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The PCR Suite

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ABSTRACT

Summary: The web application PCR Suite is an extension of the primer design program Primer3. It allows the design of primer sets encompassing single nucleotide polymorphisms, all exons of a single gene, all open reading frames in a list of cDNAs or the creation of overlapping PCR products.

Availability: www.eur.nl/fgg/kgen/primer

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INTRODUCTION

Primer design is an essential and usually time consuming step in many experiments. A large number of criteria has been devised for primer design, such as optimal GC content, maximum end stability, maximum self complementarity, primer length and so forth (see Rychlik, 1993; Breslauer *et al*., 1986). In order to optimize the process of primer design, several programs have been created. In these programs, parameters such as GC content, melting temperature and dimerization parameters for the primers can be adjusted, and the best primerset is calculated for the sequence provided. One such program is the Primer3 program written at the Whitehead Institute. Primer3 is available online (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and consists of an HTML form, a CGI script and the Primer3 core program. The software is free, downloadable and can be installed on local servers.

A drawback of the online Primer3 is that it does not allow batch uploads, or design overlapping primer sets for targets that are too large to cover by a single PCR. In this time of automation and batch processing, automatic primer design is vital for speed of experiments. We have designed a novel interface to Primer3, PCR Suite, that has a number of these options. The program consists of four interfaces to Primer3: Overlapping Primers, Genomic Primers, cDNA Primers and SNP Primers. For each of these options, a separate HTML form is available on www.eur.nl/fgg/kgen/primer/. The forms were designed to look similar to the original Primer3 form, but simplified.

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OVERVIEW

The Overlapping Primers html form is most similar to the original Primer3 form, but has the option to submit a large target sequence and request a small PCR product size. The program takes the sequence, which may be numbered or plain sequence, or sequence in FASTA format, selects the first part of the sequence, and sends this to the Primer3 core program. The resulting primerset is stored and the next part of the target is selected, allowing for an overlap which is adjustable by the user. The process is repeated until the end of the target is reached, and the primersets are presented on screen in such a way that the original target is easily recognized. If the core program was unable to find primers, this is visible in the output, allowing the user to select that region and design a separate primerset.

The Genomic Primers program designs primers around exons (Fig. 1). This part of the PCR Suite script was written to facilitate mutation analysis: for instance in screening for mutations in human disease genes, the regions around intron/exon boundaries are of interest because they often contain pathological mutations [e.g. MAPT (OMIM 157140), BRCA1 (OMIM 113705) and BRCA2 (OMIM600185)]. The identification of every exon of a gene in its genomic context and the development of primers around these exons is a time consuming task that can easily be automated. The input of Genomic Primers is a GenBank file with a genomic sequence containing the gene of interest (directions to obtaining such a file are given in the Genomic Primers html form) and the name of the gene. The user can also submit a 'minimum flanking size' of sequence surrounding the exon, to allow screening the intron/exon boundaries and nearby sequence. The GenBank file is uploaded and the program selects all the exons available for the gene. It does so by selecting all the mRNAs that correspond with the gene, a procedure that incorporates all alternative splices listed in the file. The exons are selected with flanking sequence, presented to the Primer3 core program, and the output is formatted to show the primers in the input sequence. If no suitable primers were found, exons are presented with flanking sequence, which allows the user to relax the parameters and run the program again.

SNP Primers works in a similar way, but designs primers around all the single nucleotide polymorphisms (SNPs) or

