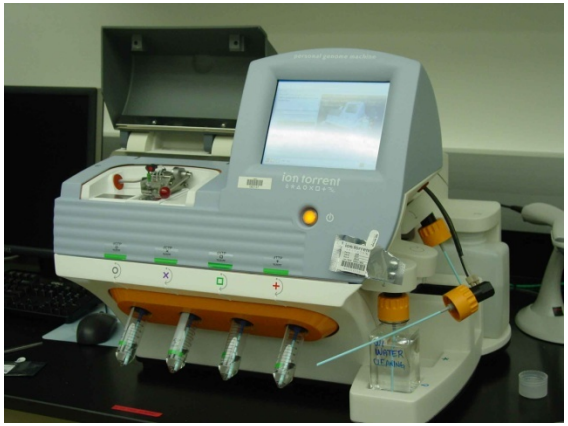
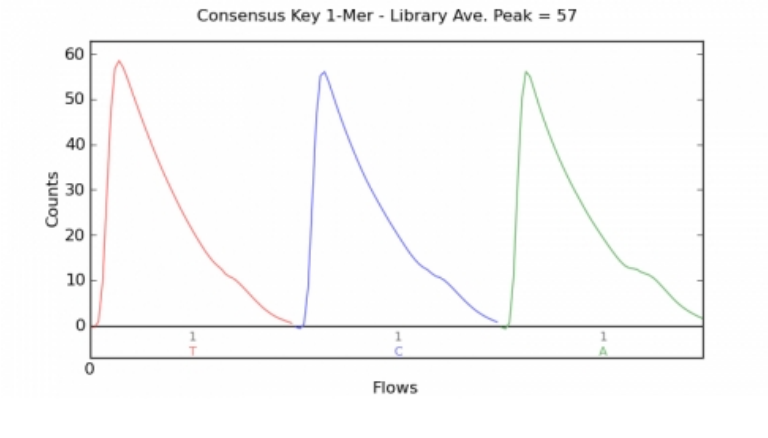
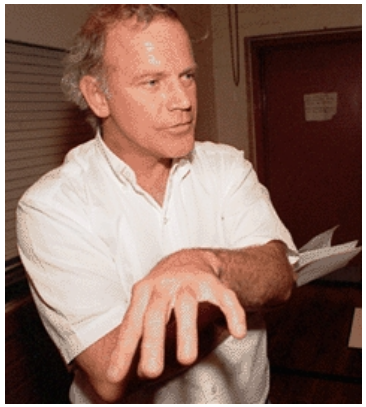
BINF 6350 ITSC 8350
Fall 2011
Biotechnology & Genomics Lab
PCR

http://webpages.uncc.edu/~jweller2





Polymerase Chain Reaction



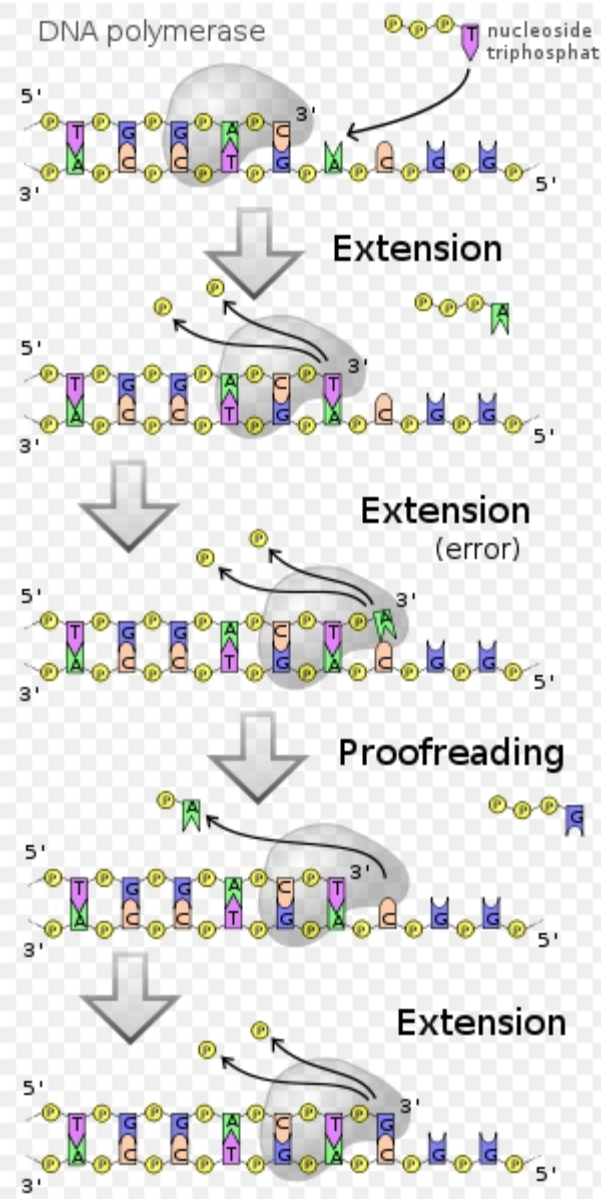
Paper 1988
Nobel: 1993

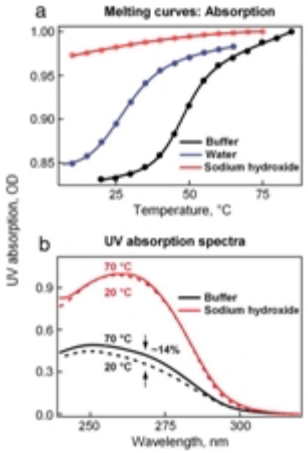
Paper 1971
Nobel: 1968

- How do you make enough genetic material to characterize it?
  - Tens of thousands of specific sequences and millions to billions of bases in any genome (besides viruses)
    - Pull out and manipulate the 3000 nucleotides of interest
- Polymerase Chain Reaction –
  - 1988, Saiki, Mullis et al. in Science
    - using a polymerase purified from an organism characterized by Brock from Yellowstone N.P.

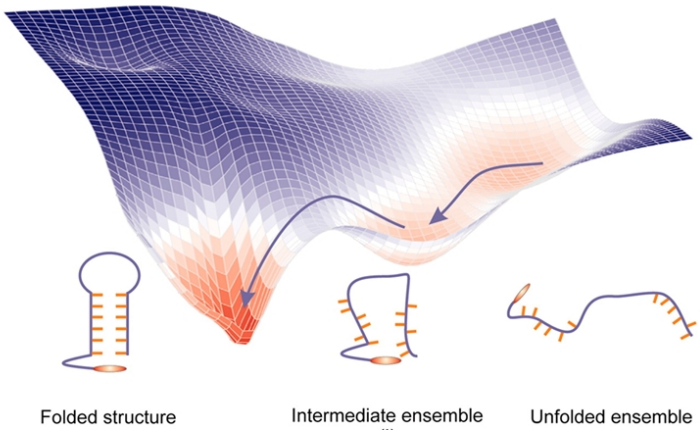
Polymerase basics

- *X*NA Polymerases
  - Substrate: DNA or RNA
  - For dsDNA- first unwind, then copy each strand
  - The new strand is made in the 5' to 3' direction
  - The pol. binding site must be ds
    - If a small oligonucleotide sits on the larger template it is called the 'primer'
      - It sits at the 3' end of template, becoming the 5' end of new strand
- Functionally: add subunits complementary to the template, forming covalent phosphodiester bonds
  - Result: two dsDNA molecules, each can be the template for two more rounds of copying
- Rinse and Repeat:
    - You will end up with $1+ 2^n$ copies– hence Chain Reaction.





PCR steps



- Template preparation:
  - Separate template strands - possibilities are thermal, enzymatic, chemical.
  - Add Primers, anneal to template
- Initiation: add polymerase. It binds to the double-stranded primer-template complex
- Bond catalysis: add nucleotides. The polymerase catalyzes the phosphodiester linkage of dNTPs to primer ends: extension
- Elongation: polymerase advances 3' → 5' down template strand, making duplex DNA
- Termination: at the end of the template the polymerase dissociates from the completed ds molecule.
  - Dissociation also occurs ~2000 bp so 'pausing' happens.

Thermo-cycling

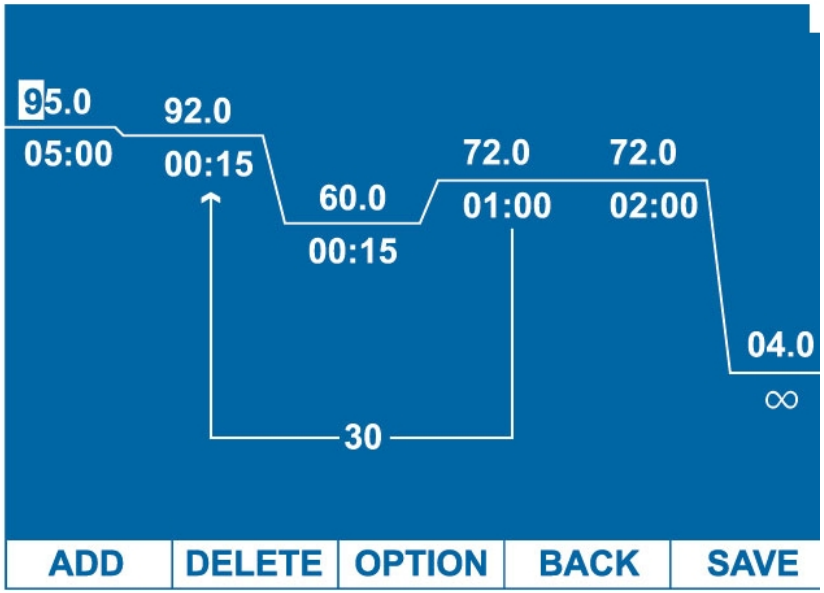
- The easiest way to separate strands and not cause template damage is to use heat. The concept :
  - Thermo-melt duplex DNA so the strands separate,
  - Cool the reaction quickly so they don’t re-anneal
  - Add short primers (in excess) that complement the single strand somewhere
  - Add polymerase and factors, and extend
  - Cycle: repeat the process.

Taq

- *Thermus aquaticus* : Great Fountain in the West Thumb Geyser Basin (photos at Flickr, Russ Finley, James Neeley)
- The heat-stable polymerase:
  - First one was from the microorganism *Thermus aquaticus* (isolated from a hot spring mat in Yellowstone Park by Brock)
  - The organism and its enzymes are stable at close to 100C, so it survives the repeated rounds of heating.
  - This allows the cycling part of the process to be a one-tube, one-step set-up procedure.

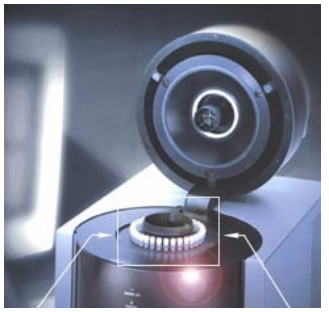
A thermocycler is the instrument in which PCR is performed.

- A carefully engineered heating/cooling block
  - Drilled holes enclose sample tubes; insulated lid for evaporation.
  - Controlled rates of heating or cooling and holding temperature give reproducible results.

