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Genome analysis

TargetFinder: a software for antisense oligonucleotide target site selection based on MAST and secondary structures of target mRNA

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ABSTRACT

Summary: TargetFinder is a PC/Windows program for interactive effective antisense oligonucleotide (AO) selection based on mRNA accessible site tagging (MAST) and secondary structures of target mRNA. To make MAST result intuitive, both the alignment result and tag frequency profile is illustrated. As theoretical reference, secondary structure and single strand probability profile of target mRNA is also represented. All of these sequences and profiles are displayed in aligned mode, which facilitates identification of the accessible sites in target mRNA. Graphical, user-friendly interface makes TargetFinder a useful tool in AO target site selection.

Availability: The software is freely available at http://www.bioit.org.cn/ao/targetfinder.htm

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INTRODUCTION

Antisense oligonucleotides (AOs) are powerful tools in life science. By binding to a target mRNA through Watson–Crick base pairing, AOs can selectively downregulate gene expression. Antisense offers a good alternative to gene knockout techniques in view of cost, time and resource requirement, and therefore has been widely used in gene function determination, drug targets validation and pathway studies (Taylor *et al*., 1999). AOs are also effective drugs. Several antisense compounds for disease treatment have been evaluated in clinical trials with promising results (Crooke, 1998).

Unfortunately, not all AOs are sufficiently effective in inhibiting protein synthesis. Even with careful design, typically <20% of AOs are effective (Stein, 1999). It is now commonly accepted that the accessibility of target sites is of great importance in determining the efficiency of AOs (Ho *et al*., 1998). Several experimental methods for accessible site identification have been developed in recent years. Among these methods, mRNA accessible site tagging (MAST) (Zhang *et al*., 2003) has a greater advantage over RNase H mapping, gel shift, oligonucleotide array and random reverse transcriptase (RT) priming in throughput, cost, complexity and efficiency. We have also developed a similar method.

Since the result of the MAST experiment is not as intuitive as that of other AO screening methods, a special software tool is needed to analyze the tagged mRNA accessible sites. However, at present there are no appropriate web tools or free softwares for MAST analysis. Zhang *et al*. (2003) used DNAStrider™ and RiboAlign™ software developed by Neuromics Corporation to accomplish this work. Here we present a software named TargetFinder which is specially designed for AO selection after MAST. At the same time, secondary structures of target mRNA presented in the program can also be used as a theoretical reference.

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SYSTEMS AND METHODS

TargetFinder is written in Borland Delphi 7.0 and works on Microsoft Windows platform without special installation. A monitor with a resolution of 1024 × 768 is required.

Visualization of MAST result

There are three steps in the MAST method. First, the target mRNA molecules are immobilized and hybridized to randomized oligonucleotide libraries; then, the oligonucleotides hybridized specifically to the target mRNA (named tags) are sequenced; and finally, the accessible site map is estimated through the alignment of tags to target mRNA. TargetFinder provides a customizable and easy-to-use solution for the last step. The target mRNA sequence is accepted in FASTA format whereas the tag sequences as plain text. The parameters of the alignment can be easily set by the users.

To facilitate identification of accessible sites, TargetFinder displays the alignment result in a compact way with color rendering. Nucleotides aligned with the target mRNA are shown in yellow letters and others in red letters. The matching number of each column in the alignment is also calculated and illustrated as a frequency profile (Fig. 1, bottom). The accessible sites are revealed by peaks with high frequency on the profile. From the viewpoint of the MAST method, relatively high frequency peaks on the profile are very likely to be effective target sites. Based on this rule, TargetFinder automatically searches the target sites with the maximum alignments. Users can quickly find the suggested target site by clicking on the corresponding item in the list of identified results.

Presentation of target mRNA secondary structure

Besides experimental methods, several theoretical approaches based on secondary structure prediction of target mRNA have also been developed to identify the accessible sites (Sczakiel, 2000). Vickers *et al*. (2000) demonstrated that mRNA structures play a significant role in determining AO efficiency *in vivo*, which motivated us to integrate the representation of RNA secondary structures in TargetFinder.

