
بسم الله

# Designing PCR Primers for Various Applications

# biotechnology

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# Workshop Outline

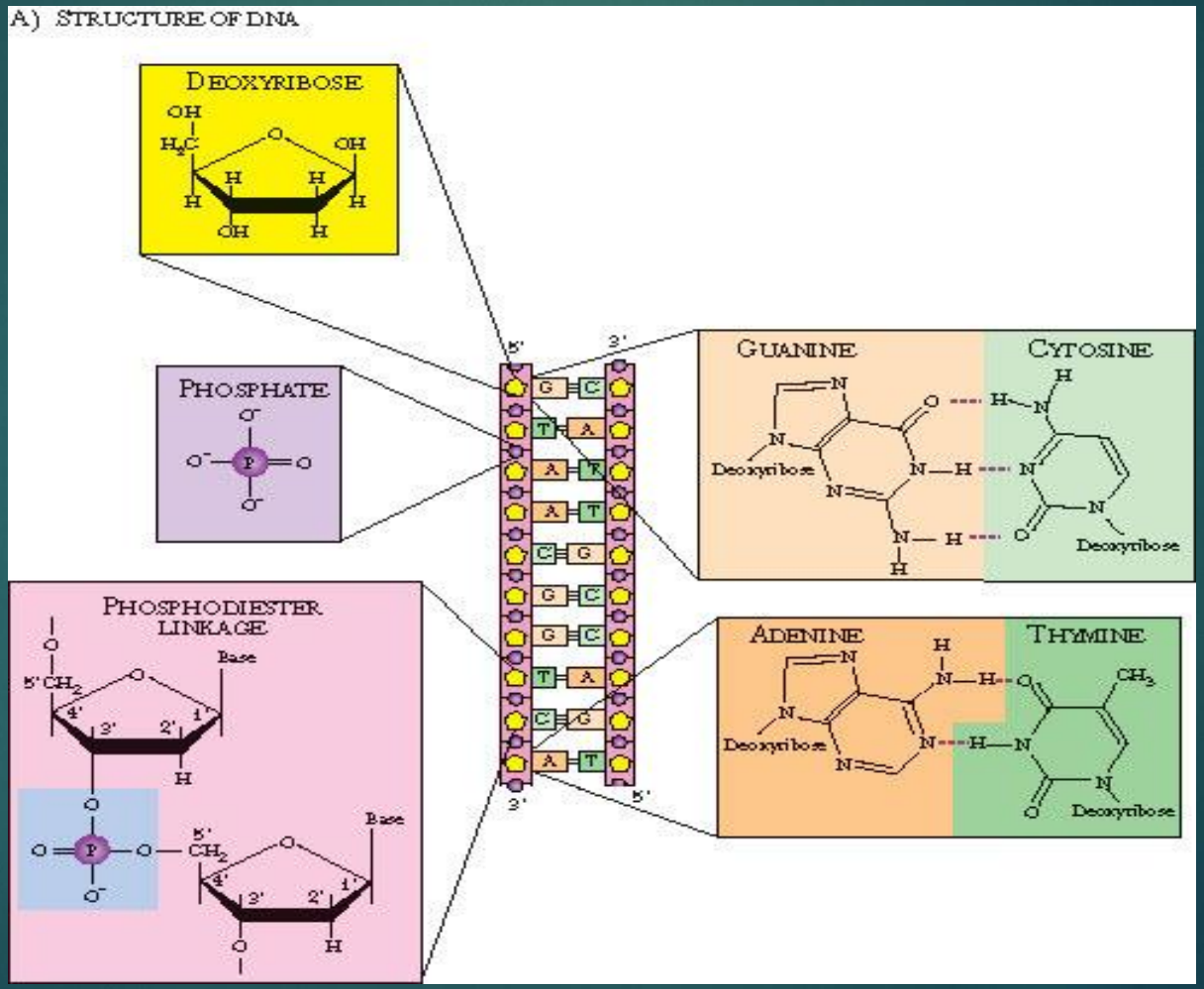
## Pourpuse

A. The General Rules for PCR Primer Design
B. Resources for General Purpose PCR Primer Design
C. Resources for Real-Time q-PCR Primer Design
D. Resources for PCR Primers/Oligos Quality Analysis

