Large scale sequence analysis with DoPrimer™

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Oligonucleotide primers play an important role in modern methods in molecular biology like PCR and DNA sequencing. With the increasing availability of automated high-throughput analysis equipment and DNA chip technologies there is also a demand for easy design of large sets of oligonucleotide primers. DoPrimer is a modular and easily extendable tool meeting these and future demands in oligonucleotide primer design.

DoPrimer is implemented using Sun's Java programming language and a highly modular architecture. This facilitates integration of additional oligonucleotide design algorithms using clearly defined interfaces. The modular design also allows to offer three different user interfaces, which are based to more than 80 percent on a common code base containing the core DoPrimer calculation modules and the program's basic infrastructure:

- The stand-alone application with GUI offers an easy-to-use graphical user interface, that enables life science researchers to perform complex tasks like batch design of primers for different applications in an intuitive environment.

- The stand-alone application with command-line control is intended for use in more complex scripted bioinformatics environments offering all primer design capabilities of the GUI version controlled by command line parameters and parameter files.

Both versions are able to do batch design of primer sets for a virtually unlimited number of target sequences. Algorithms implemented up to now are the design of PCR primers, of primers for DNA sequencing and for binding probes for Interactiva's proprietary XNA on Gold™ DNA chip system. Additionally, distributed calculation of primers in a local area network is supported. The user can run the DoPrimer calculation modules on a fast computation server and control them over the interface modules running on a workplace PC, thus avoiding unnecessary overhead on the computing server. For further performance improvements novel algorithms for primer pair assessment, e.g. in respect for self- or pair-annealing in PCR primers, have been developed [1,2].

The third version of DoPrimer, Web DoPrimer, is a web enabled system for doing online primer design of single PCR and sequencing primer sets over the Internet. Interactiva Biotechnologie GmbH offers this system combined with an SRS search system for retrieving target sequence data from the EMBL sequence database as a free service to the scientific community at http://doprimer.interactiva.de. To avoid long download times and a multitude of just too frequent problems when running client side java applets, Web DoPrimer is implemented as a server-side CGI-based system using standard HTML. However, due to DoPrimer's expendability, it will be possible to offer a DoPrimer Java applet once the need arises.

As a test application we used DoPrimer to design a complete set of proposed oligonucleotide PCR primers for the sequences in the HGBASE database of Human intragenic DNA polymorphisms. Further work contains synthesis and testing in PCR of a randomly selected subset of these oligonucleotide primers.

References

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2. Kämpke Th, Kieninger M, Mecklenburg M, Primer Design Tools 1: Fast approximation of multiple primer pairs for multiplex PCR", (submitted)