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PRISE (PRImer SElector): Software for designing sequence-selective PCR primers

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Abstract

This report presents PRImer Selector (PRISE), a new software package that implements several features that improve and streamline the design of sequence-selective PCR primers. The PRISE design process involves two main steps. In the first step, target and non-target DNA sequences are identified. In the second step, primers are designed to amplify target (but not non-target) sequences. One important feature of PRISE is that it automates the task of placing primer–template mismatches at the 3' end of the primers — a property that is crucial for sequence selectivity. Once a list of candidate primers has been produced, sorting tools in PRISE speed up the selection process by allowing a user to sort the primers by properties such as amplicon length, GC content and sequence selectivity. PRISE can be used to design primers with a range of specificities, targeting individual sequences as well as diverse assemblages of genes. PRISE also allows user-defined primers to be analyzed, enabling their properties to be examined in relation to target and non-target sequences. The utility of PRISE was demonstrated by using it to design sequence-selective PCR primers for an rRNA gene from the fungus *Pochonia chlamydosporia*.

Keywords: PCR; Primers; Sequence selective; Sequence specific; rRNA; qPCR

1. Introduction

Sequence-selective PCR primers allow the amplification and isolation of specific DNA fragments from complex mixtures. They are important in a variety of tasks, including single nucleotide polymorphism (SNP) analyses, where sequences differing by one nucleotide need to be distinguished (Ehlen and Dubeau, 1989; Nichols et al., 1989; Okayama et al., 1989; Wu et al., 1989). Sequence-selective primers are used in a variety of procedures for genomic walking (Arnold and Hodgson, 1991; Ayyadevara et al., 2000a,b; Nichols et al., 1989). In addition, such primers are also used to monitor populations of specific microorganisms in environmental samples (Jansson and Prosser, 1997; Lim et al., 2001; Scupham et al., 2006).

Designing sequence-selective PCR primers can be a cumbersome process, involving the use of several software tools and manual analyses. First, sequence-selective regions of DNA are identified. Next, PCR primers are designed to anneal to these selective regions, and, if possible, to place the selective nucleotides on the 3' ends of the primers. For rRNA gene analyses, common tools used to identify sequence-selective regions include Probe Design and Probe Match algorithms in ARB (Ludwig et al., 2004) (http://www.arb-home.de/) and Primrose (http://www.cf.ac.uk/biosi/research/biosoft/) (Ashelford et al., 2002). Primrose is a probe and primer design algorithm that utilizes the databases from the Ribosomal Database Project (http://rdp.cme.msu.edu/) (Cole et al., 2005). Although a variety of software packages for identifying sequence-selective regions and PCR primer design are readily available, to our knowledge, none have the ability to create sequence-selective primers in a systematic and automated manner from start to finish.

This report describes a new software package termed PRImer SElector (PRISE), which enables the design of sequence-selective PCR primers. At a conceptual level, the principle of PRISE is to separate the primer design process into two largely

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independent steps: (i) defining the task to be performed by the primers, and (ii) creating primers that achieve this task.

More specifically, in the first step, PRISE automates the process of identifying target and non-target DNA sequences — those that should and should not be amplified by the primers, respectively. The actual design of these primers takes place in the second step. Here, a variety of options are available to ensure that the primers meet the requirements of the application at hand. For example, when attempting to create sequence-selective primers for conserved genes, the primers frequently need to be able to distinguish DNA sequences differing by a single nucleotide. For PCR assays to optimally distinguish such sequences, the mismatches must be located at the terminal 3' nucleotide of the primers (Ayyadevara et al., 2000a,b; Huang et al., 1992; Kwok et al., 1990). The PRISE software provides this capability through a novel scoring scheme that allows the user to set the match-mismatch criteria for each nucleotide position in the primers. Once a list of candidate primers has been produced, sorting tools in PRISE speed up the selection process by allowing a user to sort the primers by properties such as amplicon length, GC content and sequence selectivity.