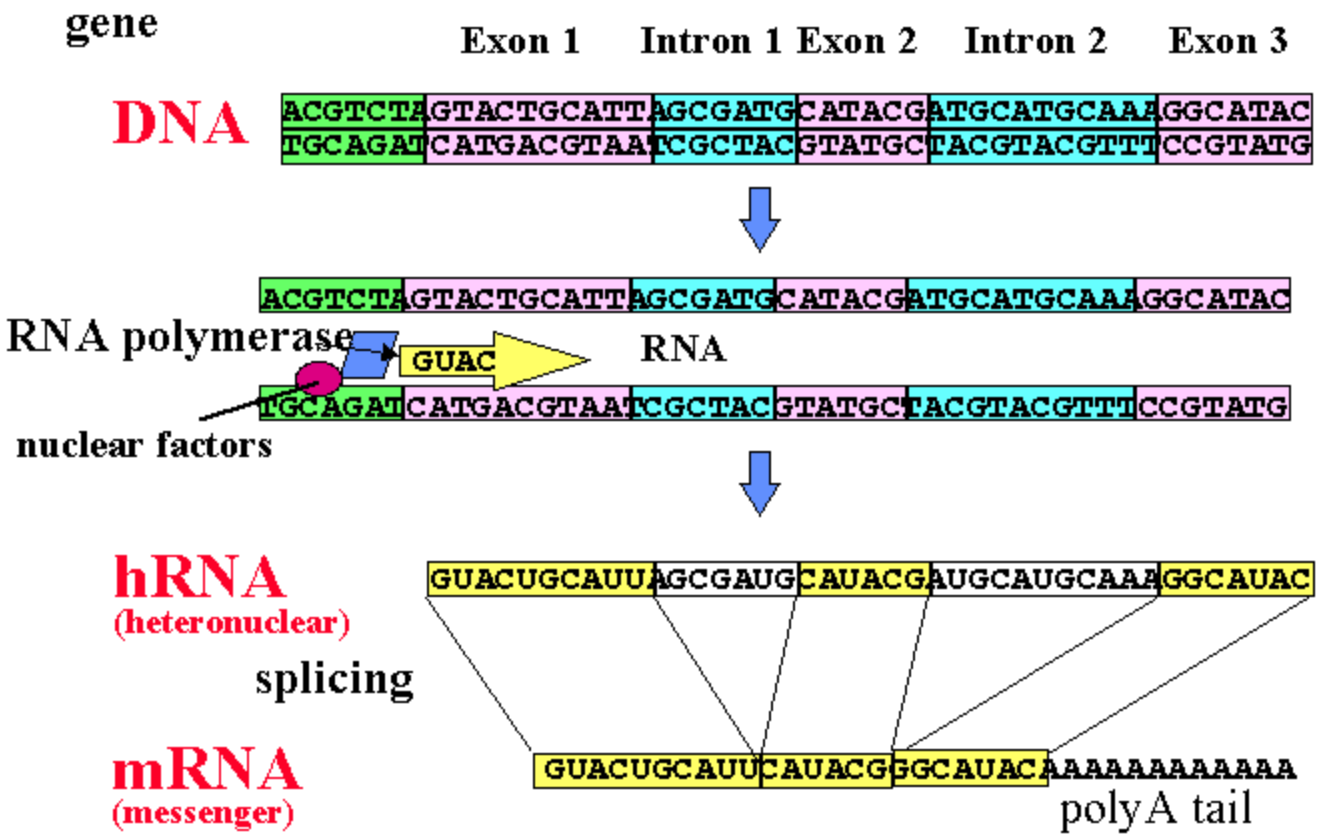
# Structure of DNA

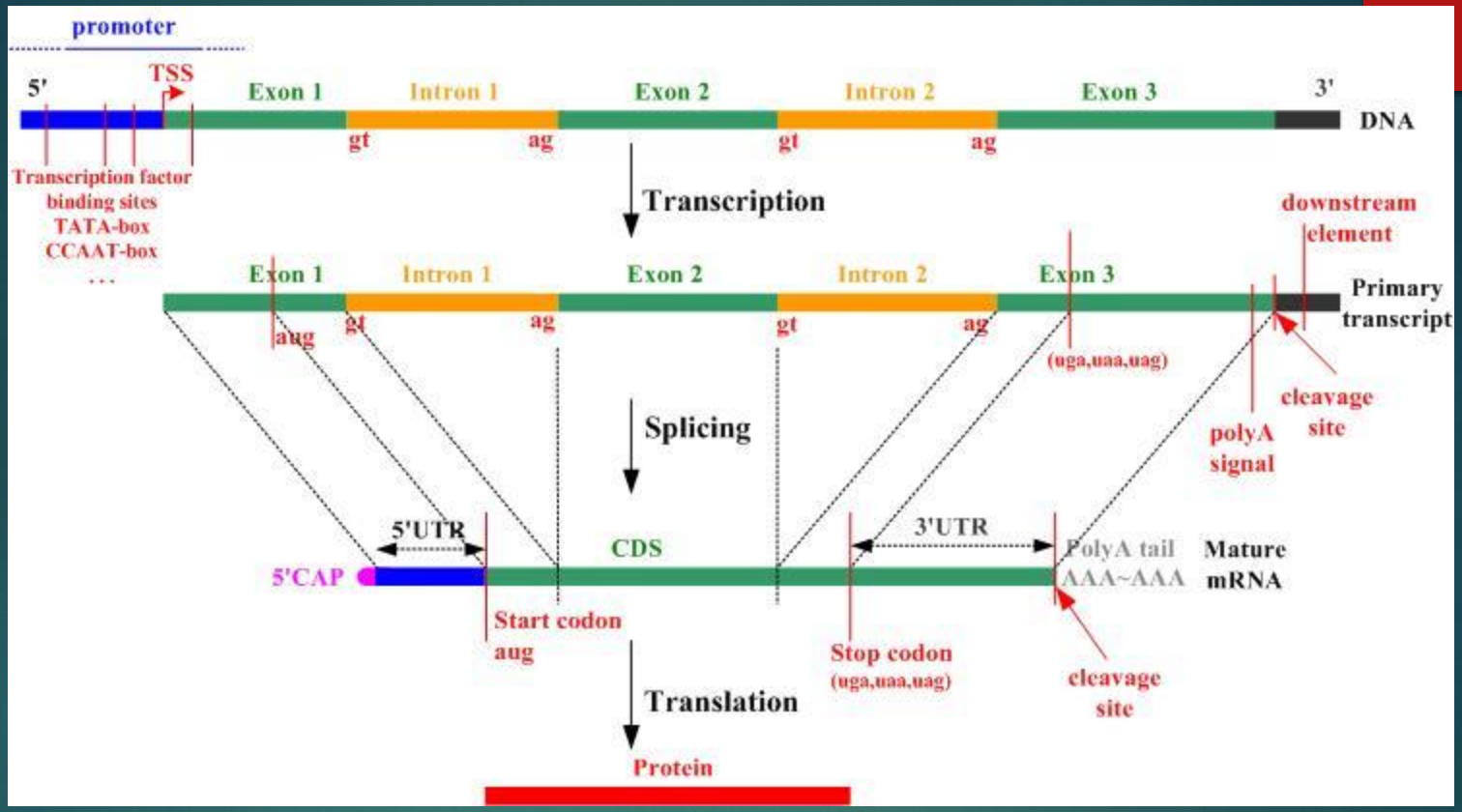
DNA

ATGCTGGCAATGCAGGCTTTTGCACTGGCTAATATAGCGTAA
TACGACCGTTACGTCCGAAAACGTGACCGATTATATCGCATT

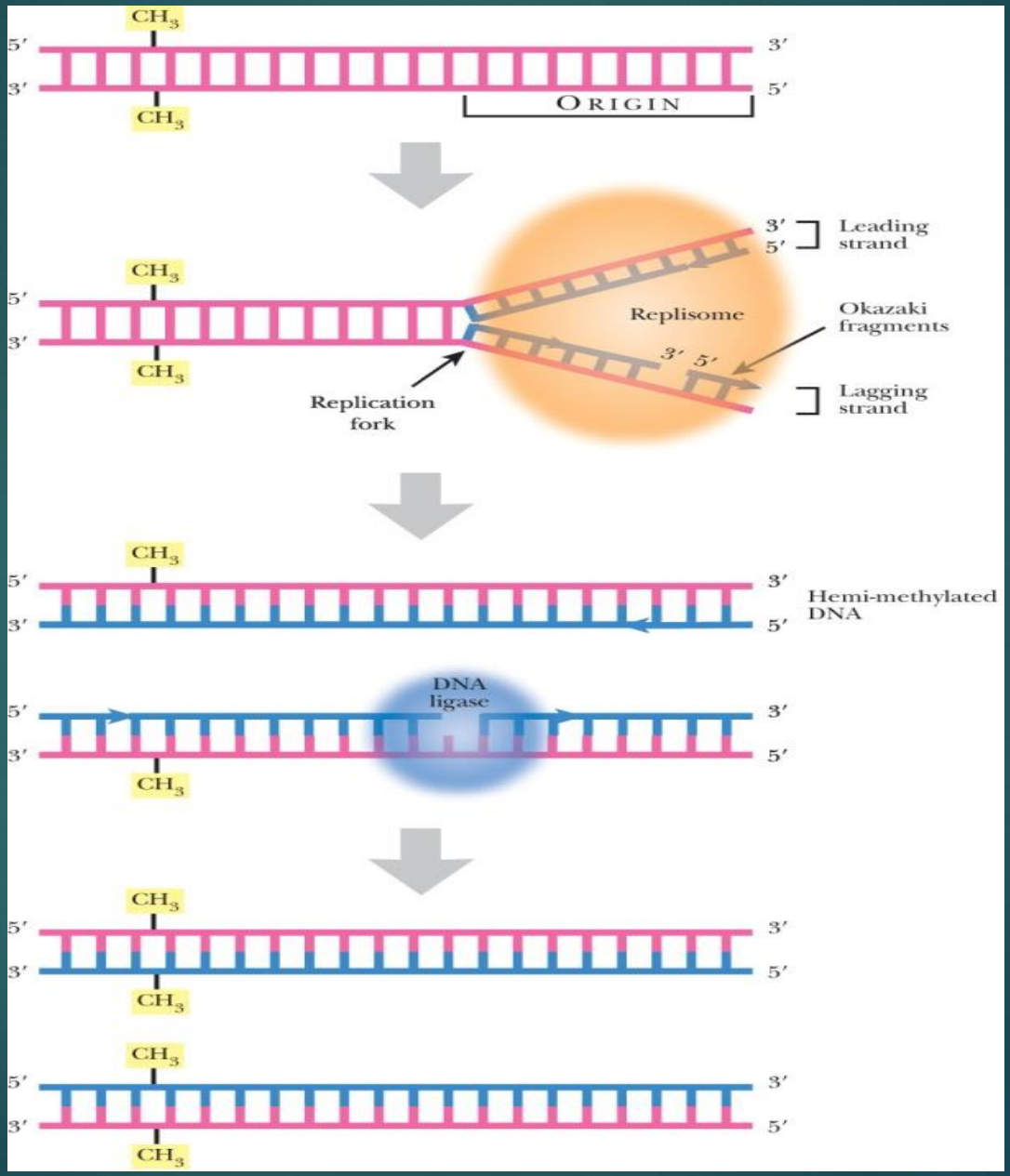


# Transcription




promoter
5'
TSS
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
3'
DNA
gt
ag
gt
ag
Transcription factor
binding sites
TATA-box
CCAAT-box
...
Transcription
downstream
element
Exon 1
Intron 1
Exon 2
Intron 2
Exon 3
Primary
transcript
aug
gt
ag
gt
ag
(uga,uaa,uag)
cleavage
site
polyA
signal
Splicing
5'UTR
CDS
3'UTR
PolyA tail
AAA~AAA
Mature
mRNA
5'CAP
Start codon
aug
Stop codon
(uga,uaa,uag)
cleavage
site
Translation
Protein

