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Designing degenerate PCR primers

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Abstract

The purpose of CODEHOP is to help in the design of degenerate PCR primers from protein sequence.

Content

The purpose of CODEHOP is to help in the design of degenerate PCR primers from protein sequence. Given a multiple alignment of a series of target proteins, CODEHOP will transform the amino-acid consensus sequence into a DNA consensus sequence, using a user-designated codon-usage table. This DNA consensus is used to design degenerate primers, which are useful for identifying new family members in the same species or for pursuing phylogenetic studies in related species.

Navigation

The easiest way to use the CODEHOP site is to start with the BLOCKMAKER program, which takes two or more related protein sequences and identifies conserved blocks of sequence within them. Once these blocks have been determined, the output can be fed directly to the CODEHOP program by following a link on the output page. Clicking on the link presents several options. You can designate which codon-usage table to use, selected from a list of over 75 precalculated codon-usage tables, including a generic average eukaryotic table or an equal weighted table (additional codon tables can be requested by e-mailing the webmaster). The other parameters that can be specified are the maximum degeneracy of the primers, the target temperature, the primer concentration, limits on the number of repeated nucleotides, and some additional limitations on primer location and stringency. Each of the sequences can be assigned a different weight, which may be useful for biasing primer design towards, or away from, a particular sequence. For each of the blocks in the set, a series of primers is generated, each with a core section and a degenerate section. Primers are generated on both the direct and the complement strands, so that the optimal PCR product size can be selected using primers from different blocks. The melting temperature (T_m) and the degeneracy of each primer are shown, as well as the relative position in each block. Caution must be used when evaluating the proposed primers. There is no checking for secondary structure, self-annealing or primer-pair compatibility, and a few of the T_m calculations are clearly wrong (how can a PCR primer have a T_m of -4.9 °C?). There is an extensive help page with explanations of the algorithms used, descriptions of the various parameters and even tips

on performing the PCR once the primers have been designed. There is also a link to the manuscript describing CODEHOP where the application of the program is demonstrated.

Reporter's comments

Timeliness

The site was last modified in December 1998.

Best feature

The ease of moving from a series of unaligned sequences to a series of degenerate primers is impressive. The most time-consuming part is using the BLOCKMAKER program to align the sequences and determine the sequence blocks. Once that is finished, the primer generation step takes only seconds.

Worst feature

The need for the sequences to be in BLOCKS format is annoying, though there is a converter that will take a Clustal multiple sequence alignment and transform it into a BLOCKS format. Unless you have a lot of sequences, or a carefully hand-edited alignment, it's probably best to use the BLOCKMAKER program to generate the necessary starting file.

Wish list

It would be useful to have the option of choosing primers and have them evaluated for possible secondary structure problems, self-annealing or primer-pair compatibility.

Related websites

There are several PCR primer design sites, including Primer3, DoPrimer, and. None of these programs, however, helps with the design of PCR primers from protein sequence. In this regard, CODEHOP appears to be unique.

Table of links

CODEHOP: consensus-degenerate hybrid oligonucleotide primers

BLOCKMAKER program

Primer3

DoPrimer

References

1. CODEHOP: consensus-degenerate hybrid oligonucleotide primers.