HISTORY OF PCR

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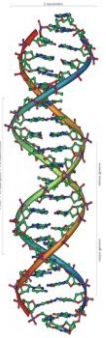
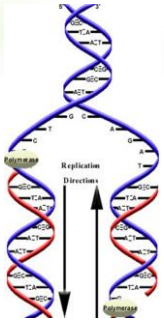
- The DNA model
  - On **April 25, 1953** James D. **Watson** and Francis **Crick** published "a radically different structure" for DNA, thereby founding the field of molecular genetics.
  - Their structural model featured two strands of complementary base-paired DNA, running in opposite directions as a double helix.
  - They concluded their report saying that "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material".
  - For this insight they were awarded the **Nobel Prize in 1962**.



- Starting in the mid **1950s**, Arthur **Kornberg** began to study the mechanism of DNA replication.
- By **1957** he identified the **first DNA polymerase**.
- The enzyme was limited, creating DNA in just one direction and requiring an existing primer to initiate copying of the template strand.
- Overall, the DNA replication process is surprisingly complex, requiring separate proteins to open the DNA helix, to keep it open, to create primers, to synthesize new DNA, to remove the primers, and to tie the pieces all together.
- Kornberg was awarded the **Nobel Prize in 1959**.

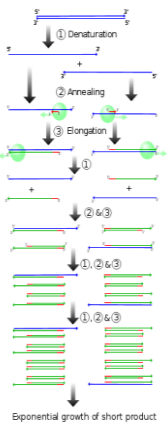


- In the early 1960s **H. Gobind Khorana** made significant advances in the elucidation of the **genetic code**.
- Afterwards, he initiated a large project to totally synthesize a **functional human gene**.
- To achieve this, Khorana pioneered many of the techniques needed to make and use **synthetic DNA oligonucleotides**.
- Sequence-specific oligonucleotides were used both as **building blocks** for the gene, and as **primers** and **templates** for DNA polymerase.
- In **1968** Khorana was awarded the **Nobel Prize** for his work on the **Genetic Code.**
  - *Born* *January 9, 1922, **Raipur, Punjab Pakistan***
  - *Residence* *India/Pakistan, United States, United Kingdom*
  - *Citizenship* *United States*
  - *Alma mater* ***University of the Punjab**; University of Liverpool*
  - ***BSC and MSc from Punjab University** in 1943 and 1945*
  - *PhD from Liverpool in 1948*

- In **1969** Thomas D. **Brock** reported the isolation of a new species of bacterium from a hot spring in Yellowstone National Park. Thermus aquaticus (**Taq**), became a standard source of enzymes able to withstand higher temperatures than those from E. Coli.
- By **1971** researchers in **Khorana's project,** concerned over their yields of DNA, began looking at "**repair synthesis**" - an artificial system of primers and templates that allows DNA polymerase to copy segments of the gene they are synthesizing.
  - Although similar to PCR in using repeated applications of DNA polymerase, the process they usually describe, employs just a single primer-template complex, and therefore would not lead to the exponential amplification seen in PCR.

- **1971** Kjell **Kleppe**, a researcher in Khorana's lab, envisioned a process very similar to PCR.
  - At the end of a paper on the earlier technique, he described how a **two-primer system** might lead to replication of a specific segment of DNA
- Also in **1971**, **Cetus Corporation** was founded in Berkeley, California
- In **1977** Frederick **Sanger** reported a method for determining the sequence of DNA.
  - The technique employed an **oligonucleotide primer**, **DNA polymerase**, and **modified nucleotide precursors** that block further extension of the primer in sequence-dependent manner.
  - For this innovation he was awarded the **Nobel Prize in 1980**.

- In **1979 Cetus Corporation** hired **Kary Mullis** to synthesize oligonucleotides for various research and development projects.
  - These oligos were used as probes for screening cloned genes, as primers for DNA sequencing and cDNA synthesis, and as building blocks for gene construction.
  - Realizing the difficulty in making the Sanger method specific to a single location in the genome, Mullis then modified the idea to add a **second primer on the opposite strand**.
  - Repeated applications - **PCR**.



