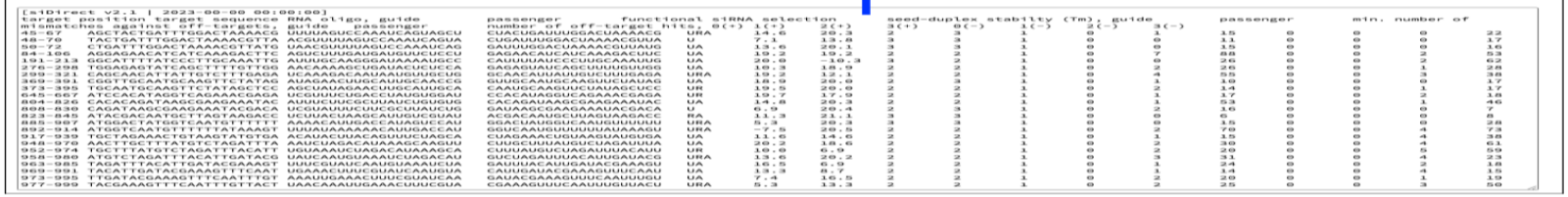
Graphical view of effective siRNA candidates

start-end Functional, off-target reduced siRNA (seed duplex Tm < 10 °C)
start-end Functional, off-target reduced siRNA (seed duplex Tm < 15 °C)
start-end Functional, off-target reduced siRNA (seed duplex Tm < 21.5 °C)
start-end Functional siRNA

H

Tab-delimited list (for data export)

I



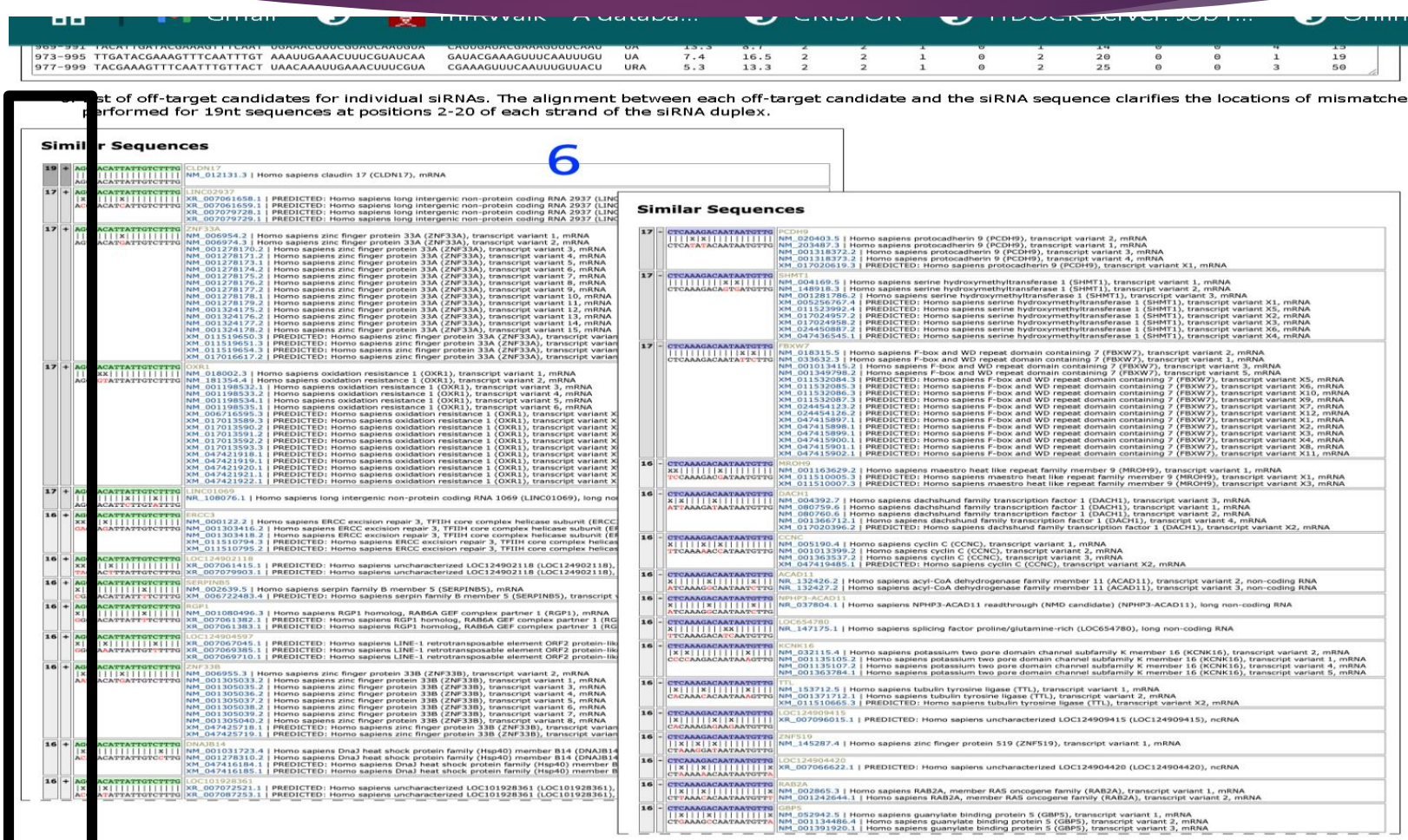
6. List of off-target candidates for individual siRNAs. The alignment between each off-target candidate and the siRNA sequence clarifies the locations of mismatches. Hits with performed for 19nt sequences at positions 2-20 of each strand of the siRNA duplex.

