RAMA UNIVERSITY

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FACULTY OF ENGINEERING AND TECHNOLOGY

Department of Biotechnology

Primer design

✓Bioinformatics has become an essential tool not only for basic research but also for applied research in biotechnology and biomedical sciences.

✓Optimal primer sequence and appropriate primer concentration are essential for maximal specificity and efficiency of PCR.

✓There are several online tools devoted to serving molecular biologist design effective PCR primers.

✓This most efficient way to design a new specific-primer by applying current publicly available links and Web services.

WEB-BASED RESOURCES FOR PRIMER DESIGN

✓There are a numerous web-based resources for PCR and primer design. Though most are freely available, they are of variable quality and not well maintained.

✓This often results in missing links and so sites that may have been useful previously may not be functional at a later date.

✓There are a number of criteria that need to be established in the design of primers and a number of these are listed below.

Program for primerdesign

Table 1. Online primer design sites.

Tool name	Description	www
CODEHOP	**C**onsensus **D**egenerate **H**ybrid **O**ligonucleotide **P**rimers; degenerate PCR primer design; will accept unaligned sequences.	http://blocks.fhcrc.org/codehop.html
Gene Fisher	Interactive primer design tool for standard or degenerate primers; will accept unaligned sequences.	http://bibiserv.techfak.uni-bielefeld.de/genefisher/
DoPrimer	Easily design primers for PCR and DNA sequencing.	http://doprimer.interactiva.de/
Primer3	Comprehensive PCR primer and hybridization probe design tool; many options but easy to accept defaults at first.	http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi http://www.basic.nwu.edu/biotools/Primer3.html http://www.justbio.com/primer/index.php
Primer Selection	Select PCR primers from nucleotide sequence.	http://alces.med.umn.edu/rawprimer.html
Web Primer	Allow alternative design of primers for either PCR or sequencing purpose.	http://genome-www2.stanford.edu/cgi-bin/SGD/web-primer
PCR Designer	For restriction analysis of sequence mutations.	http://cedar.genetics.soton.ac.uk/public_html/primer.html
Primo Pro 3.4	Reduces PCR noise by lowering the probability of random primering.	http://www.changbioscience.com/primo/primo.html
Primo Degenerate 3.4	Primo Degenerate 3.4 designs PCR primers based on a single peptide sequence or multiple alignments of proteins or nucleotides.	http://www.changbioscience.com/primo/primod.html
PCR Primer Design	An application that designs primers for PCR or sequencing purposes.	http://pga.mgh.harvard.edu/servlet/org.mgh.proteome.Primer
The Primer Generator	The program analyzes the original nucleotide sequence and desired amino acid sequence and designs a primer that either has a new restriction enzyme site or is missing an old one.	http://www.med.jhu.edu/medcenter/primer/primer.cgi
EPRIMER3	Picks PCR primers and hybridization oligos (EMBOSS).	http://bioweb.pasteur.fr/seqanal/interfaces/eprimer3.html
PRIMO	Prediction of forward and reverse oligonucleotide Primers.	http://bioweb.pasteur.fr/seqanal/interfaces/primo.html3 http://atlas.swmed.edu/primo/primo_form.html
PrimerQuest	A primer design tool.	http://www.idtdna.com/biotools/primer_quest/primer_quest.asp
MethPrimer	Design primers for methylation PCRs.	http://itsa.ucsf.edu/~urolab/methprimer/index1.html
Rawprimer	A tool for selection of PCR primers.	http://alces.med.umn.edu/rawprimer.html
MEDUSA	A tool for automatic selection and visual assessment of PCR primer pairs.	http://www.cgr.ki.se/cgr/MEDUSA/
The Primer Prim'er Project	Software suite that completely automates the PCR primer design process.	http://www-nmr.cabm.rutgers.edu/bioinformatics/Primer_Primer_Project/Primer.html
Oligonucleotides for the PCR	Seek oligonucleotides on both sides of an area.	http://www.citi2.fr/bio2/Oligo2lib.html
GAP	Genome- wide Automated Primer finder servers.	http://promoter.ics.uci.edu/Primers/

(Abd-Elsalam KA, Afr J Biotechnol 2003)

General guidelines for primer design

- Primer sizes:18-30 bp
- 40-60% of GC contents in each primer
- Optimum temperature (Tm) of each primer should be 55-66 oC and the difference of forward and reverse primers should not more than 2 oC
- 3’ end of each primer contains G or C (Last 5 bases: only 2G, 2C or GC)
- Avoid repetitive bases • Avoid mismatch between primers and template at 3’end

Primer design at a glance

✓Choose organisms or gene of interest from database such as GenBank, EMBL, DDBJ, etc.

✓Download sequence in FASTA format or using Accession No. of interested gene

✓Primer design with appropriate program such as primer-BLAST, Primer3, Primer3Plus, etc. Or manual design (universal primer multiple sequence alignment Choose conserve sequence)

✓Select suitable designed primers from primer design program

✓Consider primer characterization such as length, Tm, %GC, secondary structure of primers, etc.

✓Final checking of primer sequences and order primer synthesis

✓Use the primers inPCR or RT-PCR and optimization with reagents and PCR machine as well as genome template

Conclusion

✓The key to the PCR lies in the design of the two oligonucleotide primers.

✓It is essential that care is taken in the design of primers for PCR. Several parameters including the length of the primer, %GC content and the 3' sequence need to be optimized for successful PCR.

✓Certain of these parameters can be easily by hand optimized while others are best done with marketable computer programs.

✓The increasing use of information from the internet and the sequences held in gene databases are practical starting points when designing primers and reaction conditions for the PCR.

✓ A number or software packages such as Oligo, Primer etc. have allowed the process of primer design to be less troublesome.

✓It is also possible to include more than one set of primers in a PCR.

XYZ

abc

XYZ

abc

XYZ

abc

XYZ

abc

XYZ

abc

XYZ

abc

MCQs

1. A
2. A
3. A
4. A
5. A
6. A
7. A
8. A
9. A
10. A