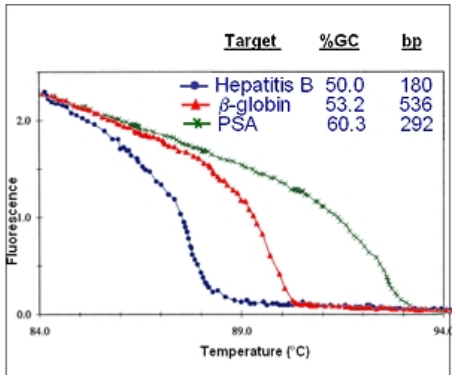


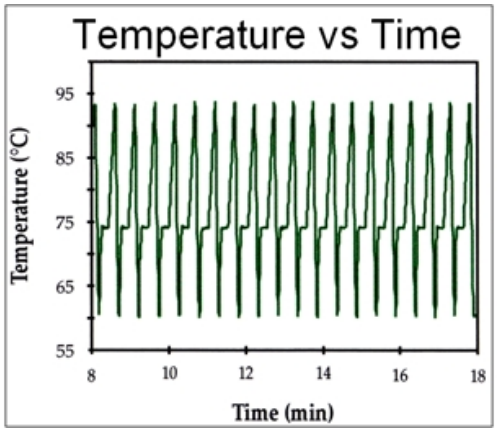
http://sentrabd.com/_borders/T1b.jpg

Instrument considerations

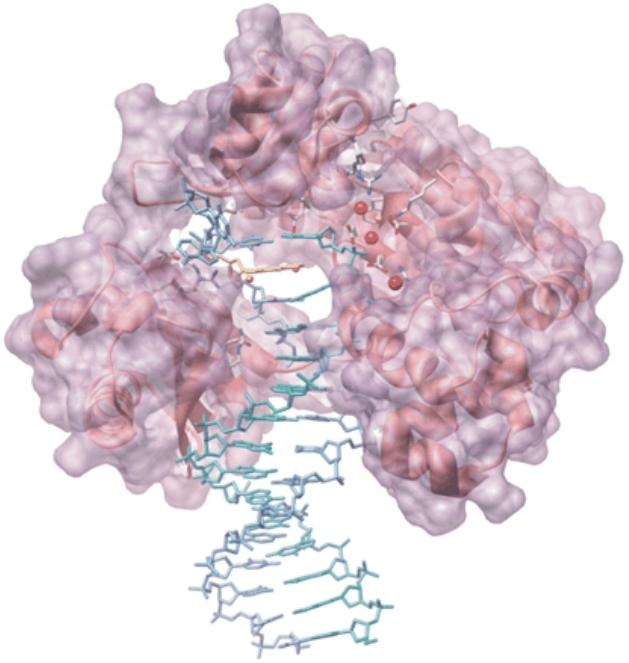


- Accuracy of thermocycling (rate of temp change, overshooting, variance in reaching and staying at a temp and accuracy).
- Accuracy/sensitivity/discrimination of optics
  - How many dyes can you use and how many together with what overlap
- Raw or massaged data available
- How much of intermediate cycle data is available for analysis (iCycler from BioRad)
- Does it sample frequently enough and have the sensitivity to look at probe behavior within a cycle (initial binding of probe as well as extension). (Light Cycler)

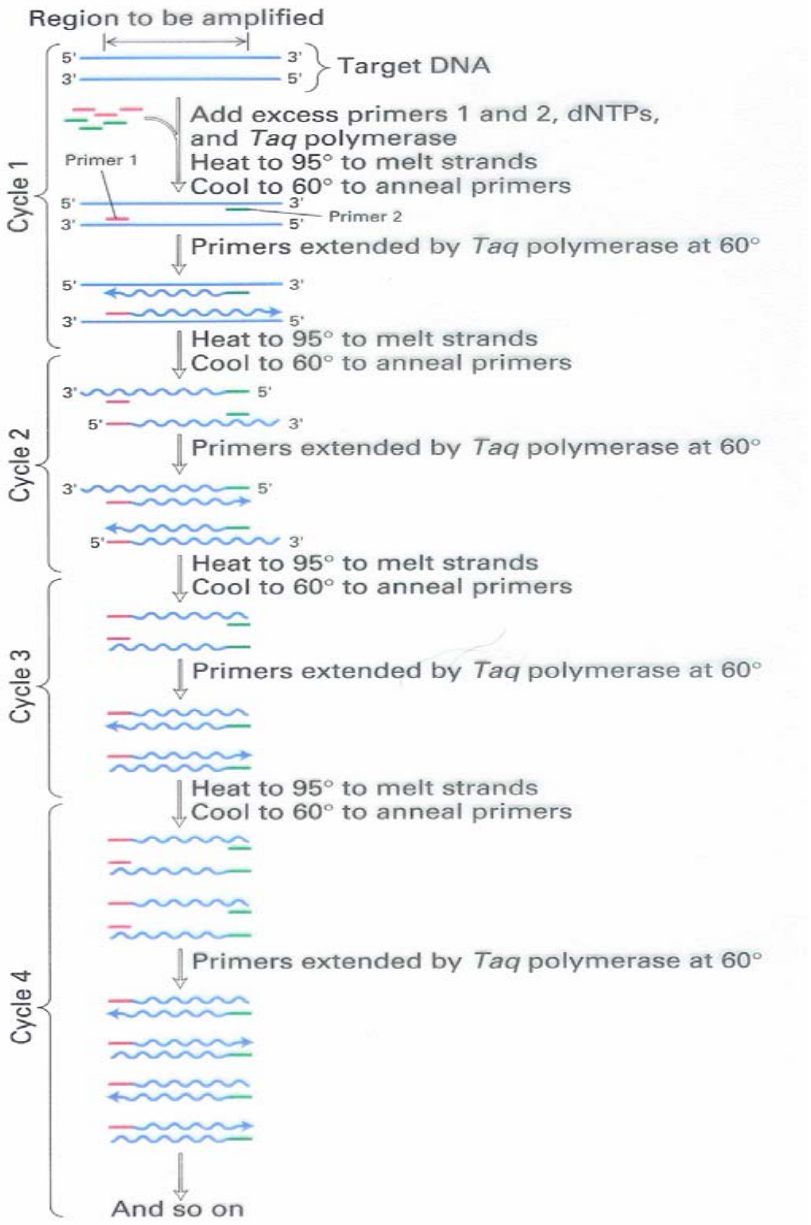




PCR methodology



- Reaction components
  - A template: DNA that has sequences complementary to the primers you will add to the reaction
  - Primers: primers of defined sequence that are complementary to specific regions of the template
  - A thermostable DNA polymerase, such as Taq with its working components (buffer, Mg++)
  - dNTPs for making new copies


Region to be amplified
5'
3'
3'
5'
Target DNA
Add excess primers 1 and 2, dNTPs, and Taq polymerase
Heat to 95° to melt strands
Cool to 60° to anneal primers
Primer 1
Primer 2
Primers extended by Taq polymerase at 60°
Heat to 95° to melt strands
Cool to 60° to anneal primers
Primers extended by Taq polymerase at 60°
Heat to 95° to melt strands
Cool to 60° to anneal primers
Primers extended by Taq polymerase at 60°
Heat to 95° to melt strands
Cool to 60° to anneal primers
Primers extended by Taq polymerase at 60°
And so on
Cycle 1
Cycle 2
Cycle 3
Cycle 4

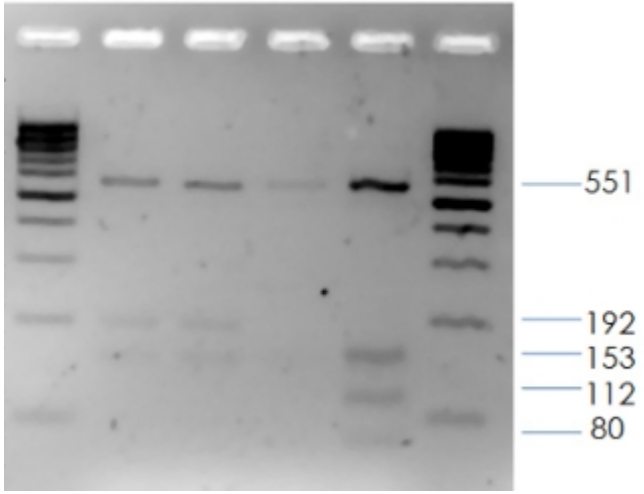
Errors from misincorporation

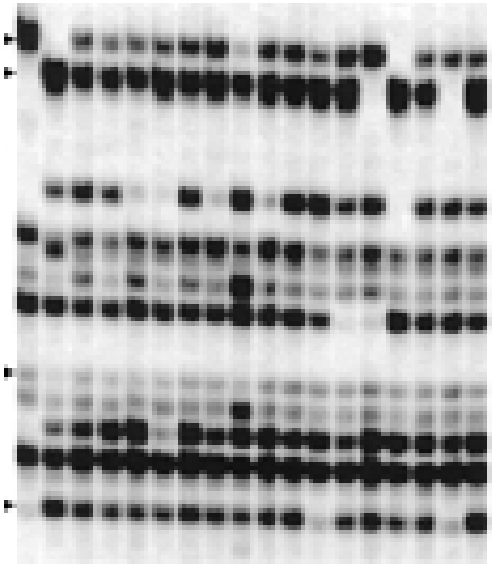
Taq	Pfu	Vent	Deep Vent	UlTMa	Exo-Pfu
8.0	1.3 *	2.8	2.7	50	50*10^-6
	2.3mM MgSO4 100-300uM dNTP pH 8.5-9.1				
Enzyme	Opt conditions				Rate is mut.freq. / bp / duplication

As pH 8→ 9 the error rate of Pfu decreased 2X, but exo- Pfu increased 9X. This increase is seen for Taq (naturally exo-) and Klenow.

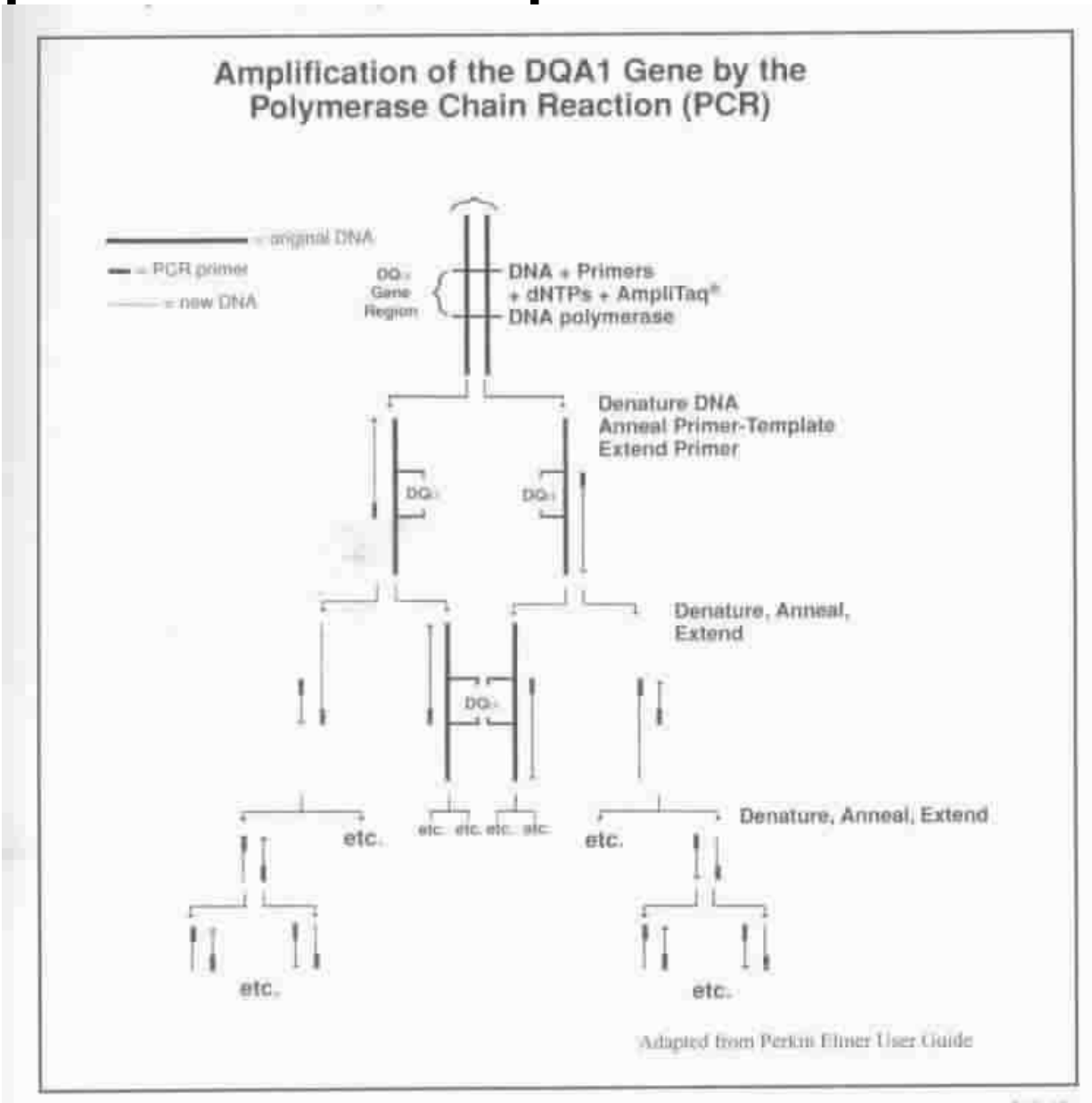
Classifying PCR Experiments

- Type I: to target amplification of a known region of nucleic acid
  - Sequence is known, primers are as specific as possible to the target region
  - Either RNA or DNA may be the target (PCR vs RT-PCR)
  - A quantitative and real-time assay may be performed
- Type II: to randomly amplify fragments given known primer(s) but unknown complement(s).
  - A genome-wide fingerprinting approach
  - Polymorphism rather than identity is sought