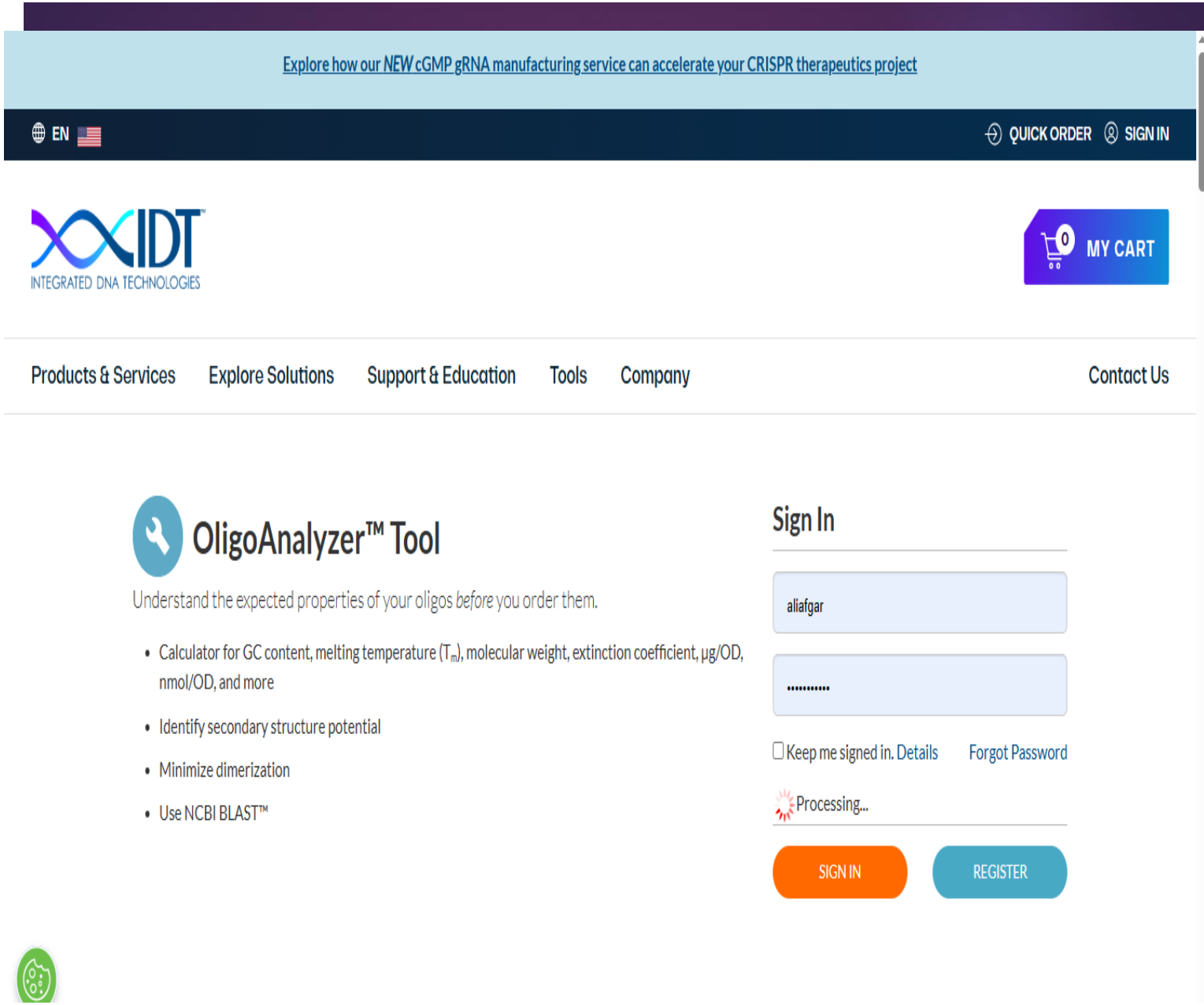
6

Similar Sequences

Type here to search

List of off-target candidates for individual siRNAs. The alignment between each off-target candidate and the siRNA sequence clarifies the locations of mismatches. Hits with a perfect match (19/19 matches), one mismatch (18/19 matches), two mismatches (17/19 matches), or three mismatches (16/19 matches) are shown. Searches are performed for 19nt sequences at positions 2-20 of each strand of the siRNA duplex

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Identify secondary structure potential
Minimize dimerization
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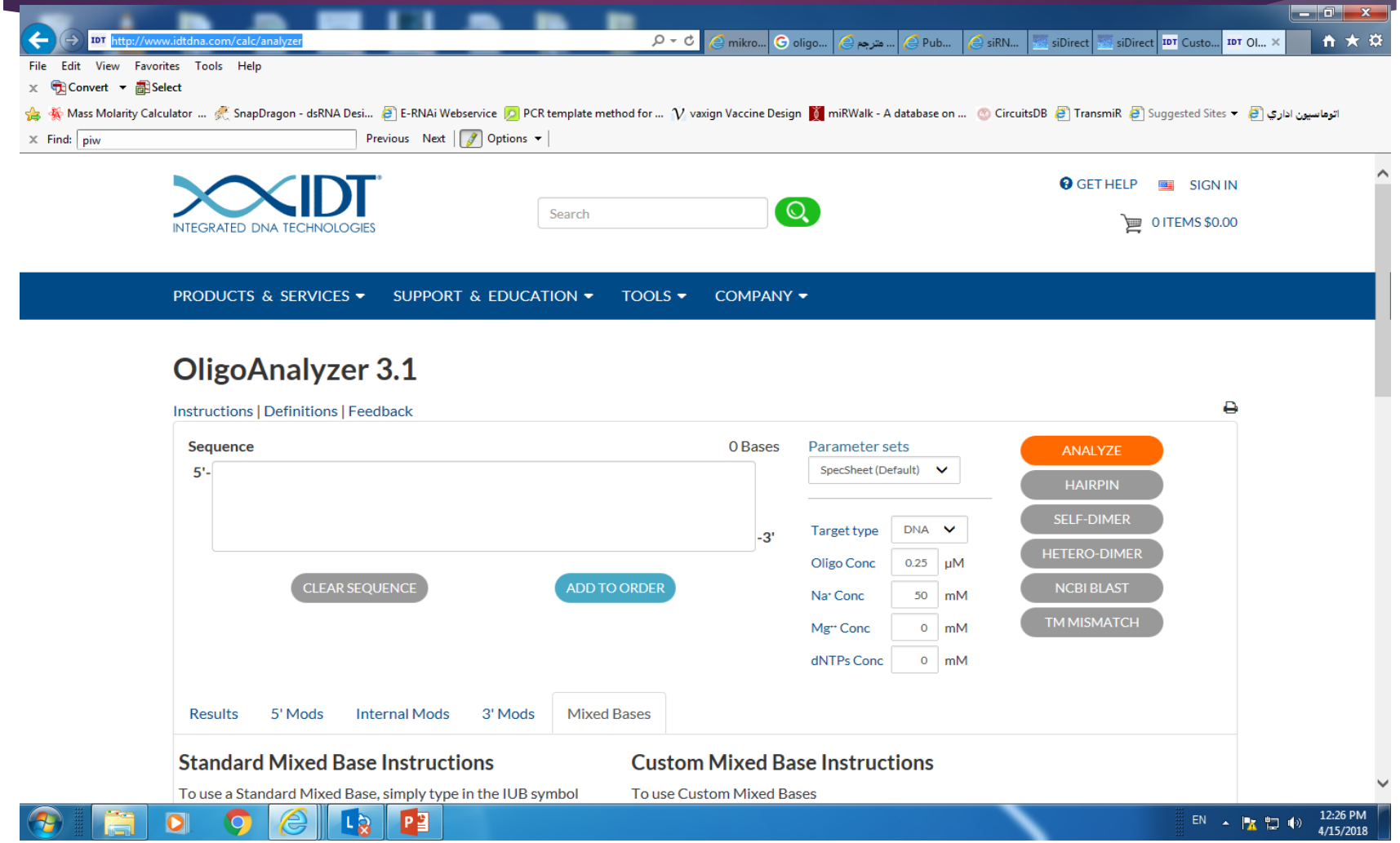

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Enter an Accession Number:

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BLAST Species

- (•) Human
- ( ) Mouse
- ( ) Rat
- ( ) Other (Manual BLAST)

