PCR Assisted Biochemistry

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Biochemistry 118

March 7, 2000

PCR Assisted Biochemistry

- History
- Background
- Parameters of successful reaction
- Practical example
- Applications
- Future

History

- Conceived by Kary Mullis while driving on the California freeways (1985)

- Received Nobel Prize for technique

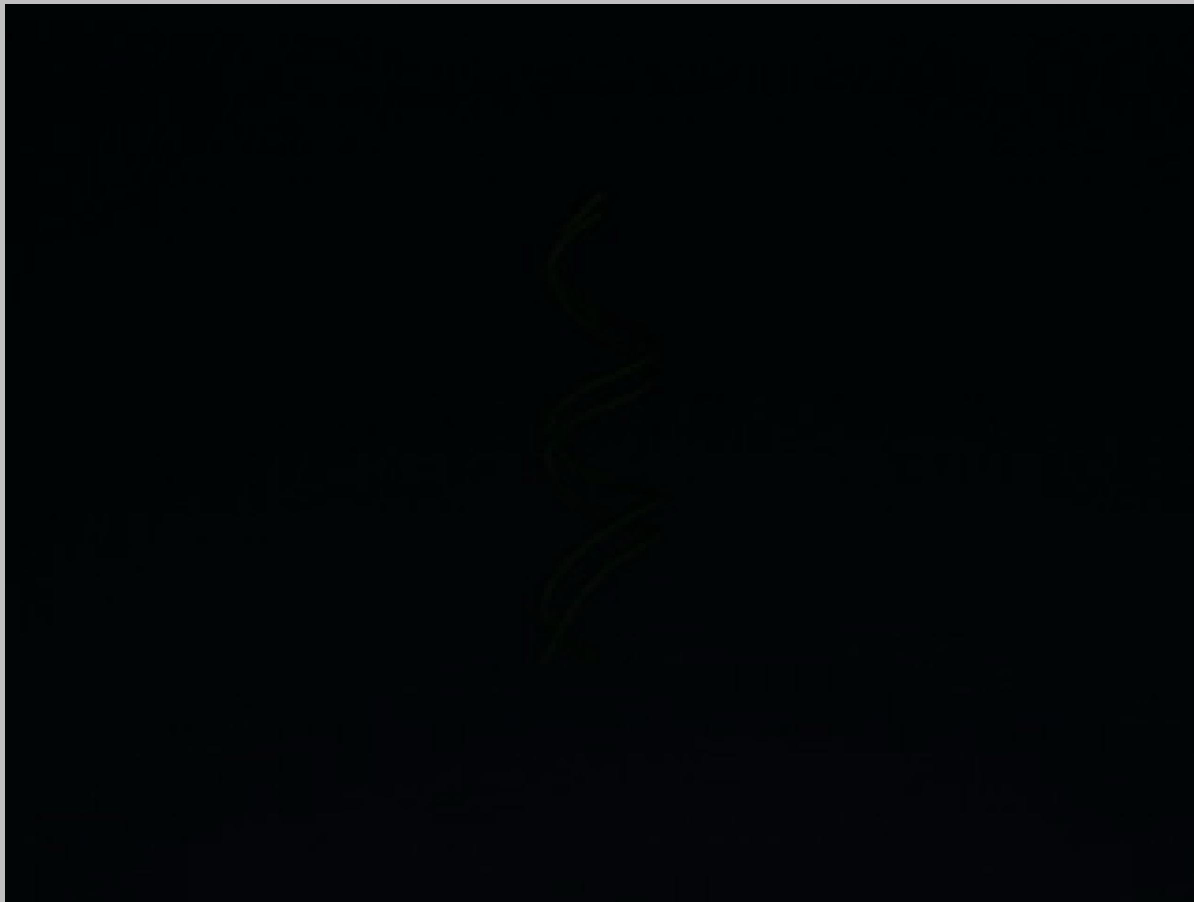
- Since then, PCR patent sold for $300 million -- highest amount ever for a patent
- Basically, a method of DNA amplification
- Technology has wide applications to fields of
    - Biology
    - Biochemistry
    - Forensics
    - Archaeology