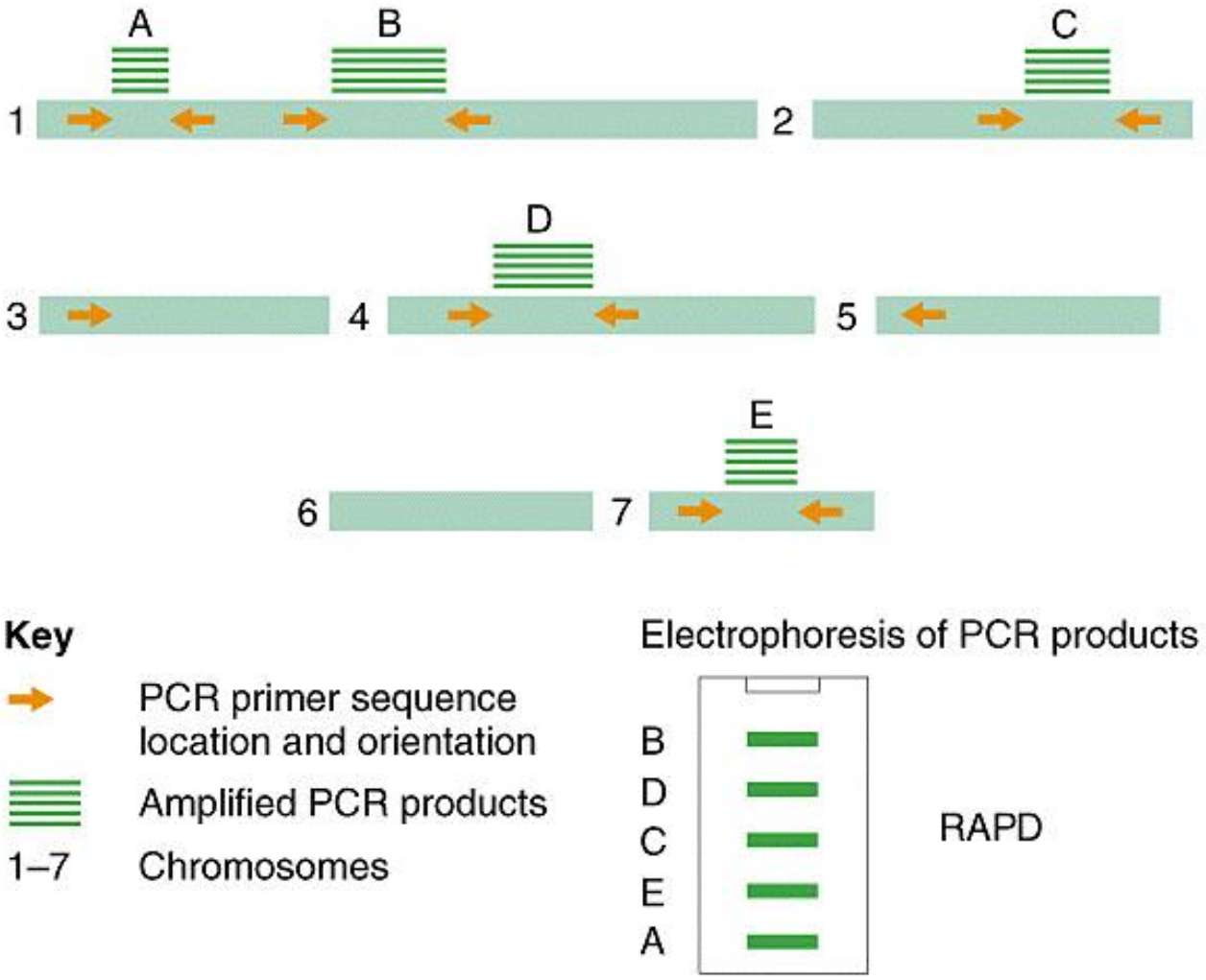




Type I: Gene-specific PCR Assay



Type II PCR



http://www.usask.ca/agriculture/plantsci/classes/plsc416/projects_2002/pawlin/resources/rapd1.jpg

PCR yields

- As product increases, primer and dNTPs are used up.
  - The reaction saturates and no more product is made
  - The amount of product depends on the amount of input template and the number of amplification cycles

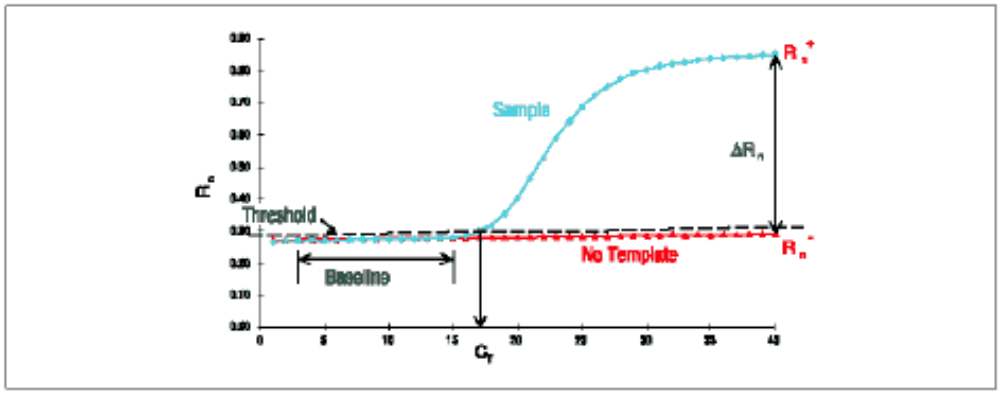
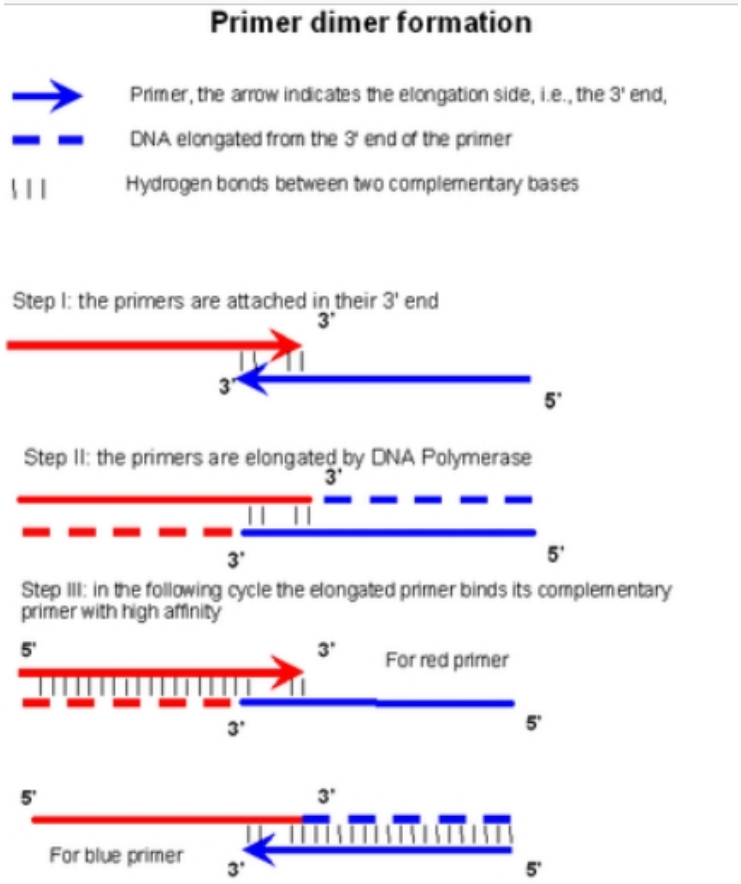


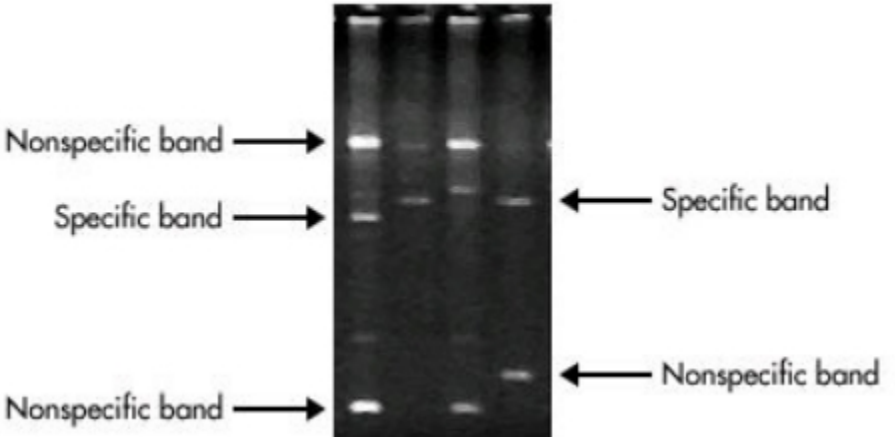
Figure 2. Model of a single amplification plot, showing terms commonly used in real-time quantitative PCR Figure from Applied Biosystems' DNA/RNA Real-Time Quantitative PCR bulletin).

Single-locus PCR

- Quality control: do you get only one product? is the correct product made? is the yield in the expected range?
  - Why would you not get one product?
    - Primers may interact to give non-template product (primer-dimer-multimer)



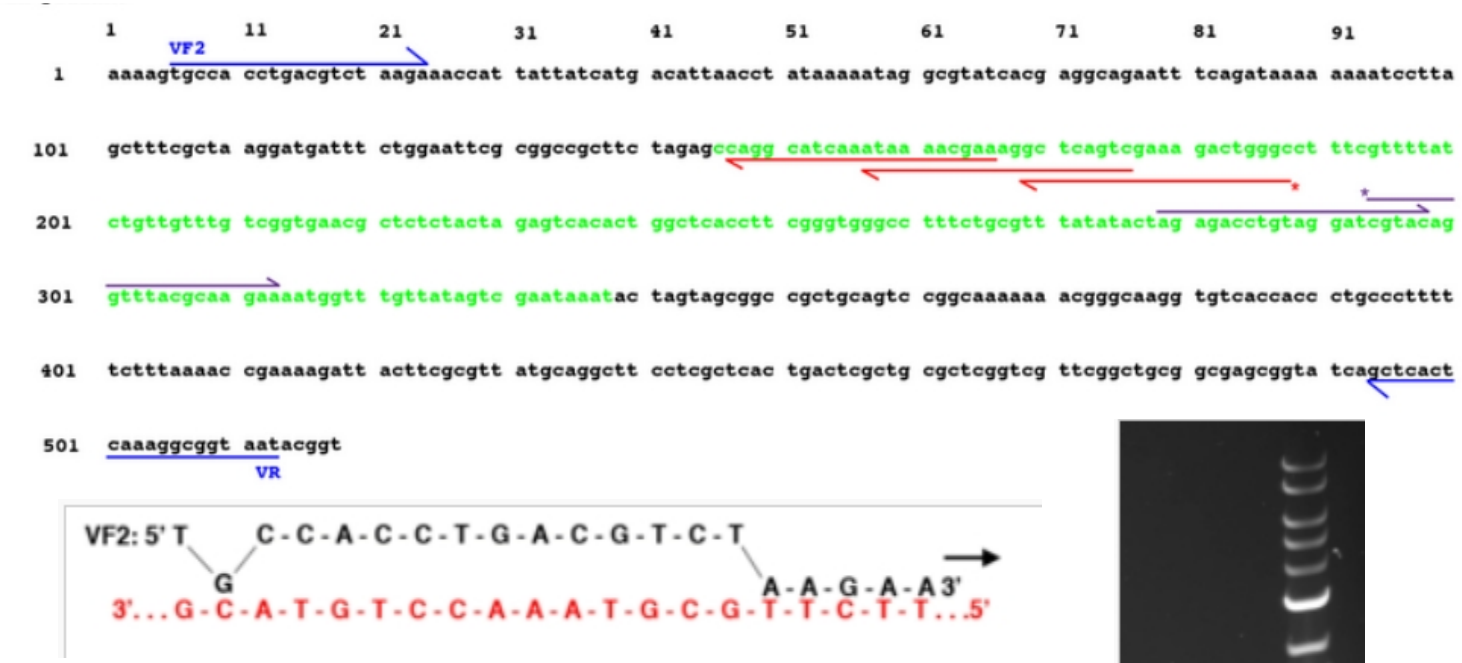
Wikipedia

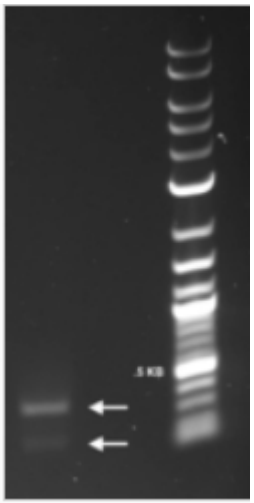


UNCC

PCR QC

- Did primers amplify an unintended locus?
  - If the length is correct: sequence or restrict the product