SEARCH

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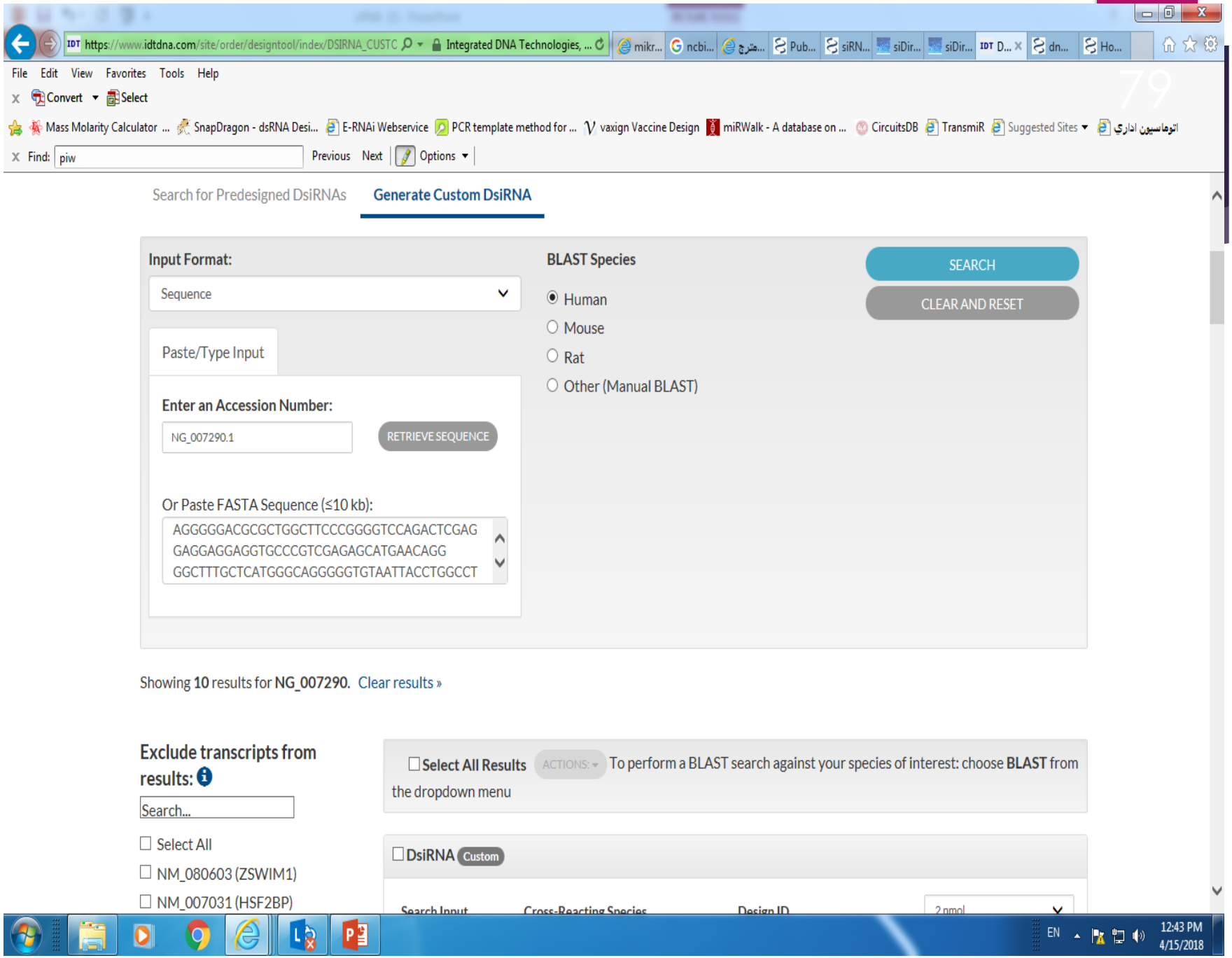
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Input Format:
Sequence
Paste/Type Input
Enter an Accession Number:
NG_007290.1
RETRIEVE SEQUENCE
Or Paste FASTA Sequence (≤10 kb):
AGGGGGACGCGCTGGCTTCCCGGGGTCCAGACTCGAG
GAGGAGGAGGTGCCCGTCGAGAGCATGAACAGG
GGCTTTGCTCATGGGCAGGGGGTGTAATTACCTGGCCT
BLAST Species
Human
Mouse
Rat
Other (Manual BLAST)
SEARCH
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Showing 10 results for NG_007290. Clear results »
Exclude transcripts from results:
Search...
Select All
NM_080603 (ZSWIM1)
NM_007031 (HSF2BP)
Select All Results
ACTIONS:
To perform a BLAST search against your species of interest: choose BLAST from the dropdown menu
DsiRNA
Custom
Search Input
Cross-Reacting Species
Design ID
2 nmol
EN
12:43 PM
4/15/2018

- **>NM_001234**
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GAGACCCCAAGAACATTAACGAGGACATAGTCAAGGTGGATTTTGAAGACGTGATCGCAGAGCCTGTGGGCACCTAC
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GCGCACTCAGGAAAGTGACCAGTGACCACTGGCGTTAGGAAGGTGGCTCCAGTAAAGGGTTTTGGCTGCATTTGGGGA
ATGCTGCATTTTGTTCGTGCCTGTAAGATTGGTTTGTGTCCTGACCAGCTCCAAAAATATACTTCACTGCCCTGAAAAACAGA
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GGAACCGTACACGTTCCTTGGAAGATCATGTTTAAGTGACTCCTGTTGCCTGAGCACAAAAATGGGCACCAATGGAGGA
AAATGACCCTTGGGCTGGCAGGGGCAGTGACCCTTCCAGGGTACCACTGAGGGAAGGGCCTGGGTTCAAGCCTCCC
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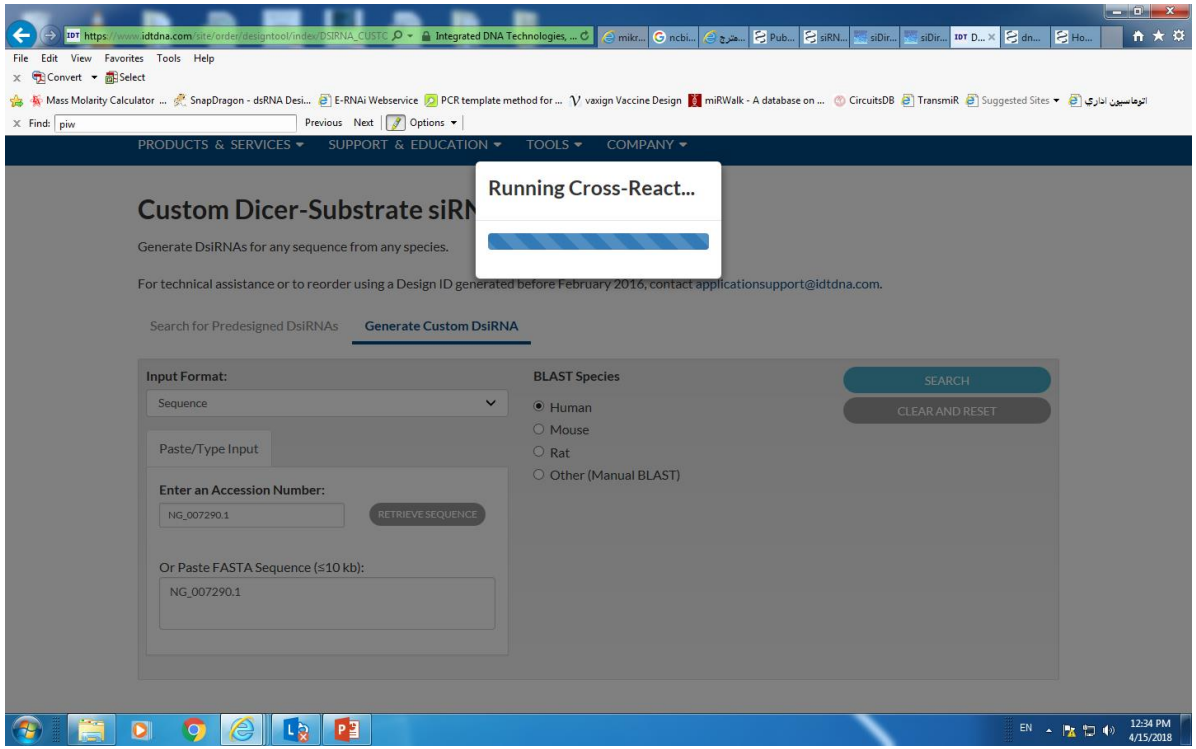
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Input Format:
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Paste/Type Input
Enter an Accession Number:
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RETRIEVE SEQUENCE
Or Paste FASTA Sequence (≤10 kb):
NG_007290.1
BLAST Species
Human
Mouse
Rat
Other (Manual BLAST)
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- [ ] Select All
- [ ] NM_001005416 (MARCH2)
- [ ] NM_001172895 (CAV1)
- [ ] NM_016496 (MARCH2)
- [ ] NM_001172897 (CAV1)
- [ ] NM_001234 (CAV3)
- [ ] NR_038882 (LOC286370)
- [ ] NM_033337 (CAV3)
- [ ] NM_001753 (CAV1)
- [ ] NM_001005415 (MARCH2)
- [ ] NM_001172896 (CAV1)
- [ ] NR_104669 (LOC101928569)
- [ ] NM_198182 (GRHL1)
- [ ] NM_001145260 (NCOA4)
- [ ] NM_001145261 (NCOA4)
- [ ] NM_001004329 (DBX2)
- [ ] NM_005437 (NCOA4)
- [ ] NM_001145262 (NCOA4)
- [ ] NM_001145263 (NCOA4)

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Search Input	Cross-Reacting Species	Design ID	2 nmol
NM_001234	Human	CD.Ri.496320.13.1	$110.00 USD