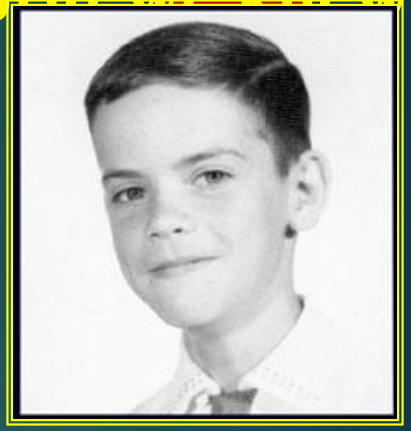
# DNA Replication and DNA Polymerase

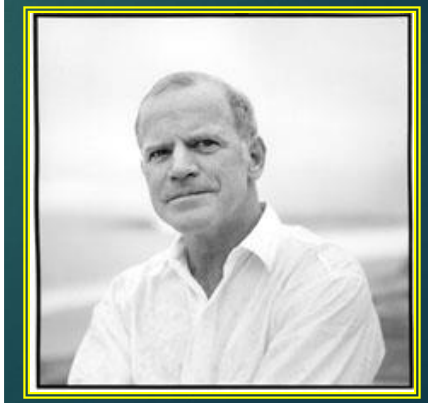


# PCR: the technology that changed the world we knew

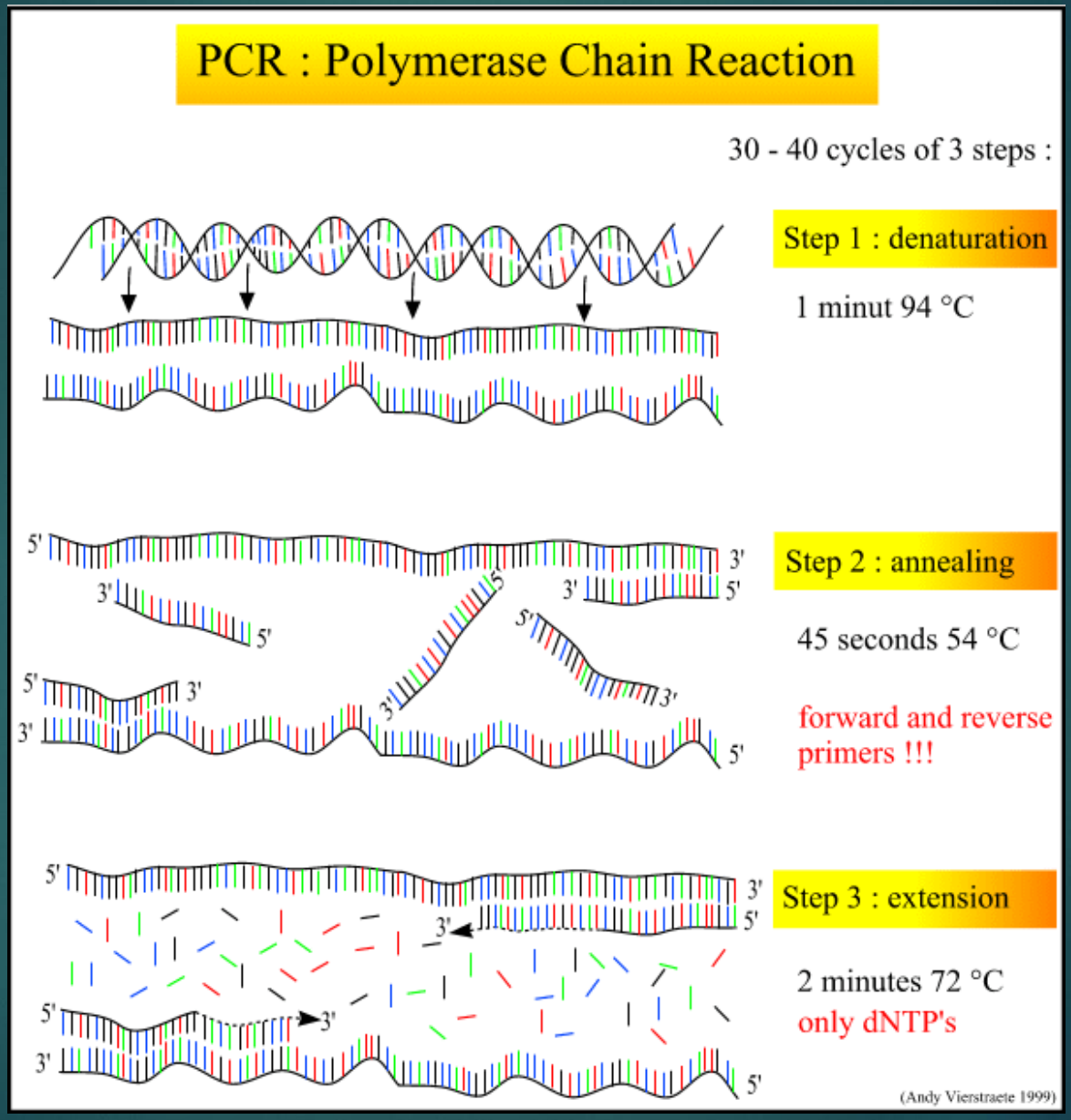
- The Polymerase Chain Reaction (PCR) revolutionized life sciences as it provides a sensitive, reliable, efficient, and convenient means of amplifying relatively large quantities of DNA
- Invented in 1983 by Kary Mullis, who won a Nobel Prize 1993
- The technique was made possible by the discovery of *Taq* polymerase, the DNA polymerase that is used by the bacterium *Thermus aquaticus,* discovered in hot springs.
- The primary materials used in PCR:

  - DNA nucleotides: the building blocks for the new DNA
  - Template DNA: the DNA sequence that you want to amplify
  - Primers: single-stranded short DNA (16--50 nucleotides long) that are complementary to a short region on either end of the template DNA
  - DNA polymerase: a heat stable enzyme that catalyzes the synthesis of new DNA