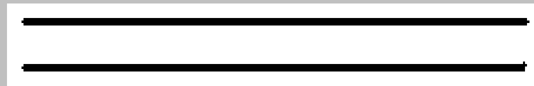
Background

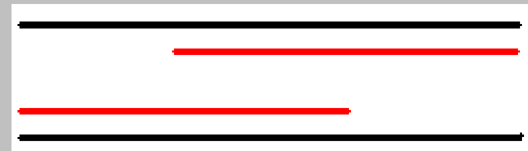
ELEMENTS OF A PCR REACTION:

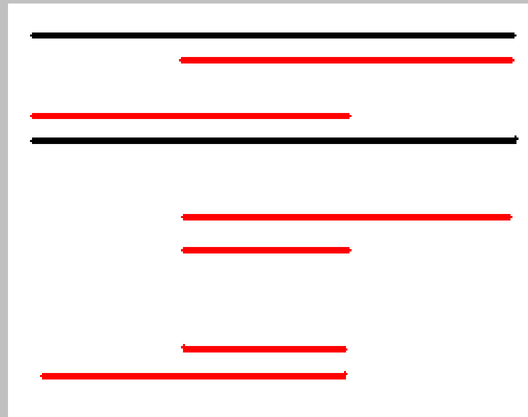
- DNA
- Primers
- Enzyme
- Nucleotides
- Thermocycler