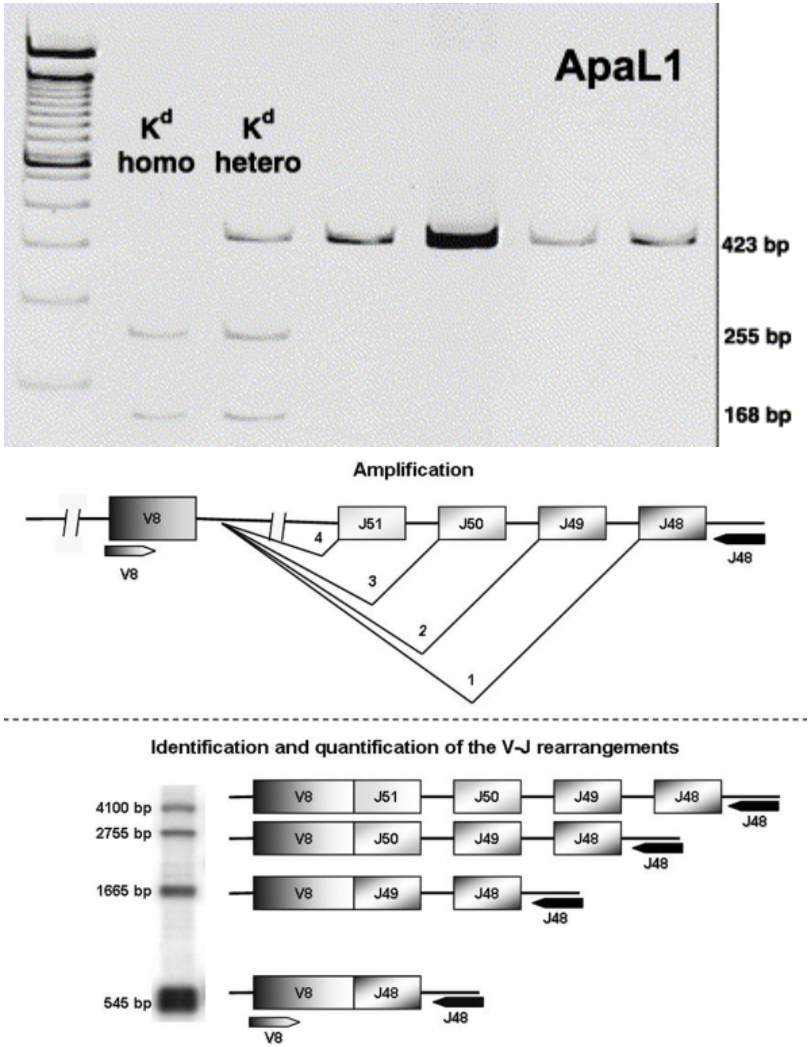




Touch-down PCR, Hot-Start PCR

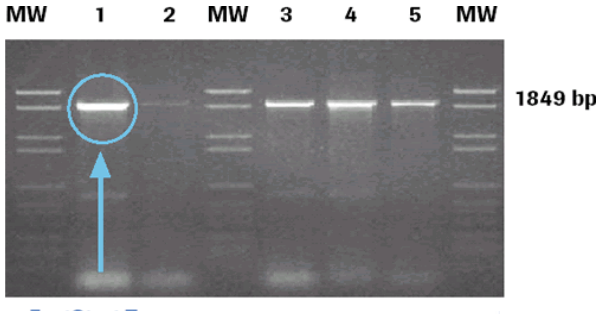
Product validation

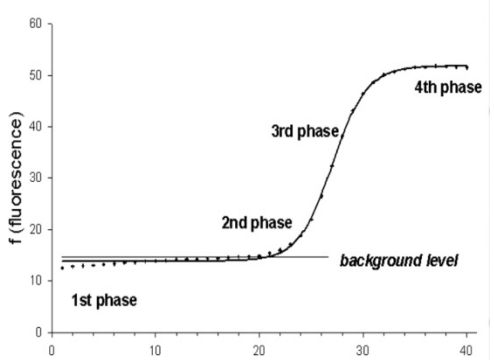
- Length may not be perfect.
  - Two alleles are naturally present
    - Clone and sequence both fragments
    - Use a restriction endonuclease
  - A gene family is present, or a common motif

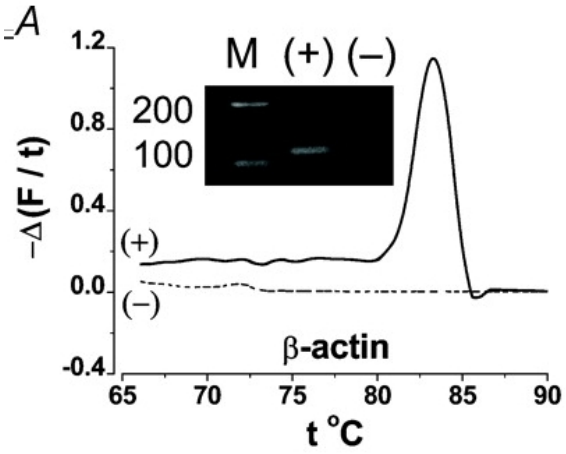


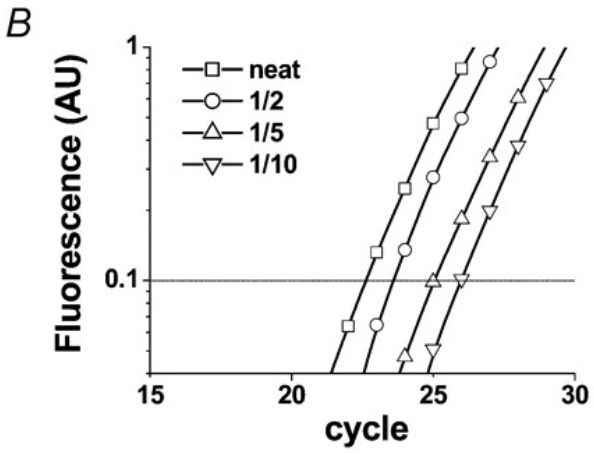
Single PCR product detection

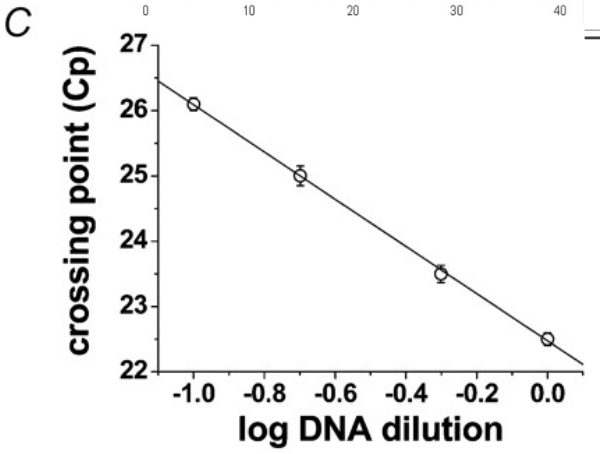
- End-point detection
  - Gel analysis – saturation versus log-phase
  - Use a dye to discriminate between ss and ds
  - Standardization - competicons





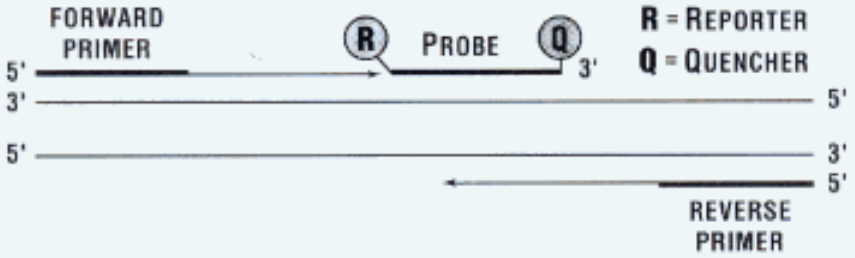






FLUOROGENIC 5' NUCLEASE CHEMISTRY

POLYMERIZATION



Two fluorescent dyes, a reporter (R) and a quencher (Q), are attached to the probes in the TaqMan PCR Reagent Kit.

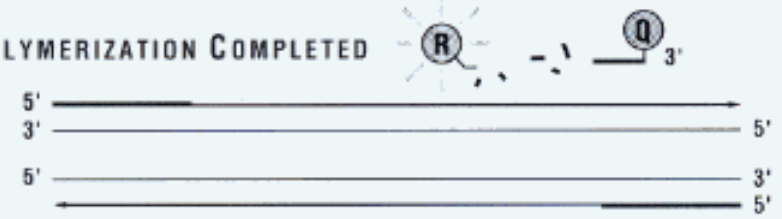
STRAND DISPLACEMENT

When both dyes are attached to the probe, reporter dye emission is quenched.

CLEAVAGE

During each extension cycle, the Taq DNA polymerase cleaves the reporter dye from the probe.

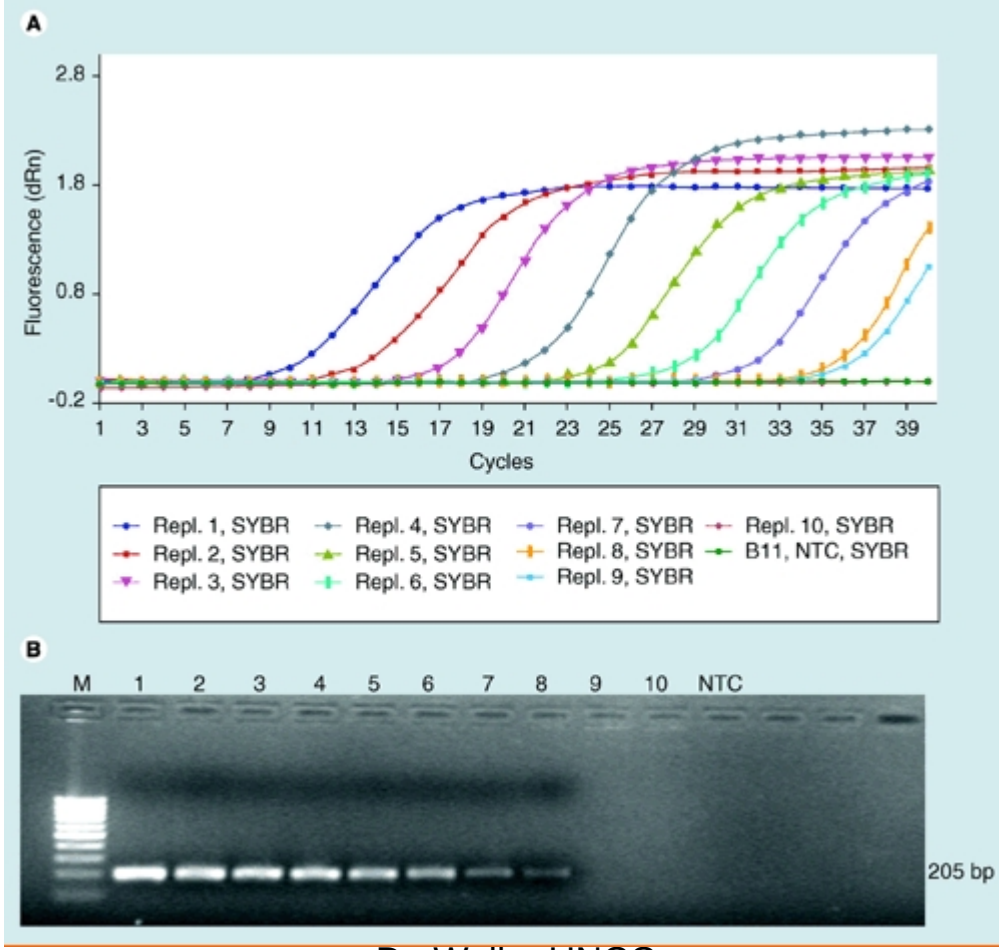
POLYMERIZATION COMPLETED



Once separated from the quencher, the reporter dye emits its characteristic fluorescence.