# Primers dictate the successfulness of a PCR




Thermocycler Condition

Initial denaturation

Denaturation

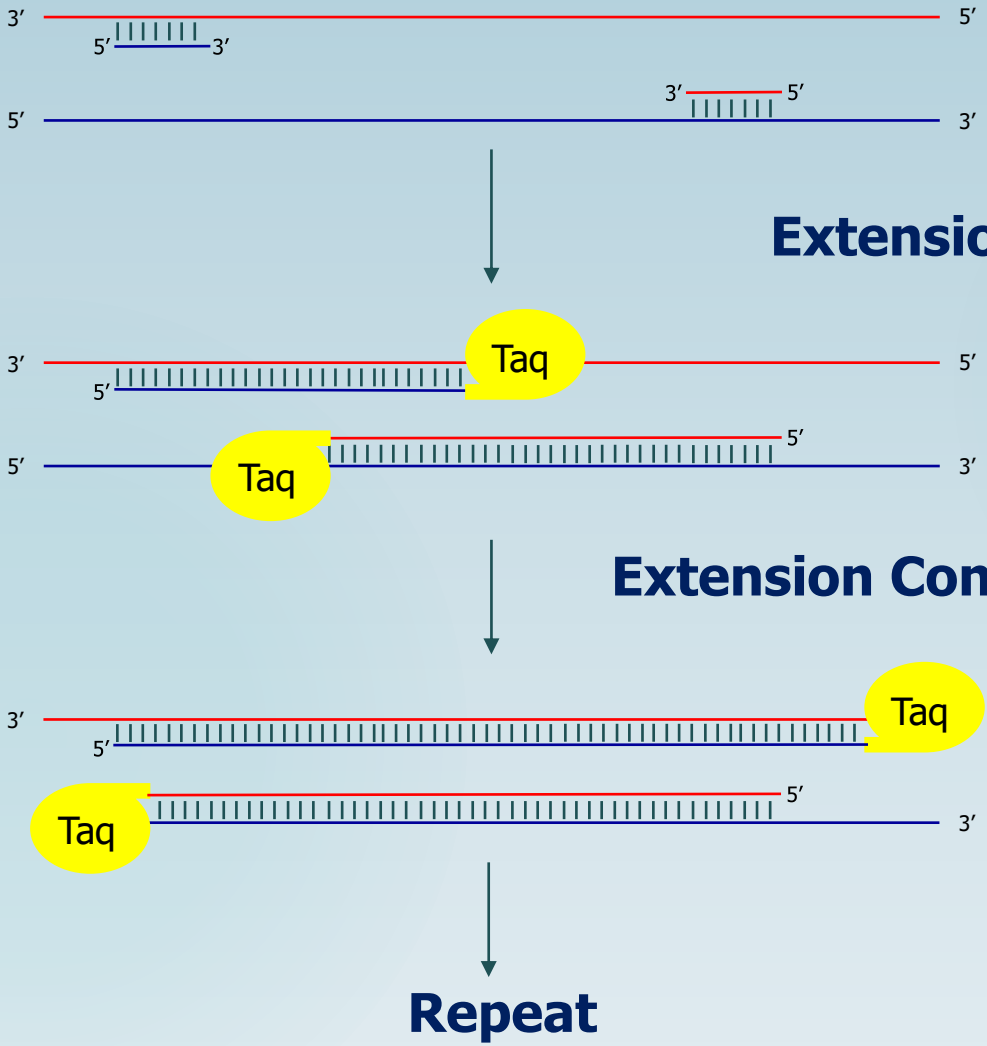
Annealing

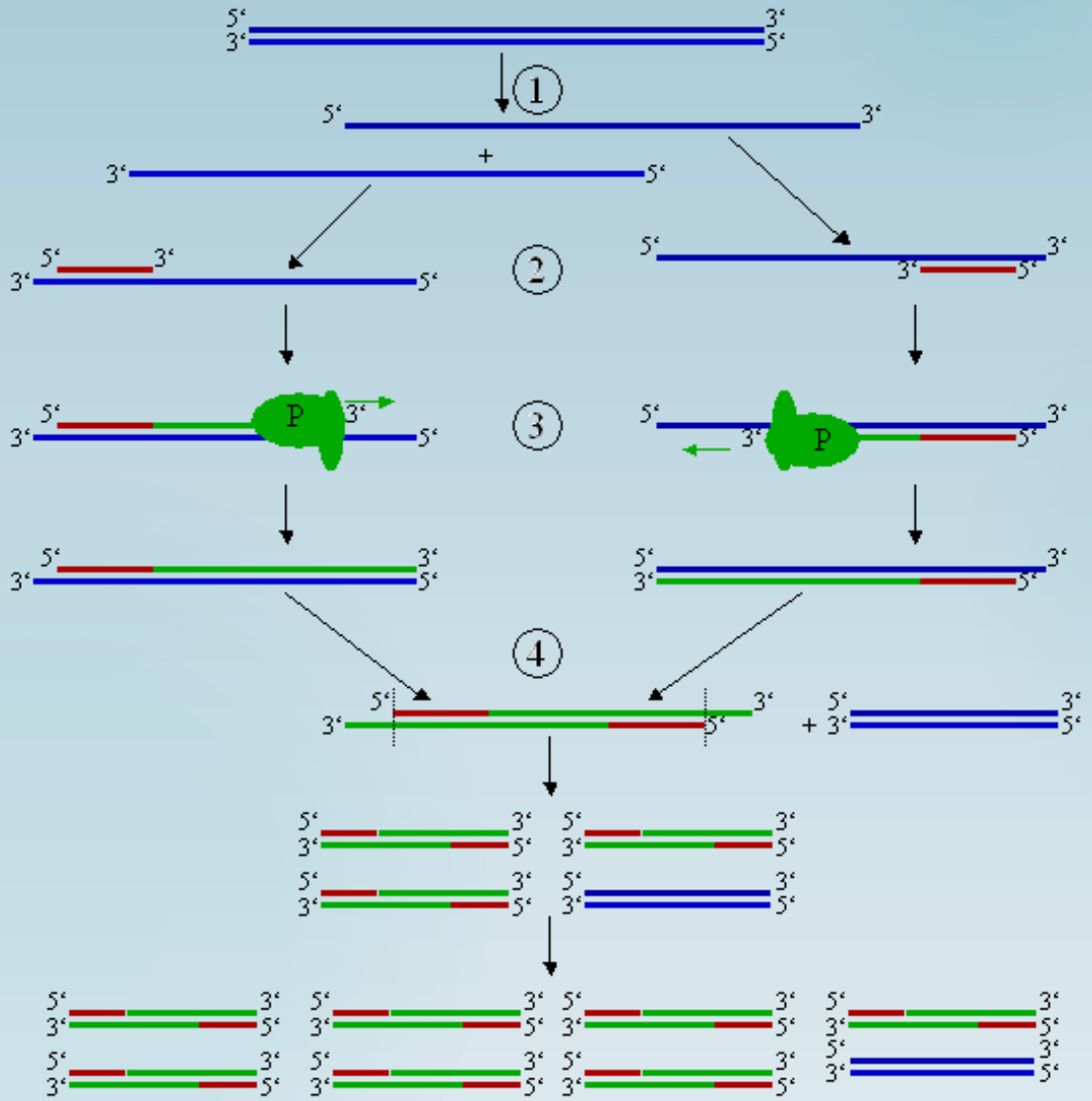
Extension

Final extension

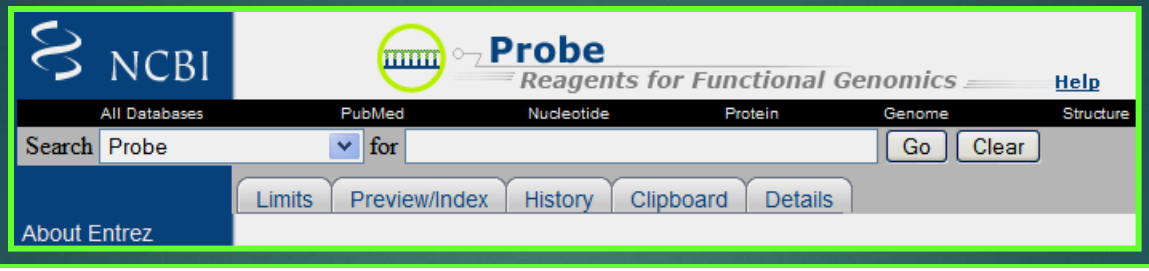
| PCR step | Temperature | Time | Repeat cycle |
|---|---|---|---|
| Initial denaturation | 95 °C | 5 min | 1 |
| Denaturation | 95 °C | 30 sec | 30 |
| Annealing | 58 °C | 30 sec | 30 |
| Extension | 72 °C | 1 min | 30 |
| Final extension | 72 °C | 5 min. | 1 |
| Hold | 4 °C | Forever | – |

PCR thermocycler conditions


3′
5′
5′
3′
3′
5′
5′
3′
Extension
3′
5′
Taq
5′
5′
Taq
3′
Extension Continued
3′
5′
Taq
5′
Taq
3′
Repeat


5'
3'
1
+
2
P
3
4

# Before you design your own primers –
# Don't reinvent the wheels!




**qPrimerDepot** ---- A quantitative real time PCR primer database

Quantitative PCR Primer Database

QPPD

RTPrimerDB
Real Time PCR Primer and Probe Database

> DNA Methylation analysis PCR Primer Database

Quick Search / Advanced Search

Gene | Substring | Exact phrase | Organism: All | Search | methPrimerDB ID

MASSACHUSETTS GENERAL HOSPITAL | CCIB The Center for Computational and Integrative Biology | HARVARD MEDICAL SCHOOL

## Primer Bank

PCR Primers for Gene Expression Detection and Quantification

# Before you start designing primers – Find and use the right resources!

- **What are the primers for?**
  - **General purpose amplification?**
  - **SNPs detection/validation?**
  - **Methylation study?**
  - **Real-time PCR?**
  - **Microarray probes?**
  - **Degenerate PCR?**
  - **Multiplex PCR?**
- **What do you have to begin with?**
  - **Single DNA/protein sequence?**
  - **Multiple DNA/protein sequence files?**
  - **GenBank ID/Gene ID/Gene Symbol/rsSNP ID?**

# After you have your primers designed – Consider a second opinion!

- **Most likely your primers can be designed by several different software**

- **Different software may vary significantly in:**
  - **Concepts and overall approaches**
  - **Designing criteria and default settings**
  - **Comprehensiveness**
  - **Usability**
  - **Accessibility and speed**

# General rules for primer design -- Primer and amplicon length

- **Optimal primer length**
  - **18-24 bp for general applications**
  - **30-35 bp for multiplex PCR**

- **Primer length determines the specificity and significantly affect its annealing to the template**
  - **Too short -- low specificity, resulting in non-specific amplification**
  - **Too long -- decrease the template-binding efficiency at normal annealing temperature due to the higher probability of forming secondary structures such as hairpins.**

- **Optimal amplicon size**
  - **300-1000 bp for general application, avoid > 3 kb**
  - **50-150 bp for real-time PCR, avoid > 400 bp**

# Probability in Genetics

- There are 4 bases in the DNA molecule A,C,G,T
- The probability of encountering any of these bases in the code is 0.25 (1/4)
- So let us look at the probability of encountering a particular sequence of bases

| Event | Probability |
|---|---|
| A | 0.25 = 0.25 |
| A,T | 0.25 x 0.25 = 0.0625 |
| A,T,A | 0.25 x0.25 x 0.25 = 0.015625 |
| A,T,A,G,G | $(0.25)^5$ = 0.0009765 |
| A,T,A,G,G,T,T,T,A,A,C | $(0.25)^{11}$ = 0.000002384 |
| A,T,A,G,G,T,T,T,A,A,C,C,T,G,G,T | $(0.25)^{16}$ =0.0000000002384 |

So it become increasing unlikely that one will get 16 bases in this particular sequence (1 chance in 4.3 billion). In this same way, one can see that as the primer increases in size, the chances of a match other than the one intended for is highly unlikely.

# General rules for primer design -- Melting temperature ($T_m$)

- **$T_m$ is the temperature at which 50% of the DNA duplex dissociates to become single stranded**
  - **Determined by primer length, base composition and concentration.**
  - **Also affected by the salt concentration of the PCR reaction mix**
  - Tm=81.5 + 0.41(%GC) - 675/N-%mismatch (N=length)
  - **Working approximation: $T_m$=2(A+T)+4(G+C) (*suitable only for 18mer or shorter*).**
  - **ATCCGGATTACGCACGATC**
  - **A+T= 9 ---------- 9*2= 18**
  - **C+G=10 ---------- 10*4= 40**
  - **Tm= 40 + 18 = 58**

- **Optimal melting temperature**
  - **52°C-- 60°C**
  - **$T_m$ above 65°C should be generally avoided because of the potential for secondary annealing.**
  - **Higher $T_m$ (75°C-- 80°C) is recommended for amplifying high GC content targets.**

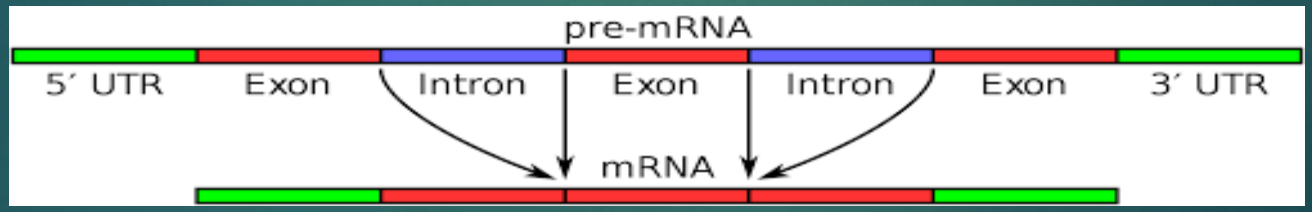
- **Primer pair $T_m$ mismatch**
  - **Significant primer pair $T_m$ mismatch can lead to poor amplification**
  - **Desirable $T_m$ difference < 5°C between the primer pair**

# General rules for primer design -- Specificity and cross homology

- **Specificity**
  - Determined primarily by primer length as well as sequence
  - The adequacy of primer specificity is dependent on the nature of the template used in the PCR reaction.

- **Cross homology**
  - Cross homology may become a problem when PCR template is genomic DNA or consists of mixed gene fragments.
  - Primers containing highly repetitive sequence are prone to generate non-specific amplicons when amplifying genomic DNA.

- **Avoid non-specific amplification**
  - **BLASTing PCR primers against NCBI non-redundant sequence database is a common way to avoid designing primers that may amplify non-targeted homologous regions.**
  - **Primers spanning intron-exon boundaries to avoid non-specific amplification of gDNA due to cDNA contamination.**
  - **Primers spanning exon-exon boundaries to avoid non-specific amplification cDNA due to gDNA contamination.**



- **Primer G/C content**
  - Optimal G/C content: **45-55%**
  - Common G/C content range: 40-60%

ATCCGGATTACGCACGATC

ATCCGGAAAACGCACGATC

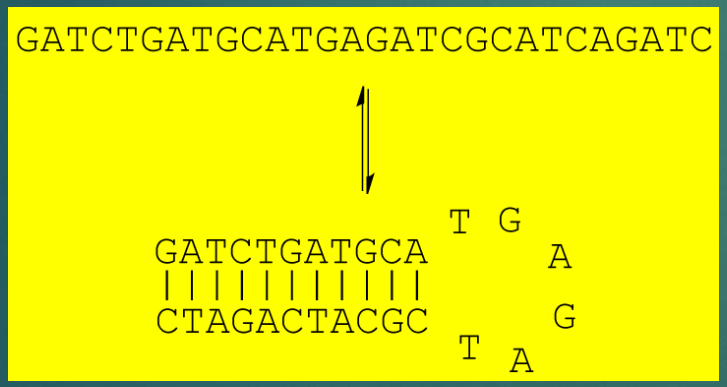
- **Runs (single base stretches)**
  - Long runs increases mis-priming (non-specific annealing) potential
  - The maximum acceptable number of runs is 4 bp

ATCCGGATATCGCACGATC

- **Repeats (consecutive di-nucleotide)**
  - Repeats increases mis-priming potential
  - The maximum acceptable number of repeats is 4 di-nucleotide