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Cross-Reacting Transcripts NM_001005416 (MARCH2), NM_001172895 (CAV1), NM_016496 (MARCH2), NM_001172897 (CAV1), NM_001234 (CAV3), NR_038882 (LOC286370), NM_033337 (CAV3), NM_001753 (CAV1), NM_001005415 (MARCH2), NM_001172896 (CAV1)

Sequence Positions 385-410

Sequence Details

Sense and antisense sequences

Sequence	Strand
5' rGrUrGrCrCrArUrGrCrArUrUrArArGrArGrCrUrArCrCrUGA 3'	+
5' rUrCrArGrGrUrArGrCrUrCrUrUrArArUrGrCrArUrGrGrCrArCrCrA 3'	-

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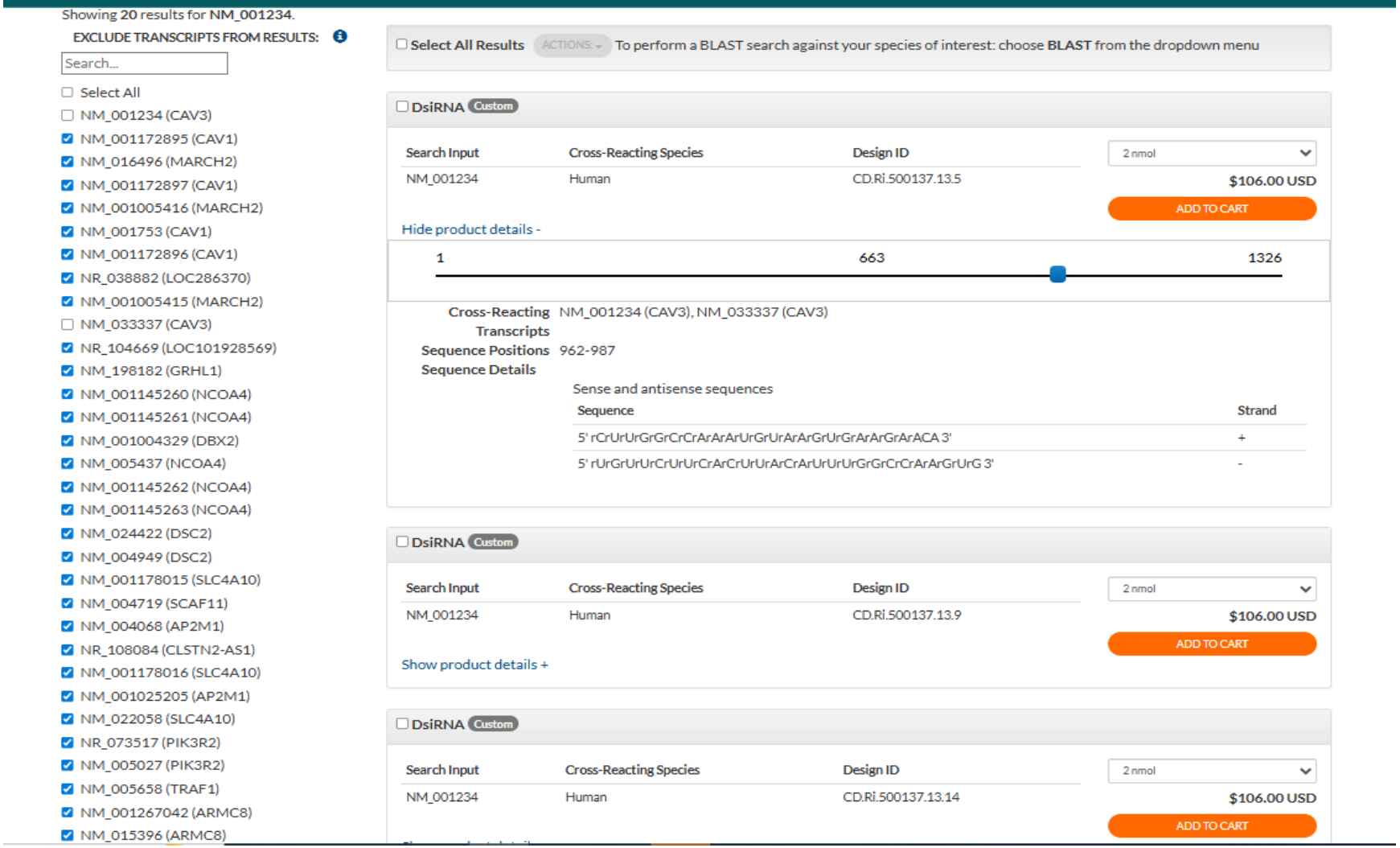
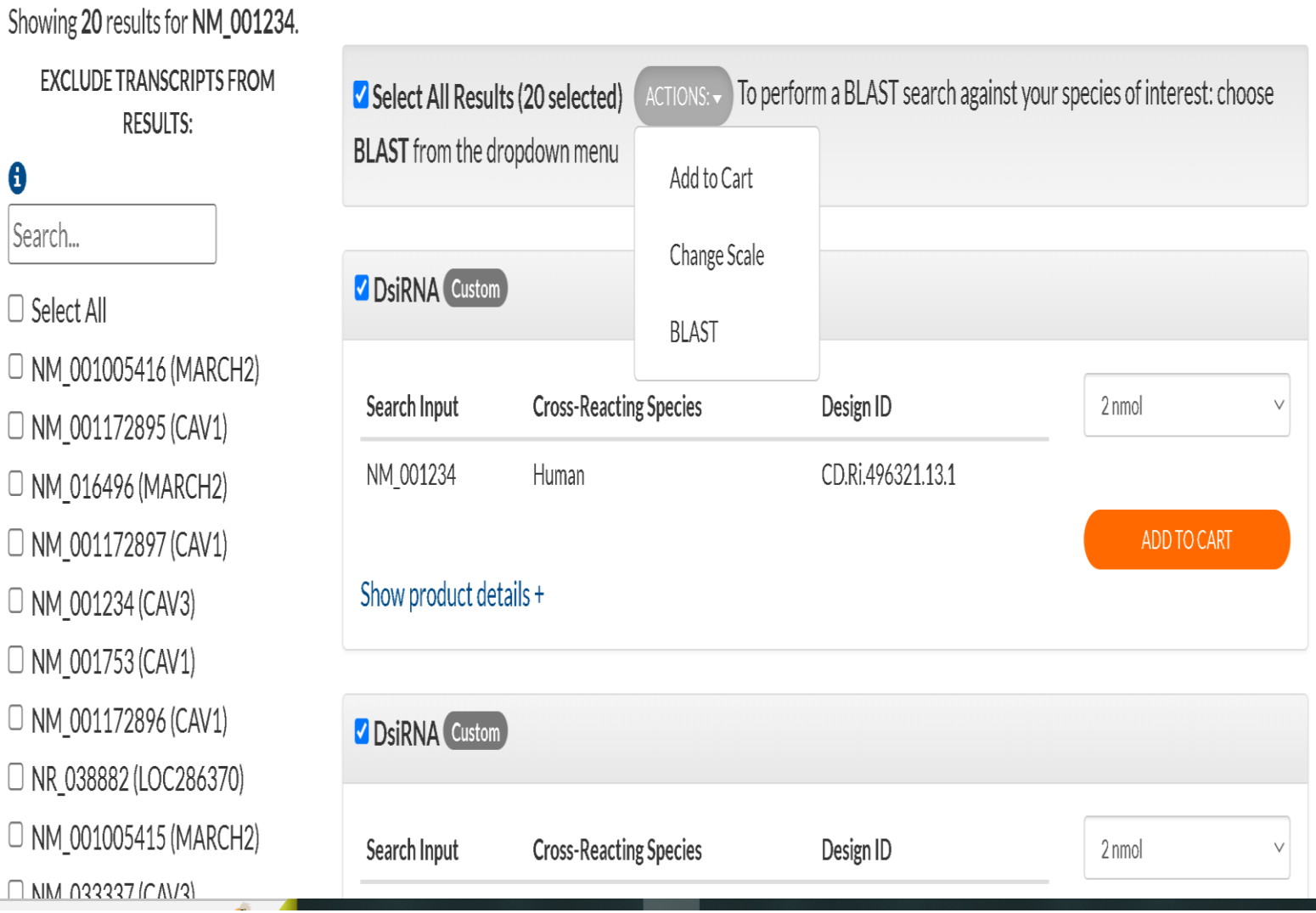
Exclude transcript from results



