Marine Genomics

Protocol

PCR
The Polymerase Chain Reaction

Polymerase Chain Reaction

Amplification of a Short DNA Stretch by Repeated Cycles of In Vitro DNA Polymerization

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Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase

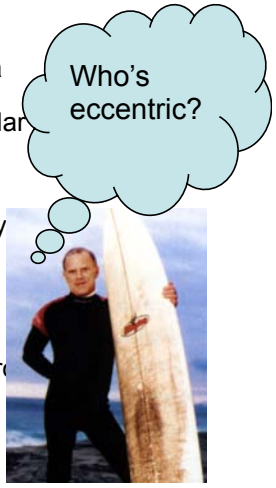
RK Saiki, DH Gelfand, S Stoffel, SJ Scharf, R Higuchi, GT Horn, KB Mullis, and HA Erlich
Cetus Corporation, Department of Human Genetics, Emeryville, CA 94608.

A **thermostable DNA polymerase** was used in an **in vitro DNA amplification procedure**, the polymerase chain reaction. The enzyme, isolated from Thermus aquaticus, greatly simplifies the procedure and, by enabling the amplification reaction to be performed at higher temperatures, significantly improves the specificity, yield, sensitivity, and length of products that can be amplified. **Single-copy genomic sequences were amplified by a factor of more than 10 million with very high specificity**, and DNA segments up to 2000 base pairs were readily amplified. In addition, the method was used to amplify and detect a target DNA molecule present only once in a sample of 10(5) cells.

✦ Mullis, K. B., and F. A. Faloona. "Specific synthesis of DNA in vitro via a polymerase catalysed chain reaction." *Methods in Enzymology* no. 155 (1987): 335-350.

What is the Polymerase Chain Reaction?

PCR is a method for performing DNA replication in a test tube.



Many, many copies of a small DNA sequence may be amplified from a few starting copies. Once the copies are made, the DNA may more easily be studied. For example, the nucleotide sequence of a particular gene can be determined.

Who uses PCR?:

- PCR is probably the most widely used technique in molecular biology
- This technique is used in biomedical research, criminal forensics, molecular archaeology.

Why is PCR so widely used?:

- It allows you to specifically amplify a single gene or DNA sequence fr a very small tissue sample (even a single cell).

Who invented PCR? When was it invented?

- PCR was invented by Kary Mullis (photo right) in 1983.
- Mullis was an employee of the Cetus Corporation.
- He won the Nobel Prize in Chemistry in 1993.
- Since then, he "has become ever more eccentric," according to James D. Watson in *DNA. The Secret of Life.* (*In my opinion, Mr. Watson knows a thing or two about being eccentric*.)

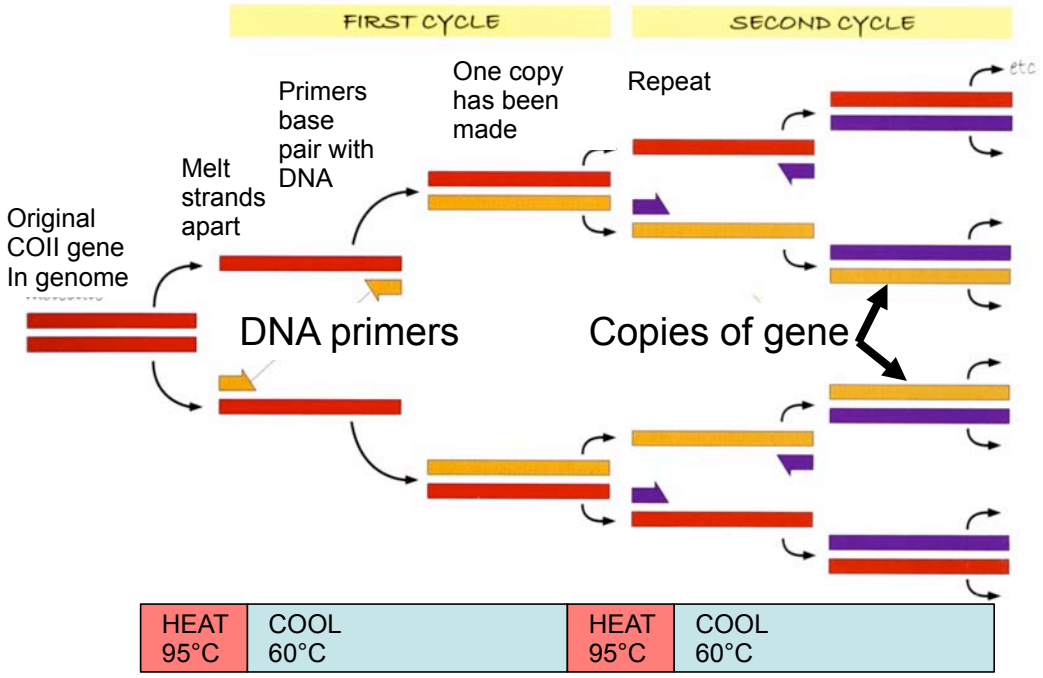
PCR: Polymerase Chain Reaction

How does it work?