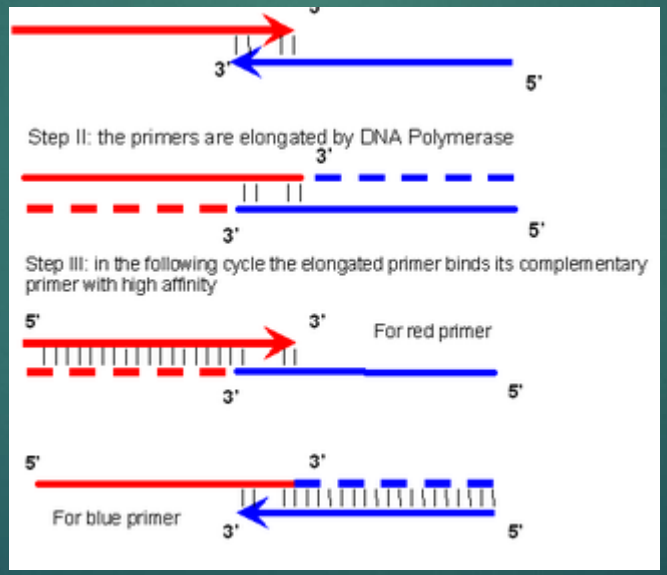
## Hairpins

- Formed via intra-molecular interactions
- Negatively affect primer-template binding, leading to poor or no amplification
- Acceptable ΔG (free energy required to break the structure): >-2 kcal/mol for 3'end hairpin; >-3 kcal/mol for internal hairpin;




- **Self-Dimer (homodimer)**
  - **Formed by inter-molecular interactions between the two same primers**
  - **Acceptable ΔG: >-5 kcal/mol for 3'end self-dimer; >-6 kcal/mol for internal self-dimer**

- **Cross-Dimer (heterodimer)**
  - **Formed by inter-molecular interactions between the sense and antisense primers**
  - **Acceptable ΔG: >-5 kcal/mol for 3'end cross-dimer; >-6 kcal/mol for internal cross-dimer;**



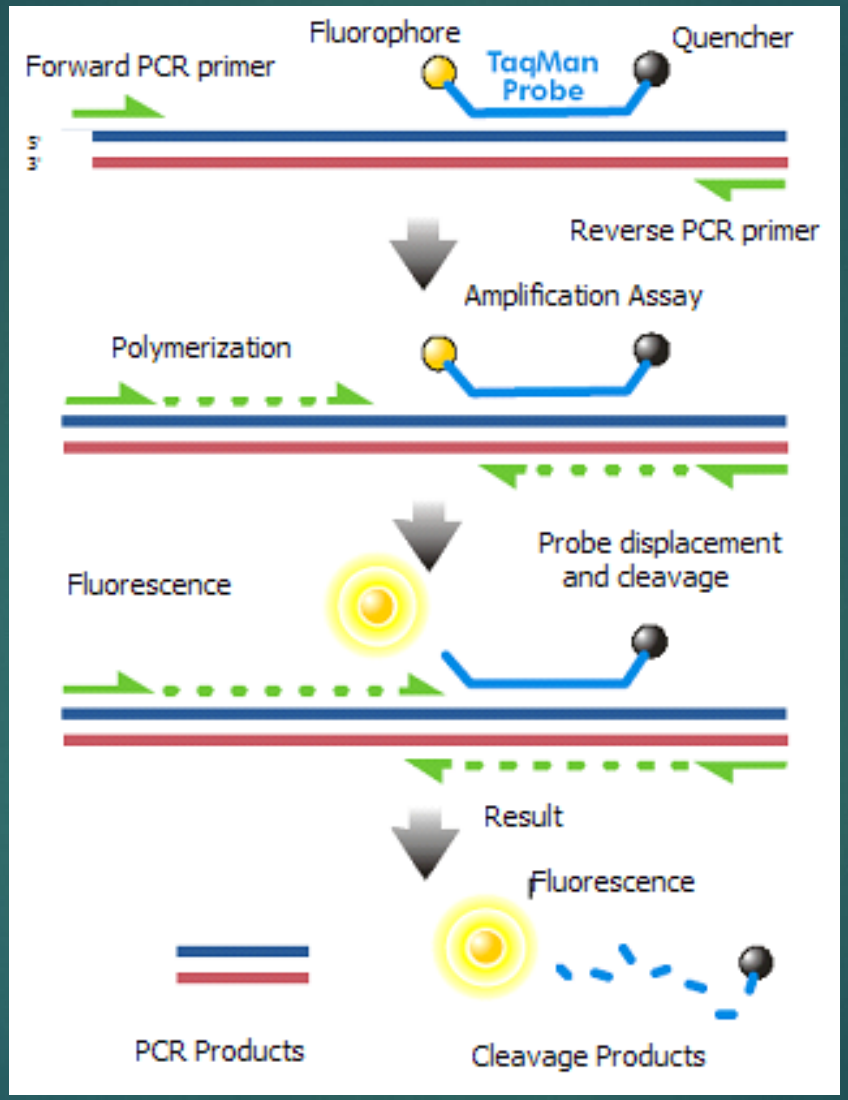
# General rules for primer design -- GC clamp and max 3' end stability

- **GC clamp**
  - Refers to the presence of G or C within the last 4 bases from the 3' end of primers
  - Essential for preventing mis-priming and enhancing specific primer-template binding
  - Avoid >3 G's or C's near the 3' end

- **Max 3'end stability**
  - Refers to the maximum $\Delta G$ of the 5 bases from the 3'end of primers.
  - While higher 3'end stability improves priming efficiency, too higher stability could negatively affect specificity because of 3'-terminal partial hybridization induced non-specific extension.
  - Avoid $\Delta G < -9$.

- **$T_a$ (Annealing temperature) vs. $T_m$**
  - **$T_a$ is determined by the $T_m$ of both primers and amplicons: optimal $T_a$=0.3 x $T_m$(primer)+0.7 x $T_m$(product)-25**
  - **General rule: $T_a$ is 5°C lower than $T_m$**
  - **Higher $T_a$ enhances specific amplification but may lower yields**
  - **Crucial in detecting polymorphisms**

- **Primer location on template**
  - **Dictated by the purpose of the experiment**
  - **For detection purpose, section towards 3' end may be preferred.**


Fluorophore
Quencher
Forward PCR primer
TaqMan Probe
5'
3'
Reverse PCR primer
Amplification Assay
Polymerization
Probe displacement and cleavage
Fluorescence
Result
Fluorescence
PCR Products
Cleavage Products

http://www.hsls.pitt.edu/guides/genetics/obrc

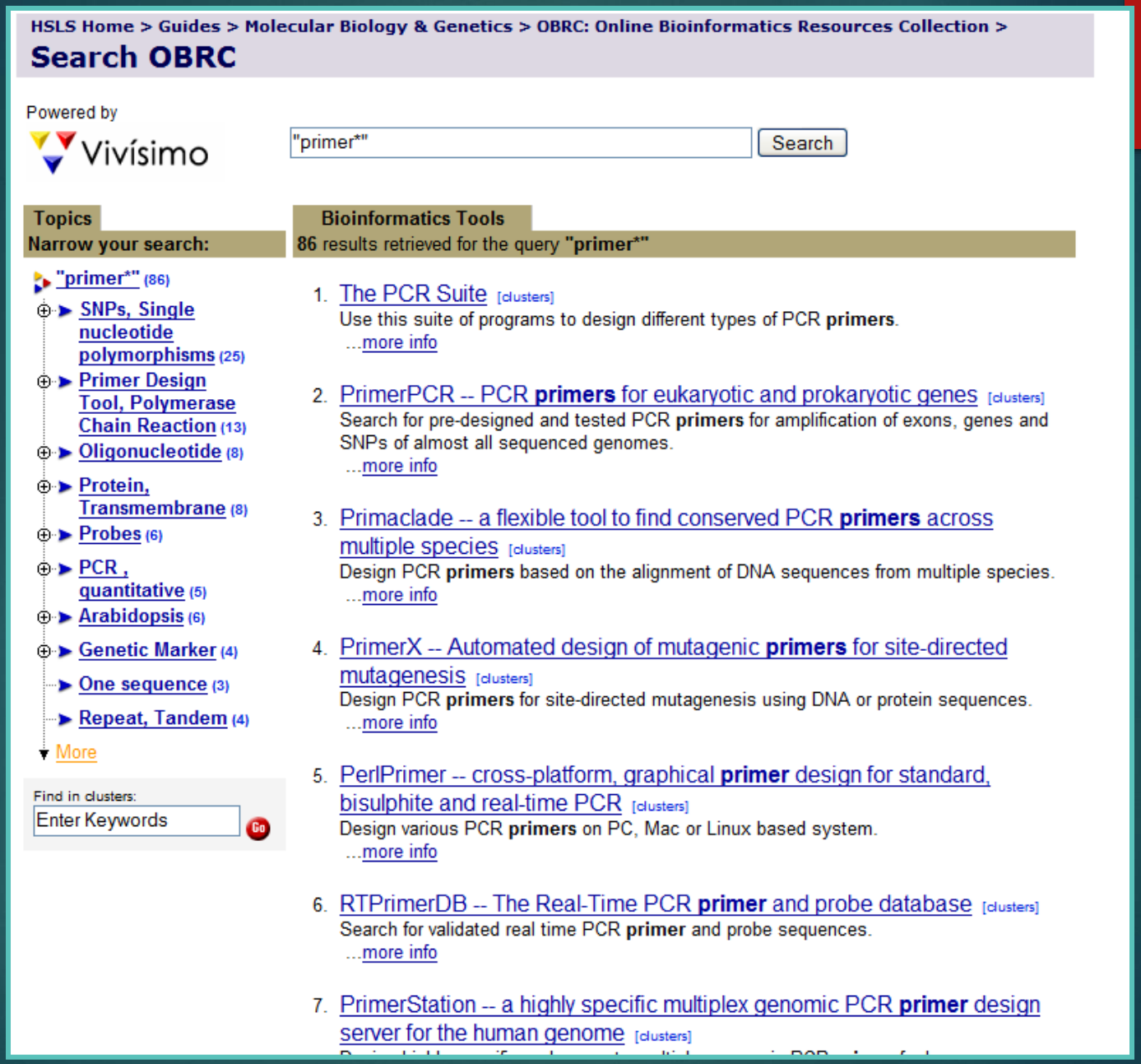

**Search the Online Bioinformatics Resources Collection (OBRC) at the Health Sciences Library System of University of Pittsburgh**

Find open-access databases and software in this collection of over 1700 bioinformatics resources.