Blast


Showing 20 results for NM_001234.
EXCLUDE TRANSCRIPTS FROM RESULTS:
Search...
Select All
NM_001005416 (MARCH2)
NM_001172895 (CAV1)
NM_016496 (MARCH2)
NM_001172897 (CAV1)
NM_001234 (CAV3)
NM_001753 (CAV1)
NM_001172896 (CAV1)
NR_038882 (LOC286370)
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Select All Results (20 selected)
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Search Input
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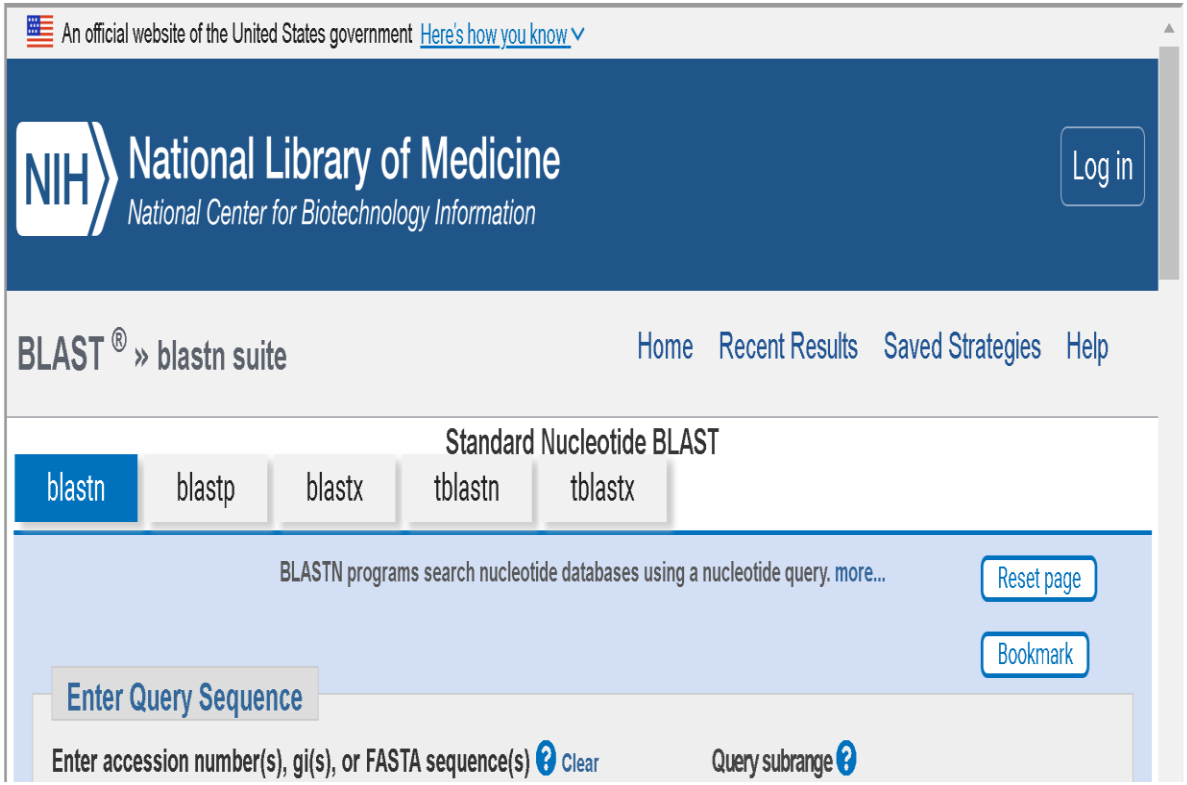

	Nucleotide Sequence
	S1TSMR7X013 Search expires on 01-11 16:23 pm Download All
Program	BLASTN Citation
Database	nt See details
Query ID	lcl|Query_4463577
Description	None
Molecule type	dna
Query Length	25
Other reports	Distance tree of results MSA viewer

Filter Results

Organism only top 20 will appear exclude

Type common name, binomial, taxid or group name

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Percent Identity to

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Query Coverage to

Filter Reset

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Sequences producing significant alignments Download Select columns Show 100

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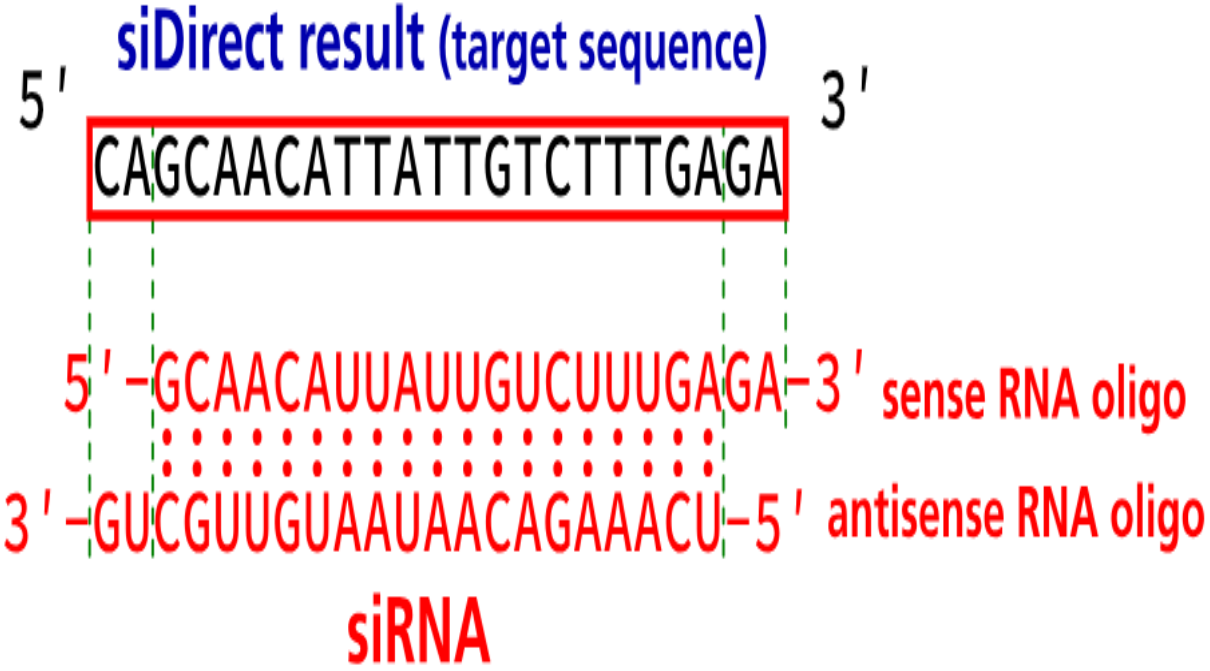
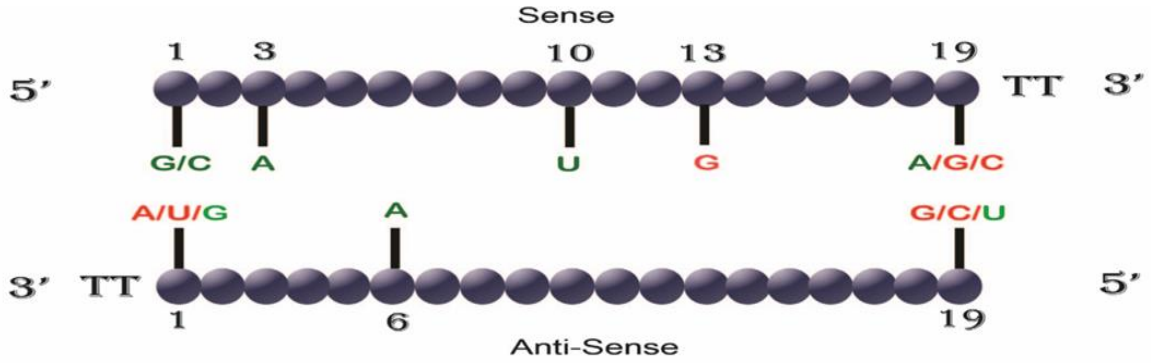
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Homo sapiens caveolin 3, mRNA (cDNA clone MGC:96959 IMAGE:7262168), complete c...	Homo sapiens	50.1	50.1	100%	5e-05	100.00%	650	gi|46854791|BC069368.1
Homo sapiens cDNA FLJ76415 complete cds, highly similar to Homo sapiens caveolin-3 ...	Homo sapiens	50.1	50.1	100%	5e-05	100.00%	559	gi|158257215|AK291892.1
Homo sapiens cavolin 3 mRNA, partial cds	Homo sapiens	50.1	50.1	100%	5e-05	100.00%	450	gi|1095447992|KU971296.1
Homo sapiens mRNA for caveolin 3 protein	Homo sapiens	50.1	50.1	100%	5e-05	100.00%	1291	gi|3059124|Y14747.1
Homo sapiens caveolin-3 (CAV3) mRNA, complete cds	Homo sapiens	50.1	50.1	100%	5e-05	100.00%	640	gi|3150444|AF036365.1
Homo sapiens chromosome 3 clone RP11-83M12 map 3p, complete sequence	Homo sapiens	50.1	50.1	100%	5e-05	100.00%	170755	gi|14861876|AF176315.2