PRISE can be used to design primers with a range of specificities, targeting individual sequences as well as diverse assemblages of genes. In this report, we demonstrate the utility of PRISE by using it to design primers that selectively amplify a specific fungal rRNA gene from environmental samples.

2. Materials and methods

2.1. Software availability and requirements

PRISE is free for non-commercial use. The program can be downloaded from http://alglab1.cs.ucr.edu/OFRG/PRISE.php. Alternatively, one can go to the OFRG website at http://algorithms.cs.ucr.edu/OFRG and follow the PRISE link. PRISE can be run on Windows 2000/NT/XP with a minimum of 512 MB of RAM (1 GB of RAM or more is recommended). Internet connectivity is also required.

2.2. Running the program

When the program is opened, a window with four selections appears (Fig. 1). These are links to instructions and modules for performing the various steps in the primer design process.

2.3. Design process

Designing PCR primers using PRISE involves two general steps. Step 1, which is divided further into two components (1.1 and 1.2), enables target and non-target DNA sequences to be identified and collected. Step 2 generates PCR primers designed to amplify target but not non-target sequences. In the following sections, we describe these steps and the respective software modules that implement them. A detailed manual (PRISE Manual) and a step-by-step protocol (PRISE Tutorial), which demonstrates how the software was used to create sequence-selective PCR primers for a specific fungal rRNA gene, can be accessed via the Instructions or Help links.

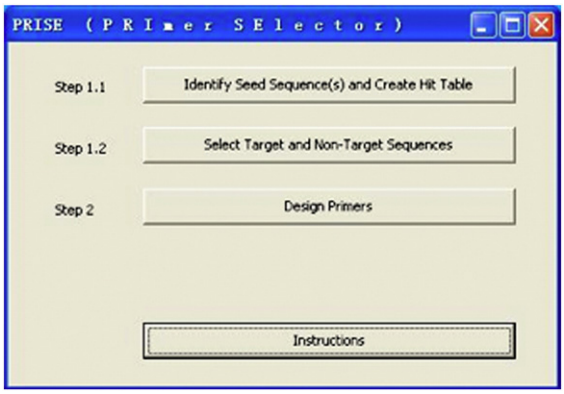

Fig. 1. Opening window with links to the instructions and modules of the PRISE software.

2.3.1. Step 1.1: identify seed sequence(s) and create hit table

In the first step of the design process, PRISE assists the user in identifying the Seed Sequence(s) and creating the Hit Table. The Seed Sequence(s) represent the DNA sequence(s) that the primers are designed to amplify. The Hit Table is a list of DNA sequences with various degrees of similarity to the Seed Sequence(s), from which the target and non-target sequences can be derived. The Hit Table is created by subjecting the Seed Sequence(s) to an analysis using BLAST (blastn) (Altschul et al., 1990).

2.3.2. Step 1.2: select target and non-target sequences

Once the Seed Sequence(s) and Hit Table have been created, the next step is to select the target and non-target sequences. This is accomplished with the Select Target and Non-Target Sequences module.

2.3.3. Using the module

After opening the module, the user loads the Seed Sequence (s) and Hit Table files into the software. After the sequences are loaded, the software downloads all of the GenBank records associated with the Seed Sequence(s) and Hit Table sequences, parses the data contained within them into separate components such as Definition or Source, performs pairwise % identity analyses between the Seed Sequence(s) and Hit Table sequences, and displays these data in a table. Once these actions have been completed, the user can select the target and non-target sequences by applying sorting tools to the sequences assembled in the table. Such tools allow the sequences to be sorted by parameters such as sequence length, % sequence identity or GenBank parameters such as Definition or Source.

2.3.4. Alternative ways to construct target and non-target sequences

Although Steps 1.1 and 1.2 are designed to select target and non-target DNA sequences, there are certainly other strategies for accomplishing this task, which the user may decide to use instead of or in combination with our steps. The only requirement for