INTEGRATED DNA TECHNOLOGIES, INC.

Dicer Substrate RNAi Design

How to design and order 27-mer Dicer-substrate Duplex RNAs for use as RNA interference reagents

The following document provides a summary of how to design and order IDT's Dicer substrate RNA duplexes for use in RNA interference (RNAi). While this tutorial is intended for the research scientist who is already familiar with RNAi, novice users should also be able to use it with minimal guidance. If you need assistance, please call IDT Technical Support at 1-800-328-2661. Note that use of this tutorial is not necessary to obtain 27-mer dicer substrate RNA reagents and the RNAi design tool on the IDT website supports automated design.
http://www.idtdna.com/Scitools/Applications/RNAi/RNAi.aspx

RNAi employs short double-stranded (ds) RNAs to specifically reduce expression of a targeted gene. In cells, long dsRNA is cleaved into short 21-23 base duplexes having 2-base 3' overhangs by the RNase-III class endoribonuclease Dicer. These short species, called "short interfering RNAs" (siRNAs) enter the RNA Induced Silencing Complex (RISC) and serve as a sequence-specific guide to target degradation of complementary mRNA species. Historically, siRNAs have been synthesized as 21-mers to directly mimic the products that are produced by Dicer *in vivo*.

It has recently been described (Kim *et al*. 2005) that chemically synthesized RNA duplexes in the 25-30 base length range can have as much as a 100-fold increase in potency compared with 21-mer siRNAs at the same location (27-mer seemed to be optimal). It is now thought that Dicer is also required to introduce the siRNA into RISC (it is not just a nuclease). Dicer is also involved in RISC assembly (Lee *et al*. 2004; Pham *et al*. 2004; Tomari *et al*. 2004). The observed increased potency obtained using longer dsRNAs in triggering RNAi is theorized to result from providing Dicer with a substrate (27-mer) instead of a product (21-mer) and that this improves the rate or efficiency of entry of the RNA duplex into RISC as facilitated by Dicer.

Unfortunately all 27-mers do not show this kind of increased potency. It is well known that shifting a 21-mer siRNA by a base or two along the mRNA sequence can change its potency by ten-fold or more. Simple 27-mers can be potent if they are "diced" into "good" 21-mers or can be weak or inactive if "diced" into "bad" 21-mers. We have recently devised design rules that allow for intelligent design of 27-mer dicer substrate RNAs where the actual diced product can be predicted and this variable eliminated from consideration. We suggest the following approach to design dicer substrate RNAs, which combines elements of both historical 21-mer design criteria and new 27-mer design criteria.

1 Select a target site for your dsRNA knockdown reagent within the transcript of interest using "standard" 21-mer design rules.

a) Use a double-stranded 19 base core region, comprised from two 21-mer RNA sequences, each with a two base 3' overhang when annealed by Watson-Crick base pairing, see Figure 1
b) Use position specific base compositions and sequence motifs that associate with high activity
c) Avoid position specific base compositions and sequence motifs that associate with low activity
d) Use thermodynamic criteria to favor 5' antisense strand uptake into RISC
e) Prefer target sites that correspond to additional RNAi design rules
f) Avoid known polymorphic regions (e.g. SNPs)
g) Avoid target sites that might cross-hybridize to additional non-target transcripts with either the sense or antisense strand of the dsRNA reagent

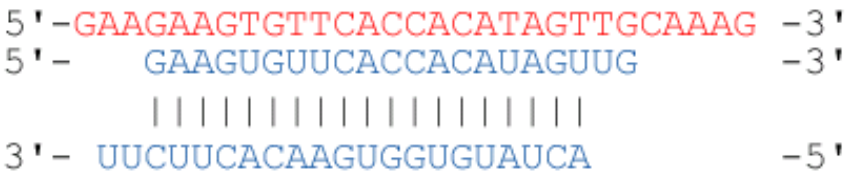

Figure 1
A single stranded DNA target site (upper-stand, DNA in red), with an associated double-stranded RNA interference molecule (lower 2 strands, RNA in blue) with the sense strand of the RNA and the antisense (or guide) strand as the lower sequence. The "|" represents the Watson-Crick base pairing between the sense and antisense strands of the dsRNA. Sense and antisense strands are oriented in an antiparallel fashion, 5' to 3'.

In finding optimal RNA interference target sites considering several factors (Reynolds *et al*. 2004) including the thermodynamic stability of the dsRNA ends is important. The 5' end of the strand having the less stable thermodynamics is preferentially incorporated into the RISC (Aza-Blanc *et al.* 2003; Khorova *et al.* 2003; Schwarz *et al.* 2003).