7. Design sense and antisense RNA oligonucleotides from the siDirect results.





functional siRNAs. To obain more information about the meaning of the design options hover your mouse over the

You can test the server using this sample sequence.

Sequence Input

Paste your sequences here (at most 20000 nts):Clear!

```
>DBI
TGCGAAGGTGCAGCGGGCGGGAGGCCCGTTGGGGGCTCAGCCGGCTGCCAGAAGCT
CTCGGGCTCTTTCCTTCCGTGCCCCTCACTTGCTCATGGGCCCATGCCTAGCCCTGATTC
GTTGGACAGAGCCTTTGTAGACCCTTGTCTGAGACCGAGCTATGTGGGGCGACCTCTGGC
TCCTCCCGCCTGCCTCTGCCAATCCGGGCACTGGGACAGAGGCTGAGTTTGAGAAAGCTG
CAGAGGAGGTTAGGCACCTTAAGACCAAGCCATCGGATGAGGAGATGCTGTTCATCTATG
GCCACTACAAACAAGCAACTGTGGGCGACATAAATACAGAACGGCCCGGGATGTTGGACT
TCACGGGCAAGGCCAAGTGGGATGCCTGGAATGAGCTGAAAGGGACTTCCAAGGAAGATG
CCATGAAAGCTTACATCAACAAAGTAGAAGAGCTAAAGAAAAAATACGGGATATGAGAGA
CTGGATTTGGTTACTGTGCCATGTGTTTATCCTAAACTGAGACAATGCCTTGTTTTTTTC
```

Accessibility Threshold for 8 nucleotides
0 = not accessibile
1 = accessible

Design Options

8nt (Seed) Accessibility Threshold	0.01157
16nt Accessibility Threshold	0.001002
Self Folding Energy	0.9022
Sequence Asymmetry	0.5
Energy Asymmetry	0.4655
Free End	0.625
Custom Sequence Rules	NNNNNNNNNNNNNNNNNNN

Output Option

Maximal Number of siRNAs	3
E-Mail address (optional):	you@where.org

REPRESS IT !

89

Job pending in Queue

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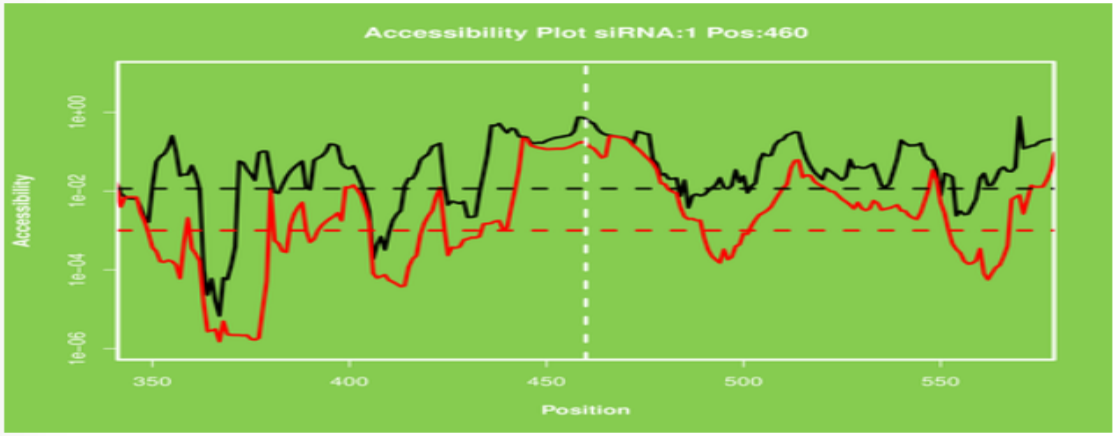
Hakim Tafer | htafer@tbi.univie.ac.at | TBI
Gregor Obernosterer | gregor.obernosterer@imba.oeaw.ac.at | IMBA
Stefan L Ameres | stefan.ameres@univie.ac.at | MFPL

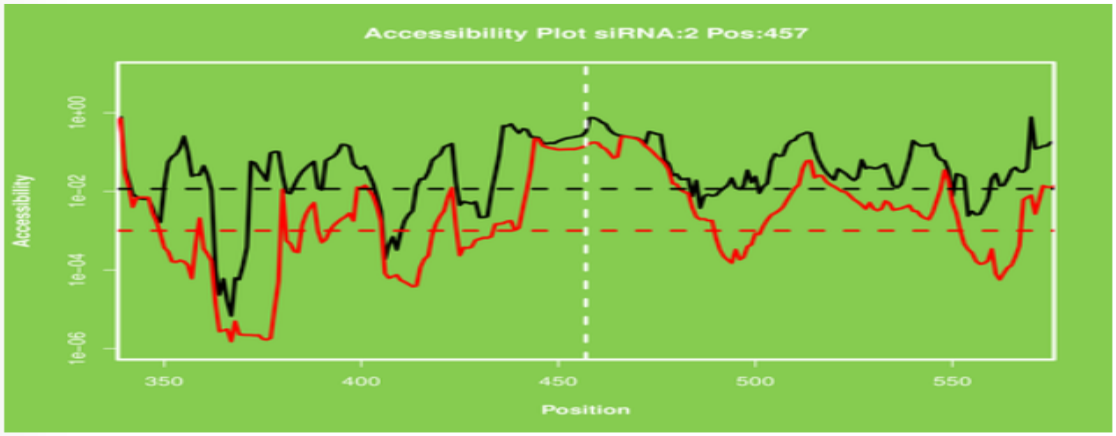
RNA XS

1 Data Input 2 View Results

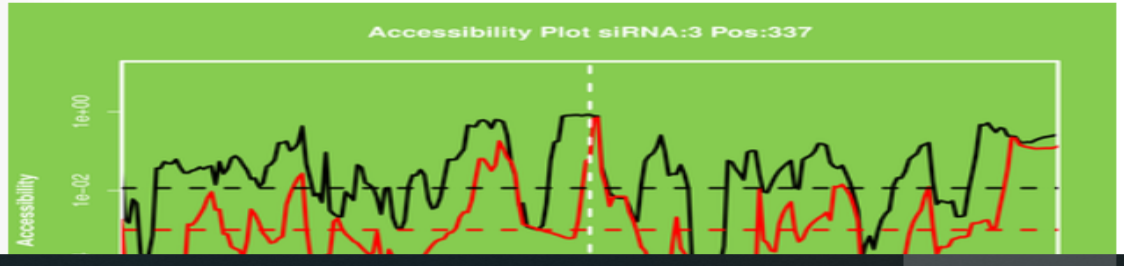
Hereunder you will find a detailed view of the 3 best siRNAs selectioned for DBI_RNA
A ranked list of all predicted siRNAs for DBI_RNA is available as text file here
Your results will be deleted on 26, 2019. Find here a Tar Zip archive of your Results