Reaction Overview: Exponential Amplification of DNA



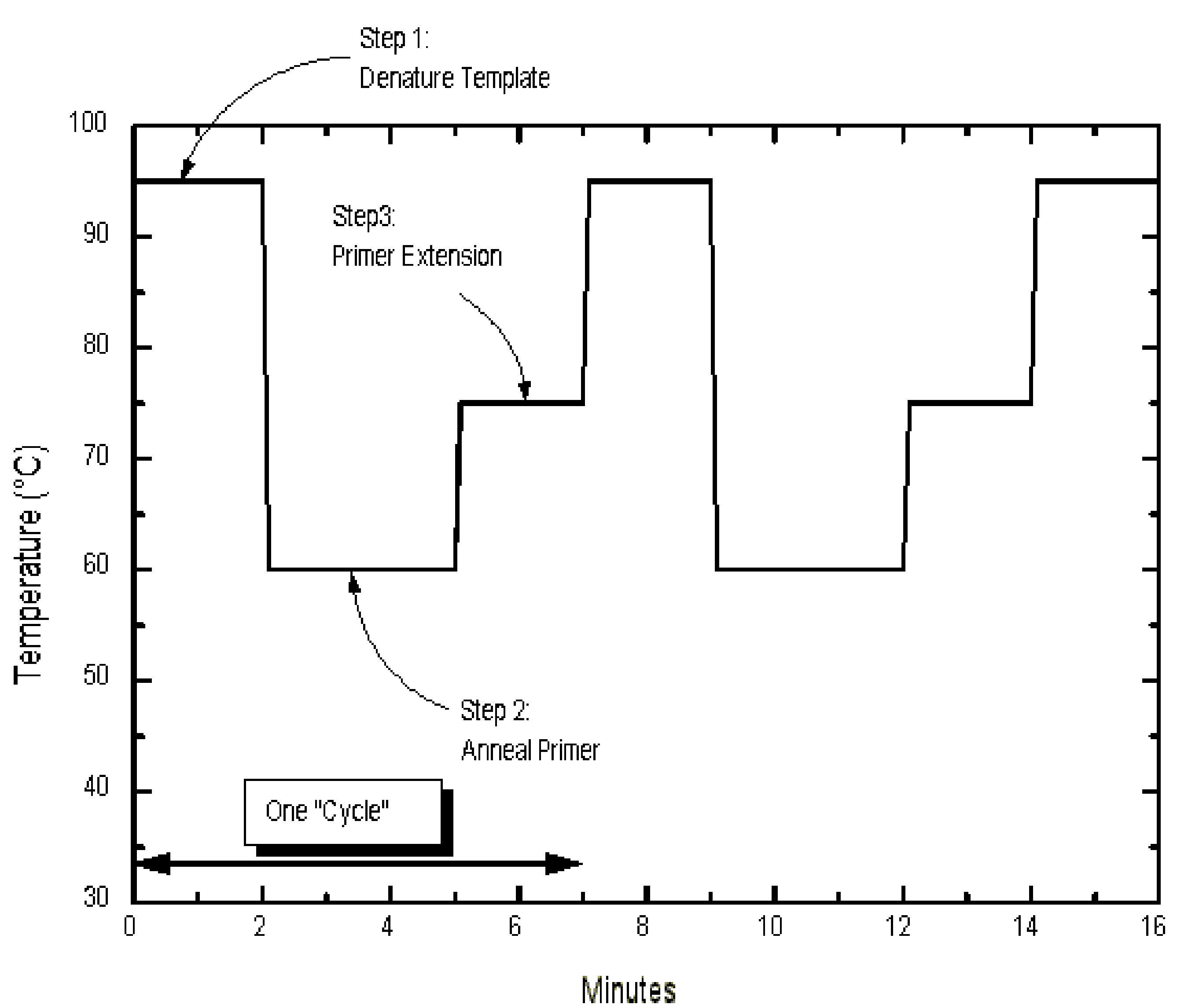

Original DNA


After Cycle 1


After Cycle 2

After Cycle 3

After N cycles, amount of target DNA is $2^N - 2N$



TAQ polymerase optimum at 72° C

DNA

- Need to know at least the beginning and end of DNA sequence
- These flanking regions have to be unique to strand interested in amplifying
- Region of interest can be present in as little as one copy
- *Enough DNA in 0.1 microliter of human saliva to use PCR*

Enzyme

- DNA polymerase from *Thermus aquaticus*--Yellowstone
- Alternatives: *Thermococcus litoralis* , *Pyrococcus furiosus*

Thermocycler

Primers and Design

Primer sequence:

Length: 20-30 base pairs long ($1/4^N$)

50% ± 15% G-C

Avoid motifs and poly-N sequences

Avoid inverted repeating sequences

Two primers should have little complementarity

3’ end of primer should be G/C

Melting Temperature

T_m = [(number of A+T residues) x 2 °C] + [(number of G+C residues) x 4 °C]

Both primers should have comparable melting temperatures

Annealing temperature is about 5° C lower than melting temp

1 ccgtaacgga ggatgttttt cagaatgtgg ttgggattga tggatgggag gtagaacgag
61 ttcgagttga aaaggttttg tgtgccatgt gaaaaggtta gcatctatta cgtagacgag
121 agaaattcat tggaaatttg agaaggagat tgagcataat gaaacttgtt ttggaaaaat
181 atgttgttat taatgtggag gtgggcaaga atgagaataa tcagtagcaa tgaggtgtca
241 ataatttgat actgtctaca tggaagacgg cgaccagagc catggaagtc agaatgaaaa
301 atgataaatg tgaaaacatt ctagagaaga aatgaatacg cgaaggcccg tggtgggtga
361 tgacatgatg tgatttctgc ccagtgctct gaatgtcaaa gtgaagaaat tcaatgaagg
421 acgggtaaac ggcgggagta actatgactc tcttaaggta gccaaatgca tagtcatcta
481 attagtgacg ttcatgaatg gatgaacgag attcccactg tccctaccta ctatccagcg
541 aaaccacagc caaggtaacg ggcttggtgg aatccgcggg gaaagaagac cctgttgagc
601 ttgactctag tctggcacgg tgaagagcca tgagaagtgt agaataagtg ggaggcccct
661 gggccccccct gcccagcaag gggacagagt ggggcaaggc cagaggtgaa ataccactac
721 tctgattgtt tattcactga cccgtgaggt gccccaaggg gctcttgctt ctggcgccga
781 gtgcccggcc acatgcacat gccaaattgt aaagaccatc gat

GeneFisher

http://bibiserv.techfak.uni-bielefeld.de/genefisher/

	Forward Primer Data	Reverse Primer Data
Sequence	GATTGATGGATGGGAGGTA	CTGTGGTTTCGCTGGA
GC Content	47	56
Position	34	533
Degeneracy	0	0
3' GC	50	50
3' Degeneracy	0	0
Tm	52.7996	51.349
Location	34	533