Search OBRC (e.g. **PCR primer**, **transcription factor**)

**Search tips and more information on OBRC**

http://www.usc.edu/hsc/nml/lib-services/bioinformatics/index.html




http://search.hsls.pitt.edu/vivisimo/cgi-bin/query-meta?input-form=molbio-simple&query=pcr+primer&v%3Asources=OBRC&v%3Aproject=molbio

# Resources for General Purpose PCR Primer Design

- Primer3
- Primer3Plus
- PrimerZ
- PerlPrimer
- Vector NTI Advantage 10

# General Purpose PCR Primer Design Tool– Primer3

| | |
|---|---|
| **Name** | **Primer3 -- an online tool for PCR primer design** |
| **Type** | Web-based software |
| **Key Functions** | Design PCR primers and hybridization probes. |
| **Publication Info** | Methods Mol Biol 2000 |
| **Times Cited** | 823 |
| **Pros** | The original and most widely used PCR primer design program; uses sequence as input; a huge number of options for customizing primer design; |
| **Cons** | busy interface; |
| **Note** | In OBRC; the program has been widely adopted by many primer design software. |
| **YiBu's Rating** | **4 out of 5** |

**Web Site:**

http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1043858198/info

# General Purpose PCR Primer Design Tool– Primer3Plus

| | |
|---|---|
| **Name** | **Primer3Plus, an enhanced web interface to Primer3** |
| **Type** | Web-based software |
| **Key Functions** | Design PCR primers for a given DNA sequence. |
| **Publication Info** | NAR 2007 |
| **Times Cited** | N/A |
| **Pros** | Uses sequences or sequence file as input; a huge number of configuration options; automates specific tasks such as designing primers for cloning or step-wise sequencing; primers can be sent to an order form; clean, intuitive and well organized interface; |
| **Cons** | |
| **Note** | in OBRC. It is an updated, task-oriented web-interface to the original Primer3. |
| **YiBu's Rating** | **4.5 out of 5** |

**Web Site:**

http://www.bioinformatics.nl/primer3plus

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1191263055/info

# General Purpose PCR Primer Design Tool– PrimerZ

| | |
|---|---|
| **Name** | **PrimerZ -- streamlined primer design for promoters, exons and human/mouse SNPs** |
| **Type** | Web-based software |
| **Key Functions** | Design PCR primers for promoters, exons, and human/mouse SNPs |
| **Publication Info** | Nucleic Acid Research 2007 |
| **Times Cited** | 0 (too new) |
| **Pros** | Uses gene name, Ensembl ID, rs# as input; settings for amplicon region and length, as well as PCR product sizes; allow batch rsSNP processing; frequently updated; offers many advance design settings; interactive results output |
| **Cons** | reported successful rate over 70%; only for human and mouse |
| **Note** | built on Primer3; in OBRC. |
| **YiBu's Rating** | **4.5 out of 5** |

**Web Site:**

http://genepipe.ngc.sinica.edu.tw/primerz/beginDesign.do

**More Info:**
http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1190992855/info

# General Purpose PCR Primer Design Tool – PerlPrimer

| | |
|---|---|
| **Name** | **PerlPrimer -- cross-platform, graphical primer design for standard, bisulphite and real-time PCR** |
| **Type** | Desktop software (for Windows, Linux and Mac OS-X) |
| **Key Functions** | Design primers for standard, bisulphite and real-time PCR, and sequencing. |
| **Publication Info** | Bioinformatics 2004 |
| **Times Cited** | 8 |
| **Pros** | Cross-platform; versatile applications; retrieving sequences from Ensembl as input; QPCR primer design without manual intron-exon boundary entry; BLAST search primers; primer pair quality analysis; ORF and CpG island detection; |
| **Cons** | Requires local installation of the software; automatic sequence retrieval only through Esemble (selected eukaryotic genomes) not NCBI (more comprehensive). |
| **Note** | In OBRC |
| **YiBu's Rating** | **4 out of 5** |

**Web Site:**

http://perlprimer.sourceforge.net/index.html

**PerlPrimer screenshots:**

http://perlprimer.sourceforge.net/screenshots.html

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1167845497/info

# General Purpose PCR Primer Design Tool– Vector NTI Advance 10

| | |
|---|---|
| **Name** | **Vector NTI Advance 10 -- design primers and oligos for regular/multiplex PCR, sequencing, hybridization.** |
| **Type** | Commercial Desktop software (free to nonprofit users) |
| **Key Functions** | Design PCR primers and oligos for routine molecular applications |
| **Publication Info** | N/A |
| **Times Cited** | N/A |
| **Pros** | Uses user sequence, multiple DNA sequence alignment as input; commercial quality features/functions; integrated with other Vector NTI applications; many design settings; analyzes and ranks primer quality; |
| **Cons** | requires software installation/licensing; |
| **Note** | NML offers workshop and tutorials; in OBRC. |
| **YiBu's Rating** | **4 out of 5** |

**Web Site for NML Workshop:**

http://www.usc.edu/hsc/nml/lib-services/bioinformatics/vector_nti_advance_10_workshop.html

**More Info On Vector NTI Advance 10:**

http://www.usc.edu/hsc/nml/lib-services/bioinformatics/vector_nti_advance_10.html

# Primer Design Resources for Real-time PCR

- NCBI Probe Database
- RTPrimerDB
- Primer Bank
- qPrimerDepot
- PCR-QPPD
- PerlPrimer

# Public PCR Primers/Oligo Probes Repository – The NCBI Probe Database

| | |
|---|---|
| **Name** | **NCBI Probe Database** |
| **Type** | Web-based database |
| **Key Functions** | Search for documented PCR primers and oligo probes for genotyping, gene expression, SNP discovery, gene silencing and genome mapping applications. |
| **Publication Info** | Unpublished |
| **Times Cited** | n/a |
| **Pros** | The largest database of its kind; results including information on reagent distributors, probe effectiveness, and computed sequence similarities; Entrez allows search to be limited by applications, probe type, and model organism; |
| **Cons** | n/a |
| **Note** | |
| **YiBu's Rating** | **4.5 out of 5** |

**Web Site:**

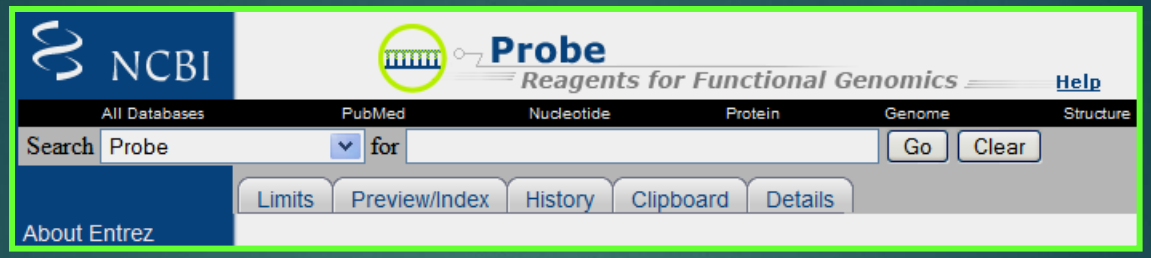
**Database Overview:**

http://www.ncbi.nlm.nih.gov/genome/probe/doc/Overview.shtml

**Database Query Tips:**

http://www.ncbi.nlm.nih.gov/genome/probe/doc/QueryTips.shtml

# http://www.ncbi.nlm.nih.gov/sites/entrez?db=probe




**Number of ProbeDB entries by experimental application.**

| Application | Probes |
|---|---|
| Genotyping | 7,269,325 |
| Gene Expression | 783,816 |
| SNP Discovery | 429,931 |
| Gene Silencing | 274,357 |
| Genome Mapping | 35,162 |

**Number of ProbeDB entries by probe type.**

| Probe Types | Probes |
|---|---|
| Sequence-specific Oligonucleotide (SSO) | 4,796,642 |
| Bead Microarray Element | 2,472,542 |
| TaqMan Gene Expression (TaqMan) | 780,053 |
| Resequencing Amplicon (RSA) | 429,931 |
| Small Hairpin RNA (shRNA) | 270,266 |
| Restriction Fragment Length Polymorphism (RFLP) | 23,391 |
| Simple Sequence Repeats (SSR) | 7,961 |
| Small Interfering RNA (siRNA) | 4,091 |
| Ribonucleic Acid Probe (riboprobe) | 3,763 |
| Amplified Fragment Length Polymorphism (AFLP) | 1,843 |
| Random Amplified Polymorphic DNA (RAPD) | 1,262 |
| Sequence Tagged Site (STS) | 285 |
| Cleaved Amplified Polymorphic Sequence (CAPS) | 229 |
| DNA Microarray Element (DNA microarray) | 141 |
| SNP Marker (SNP marker) | 103 |
| SCAR (SCAR) | 53 |
| Derived Cleaved Amplified Polymorphic Sequence (dCAPS) | 22 |
| Single-Strand Conformation Polymorphisms (SSCP) | 6 |
| Overlapping Oligonucleotide (overgo) | 6 |
| EST-UTR (EST-UTR) | 1 |