- PCR is an *in vitro* process (occurring in a test tube) that imitates the *in vivo* process (occurring in a living cell) of DNA replication.
- There are some similarities and some differences between PCR and DNA replication.

	DNA replication (in vivo)	**PCR (in vitro)**
What does it accomplish?	Copies the whole genome once.	Makes 2^{30}- 2^{40} copies of one gene.
STEPS OF REPLICATION	**HOW IS IT ACCOMPLISHED?**	**HOW IS IT ACCOMPLISHED?**
Step 1. Open up double strand of DNA helix.	Enzyme called **helicase.**	Melt strands apart (heat test tube to near boiling; >90°C)
Step 2. "Prime" the copying process by annealing a small primer to the exposed bases.	Millions of different **RNA primers** produced by **primase -** bind throughout the genome.	**1 specific pair of DNA primers** devised by scientist to match one particular gene.
Step 3. Copy DNA using an enzyme to catalyze the polymerization of individual nucleotide bases.	DNA polymerase + A, C, G, T	DNA polymerase (from hot-spring extremophile). + A, C, G, T.
Is that it?	That's essentially it!	Repeat process 30-40 times, each time doubling the number of copies.

PCR: Polymerase Chain Reaction



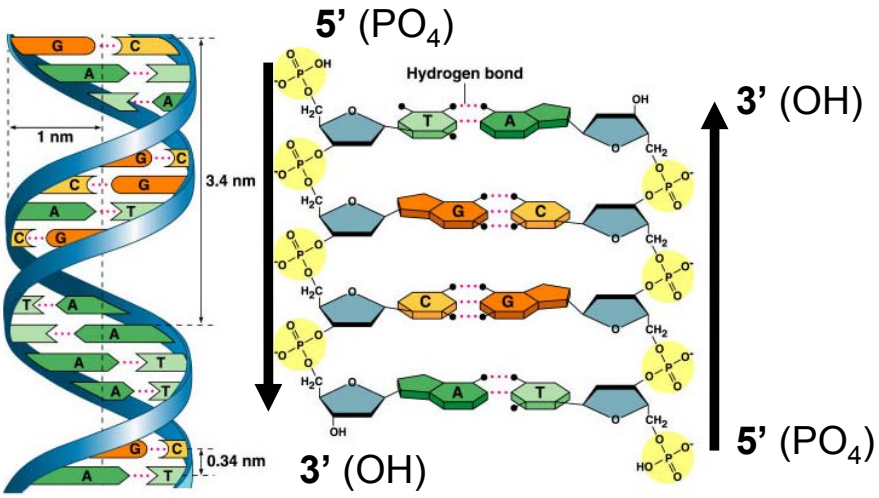
Rules of DNA Replication (*in vivo* and *in vitro*)

Base Pairing

- Two simple rules ensure that the newly copied strand has the same sequence as the original strand that it displaced. (1) **A** pairs with **T** via two hydrogen bonds (2) **G** pairs with **C** via three hydrogen bonds.

Directionality of DNA Replication

- Also, remember that DNA replication proceeds from 5' to 3' on each strand, and that the two strands are anti-parallel, so replication is headed in opposite directions on either strand.



Assembling the PCR reaction

For each 20-microliter PCR reaction, in a separate 200-microliter microcentrifuge tube, assemble the following reagents:

Reagents	Stock concentration	Final amount or concentration	Volume per 20-microliter reaction
Reaction buffer	10X	1X	2.0 microliters (µL)
dNTPs	2 mM (10X)	200 µM	2.0 microliters (µL)
thermostable polymerase	5 units / µL	1 unit	0.2 microliters (µL)
forward primer	10 picomoles / µL	20 picomoles	2.0 microliters (µL)
reverse primer	10 picomoles / µL	20 picomoles	2.0 microliters (µL)
template DNA	10-500 nanograms / µL	100-500 ng	1-11.8 microliters (µL)

Troubleshooting PCR

Amplification of the wrong product	**Annealing temperature is too low.**	Check primer melting temperature. Set annealing temperature to Tm-5C°
	Mispriming at the correct annealing temperature	Check that primers don't match to multiple sequences in the genome.
	Too much dNTPs	Reduce dNTPs to 25-50 µM

No Product	**Annealing temperature is too high.**	Check primer melting temperature. Set annealing temperature to Tm-5C°
	Poor primer design	Verify that primer sequence is correct.
	Missing reagent or not enough of a reagent	Be sure that you are using 20 picomoles of each primer.
	Too few cycles of PCR.	Increase cycle number up to 40.