Fluorogenic 5’nuclease PCR assays

- End-point versus real-time results

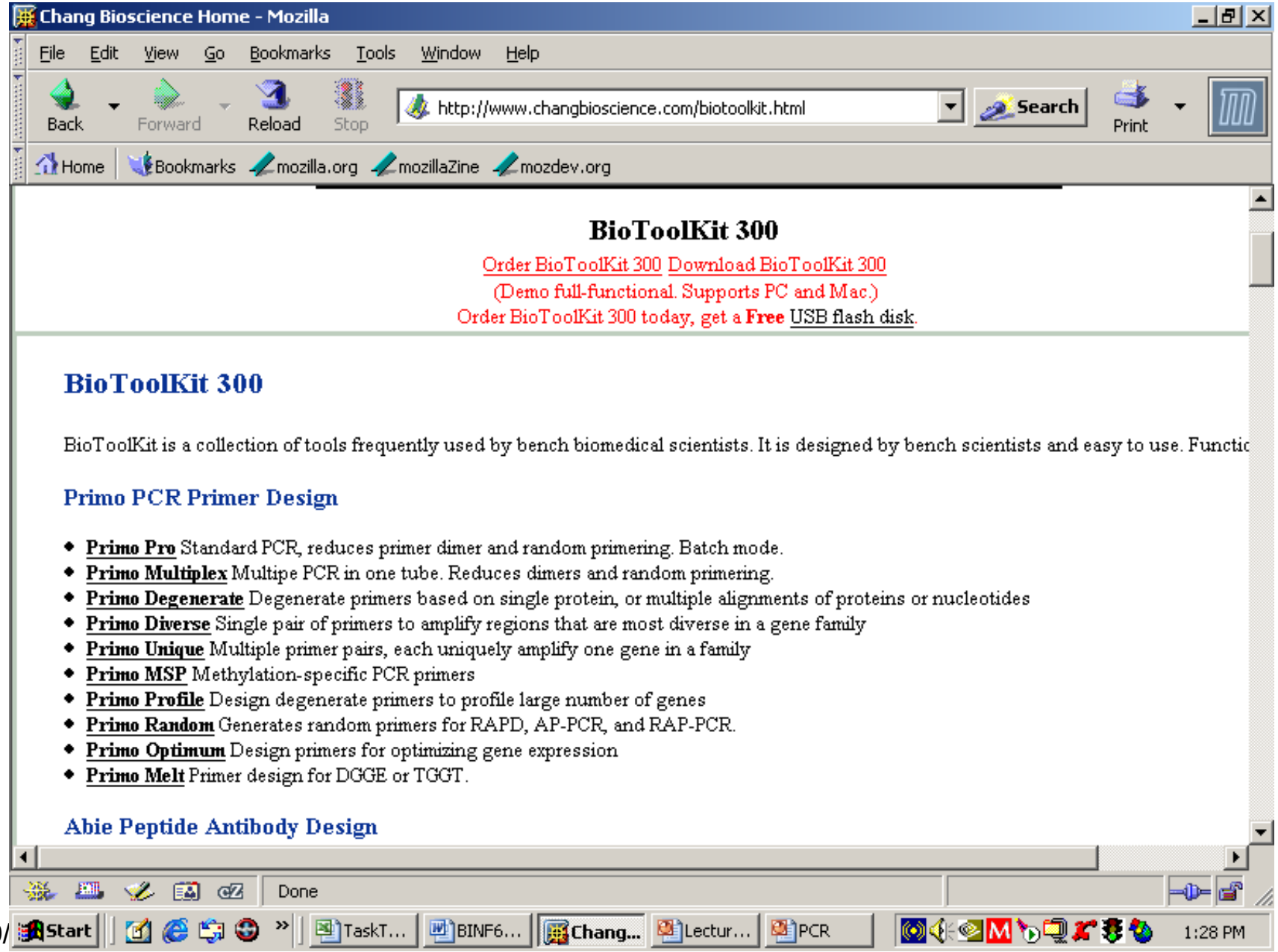


PCR assay design

- The success of PCR lies in the combination of the primer and the annealing conditions.
  - For a single target, each primer must *uniquely* bind a single site in the target genome, flanking the desired region
  - The polymerase is most active at ~72C
  - Primers come as a pair per target.
  - Primers should form stable duplexes with target between 55-72C.
    - usually 18-20 nucleotides in length
  - Primers need not have identical length
    - Binding constants should match (approx by T_m) – approx equal GC
    - no internal H-bonding
    - Accessible target sequence
  - Sufficient primer and dNTPs must be added to fuel the desired level of product – both are incorporated into the product
    - Above 1ug/ml product the reaction will saturate.

Rules: Primer/Probe design

- For multiple product yields to be balanced: a*mplicons* should be 50-150 bp in length.
  - GC proportion at 20-80%, and primers as similar as possible
  - Avoid runs (>4 in a row) of the same nt, especially Gs
  - The last 5 of the 3'-end nts should have at most 2 of either G or C
  - The T_m of the primers is usually 58-60C.
  - For fluorogenic assays
    - The 5' most base is not a G
    - The probe is close to, but not overlapping, the 3' end of the 5' primer.
    - Design the probe to the strand that lets C rather G predominate.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File Edit View Go Bookmarks Tools Window Help
Back Forward Reload Stop
http://www.changbioscience.com/biotoolkit.html
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Print
Home Bookmarks mozilla.org mozillaZine mozdev.org
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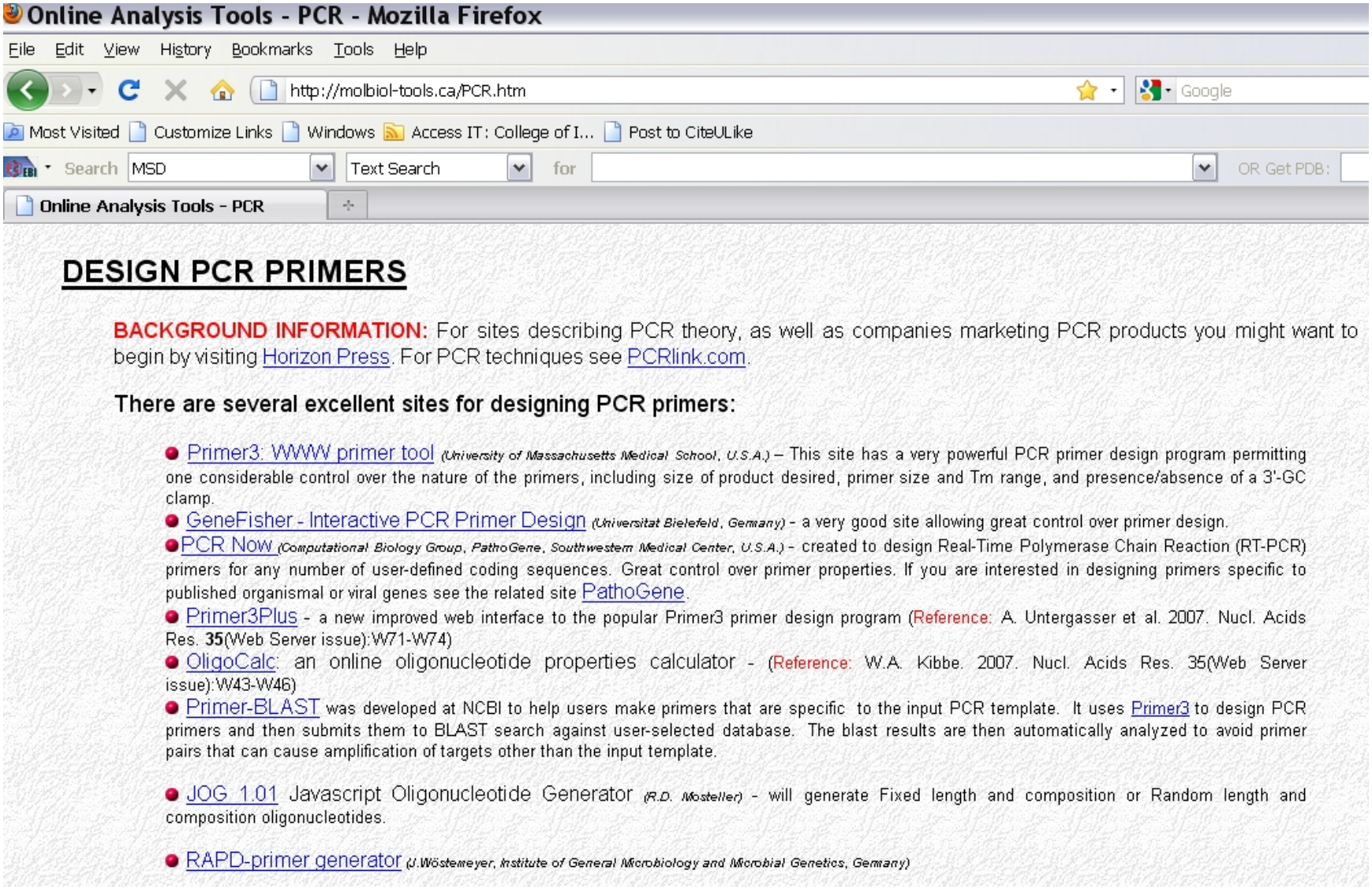
BioToolKit 300
BioToolKit is a collection of tools frequently used by bench biomedical scientists. It is designed by bench scientists and easy to use. Functic
Primo PCR Primer Design
Primo Pro Standard PCR, reduces primer dimer and random primering. Batch mode.
Primo Multiplex Multipe PCR in one tube. Reduces dimers and random primering.
Primo Degenerate Degenerate primers based on single protein, or multiple alignments of proteins or nucleotides
Primo Diverse Single pair of primers to amplify regions that are most diverse in a gene family
Primo Unique Multiple primer pairs, each uniquely amplify one gene in a family
Primo MSP Methylation-specific PCR primers
Primo Profile Design degenerate primers to profile large number of genes
Primo Random Generates random primers for RAPD, AP-PCR, and RAP-PCR.
Primo Optimum Design primers for optimizing gene expression
Primo Melt Primer design for DGGE or TGGT.
Abie Peptide Antibody Design
Done
Start
TaskT...
BINF6...
Chang...
Lectur...
PCR
1:28 PM


Online Analysis Tools - PCR - Mozilla Firefox
File Edit View History Bookmarks Tools Help
http://molbiol-tools.ca/PCR.htm
Google
Most Visited Customize Links Windows Access IT: College of I... Post to CiteULike
Search MSD Text Search for OR Get PDB:
Online Analysis Tools - PCR
DESIGN PCR PRIMERS
BACKGROUND INFORMATION: For sites describing PCR theory, as well as companies marketing PCR products you might want to begin by visiting Horizon Press. For PCR techniques see PCRlink.com.
There are several excellent sites for designing PCR primers:
Primer3: WWW primer tool (University of Massachusetts Medical School, U.S.A.) – This site has a very powerful PCR primer design program permitting one considerable control over the nature of the primers, including size of product desired, primer size and Tm range, and presence/absence of a 3'-GC clamp.
GeneFisher - Interactive PCR Primer Design (Universitat Bielefeld, Germany) - a very good site allowing great control over primer design.
PCR Now (Computational Biology Group, PathoGene, Southwestern Medical Center, U.S.A.) - created to design Real-Time Polymerase Chain Reaction (RT-PCR) primers for any number of user-defined coding sequences. Great control over primer properties. If you are interested in designing primers specific to published organismal or viral genes see the related site PathoGene.
Primer3Plus - a new improved web interface to the popular Primer3 primer design program (Reference: A. Untergasser et al. 2007. Nucl. Acids Res. 35(Web Server issue):W71-W74)
OligoCalc: an online oligonucleotide properties calculator - (Reference: W.A. Kibbe. 2007. Nucl. Acids Res. 35(Web Server issue):W43-W46)
Primer-BLAST was developed at NCBI to help users make primers that are specific to the input PCR template. It uses Primer3 to design PCR primers and then submits them to BLAST search against user-selected database. The blast results are then automatically analyzed to avoid primer pairs that can cause amplification of targets other than the input template.
JOG 1.01 Javascript Oligonucleotide Generator (R.D. Mosteller) - will generate Fixed length and composition or Random length and composition oligonucleotides.
RAPD-primer generator (J.Wöstemeyer, Institute of General Microbiology and Microbial Genetics, Germany)