Applications

Forensics

• assessment/reassessment of crimes

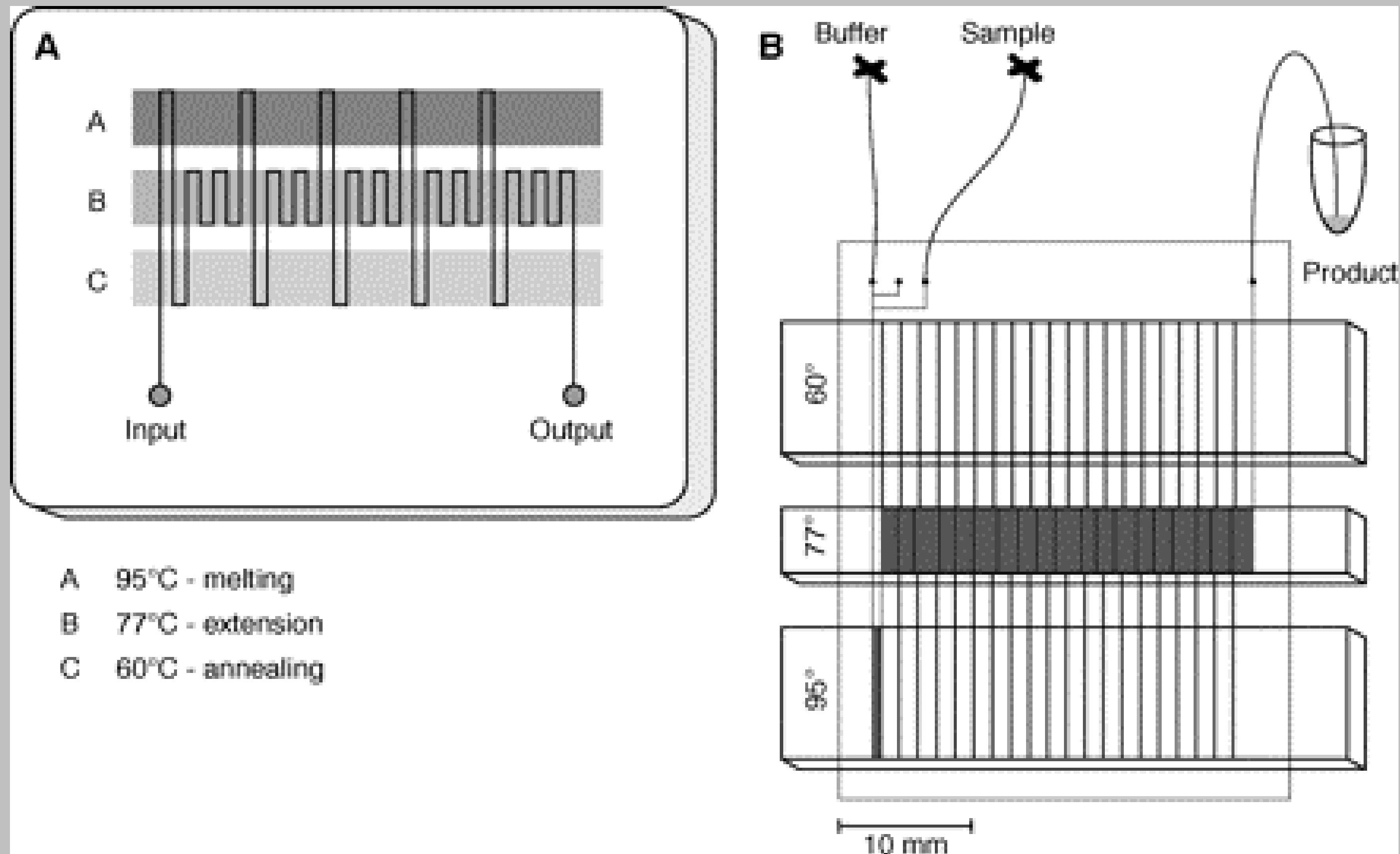
Archaeology

• determine gene sequences of ancient organisms
• rethinking the past, human origins