Three typical methods for RNA secondary structure representation have been implemented in TargetFinder to help users explore the structure of the target mRNA predicted by mfold (Zuker, 2003). A secondary structure stored in .ct file format is represented as dot–parenthesis notation and mountain plot. Considering that mfold now provides many results with different free energies

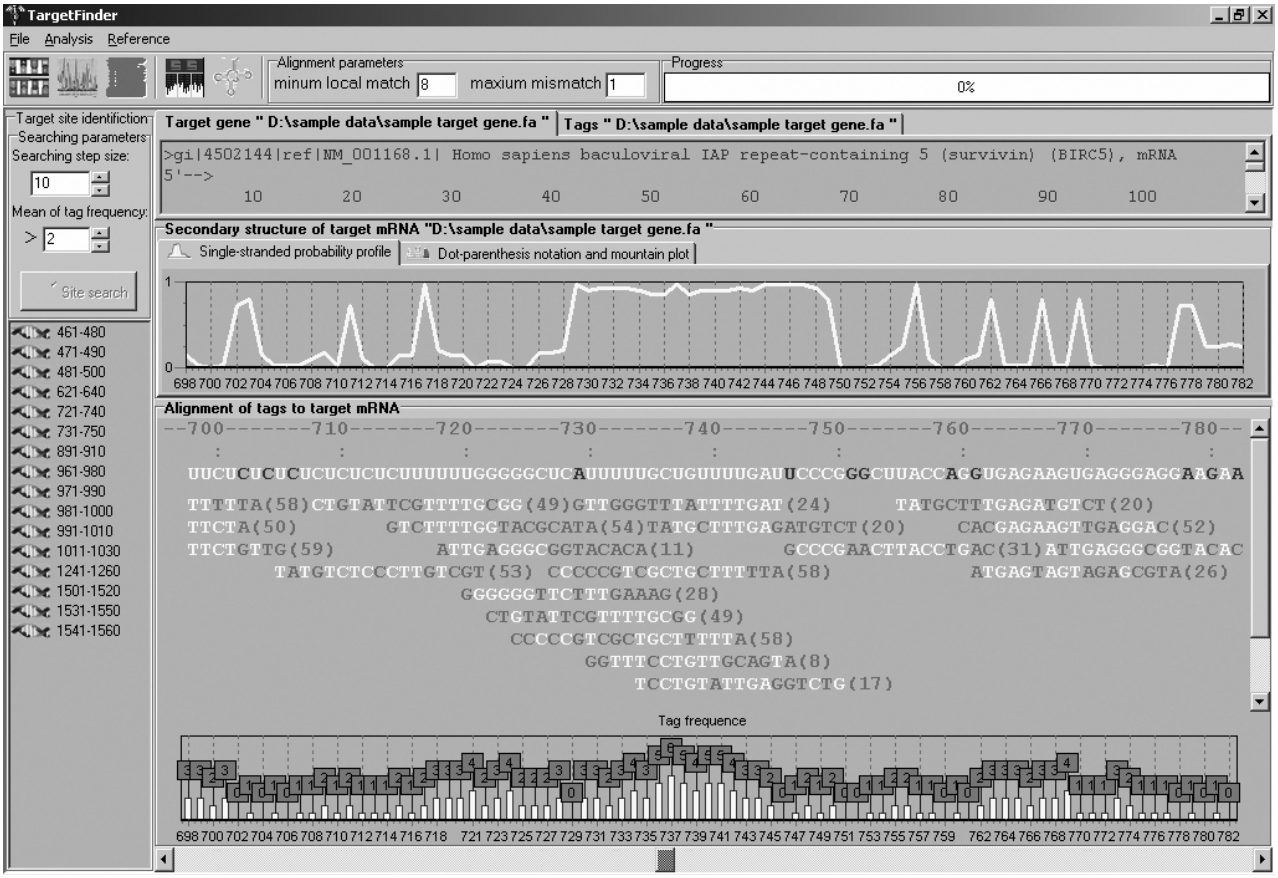

Fig. 1. A typical view of TargetFinder window.

to mitigate the uncertainty of the prediction, TargetFinder can also calculate and output the single strand probability profile from the ss-count file, which is an integration of all predicted structures. This is also helpful for target site selection, as single-stranded regions are more likely to be accessible sites than double-stranded regions (Lima *et al*., 1992). Both the MAST result and the secondary structure information, including target mRNA sequence, tags, frequency profile, dot–parenthesis representation, mountain plot and single strand probability profile are displayed in the aligned mode (Fig. 1, top). All the aforementioned results of analysis can be saved easily as a text or image file by clicking the right button of the mouse and then selecting the corresponding item in the popup menu.

DISCUSSION

TargetFinder described above is a freely available tool for interactive AO target site selection. A user-friendly interface is provided for MAST result analysis and mRNA secondary structure presentation. Future versions of the software will incorporate other methods of AO target site selection—both experimental and theoretical.

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