Realtime PCR primer design:

- RealTimeDesign *(Biosearch Technologies)* - free but requires registration.
- QuantPrime - is a flexible program for reliable primer design for use in larger qPCR experiments. The flexible framework is also open for simple use other quantification applications, such as hydrolyzation probe design for qPCR and oligonucleotide probe design for quantitative *in situ* hybridizati (Reference: S. Arvidsson et al. 2008. BMC Bioinformatics **9:**465)
- Real-time PCR (TaqMan) Primer Design *(GenScript USA Inc.)*

For additional physicochemical data on the primers the following six sites are useful:

- NetPrimer *(Premier Biosoft International, U.S.A.)* - In my opinion the best site since it provides one with Tm, thermodynamic properties and most stable hairpin & dimers.BUT it takes a while for the program to load.
- dnaMATE - calculates a consensus Tm) for short DNA sequence (16-30 nts) using a merged method that is based on three different thermodynamic tables. The consensus Tm value is a robust and accurate estimation of melting temperature for short DNA sequences of practical application in molecular biology. Accuracy benchmarks using all experimental data available indicate that the consensus Tm prediction errors will be within 5 ºC from the experimental value in 89% of the cases. (Reference: A. Panjkovich et al. 2005. Nucl. Acids Res. **33:** W570-W572.).
- OligoAnalyzer *(Integrated DNA Technologies, Inc., U.S.A.)* - in addition to hairpin and self-dimer analysis of existing primers this site provides one with the opportunity to BLAST the sequence against NCBI's database and measure the impact of incorporating 5'-modifications into the sequence. The oligos can then be ordered directly.
- Another excellent site is Oligonucleotide Properties Calculator (*Northwestern University Medical School, Chicago, U.S.A.*) which provides one with detailed information on the calculations. Also permits analysis of 6-FAM, HEX, or TAMRA-labelled oligos.
- Biopolymer Calculator *(Yale University, U.S.A.)* - Not yet functional.
- Melting: enthalopy, entropy and melting temperature *(N. Le Novere, Pasteur Institute, Paris, France).*

Introduction of silent mutations:

- WatCut *(Michael Palmer, University of Waterloo, Canada)* - takes an oligonucleotide and introduces silent mutations in potential restriction sites such that the amino acid sequence of the protein is unaltered.

When you are ready to set-up your PCR reaction see:

- PCR Box Titration Calculator *(Allotron Biosensor Corporation)* - for figuring out the amounts of each reagent to use in a two-dimensional box titration for PCR. For standard PCR reactions adjust volume, and change "row" and "column" number to "1", click on all the "top" or "bottom" and "done".
- PCR Reaction Mixture Setup *(R. Kalendar, University of Helsinki, Finland)* - very nice site.

Primer presentation on the DNA sequence:

- Sequence Extractor *(Paul Stothard)* - generates a clickable restriction map and PCR primer map of a DNA sequence (Accepted formats are: raw, GenBank, EMBL, and FASTA) offering a great deal of control on output. Protein translations and intron/exon boundaries are also shown. Use Sequence Extractor to build DNA constructs *in silico*.

Primer 3

- Good for sequence specificity
- Free
- The most-used non-proprietary package
  - Does not incorporate newest information about biophysical properties of duplex
  - Depending on site, may not access most recent reference sequence versions

Primer3 Input Help

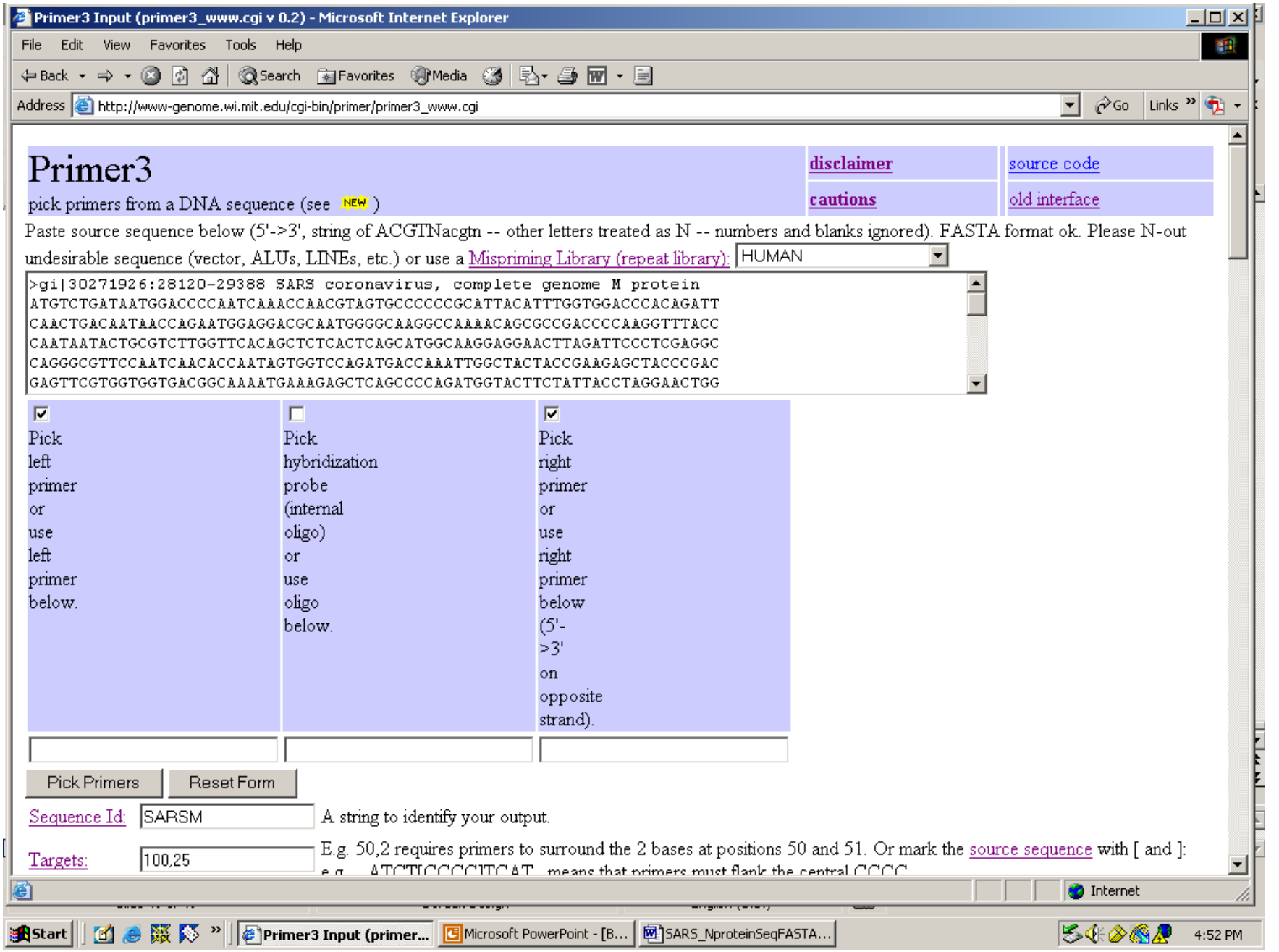
- Requirements:
  - avoid sequence of low or uncertain quality,
  - remove vector sequences, and scan for repetitive sequences.
  - Avoid partially homologous sequences
    - screen candidate oligos against a Mispriming Library
- **Source Sequence**
  - The template sequence: the basis from which to select primers or hybridization oligos.
- **Sequence Id**
  - An identifier that is reproduced in the output to enable you to identify the chosen primers.

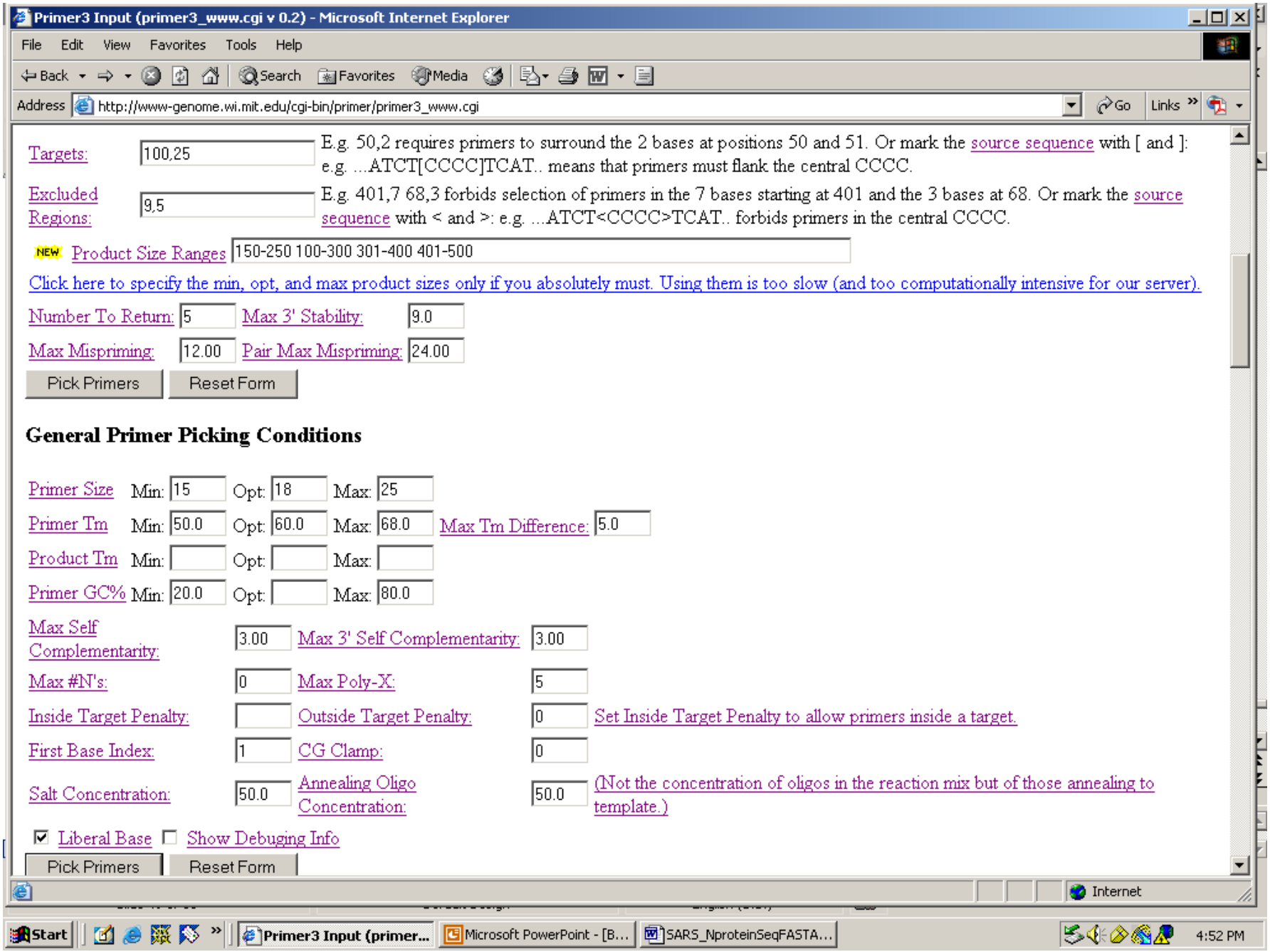
Primer 3 vocabulary

- **Targets** – a region on the source that *must be in* the PCR product
  - If one or more Targets is specified then a primer pair must flank at least one of them.
  - A Target might be a simple sequence repeat site (for example a CA repeat) or a single-base-pair polymorphism.
  - Specify with a space-separated list of *start*,*length* pairs
    - *start* is the index of the first base of a Target
    - *length* is its extent in nt.
- **Excluded Regions**
  - A region that primer oligos *may not* overlap.
    - Specify with an associated value that is a space-separated list of *start*,*length* pairs

Primer 3 parameters

- **Product Size Range**
  - A list of product size ranges, for example 150-250 100-300 301-400 Primer3 first tries to pick primers in the first range. If that is not possible, it goes to the next range and tries again.
- **Product Size**
  - Minimum, Optimum, and Maximum lengths (in bases) of the PCR product. Primer3 will not generate primers with products shorter than Min or longer than Max, and with default arguments Primer3 will attempt to pick primers producing products close to the Optimum length.

