dsCheck: highly sensitive off-target search software for double-stranded RNA-mediated RNA interference

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ABSTRACT

Off-target effects are one of the most serious problems in RNA interference (RNAi). Here, we present dsCheck (http://dsCheck.RNAi.jp/), web-based online software for estimating off-target effects caused by the long double-stranded RNA (dsRNA) used in RNAi studies. In the biochemical process of RNAi, the long dsRNA is cleaved by Dicer into short-interfering RNA (siRNA) cocktails. The software simulates this process and investigates individual 19 nt substrings of the long dsRNA. Subsequently, the software promptly enumerates a list of potential off-target gene candidates based on the order of off-target effects using its novel algorithm, which significantly improves both the efficiency and the sensitivity of the homology search. The website not only provides a rigorous off-target search to verify previously designed dsRNA sequences but also presents ‘off-target minimized’ dsRNA design, which is essential for reliable experiments in RNAi-based functional genomics.

INTRODUCTION

RNA interference (RNAi) is now widely used to knockdown gene expression in a sequence-specific manner, making it a powerful tool for studying gene function (1–3). The process of RNAi is mediated by double-stranded RNA (dsRNA) that contains a sequence homologous to the target mRNA. Long dsRNA introduced into the cell is cleaved by the enzyme Dicer into short-interfering RNA (siRNA) followed by incorporation into the RNA-induced silencing complex (RISC), which is responsible for target mRNA degradation (4).

One of the most serious problems in RNAi is ‘off-target’ silencing effects (5). Off-target silencing effects are caused by siRNA (introduced directly into cells, or produced in vivo from long dsRNA) that has sequence similarities with unrelated genes. In Caenorhabditis elegans, Drosophila or plants, RNAi experiments are usually performed using long dsRNAs. In these cases, there is a high risk of cross-suppression or co-suppression between closely related genes that share a highly conserved region.

To minimize the possibility of off-target effects, it is necessary to perform an off-target search to design dsRNA or siRNA that has limited sequence similarities with unrelated genes. Recently, fast and sensitive off-target search software for siRNA design has been reported (6,7), but commonly used siRNA design servers are not useful in performing off-target searches for long dsRNAs. DEQOR server uses BLAST to perform off-target searches for endoribonuclease-prepared siRNAs (8), although BLAST frequently fails to identify off-targets (6). Therefore, we have developed a new web-based online software system, dsCheck, to provide fast and accurate off-target searches for long dsRNA sequences. The software ‘dices’ the input sequence into an siRNA cocktail and performs an exhaustive scan for each siRNA to find off-target gene candidates, simulating the biochemical process of dsRNA-mediated RNAi in vivo. dsCheck also provides efficient design of ‘off-target minimized’ dsRNA by avoiding regions that share a considerable number of diced siRNAs with a specific off-target gene, and monitoring the total number of off-target hits. The software should be especially useful for checking whether previously designed dsRNAs have off-target gene candidates, as well as for designing target-specific dsRNA when off-target effects are suspected.

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The authors wish it to be known that, in their opinion, the first two authors should be regarded as joint First Authors

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METHODS

Off-target search strategies for long dsRNA

The key idea of the program follows the biochemical process of dsRNA-mediated RNAi shown in Figure 1A. The input dsRNA sequence is diced into 19 nt substrings of an siRNA cocktail, and an exhaustive off-target search is performed for all individual siRNAs using the siDirect engine, which makes it possible to enumerate the complete set of off-targets in a reasonable amount of time (7). In dsCheck, the in silico dicing size is set to 19, as a complete match at the 19 nt double-stranded region of an siRNA is sufficient for the target mRNA degradation. For example, an input 500 bp dsRNA sequence is processed into 482 substrings each 19 nt in length, which are subjected to the off-target search individually. In the next step, all the hits with a complete match (i.e. 19/19 matches), one mismatch (18/19 matches) or two mismatches (17/19 matches) are counted individually for every off-target gene candidate and sorted in descending lexicographic order for the output.

Figure 1B shows a typical output for a 1497 bp query sequence of the Drosophila POU domain protein, pdm2 (NM_078834, coding region). The result shows significant hits against pdm2 (two splicing variants: NM_078834 and NM_165017), and two unrelated genes, nub (NM_057311) and vvl (NM_079224). These proteins share the highly conserved POU domain shown in Figure 1C, indicating a high risk of cross-suppression by dsRNA targeting this region.

Designing off-target minimized dsRNA sequences

To design off-target minimized dsRNA sequences, one approach would be to suppose that the off-target effects are caused by a considerable number of collaborative hits by diced siRNAs on the same gene, and to select a region that minimizes the maximum number of collaborative off-target hits, which are defined as complete or partial matches of multiple 19 nt substrings against the same off-target gene. According to this criterion, dsCheck starts by selecting a region that minimizes the maximum number of ‘complete match’ collaborative off-target hits. If multiple regions are optimal, it also examines the maximum number of ‘partial match’ collaborative off-target hits to select the best one. If the complete match, collaborative hits on a sequence exceed 80% of the total number of diced 19 nt substrings, dsCheck regards the sequence as the intended target gene.

Some dsRNA sequences include 19 nt substrings that may react with a large number of off-target genes, which differs from the collaborative silencing effects acting on a single off-target gene. An additional criterion is necessary to evaluate the silencing effect of one siRNA sequence on many off-targets,
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