PCR Assisted Biochemistry

Farzad Alemi
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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
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

\[ T_m = [(\text{number of A+T residues}) \times 2 \degree C] + [(\text{number of G+C residues}) \times 4 \degree C] \]

Both primers should have comparable melting temperatures
Annealing temperature is about 5\(^\circ\)C lower than melting temp
1 ccgtaacgga ggtggttttt cagaatggtta tgggtgggtg ggtagaacgag
gtagaacgag
61 ttcgaagaaga agaagtttta agtgccagtgt gaaaggtta gcaatctaga ctagacagag
gtagaacgag
121 aaaaactcag tggaaatttt cgaagatctta gaacttggtt ctaggtgtcag
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tgggtgtt
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421 atcgatagc cttggtgaa ttcgcttgta gaaaggtta gcatctatta ctaggtgtcag
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541 aaacccacag cagatgtaacg ggtggttttt aatcccgagg gcacaagac ccctgtgcgc
tgggtgtt
601 tggactactg tcggccaggg tggagagcag tggagagcag tggagagcag cggtgcgtgc
tgggtgtt
661 gggcccggg ggggccgaggt gggcagacag gggcagacag gggcagacag gggcagacag
tgggtgtt
721 tgggtggtt tatctactga cccgtgaggt gccccaaggg gctcttgctt ctggcgccga
tgggtgtt
781 ggggcccggc acagtcacat gccaaattgt aagacccct cat

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**Forward Primer Data**

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**Reverse Primer Data**

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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