Total Number of Probes is 8,792,591 (Last updated on: 09/16/2007 20:39:29)

# Resources for real time PCR– RTPrimerDB

| | |
|---|---|
| **Name** | **RTPrimerDB: the real-time PCR primer and probe database** |
| **Type** | Database |
| **Key Functions** | Search for validated primers and probes used in real-time PCR assays employing popular chemistries (SYBR Green I, Taqman, Hybridisation Probes, Molecular Beacon); map primers/probes onto different transcript variants |
| **Publication Info** | Nucleic Acids Research 2003, 2006 (update) |
| **Times Cited** | 62 |
| **Pros** | Primers/probes experimentally validated; search with gene name/symbol, Entrez/Ensembl Gene identifier, SNP ID, or oligo sequence; queries can be limited to a specific application (gene expression, DNA copy number, SNP detection, mutation analysis, fusion gene) or **organisms** (**20**); results linked to PubMed record and BLAST; frequently updated; |
| **Cons** | Gene expression assay viewer requires Adobe SVG viewer plug-in, and only available for human, mouse, rat. |
| **Note** | In OBRC; 3845 real-time PCR assays for 2373 genes as of Nov. 2007. |
| **YiBu's Rating** | **4.8 out of 5** |

**Web Site:**

http://medgen.ugent.be/rtprimerdb/

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1099597360/info

# Resources for real time PCR– Primer Bank

| | |
|---|---|
| **Name** | **A PCR primer bank for quantitative gene expression analysis** |
| **Type** | Web-based database |
| **Key Functions** | Search for pre-designed transcript-specific PCR primers for genome-scale real time PCR assay |
| **Publication Info** | Nucleic Acids Research 2003 |
| **Times Cited** | 67 |
| **Pros** | Searchable by GenBank Accession number, gene ID/symbol, keywords etc.; design algorithm extensively tested and validated with success rate of 82.6%; results output containing positions of primers and amplicons in the sequence context of the queried gene |
| **Cons** | Only for human and mouse genes; algorithm not designed to span introns; no graphic display; |
| **Note** | Contains 306,800 real-time PCR primers for 33741 human genes and 27681 mouse genes as of Nov. 2007 |
| **YiBu's Rating** | **4.8 out of 5** |

**Web Site:**

http://pga.mgh.harvard.edu/primerbank/

**More Info:**

http://www.ncbi.nlm.nih.gov/sites/entrez?Db=pubmed&Cmd=ShowDetailView&TermToSearch=14654707

# Resources for real time PCR– qPrimerDepot

| | |
|---|---|
| **Name** | **qPrimerDepot -- a primer database for quantitative real time PCR** |
| **Type** | Web-based database |
| **Key Functions** | Provides optimized qRT-PCR primers for all human/mouse RefSeq genes; |
| **Publication Info** | NAR 2005 |
| **Times Cited** | 19 |
| **Pros** | Uses GenBank RefSeq ID or gene name as input; primers are designed to amplify desired templates under unified annealing temperature; avoids genomic DNA contamination (for intron-bearing genes); simple user interface; Validation results show 70-94% successful rate; |
| **Cons** | Results output without sequence context display of primer/amplicons positions |
| **Note** | In OBRC; built on Primer3; |
| **YiBu's Rating** | **4.5 out of 5** |

**Web Site for Human Genes:**

http://primerdepot.nci.nih.gov/

**Web Site for Mouse Genes:**

http://mouseprimerdepot.nci.nih.gov/

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1174922412/info

# Resources for real time PCR– QPPD

| | |
|---|---|
| **Name** | **QPPD -- Quantitative PCR Primer Database for human and mouse** |
| **Type** | Web-based database |
| **Key Functions** | Search for published quantitative/real time RT-PCR primer and probes for studying of human and mouse gene expression. |
| **Publication Info** | Unpublished |
| **Times Cited** | n/a |
| **Pros** | All primers were experimentally validated with literature references; uses gene name as input; searches can be limited by different assay types; output includes graphic display of primers/amplicons positions and sizes. |
| **Cons** | Only human and mouse genes available; no user documentations and database stats. |
| **Note** | In OBRC |
| **YiBu's Rating** | **4 out of 5** |

**Web Site:**

http://web.ncifcrf.gov/rtp/GEL/primerdb/default.asp

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1152117830/info

# Resources for real time PCR– PerlPrimer

| | |
|---|---|
| **Name** | **PerlPrimer -- cross-platform, graphical primer design for standard, bisulphite and real-time PCR** |
| **Type** | Desktop software (for Windows, Linux and Mac OS-X) |
| **Key Functions** | Design primers for standard, bisulphite and real-time PCR, and sequencing. |
| **Publication Info** | Bioinformatics 2004 |
| **Times Cited** | 8 |
| **Pros** | Cross-platform; versatile applications; retrieving sequences from Ensembl as input; QPCR primer design without manual intron-exon boundary entry; BLAST search primers; primer pair quality analysis; ORF and CpG island detection; |
| **Cons** | Requires local installation of the software; automatic sequence retrieval only through Esemble (selected eukaryotic genomes) not NCBI (more comprehensive). |
| **Note** | In OBRC |
| **YiBu's Rating** | **4 out of 5** |

**Web Site:**

http://perlprimer.sourceforge.net/index.html

**PerlPrimer screenshots:**

http://perlprimer.sourceforge.net/screenshots.html

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1167845497/info

# Resources for Site-Directed Mutagenesis PCR – PrimerX

| | |
|---|---|
| **Name** | **PrimerX -- Automated design of mutagenic primers for site-directed mutagenesis** |
| **Type** | Web-based software |
| **Key Functions** | Design mutagenic primers for site-directed mutagenesis. |
| **Publication Info** | unpublished |
| **Times Cited** | n/a |
| **Pros** | Uses either DNA or protein sequences as input; results can be customized for three different commercial mutagenesis kits; straightforward user interface, |
| **Cons** | No data on evaluation and user feedback; |
| **Note** | In OBRC |
| **YiBu's Rating** | **4 out of 5** |

**Web Site:**

http://www.bioinformatics.org/primerx/

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1175091818/info

# Resources for PCR Primer or Oligo Analysis

- **AutoDimer**
- **IDT OligoAnalyzer 3.0**
- **PUNS**
- **NCBI BLAST**
- **UCSC In-Silico PCR**

# Resources for PCR Primer or Oligo Analysis – AutoDimer

| | |
|---|---|
| **Name** | **AutoDimer -- a screening tool for primer-dimer and hairpin structures** |
| **Type** | Web-based or desktop software |
| **Key Functions** | Rapidly screen PCR primers for primer-dimer and hairpin interactions in short DNA oligomers (< 30 nucleotides) |
| **Publication Info** | Biotechniques 2004 |
| **Times Cited** | 17 |
| **Pros** | Suited for screening multiplex PCR primers; output has alignment; |
| **Cons** | Requires manually formatted primers file as input; crude web layout; the web-based version is limited 100 sequences/run and maximum oligomer length of 75 nucleotides or less. |
| **Note** | In OBRC |
| **YiBu's Rating** | **4 out of 5** |

**Web Site:**

http://www.cstl.nist.gov/div831/strbase/AutoDimerHomepage/AutoDimerProgramHomepage.htm

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1154964478/info

# Resources for PCR Primer or Oligo Analysis –IDT OligoAnalyzer 3.0

| | |
|---|---|
| **Name** | **IDT OligoAnalyzer 3.0** |
| **Type** | Web-based software |
| **Key Functions** | Analyze primer/oligo sequence and structure. |
| **Publication Info** | Unpublished |
| **Times Cited** | n/a |
| **Pros** | Includes analysis of hairpins, self-dimer, heterodimer; customizable primer/oligo and salt concentrations; many options for various sequence modifications; direct submission for NCBI BLAST; allows direct order of primer/oligo; |
| **Cons** | No evaluation data. |
| **Note** | |
| **YiBu's Rating** | **4 out of 5** |

**Web Site:**

http://www.idtdna.com/analyzer/Applications/OligoAnalyzer/

**Online Instruction:**

http://www.idtdna.com/Analyzer/Applications/Instructions/Default.aspx?AnalyzerInstructions=true

# Resources for PCR Primer Specificity Analysis – PUNS

| | |
|---|---|
| **Name** | **PUNS -- Transcriptomic- and genomic-in silico PCR for enhanced primer design** |
| **Type** | Web-based or desktop software (Windows, Linux) |
| **Key Functions** | Use in silico PCR to verify primer specificity by comparing the primers against the entire transcriptome/genome and looking for alternate binding and potential alternate amplicons. Particularly suited for the identification of highly selective primers for quantitative microarray validation; |
| **Publication Info** | Bioinformatics 2004 |
| **Times Cited** | 4 |
| **Pros** | Focuses on primer specificity analysis; capable of design cross-species primers; uses pre-designed primers as input; good online documentation |
| **Cons** | For specificity check against transcriptome, works only with model organisms whose transcriptome info are available in UniGene; requires special input file format; unnecessary complicated interface; |
| **Note** | in OBRC |
| **YiBu's Rating** | **4 out of 5** |