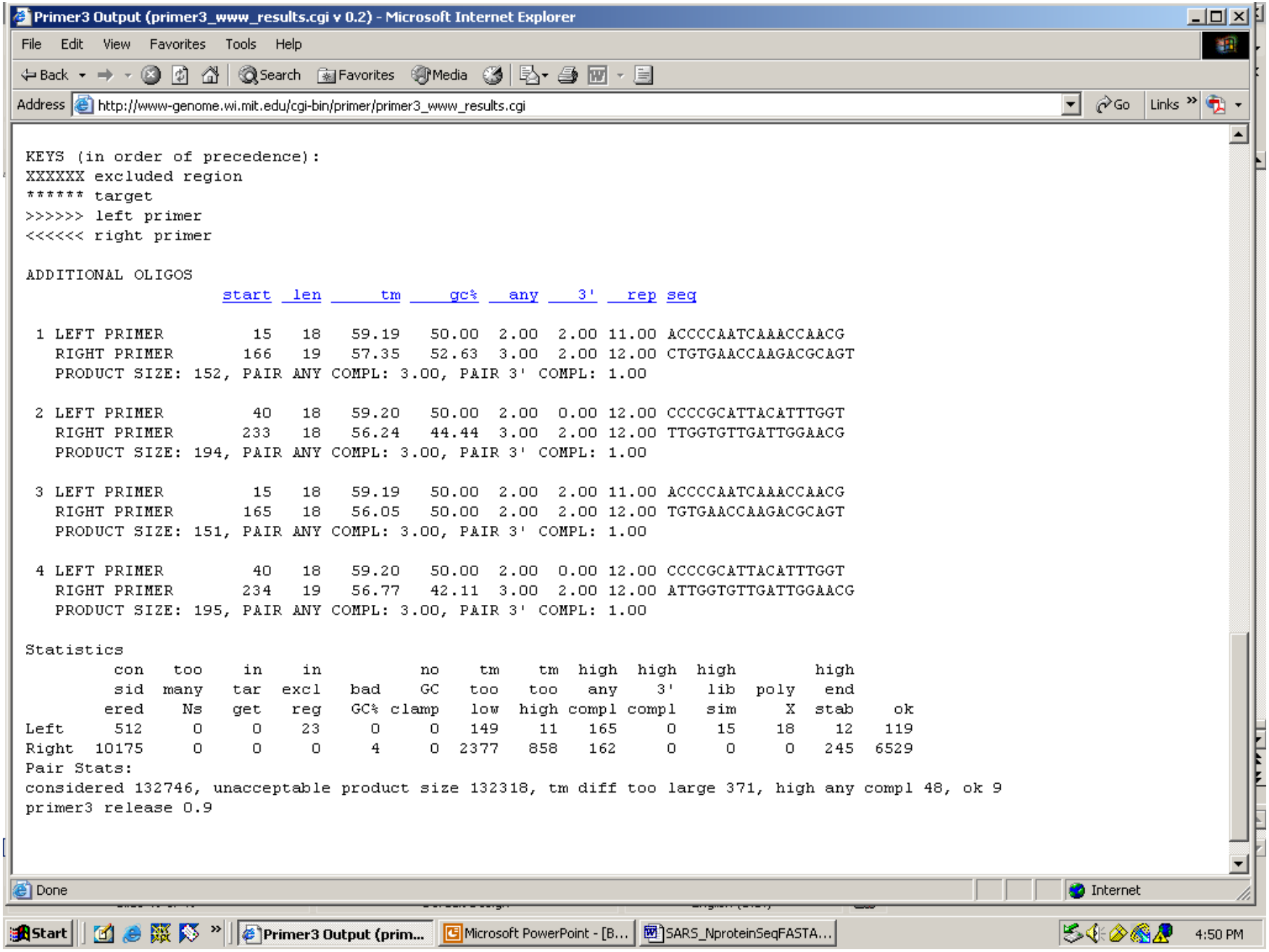

Primer3 Input (primer3_www.cgi v 0.2) - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites Media
Address http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi
Go Links
Primer3
pick primers from a DNA sequence (see NEW)
disclaimer
cautions
source code
old interface
Paste source sequence below (5'->3', string of ACGTNacgtn -- other letters treated as N -- numbers and blanks ignored). FASTA format ok. Please N-out undesirable sequence (vector, ALUs, LINEs, etc.) or use a Mispriming Library (repeat library): HUMAN
>gi|30271926:28120-29388 SARS coronavirus, complete genome M protein
ATGTCTGATAATGGACCCCAATCAAACCAACGTAGTGCCCCCCGCATTACATTTGGTGGACCCACAGATT
CAACTGACAATAACCAGAATGGAGGACGCAATGGGGCAAGGCCAAAACAGCGCCGACCCCAAGGTTTACC
CAATAATACTGCGTCTTGGTTCACAGCTCTCACTCAGCATGGCAAGGAGGAACTTAGATTCCCTCGAGGC
CAGGGCGTTCCAATCAACACCAATAGTGGTCCAGATGACCAAATTGGCTACTACCGAAGAGCTACCCGAC
GAGTTCGTGGTGGTGACGGCAAAATGAAAGAGCTCAGCCCCAGATGGTACTTCTATTACCTAGGAACTGG
Pick left primer or use left primer below.
Pick hybridization probe (internal oligo) or use oligo below.
Pick right primer or use right primer below (5'->3' on opposite strand).
Pick Primers
Reset Form
Sequence Id: SARSM
A string to identify your output.
Targets: 100,25
E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]:
Internet
Start
Primer3 Input (primer...
Microsoft PowerPoint - [B...
SARS_NproteinSeqFASTA...
4:52 PM


Primer3 Input (primer3_www.cgi v 0.2) - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites Media
Address http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi
Go Links
Targets: 100,25
E.g. 50,2 requires primers to surround the 2 bases at positions 50 and 51. Or mark the source sequence with [and]: e.g. ...ATCT[CCCC]TCAT.. means that primers must flank the central CCCC.
Excluded Regions: 9,5
E.g. 401,7 68,3 forbids selection of primers in the 7 bases starting at 401 and the 3 bases at 68. Or mark the source sequence with < and >: e.g. ...ATCT<CCCC>TCAT.. forbids primers in the central CCCC.
NEW Product Size Ranges 150-250 100-300 301-400 401-500
Click here to specify the min, opt, and max product sizes only if you absolutely must. Using them is too slow (and too computationally intensive for our server).
Number To Return: 5 Max 3' Stability: 9.0
Max Mispriming: 12.00 Pair Max Mispriming: 24.00
Pick Primers Reset Form
General Primer Picking Conditions
Primer Size Min: 15 Opt: 18 Max: 25
Primer Tm Min: 50.0 Opt: 60.0 Max: 68.0 Max Tm Difference: 5.0
Product Tm Min: Opt: Max:
Primer GC% Min: 20.0 Opt: Max: 80.0
Max Self Complementarity: 3.00 Max 3' Self Complementarity: 3.00
Max #N's: 0 Max Poly-X: 5
Inside Target Penalty: Outside Target Penalty: 0 Set Inside Target Penalty to allow primers inside a target.
First Base Index: 1 CG Clamp: 0
Salt Concentration: 50.0 Annealing Oligo Concentration: 50.0 (Not the concentration of oligos in the reaction mix but of those annealing to template.)
Liberal Base Show Debuging Info
Pick Primers Reset Form
Internet
Start
Primer3 Input (primer...
Microsoft PowerPoint - [B...
SARS_NproteinSeqFASTA...
4:52 PM

Primer3 Output

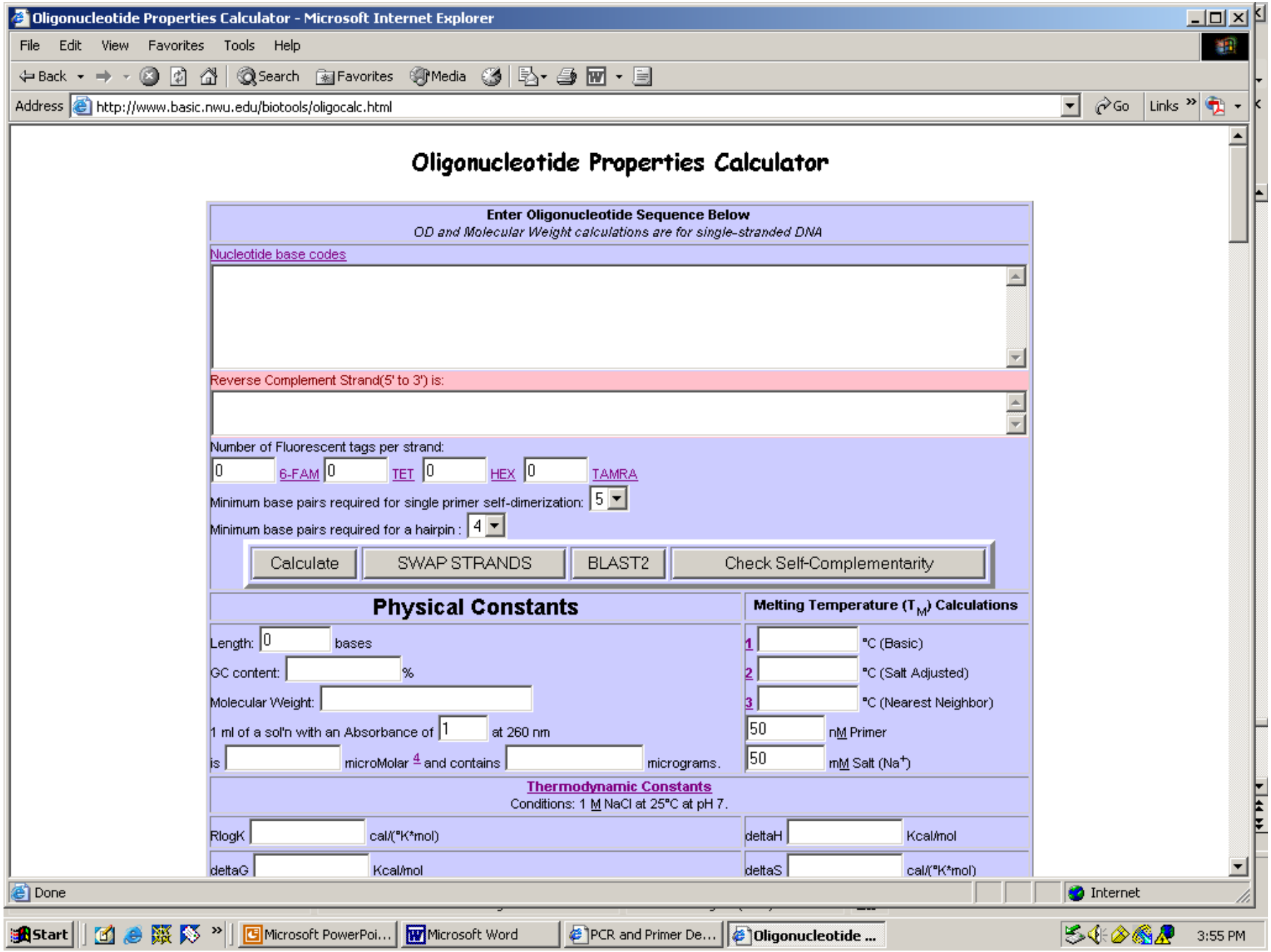
WARNING: 2 Sequence Ids provided: SARSM and gi|30271926:28120-29388 SARS coronavirus, complete genome M protein; using SARSM|gi|30271926:28120-29388 SARS coronavirus, complete genome M protein

```
PRIMER PICKING RESULTS FOR SARSM|gi|30271926:28120-29388 SARS coronavirus, complete genome M protein

Using mispriming library humrep_and_simple.txt
Using 1-based sequence positions
OLIGO            start  len       tm      gc%   any   3'   rep seq
LEFT PRIMER         15   18    59.19    50.00  2.00  2.00 11.00 ACCCCAATCAAACCAACG
RIGHT PRIMER       167   20    61.46    55.00  3.00  2.00 12.00 GCTGTGAACCAAGACGCAGT
SEQUENCE SIZE: 1269
INCLUDED REGION SIZE: 1269

PRODUCT SIZE: 153, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00
TARGETS (start, len)*: 100,25
EXCLUDED REGIONS (start, len)*: 9,5

    1 ATGTCTGATAATGGACCCCAATCAAACCAACGTAGTGCCCCCCGCATTACATTTGGTGGA
              XXXXX >>>>>>>>>>>>>>>>>>>

   61 CCCACAGATTCAACTGACAATAACCAGAATGGAGGACGCAATGGGGCAAGGCCAAAACAG
                                          **********************

  121 CGCCGACCCCAAGGTTTACCCAATAATACTGCGTCTTGGTTCACAGCTCTCACTCAGCAT
      ****                        <<<<<<<<<<<<<<<<<<<<

  181 GGCAAGGAGGAACTTAGATTCCCTCGAGGCCAGGGCGTTCCAATCAACACCAATAGTGGT


  241 CCAGATGACCAAATTGGCTACTACCGAAGAGCTACCCGACGAGTTCGTGGTGGTGACGGC
```