siRNA	1
Worst Rank	34
Position	460
Access 8nt	0.6771
Access 16nt	0.1712
Assymetry (S)	0.7500
Assymetry (E)	0.7241
Self Folding	1.0000
Free End	1.0000
Target Seq.	AGAAGAGCTAAAGAAAAAA
siRNA Seq.	TTTTTTTCTTTAGCTCTTCT
BLAST	NCBI BLAST



siRNA	2
Worst Rank	40
Position	457
Access 8nt	0.3280
Access 16nt	0.1476
Assymetry (S)	0.7500
Assymetry (E)	0.7069
Self Folding	1.0000
Free End	1.0000
Target Seq.	AGTAGAAGAGCTAAAGAAA
siRNA Seq.	TTTCTTTAGCTCTTCTACT
BLAST	NCBI BLAST

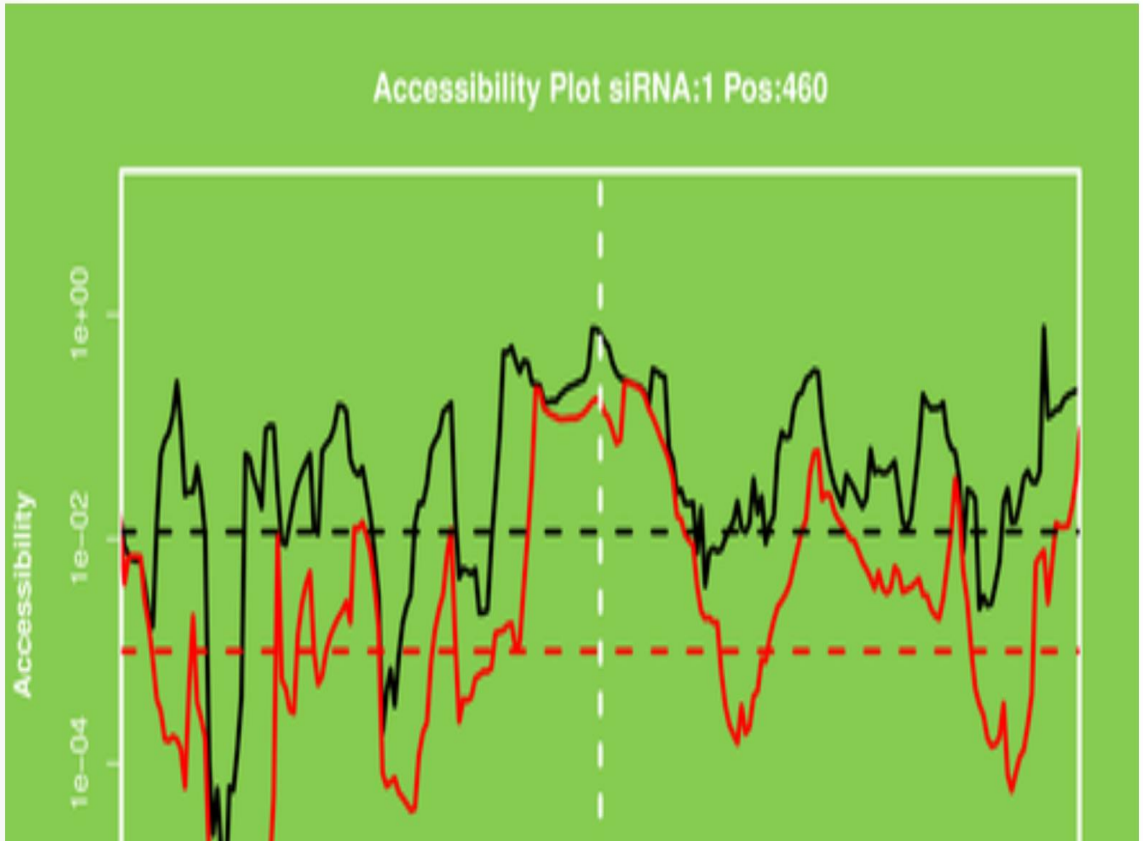


siRNA	3
Worst Rank	41
Position	337
Access 8nt	0.8360
Access 16nt	0.0625
Assymetry (S)	1.0000
Assymetry (E)	0.8190
Self Folding	1.0000
Free End	1.0000
Target Seq.	GGGCGACATAAATACAGAA
siRNA Seq.	TTCTGTATTTATGTCGCCC
BLAST	NCBI BLAST

Hereunder you will find a detailed view of the 3 best siRNAs selectioned for DBI_RNA

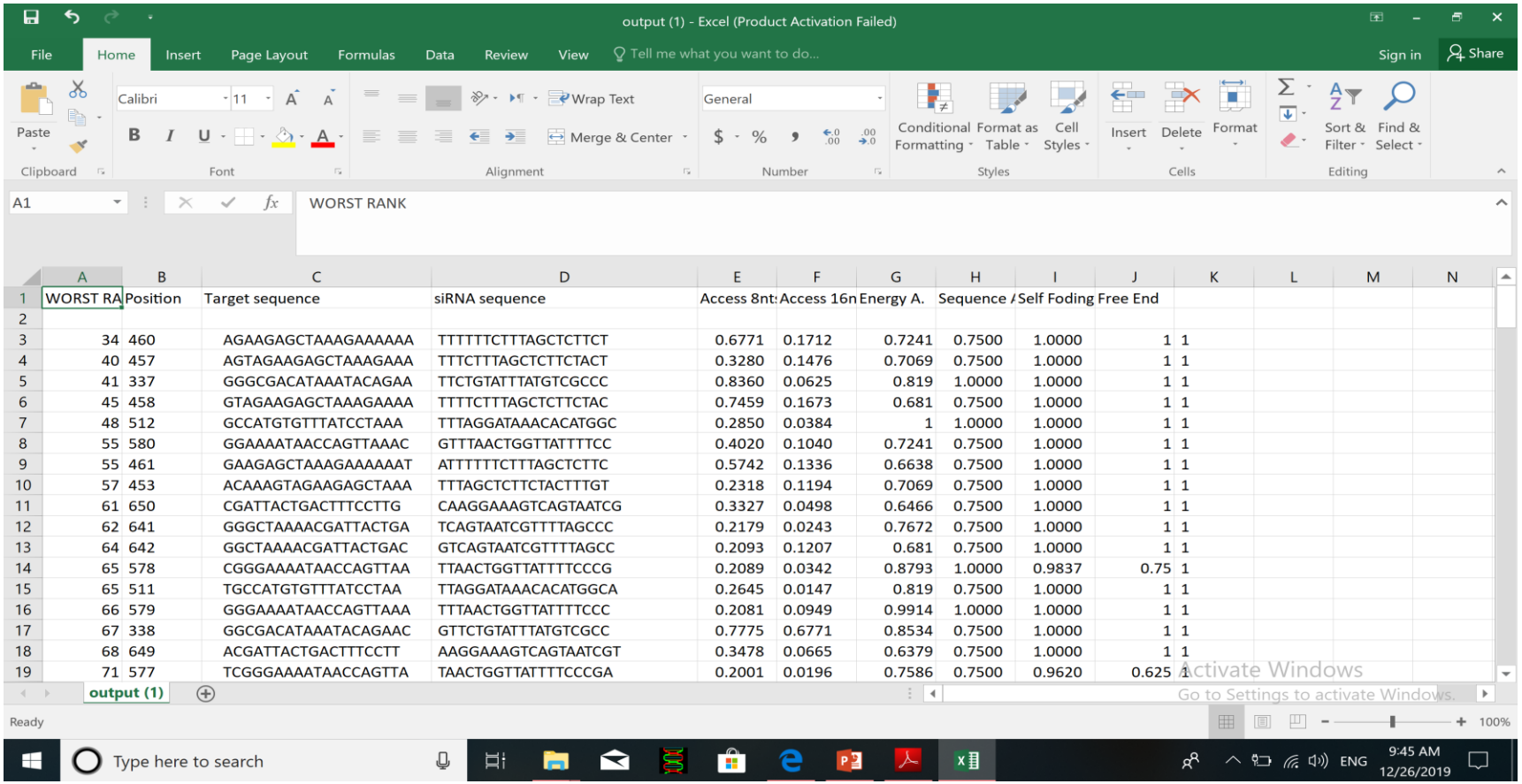
A ranked list of all predicted siRNAs for DBI_RNA is available as text file here

