Amplicon: software for designing PCR primers on aligned DNA sequences

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ABSTRACT
Summary: Amplicon is a program for designing PCR primers on aligned groups of DNA sequences. The most important application for Amplicon is the design of ‘group-specific’ PCR primer sets that amplify a DNA region from a given taxonomic group but do not amplify orthologous regions from other taxonomic groups.

Availability: Amplicon is freely available as a script that will run on any platform with Python 2.3 installed (http://www.python.org). It is also available as a Windows executable. Free downloads that do not require registration can be found at http://www.aad.gov.au/amplicon
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INTRODUCTION
PCR primers for amplifying a given DNA region can often be placed at a number of possible sites in a sequence. Numerous programs have been developed to assist in the selection of primer binding sites that will most effectively produce the desired product (e.g. Haas et al., 1998; Rozen and Skaletsky, 2000; Nielsen and Knudsen, 2002). However, most programs available focus on designing primers to amplify DNA regions from within a single known sequence.

It is often desirable to PCR amplify a given DNA region while excluding very similar DNA sequences from amplification. A simple example is allele-specific PCR. A PCR product for identifying one allele of a gene is required, and so the PCR should not produce a product from the other allele, which may also be present in the DNA template solution for the PCR. Primers are chosen that exploit the nucleotide differences between the alleles so that a PCR product is only produced from one allele.

A more challenging situation is the use of group-specific PCR primers to amplify orthologous DNA regions from a given range of taxa. Primer binding sites from species within the target group of taxa may vary, and the orthologous binding sites in non-target taxa may also vary. Group-specific primers must therefore be very carefully designed to ensure that PCR products are generated from all members of the target group and never from members of non-target groups.

Tools for designing group-specific PCR primers are in demand because these types of primer sets are becoming increasingly popular for investigating species diversity in environmental samples (Boom et al., 2002; Van Borm and Boomsma, 2002; Jarman et al., 2002, 2003). Amplicon is a primer design program specifically aimed at group-specific primer design on sets of aligned DNA sequences. Amplicon has been used to design group-specific primer sets that have proven to be useful for identifying species from environmental samples (Jarman et al., 2003). The PCR primers were successfully used to amplify products from target taxa and not from non-target taxa to allow the identification of species present in samples containing a wide range of organisms.
If primers or primer binding sites are degenerate, then all possible combinations of real sequences represented by the degenerate sequences are tested. Gaps are removed from primers and from primer binding sites before such tests are made. When gaps are removed from primer binding site sequences in the ‘target’ sequence, the sequence region to either side of the selected primer binding site in the ‘excluded’ sequence is checked for primer binding in case alignment has skewed the position of the possible binding site in the excluded sequence.

Sections of alignments found between the chosen primer binding sites can be saved for phylogenetic analysis by other software packages. This is useful when designing group-specific PCR primers for producing a product that varies in sequence between species in a given group. If there is enough information in the form of sequence variation within the fragment, then it may be used to identify the species. Files can be saved for sequence similarity analysis in NEXUS (Swofford, 2003), MEGA (Kumar et al., 2001), PHYLIP (Felsenstein, 1993), CLUSTAL (Thompson et al., 1997) or FASTA formats.

COMPARISON WITH SIMILAR TOOLS

A large number of general PCR primer design packages and Web sites are available. However, very few packages other than Amplicon are capable of group-specific PCR primer design. PROBEmer is a Web-based program that will compare sequences provided by the user to find primer binding sites that are unique to one of them (Emrich et al., 2003). PrimerPremier is a commercial package available from Premier Biosoft International that is very highly featured and includes methods for allele-specific PCR primer design, but it is commercial software and the source code is not available. Amplicon is the only free, open source, stand-alone software for group-specific primer design and includes several features not available in any of the other similar programs.

IMPLEMENTATION

Amplicon is written in Python 2.3 and uses the Tkinter GUI tool-kit (version 8.4), which is part of the standard Python distribution. It should run as a script on any platform with Python installed. For Windows users, an executable version that does not require a Python installation is available. Amplicon is distributed with a manual and three example alignment files. The packages are distributed under the GNU General Public Licence (Free Software Foundation 1991; http://www.gnu.org).

REFERENCES


IUPAC-IUB (1970) Abbreviations and symbols for nucleic acids, polynucleotides and their constituents.