Molecular Biology

• cloning genes
• RT-PCR
• Amplification of DNA from tissues

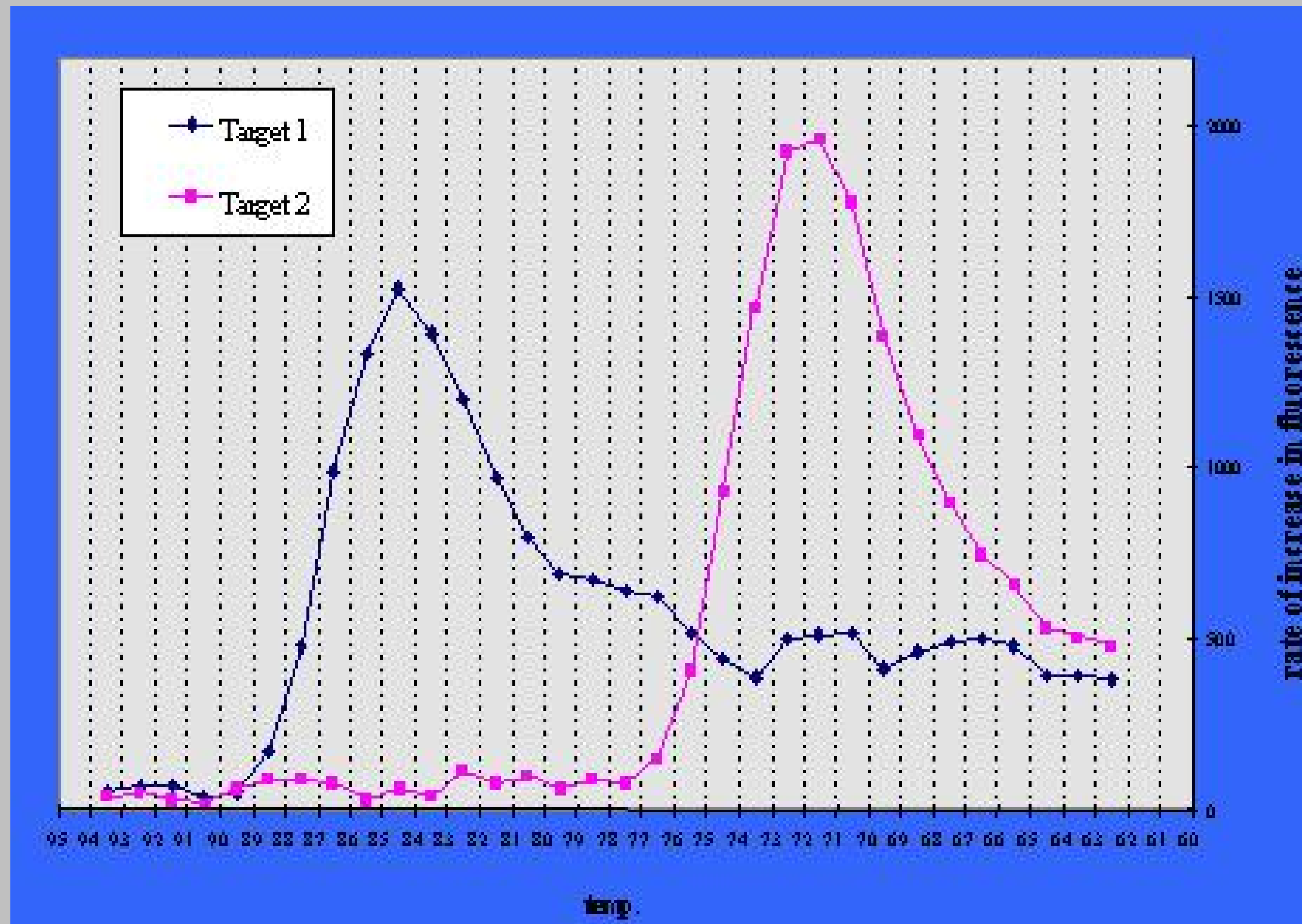
PCR on a Chip



Uses: Reaction complete in 2-20 minutes

Extremely portable

Fluorescence PCR



Uses: Identification purposes

Real-Time PCR

Uses:
- Portable means to diagnose bacteria
- Military, medical, and municipal applications
- Fast: Results in less than seven minutes

Acknowledgements

Professor Brutlag

Gene Fisher

http://bibiserv.techfak.uni-bielefeld.de/genefisher/

Molecular Biology Techniques Manual, Third Edition

Edited by: Coyne, V. et al

"PCR Detection of Bacteria in Seven Minutes"

Phillip Belgrader. Science April 1999