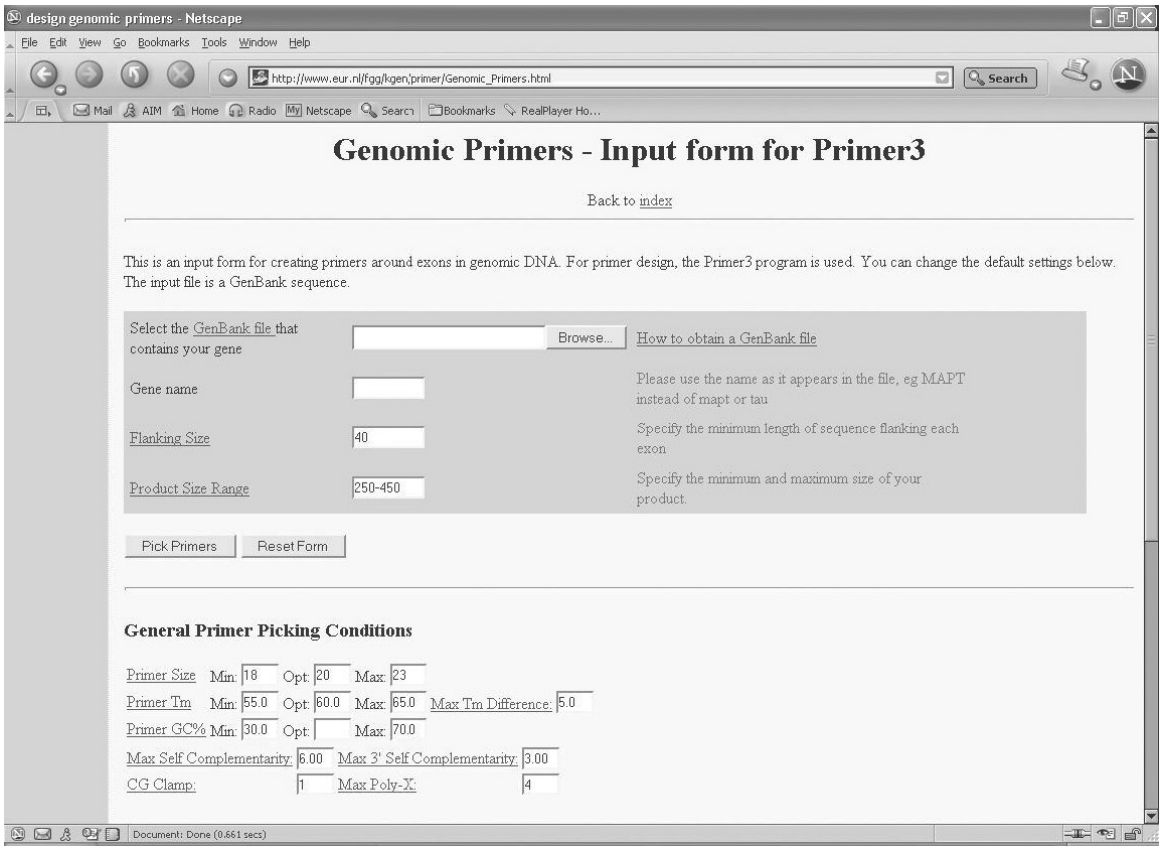

Fig. 1. The Genomic Primers input form.

'variations' in a GenBank file. The SNPs marked 'ambiguous' are skipped, because their physical position within the genome is unknown and this may confuse later analysis of the SNP data. The program also avoids incorporating SNPs in primers, by masking all SNPs in the region before sending the sequence to the Primer3 core program.

The last form, cDNA Primers, designs primers around the open reading frames (ORFs) of a list of cDNA sequences. The input is a list of GenBank files containing the cDNA sequences of interest. If the ORF is the same size as the sequence, which happens with many predicted genes, primers are designed as close to the start and end of the sequence as possible.

To generate primer sets with a higher PCR success rate, the default settings of all four programs are more stringent than in the original Primer3 form. The algorithms to compute these parameters are discussed in the Primer3 article by Rozen and Skaletsky (2000).

In our project on finding a gene for Parkinson's disease, we needed to screen 90 genes for the presence of mutations. The PCR Suite allowed us to quickly test for presence of all their corresponding cDNAs in our patients, and we found that indeed one of these appeared to be incomplete: DJ1, the causative gene of Park7 (Bonifati *et al*., 2003). We designed genomic primers around each exon and found another mutation in a second family. This shows that the PCR Suite is very useful in positional cloning efforts, where large numbers of genes are commonly seen.

IMPLEMENTATION

The PCR Suite CGI application was written in Perl and runs on a Linux server. It is available at www.eur.nl/fgg/kgen/primer and can be downloaded from this site. The Primer3 core program is available at the Whitehead Institute: http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi. A detailed description of how the PCR Suite interacts with Primer3 can be found at the website.

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