Your results will be deleted on 26, 2019. Find here a Tar Zip archive of your Results



siRNA	1
Worst Rank	34
Position	460
Access 8nt	0.6771
Access 16nt	0.1712
Assymetry (S)	0.7500
Assymetry (E)	0.7241
Self Folding	1.0000
Free End	1.0000
Target Seq.	AGAAGAGCTAAAGAAAAAA
siRNA Seq.	TTTTTTCTTTAGCTCTTCT
BLAST	NCBI BLAST

لیستی از دیگر siRNA های طراحی شده را می توان مشاهده کرد



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A1 WORST RANK

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	WORST RA	Position	Target sequence	siRNA sequence	Access 8nt	Access 16n	Energy A.	Sequence A	Self Foding	Free End				
2														
3	34	460	AGAAGAGCTAAAGAAAAAA	TTTTTTCTTTAGCTCTTCT	0.6771	0.1712	0.7241	0.7500	1.0000	1	1			
4	40	457	AGTAGAAGAGCTAAAGAAA	TTTCTTTAGCTCTTCTACT	0.3280	0.1476	0.7069	0.7500	1.0000	1	1			
5	41	337	GGGCGACATAAATACAGAA	TTCTGTATTTATGTCGCCC	0.8360	0.0625	0.819	1.0000	1.0000	1	1			
6	45	458	GTAGAAGAGCTAAAGAAAA	TTTTCTTTAGCTCTTCTAC	0.7459	0.1673	0.681	0.7500	1.0000	1	1			
7	48	512	GCCATGTGTTTATCCTAAA	TTTAGGATAAACACATGGC	0.2850	0.0384	1	1.0000	1.0000	1	1			
8	55	580	GGAAAATAACCAGTTAAAC	GTTTAACTGGTTATTTTCC	0.4020	0.1040	0.7241	0.7500	1.0000	1	1			
9	55	461	GAAGAGCTAAAGAAAAAAT	ATTTTTTCTTTAGCTCTTC	0.5742	0.1336	0.6638	0.7500	1.0000	1	1			
10	57	453	ACAAAGTAGAAGAGCTAAA	TTTAGCTCTTCTACTTTGT	0.2318	0.1194	0.7069	0.7500	1.0000	1	1			
11	61	650	CGATTACTGACTTTCCTTG	CAAGGAAAGTCAGTAATCG	0.3327	0.0498	0.6466	0.7500	1.0000	1	1			
12	62	641	GGGCTAAAACGATTACTGA	TCAGTAATCGTTTTAGCCC	0.2179	0.0243	0.7672	0.7500	1.0000	1	1			
13	64	642	GGCTAAAACGATTACTGAC	GTCAGTAATCGTTTTAGCC	0.2093	0.1207	0.681	0.7500	1.0000	1	1			
14	65	578	CGGGAAAATAACCAGTTAA	TTAACTGGTTATTTTCCCG	0.2089	0.0342	0.8793	1.0000	0.9837	0.75	1			
15	65	511	TGCCATGTGTTTATCCTAA	TTAGGATAAACACATGGCA	0.2645	0.0147	0.819	0.7500	1.0000	1	1			
16	66	579	GGGAAAATAACCAGTTAAA	TTTAACTGGTTATTTTCCC	0.2081	0.0949	0.9914	1.0000	1.0000	1	1			
17	67	338	GGCGACATAAATACAGAAC	GTTCTGTATTTATGTCGCC	0.7775	0.6771	0.8534	0.7500	1.0000	1	1			
18	68	649	ACGATTACTGACTTTCCTT	AAGGAAAGTCAGTAATCGT	0.3478	0.0665	0.6379	0.7500	1.0000	1	1			
19	71	577	TCGGGAAAATAACCAGTTA	TAACTGGTTATTTTCCCGA	0.2001	0.0196	0.7586	0.7500	0.9620	0.625	1			

output (1)

Ready

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- **Worst Rank: 31**
- **رتبه‌ی بدترین معیار در بین تمام siRNAهای طراحی‌شده برای این توالی. عدد پایین‌تر بهتره؛ یعنی این توالی در اکثر معیارها عملکرد خوبی داشته.**
- _____
- **Position: 460**
- **موقعیت شروع توالی siRNAروی mRNAهدف. یعنی این siRNAاز نوکلئوتید شماره ۴۶۰ در توالی هدف شروع می‌شه.**
- _____
- **Access 8nt: 0.6771**
- **دسترسی‌پذیری ساختار دوم mRNAدر ناحیه‌ی) seedنوکلئوتیدهای ۲ تا ۹ از :(siRNA**
  - **عدد بین ۰ تا ۱ هست.**
  - **عدد بالاتر یعنی این ناحیه بیشتر single-strandedو قابل‌دسترس برای اتصال به siRNAهست.**
  - **مقدار ۰.۶۷۷۱ یعنی ناحیه‌ی seedنسبتاً خوب بازه و احتمال اتصال بالاست.**
- _____

🔓 Access 16nt: 0.1712

دسترسی‌پذیری ناحیه‌ی کامل اتصال (نوکلئوتیدهای ۲ تا ۱۷ از siRNA):

- عدد پایین‌تر یعنی ساختار دوم mRNA در این ناحیه بسته‌تره.
- مقدار ۰.۱۷۱۲ نشون می‌ده که ناحیه‌ی کامل اتصال نسبتاً محدودتره، ولی هنوز قابل‌قبوله.

⚖️ Asymmetry (S): 0.7500

عدم تقارن توالی siRNA از نظر سکانس:

- عدد بالاتر یعنی تفاوت بین دو انتهای siRNA بیشتره، که باعث می‌شه رشته‌ی راهنما راحت‌تر وارد RISC بشه.
- مقدار ۰.۷۵ نشون‌دهنده‌ی تقارن مناسب برای انتخاب رشته‌ی راهنماست.

⚡ Asymmetry (E): 0.7241

عدم تقارن از نظر انرژی ترمودینامیکی بین دو انتهای siRNA:

- تفاوت انرژی بین انتهای ۵' و ۳' باعث می‌شه رشته‌ی راهنما بهتر انتخاب بشه.
- مقدار ۰.۷۲ یعنی انرژی انتهاها به‌خوبی تنظیم شده.

Self Folding: 1.0000

توانایی siRNAبرای تشکیل ساختار دوم خودش:

- **عدد ۱ یعنی siRNAساختار دوم داخلی نداره و کاملاً بازه.**
- **این عالیه چون siRNAباید بدون مانع به mRNAهدف متصل بشه.**

Free End: 1.0000

آزاد بودن انتهای siRNAبرای ورود به RISC:

- **عدد ۱ یعنی انتهای siRNAکاملاً آزاد و بدون ساختار مزاحم هست.**
- **این ویژگی برای عملکرد مؤثر در مسیر RNAiبسیار مهمه**

جرج برنارد شاو

یک عمر اشتباه کردن، نه تنها افتخار آمیز است، که بسیار سودمند تر از یک عمر نشستن بیهوده است.