- Later in **1983 Mullis** began to test his idea. His first experiment did not involve thermal cycling - he hoped that the polymerase could perform continued replication on its own.
  - Later experiments that year included repeated thermal cycling, and targeted small segments of a cloned gene.
  - Mullis considered these experiments a success, but could not convince other researchers.
- In June **1984 Cetus** held its annual meeting in Monterey, California.
  - Its scientists and consultants presented their results, and considered future projects.
  - Mullis presented a poster on the production of oligonucleotides by his laboratory, and presented some of the results from his experiments with **PCR**. Only Joshua Lederberg, a Cetus consultant, showed any interest.
  - Later at the meeting, Mullis was involved in a physical altercation with another Cetus researcher, over a dispute unrelated to PCR. The other scientist left the company, and Mullis was removed as head of the oligo synthesis lab.

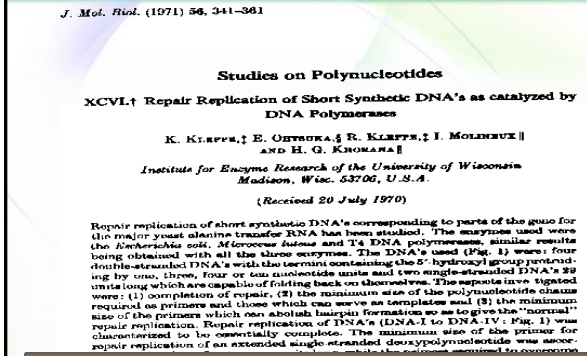
PCR – a Norwegian invention?

Dr. Kjell Kleppe

H.G. Khorana

"Repair replication"

Study on polynucleotides: repair replication of short synthetic DNA's as catalyzed by DNA polymerases. J Mol Biol, 1971, **56**:341



J. Mol. Biol. (1971) 56, 341–361

Studies on Polynucleotides

XCVI.† Repair Replication of Short Synthetic DNA's as catalyzed by DNA Polymerases

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(*Received 20 July 1970*)

Repair replication of short synthetic DNA's corresponding to parts of the gene for (in major yeast alanine transfer RNA has been studied. The enzymes used were (the *Escherichia coli*, *Micrococcus luteus* and T4 DNA polymerases, similar results being obtained with all the three enzymes. The DNA's used (Fig. 1) were: four double-stranded DNA's with the termini containing the 5'-hydroxyl group revealed ing by one, three, four or ten nucleotide units and two single-stranded DNA's 29 initiating which are capable of folding back on themselves. The aspects investigated were: (1) completion of repair, (2) the minimum size of the polynucleotide chains required as primers and those which can serve as templates and (3) the minimum size of the primers which can abolish hairpin formation so as to give the "normal" repair replication. Repair replication of DNA's (DNA-I to DNA-IV; Fig. 1) was characterized to be essentially complete. The minimum size of the primer for repair replication of an extended single stranded deoxypolynucleotide was accor-

The article from 1971 in which Kleppe et al. describe Repair replication – basically has the same principle as PCR!

J. Mol. Biol. (1971) **56** 341-361

Did Mullis Really Invent PCR?

- The basic principle of replicating a piece of DNA using two primers had already been described by Gobind Khorana in 1971:

  Kleppe *et al.* (1971) J. Mol. Biol. 56, 341-346.

- Progress was limited by primer synthesis and polymerase purification issues.
- Mullis properly exploited amplification.

- **Invented by Kary Mullis in 1983.**
- **First published account appeared in 1985.**
- **Awarded Nobel Prize for Chemistry in 1993.**

Applications of PCR

PCR is for Everyone

Detection of pathogens
Classification of organisms
Genotyping
Molecular archaeology
Mutagenesis
Mutation detection
Cancer research
Sequencing
Gene Expression
Drug discovery
Genetic matching
Genetic engineering
Prenatal diagnosis
De-extinction