Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase

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Background:

Kary B. Mullis (1944-current)
won Nobel Prize 1993 in Chemistry
Randall K. Saiki

Before Polymerase Chain Reaction:

1865 - Gregor Mendel's law of inheritance gave rise to genetics "Father of Modern Genetics"
Mendel coined the terms dominant and recessive traits
1902 - Walter Sutton used the term "gene" when describing factors located on chromosomes
1933 - Arne Tiselius introduced the technique, electrophoresis, to separate proteins in a solution
1950 - "Chargaff's Rules" by Erwin Chargaff states the relationship between Adenine\Thymine and Cytosine\Guanine in DNA
1952 - Rosalind Franklin and Maurice Wilkins used X-ray crystallography to study the structure of DNA
1953 - James Watson and Francis Crick proposed a double stranded helical structure of DNA
1955 - Arthur Kornberg discovered and isolated DNA polymerase from *E. coli* bacteria
1972 - First successful DNA cloning experiments were preformed by Paul Berg using a restriction enzyme and ligase to form the first recombinant DNA molecule
1975 - Edwin Southern published his experimental details for the Southern Blot technique to identify DNA fragments
1977 - Richard Roberts and Phillip Sharp proved that eukaryotic genes are split into introns and extrons
1983 - Kary Mullis invented the Polymerase Chain Reaction (PRC), used to multiply DNA sequences in vitro
Klenow polymerase (DNA polymerase I) of *E. coli* was the first enzyme used
Discovery of thermostable DNA polymerase (*Taq* polymerase)

PCR is an in vitro technique used for amplifying specific DNA segment that uses certain features of DNA replication

Video - Polymerase Chain Reaction

Material and Methods:

Polymerase Chain Reaction (PCR) uses the following materials:

- 2 oligonucleotide primers
- segment of DNA
- primers
- DNA Polymerase
- enzymes

Objectives:

To prove that using *Taq* polymerase from *Thermus aquaticus* is more efficient than Klenow polymerase (DNA polymerase I) of *E. coli*

Hypothesis:

Taq polymerase increases the overall performance of amplifying DNA by increasing the specificity, yield, sensitivity, and length of targets that can be amplified.

Results:

Figure 1: Comparison of Klenow and *Taq* DNA polymerase-catalyzed PCR amplification products of the human 3-globin gene

Figure 2: Factors that influence the specificity *Taq* polymerase-catalyzed amplifications

Figure 3: Poisson distribution of single target sequences in samples of 10^5 cells

Figure 4: Examination of the ability of Klenow and *Taq* DNA polymerases to amplify longer target segments

Figure 5: Amplification of inserts in a phage X cDNA library

Figure 6: Direct sequencing of a PCR-amplified human genomic target

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Discussion\Conclusion:

Assessing the fidelity of *Taq* polymerase

- Cloned and sequenced individual amplification products with primers that define a region of the HLA-DPβ gene.
- In 28 separate clones each with 239 bp of amplified DPβ genomic DNA, 17 misincorporated bases which represents an overall error frequency of 0.25%
- No insertions or deletions
- If constant over 30 cycles, misincorperation rate per nucleotide per cycle is 2×10^{-4} (Larger than the 8×10^{-5} rate of Klenow polymerase)
- This misincorporation rate could be a problem for PCR amplifications with only a few copies of the target gene, but with comparison of several samples this would resolve any inconsistencies
- Greater problems arise from the Klenow polymerase: "Shuffled" clones may arise and a mosaic of genes may be amplified
- This is not an issue with *Taq* polymerase

Taq polymerase has the ability to amplify and manipulate a target sequence that is only present once in a same of 10^5 to 10^6 cells

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PCR Today:

Some important practical applications:

- Gene expression or rearrangement in single cells
- DNA cloning for sequencing
- Forensic applications
  - detection and identification of specific DNA fragments that can be traced to a particular individual
- Medical diagnostics
  - detection of mutations causing human genetic diseases
- Detection of pathogenic microorganisms in a clinical specimen
  - important for organisms that are difficult to culture, like viruses
- Extension of automation using robotics = large number of PCRs performed at one time
  = total set up time can be reduced to 15 minutes
- Real-time PCR

"Because PCR amplifies DNA, the amount of DNA needed to initiate a PCR is very small-even a single DNA molecule will suffice. Remarkable sources of DNA for PCR included biopsies embedded in Parafin for more than 40 years; blood samples, taken by heel prick of new borns, for the neonatal detection of PKU and stored as dried spots on cards; and even the tooth of a 10-11 year old Neanderthal child dating from 100,000 years ago."

- Watson et al.