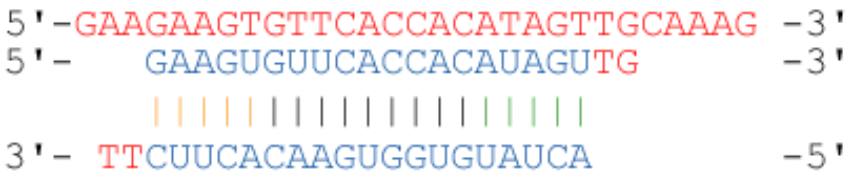

Figure 2
A standard synthetic RNAi reagent has the terminal two 3' nucleotides as DNA (shown in red), and the remainder being RNA (shown in blue), and for preferential uptake of the antisense strand into RISC the weaker Watson-Crick bonding (shown in green) occurs on the 5' side of the strand desired to be incorporated into RISC, whereas the stronger Watson-Crick (shown in orange) occurs on the 3' end of the antisense strand.

2 To create a Dicer substrate RNAi from the standard RNAi shown in Figure 2, extend the target site of the dsRNA to be a Dicer substrate by adding bases to the 3' end of the sense strand and to the 5' end of the antisense strand. Specifically 4 bases to the 3' end of the sense strand and 6 bases to the 5' end of the antisense strand to create an asymmetic blunt ended and 3' overhang molecule illustrated in Figure 3.

a) Add RNA to lengthen the sense strand to 25 nucleotides and the antisense strand to 27 nucleotides, new base positions shown in light blue, Figure 3. Compare Figure 3 to Figure 1.

version updated 2005/06/14

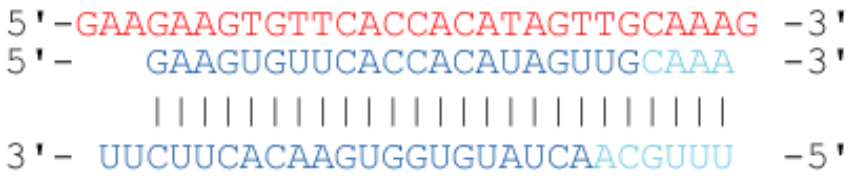

Figure 3
A standard double-stranded RNA interference molecule (in blue) with additional sequence added to the 3' end of the sense strand and 5' end of the antisense strand (in light blue) to preferentially act as a Dicer substrate.

b) Replace the two terminal RNA bases on the 3' end of the sense strand with DNA bases (shown in red) for preferential processing (data not shown). Use of the asymmetric design with 2-base 3'-overhang on one side and blunt on the other side helps direct dicing. Further, DNA base modification as shown also helps to direct dicing.

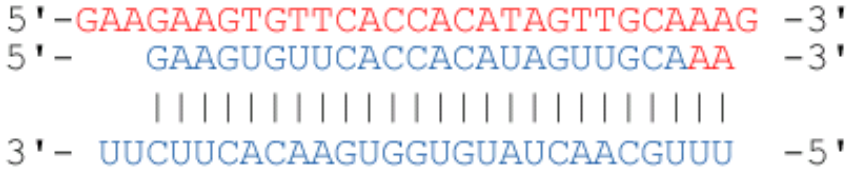

Figure 4
A Dicer substrate RNA interference molecule with RNA bases in blue and DNA bases in red.

3 The final Dicer substrate RNA interference reagent should consist of a 25 base sense strand and a 27 base antisense strand (Figure 4) that when acted upon by the Dicer enzyme would become the mature dsRNA reagent and is expected to have the antisense strand incorporated into the mature RISC (Figure 1). Expected Dicer sense and antisense strand cleavage sites are shown in Figure 5.

Figure 5
A Dicer substrate RNA interference molecule with RNA bases in blue and DNA bases in red and the expected Dicer cleavage sites.

4 Additionally, presence of a 5' phosphate group on the sense strand is needed for RISC entry and adding it to the RNA at synthesis can improve potency but is not required as phosphorylation can occur intracellularly (Tomari *et al*. 2004). The final dicer substrate RNA duplex is shown below in Figure 6.

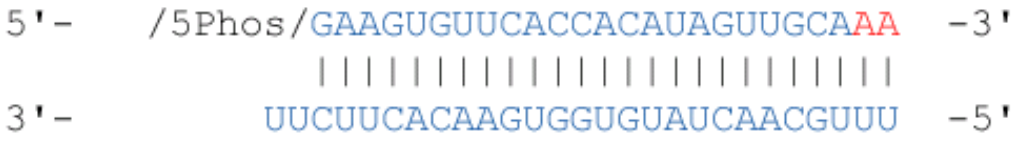

Figure 6
A Dicer substrate RNA interference molecule with RNA bases in blue and DNA bases in red and 5' sense strand phosphorylation.

References

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IDT Contacts

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IDT Technical Support

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