Primer3 Output (primer3_www_results.cgi v 0.2) - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites Media
Address http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www_results.cgi
Go Links
KEYS (in order of precedence):
XXXXXX excluded region
****** target
>>>>>> left primer
<<<<<< right primer
ADDITIONAL OLIGOS
start len tm gc% any 3' rep seq
1 LEFT PRIMER 15 18 59.19 50.00 2.00 2.00 11.00 ACCCCAATCAAACCAACG
RIGHT PRIMER 166 19 57.35 52.63 3.00 2.00 12.00 CTGTGAACCAAGACGCAGT
PRODUCT SIZE: 152, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00
2 LEFT PRIMER 40 18 59.20 50.00 2.00 0.00 12.00 CCCCGCATTACATTTGGT
RIGHT PRIMER 233 18 56.24 44.44 3.00 2.00 12.00 TTGGTGTTGATTGGAACG
PRODUCT SIZE: 194, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00
3 LEFT PRIMER 15 18 59.19 50.00 2.00 2.00 11.00 ACCCCAATCAAACCAACG
RIGHT PRIMER 165 18 56.05 50.00 2.00 2.00 12.00 TGTGAACCAAGACGCAGT
PRODUCT SIZE: 151, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00
4 LEFT PRIMER 40 18 59.20 50.00 2.00 0.00 12.00 CCCCGCATTACATTTGGT
RIGHT PRIMER 234 19 56.77 42.11 3.00 2.00 12.00 ATTGGTGTTGATTGGAACG
PRODUCT SIZE: 195, PAIR ANY COMPL: 3.00, PAIR 3' COMPL: 1.00
Statistics
con too in in no tm tm high high high high
sid many tar excl bad GC too too any 3' lib poly end
ered Ns get reg GC% clamp low high compl compl sim X stab ok
Left 512 0 0 23 0 0 149 11 165 0 15 18 12 119
Right 10175 0 0 0 4 0 2377 858 162 0 0 0 245 6529
Pair Stats:
considered 132746, unacceptable product size 132318, tm diff too large 371, high any compl 48, ok 9
primer3 release 0.9
Done
Internet
Start
Primer3 Output (prim...
Microsoft PowerPoint - [B...
SARS_NproteinSeqFASTA...
4:50 PM

Other Primer Design Software

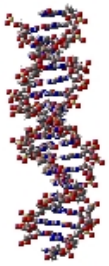
- Oligowiz, OligoArray are for microarray design, but have useful features for PCR design as well.
- Oligonucleotides Properties Calculator
- There is also a server with some free software at the MolBioNet site (next slide)


Oligonucleotide Properties Calculator - Microsoft Internet Explorer
File Edit View Favorites Tools Help
Back Search Favorites Media
Address http://www.basic.nwu.edu/biotools/oligocalc.html
Go Links
Oligonucleotide Properties Calculator
Enter Oligonucleotide Sequence Below
OD and Molecular Weight calculations are for single-stranded DNA
Nucleotide base codes
Reverse Complement Strand(5' to 3') is:
Number of Fluorescent tags per strand:
0 6-FAM 0 TET 0 HEX 0 TAMRA
Minimum base pairs required for single primer self-dimerization: 5
Minimum base pairs required for a hairpin : 4
Calculate
SWAP STRANDS
BLAST2
Check Self-Complementarity
Physical Constants
Length: 0 bases
GC content: %
Molecular Weight:
1 ml of a sol'n with an Absorbance of 1 at 260 nm
is microMolar 4 and contains micrograms.
Melting Temperature (T_M) Calculations
1 °C (Basic)
2 °C (Salt Adjusted)
3 °C (Nearest Neighbor)
50 nM Primer
50 mM Salt (Na+)
Thermodynamic Constants
Conditions: 1 M NaCl at 25°C at pH 7.
RlogK cal/(°K*mol)
deltaH Kcal/mol
deltaG Kcal/mol
deltaS cal/(°K*mol)
Done
Internet
Start
Microsoft PowerPoi...
Microsoft Word
PCR and Primer De...
Oligonucleotide ...
3:55 PM

In silico simulation of molecular biology experiments

About, Citing this site

Last update: 2010/01/21 (1021 prokaryotic and 12+10 eukaryotic genomes)



insilico.ehu.es

Experiments against prokaryotic genomes

- PCR amplification
- Restriction digest and PFGE
- PCR-RFLP
- T-RFLP
- Double Digestion fingerprinting
- AFLP-PCR
- SAMPL
- SRF
- DDSL
- resAP-PCR
- DNA fingerprinting
- cDNA-AFLP
- Microsatellite Repeats
- Find ORF by name
- Sort sequence locator

Experiments against eukaryotic genomes

- Main

Experiments against user's sequences

- Main

Restriction digest of DNA

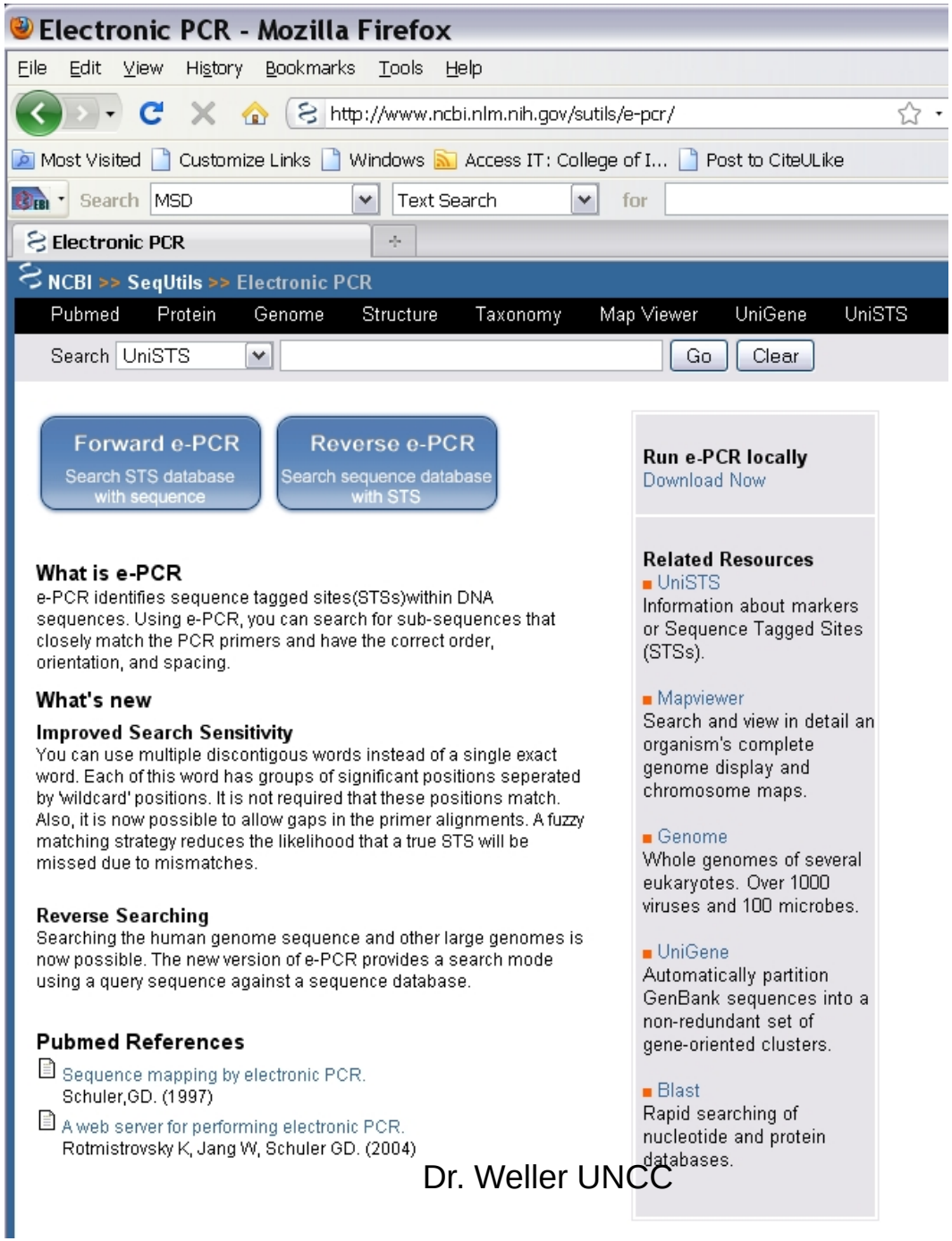
- Translate DNA to protein
- Coloured sequences for presentations
- Discriminatory Power Calculator
- Molecular Weight Calculator
- Basic Tm calculation
- RCF / rpm conversion
- Dice + UPGMA analysis of PFGE patterns
- DNA/Protein Alignment (Smith-Waterman)
- Multiple Sequence Alignment (ClustalW)

OligoWeb: **oligonucleotide frequencies in prokaryotes**

Online exercise
Design of PCR and PCR-RFLP experiments

Recommended sites:
BacterialGenomics.org
Biophp.org

Optimized for Mozilla/Firefox


Electronic PCR - Mozilla Firefox
File Edit View History Bookmarks Tools Help
http://www.ncbi.nlm.nih.gov/sutils/e-pcr/
Most Visited Customize Links Windows Access IT: College of I... Post to CiteULike
Search MSD Text Search for
Electronic PCR
NCBI >> SeqUtils >> Electronic PCR
Pubmed Protein Genome Structure Taxonomy Map Viewer UniGene UniSTS
Search UniSTS Go Clear
Forward e-PCR
Search STS database with sequence
Reverse e-PCR
Search sequence database with STS
What is e-PCR
e-PCR identifies sequence tagged sites(STSs)within DNA sequences. Using e-PCR, you can search for sub-sequences that closely match the PCR primers and have the correct order, orientation, and spacing.
What's new
Improved Search Sensitivity
You can use multiple discontigous words instead of a single exact word. Each of this word has groups of significant positions seperated by 'wildcard' positions. It is not required that these positions match. Also, it is now possible to allow gaps in the primer alignments. A fuzzy matching strategy reduces the likelihood that a true STS will be missed due to mismatches.
Reverse Searching
Searching the human genome sequence and other large genomes is now possible. The new version of e-PCR provides a search mode using a query sequence against a sequence database.
Pubmed References
Sequence mapping by electronic PCR.
Schuler,GD. (1997)
A web server for performing electronic PCR.
Rotmistrovsky K, Jang W, Schuler GD. (2004)
Run e-PCR locally
Download Now
Related Resources
UniSTS
Information about markers or Sequence Tagged Sites (STSs).
Mapviewer
Search and view in detail an organism's complete genome display and chromosome maps.
Genome
Whole genomes of several eukaryotes. Over 1000 viruses and 100 microbes.
UniGene
Automatically partition GenBank sequences into a non-redundant set of gene-oriented clusters.
Blast
Rapid searching of nucleotide and protein databases.

Design a Primer!

- We need to test the Phaseolus DNA and the Glycine primers do not work.
- Design primers for the first exon in actin and tubulin – beta subunit.