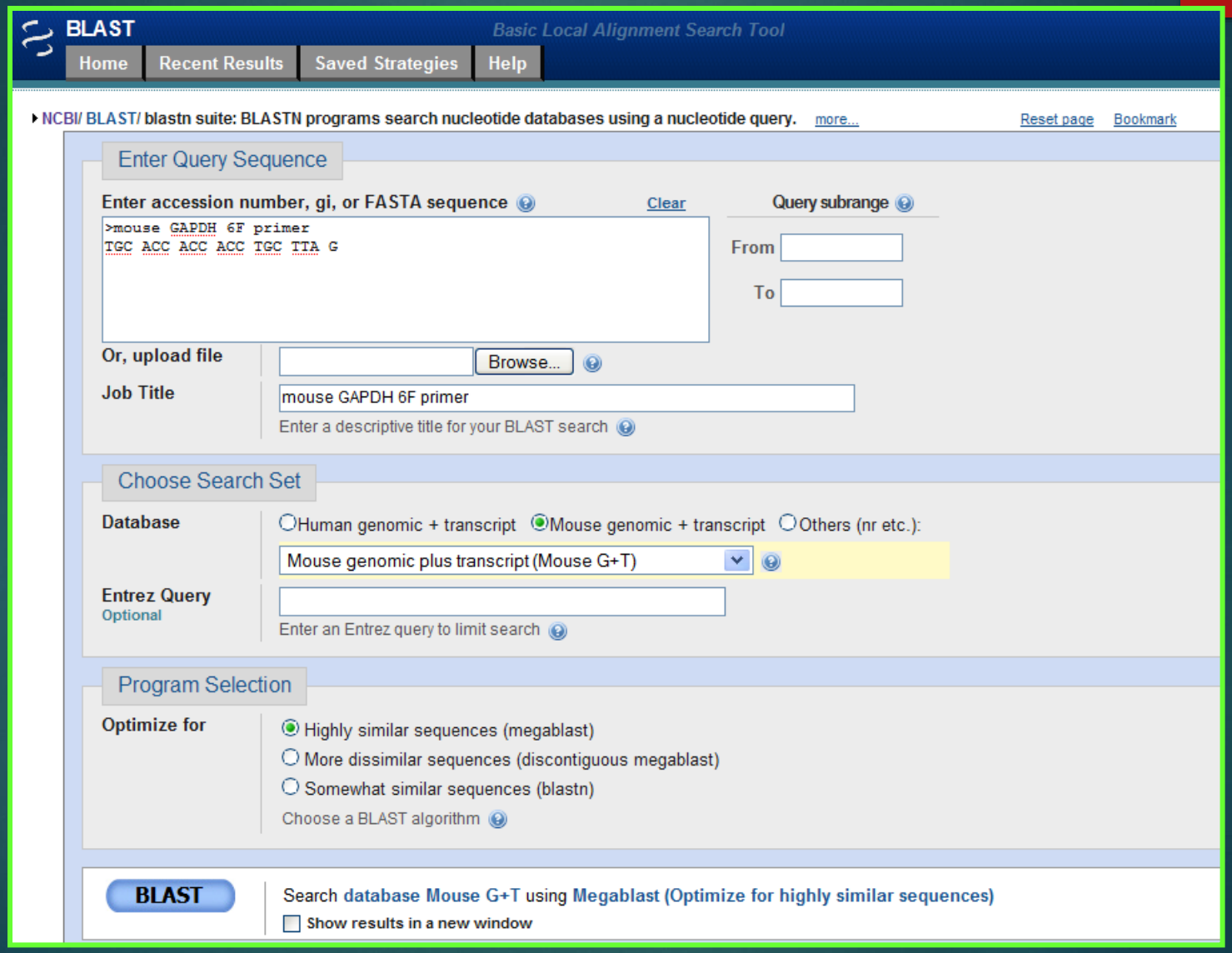
**Web Site:**

http://okeylabimac.med.utoronto.ca/PUNS/

**More Info:**

http://www.hsls.pitt.edu/guides/genetics/obrc/dna/pcr_oligos/URL1175092441/info

# Resources for PCR Primer Specificity Analysis – NCBI BLAST




http://www.ncbi.nlm.nih.gov/blast/Blast.cgi?PAGE=Nucleotides&PROGRAM=blastn&MEGABLAST=on&BLAST_PROGRAMS=megaBlast&PAGE_TYPE=BlastSearch&SHOW_DEFAULTS=on

# Resources for PCR Primer Mapping – UCSC In-Silico PCR

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## UCSC In-Silico PCR

Genome: Mouse | Assembly: Jul. 2007 | Forward Primer: TGCACCACCAaCTGCTT | Reverse Primer: GGATGCAGGGATGATG | submit

Max Product Size: 50000 | Min Perfect Match: 18 | Min Good Match: 18 | Flip Reverse Primer: ☐

## About In-Silico PCR

In-Silico PCR searches a sequence database with a pair of PCR primers, using an indexing strategy for fast performance.

### Configuration Options

**Genome and Assembly** - The sequence database to search.
**Forward Primer** - Must be at least 15 bases in length.
**Reverse Primer** - On the opposite strand from the forward primer. Minimum length of 15 bases.
**Max Product Size** - Maximum size of amplified region.
**Min Perfect Match** - Number of bases that match exactly on 3' end of primers. Minimum match size is 15.
**Min Good Match** - Number of bases on 3' end of primers where at least 2 out of 3 bases match.
**Flip Reverse Primer** - Invert the sequence order of the reverse primer and complement it.

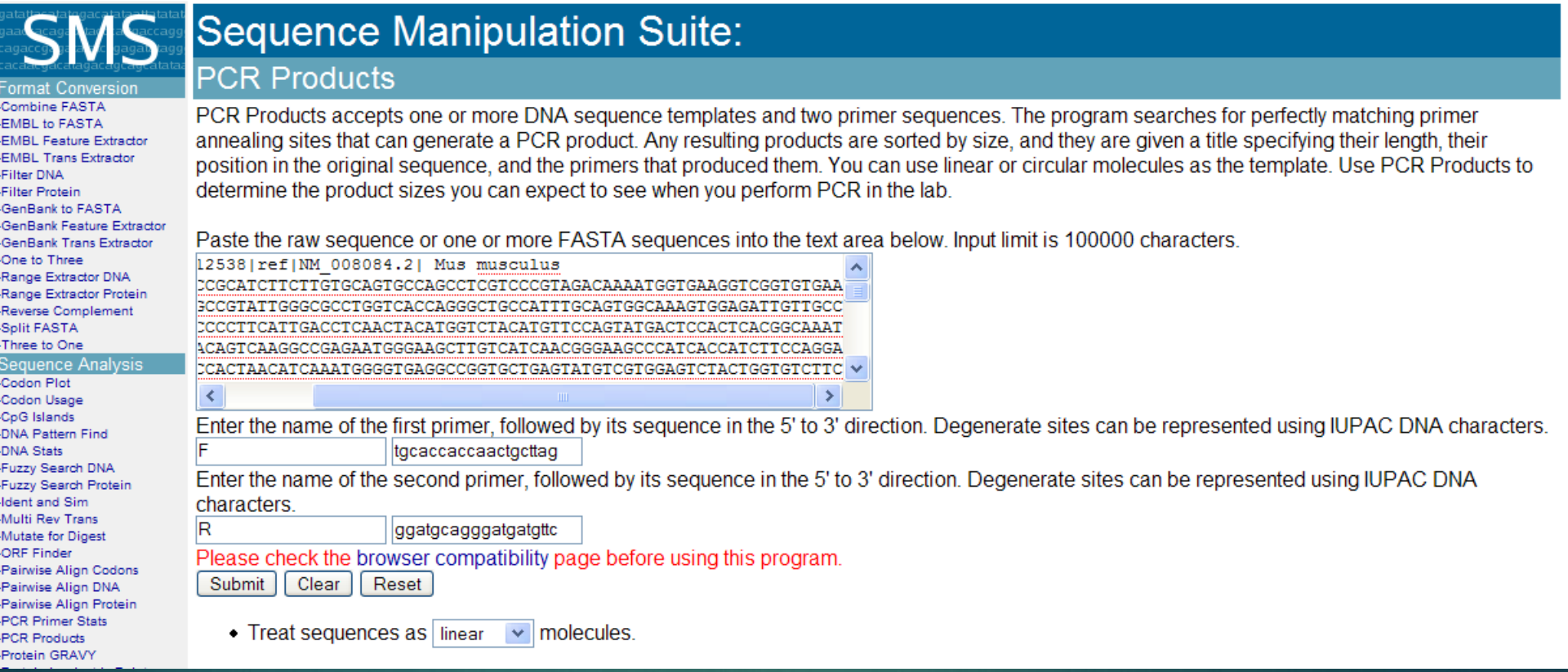
### Output

When successful, the search returns a sequence output file in fasta format containing all sequence in the database that lie between and include the primer pair. The fasta header describes the region in the database and the primers. The fasta body is capitalized in areas where the primer sequence matches the database sequence and in lower-case elsewhere. Here is an example:

```
>chr22:31000551+31001000  TAACAGATTGATGATGCATGAAATGGG CCCATGAGTGGCTCCTAAAGCAGCTGC
TtACAGATTGATGATGCATGAAATGGGgggtggccaggggtgggggggtga
gactgcagagaaaggcagggctggttcataacaagctttgtgcgtcccaa
tatgacagctgaagttttccaggggctgatggtgagccagtgagggtaag
tacacagaacatcctagagaaaccctcattccttaaagattaaaaataaa
```

http://genome.ucsc.edu/cgi-bin/hgPcr?db=mm9

# Resources for PCR Primer Mapping/Amplicon Size – SMS Tool




http://www.bioinformatics.org/sms2/pcr_products.html

http://www.bioinformatics.org/sms2/index.html


با تشکر از توجه شما