NEXT-RNAi features

NEXT-RNAi prediction of reagent specificity

An RNAi reagent is said to be specific if it interferes with the intended target mRNA(s) only ('on-target') but with no other, unintended target mRNA(s) ('off-target').

NEXT-RNAi tries to optimize the reagent's specificity the following way:

- For the design of long dsRNAs any perfect homologies of contained siRNAs (by default 19 bp long with an offset of 1) to other than the intended target mRNA(s) are avoided. For the design of single siRNAs perfect homologies of this sequence to unintended target mRNA(s) are avoided.
- NEXT-RNAi can also compute the number of siRNA seed matches (positions 12/13-18 in the siRNA sense strand) to a user-defined FASTA databases (containing e.g. sequences of 3'-UTRs). A high number of seed matches to 3'-UTRs was associated with stronger 'off-target' signatures.
- 'Off-target' effects can also arise from imperfect homologies to unintended target mRNA(s). To address this NEXT-RNAi maps the full reagent sequence (long dsRNAs or siRNA) to the transcriptome and reports homologies of all targets identified (with E-values below a selected cutoff). NEXT-RNAi does not include this step during the design, but only as evaluation of the reagent specificity. RNAi reagents with high homology to others than the intended target mRNA(s) should be used with caution.
- Regions of low sequence complexity can lead to unwanted 'off-target' effects. NEXT-RNAi avoids several kinds of low-complexity regions in the design process described here.

NEXT-RNAi prediction of reagent efficiency

NEXT-RNAi enables the prediction of siRNA efficiencies according to two published sets of criteria:

- 'Rational' prediction according to Reynolds et al.
- 'Weighted' prediction according to Shah et al.

Rational efficiency prediction

The 'rational' prediction includes 8 criteria to predict the efficiency of an siRNA (sense strand):

- G/C content (+1 if between 30% and 52%)
- A/T bases at positions 15-19 (+1 each)
- Absence of internal repeats (+1 if melting temperature of potential internal hairpin < 20°C)
- A base at position 19 (+1)
- A base at position 3 (+1)
- T base at position 10 (+1)
- G/C at position 19 (-1)
- G at position 13 (-1)

This scoring provides a score range from -2 to 10. In the studies done by Reynolds et al. a cut-off of 6 was used to define an efficient silencer (siRNAs with scores equal or higher than 6 are considered efficient).

For NEXT-RNAi the obtained scores are then normalized in the range of 0 – 100 using the following formula:

FinalScore = ((Score of siRNA - minimal possible score) / (maximal possible score - minimal possible score)) * 100

For an siRNA considered as efficient silencer this means:

FinalScore = ((6 + 2) / (10 + 2)) * 100 = 66.67

'Weighted' efficiency predicition

The 'weighted' prediction includes 12 criteria to predict the efficiency of an siRNA (sense strand):

- A/T base at position 1 (-1.4)
- G/C base at position 1 (+1.11)
- A base at position 6 (+0.7)
- T base at position 10 (+0.25)
- G base at position 13 (-1.66)
- T base at position 13 (+0.31)
- A/T base at position 16 (+0.74)
- A/T base at position 17 (+1.2)
- A/T base at position 18 (+1.44)
- A/T base at position 19 (+0.87)
- G/C base at position 19 (-1.02)
- G/C content (+0.42 if between 30% and 55%)

This scoring provides a score range from -4.08 and 7.04.

For NEXT-RNAi the obtained scores are then normalized in the range of 0 – 100 using the following formula:

FinalScore = ((Score of siRNA - minimal possible score) / (maximal possible score - minimal possible score)) * 100

Shah et al. found in their study that the average score of the most potent siRNAs was 63.

Assessing regions of low sequence complexity

NEXT-RNAi avoids sequence-regions of low complexity (regarding base composition) from the RNAi reagent. Those were associated with 'off-target' effects and cytotoxic effects. Two filters are applied in the design process:

- regions of low complexity (such as sequences with simple base repeats) are filtered using mdust,
- sequences with at least 6 contiguous CA[ACGT] (CAN) repeats are filtered.

Standardized primer designs

NEXT-RNAi uses primer3 for the design of primer pairs required to amplify templates for long dsRNAs by PCR. All primer design parameters can be adjusted by the user, such as melting temperature, GC content, primer pair penalty and desired amplicon length. These settings are applied to all RNAi reagents designed in a single run, which facilitates efficient (high-throughput) PCR of all templates. It is recommended to use the same primer design settings for all reagents that are going to be synthesized side by side.

Design of independent RNAi reagents with NEXT-RNAi

E-RNAi provides two ways to design independent RNAi reagents:

- The sequences of previously designed reagents can be submitted to be excluded from the design of new reagents. This can be set with the INDEPENDENT parameter in the NEXT-RNAi options file (see here for further documentation)
- Since NEXT-RNAi can be queried with many sequences for one run, e.g. multiple exons of the same transcript can be queried and the resulting designs will be independent straightaway.