Polymerase Chain Rection

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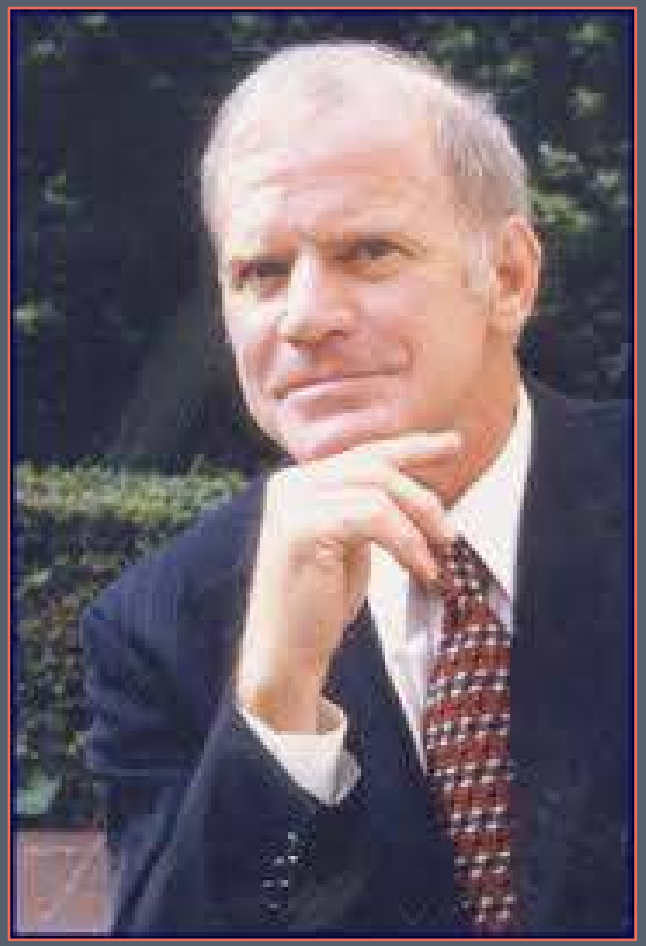
- The polymerase chain reaction (PCR) is a technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence.

Invent By

- Developed in 1983 by Kary Mullis
- In 1993, Mullis was awarded the Nobel Prize in Chemistryalong with Michael Smith for his work on PCR



If the K-T boundary isotopic spike is indeed the result of impact-related acid rain, the oceanic strontium isotope record may reveal other large impacts. The seawater strontium curve of Burke *et al.* (*9*), which spans the past 500 million years, shows at least two other prominent high spikes in the $^{87}Sr/^{86}Sr$ ratio, one in the mid-Cretaceous, at ~100 million years, and the other in the Pennsylvanian, at ~290 million years. The first appears to precede by a few million years the mass extinction event at the Cenomanian-Turonian boundary. There is also a large increase in $^{87}Sr/^{86}Sr$ across the Permian-Triassic boundary (*9*), the time of the most extreme mass extinction in the Phanerozoic record (*17*). However, the increase appears to be rather gradual, extending over 20 million to 25 million years, and is thus quite different in character from the K-T spike. Nevertheless, data are sparse for this interval, and more work will be required to determine the exact nature of the increase.

The occurrence of a spike toward higher values in the seawater $^{87}Sr/^{86}Sr$ record at the K-T boundary is tantalizing evidence for enhanced continental weathering, possibly due to impact-related acid rain. Detailed strontium isotopic studies through this and other intervals where such spikes appear are required to determine precisely the nature of the isotopic variations with respect to stratigraphy, and particularly with respect to

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

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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.

THE ANALYSIS OF SPECIFIC NUCLEOTIDE sequences, like many analytic procedures, is often hampered by the presence of extraneous material or by the extremely small amounts available for examination. We have recently described a meth-

fragment, approximately 2^n, where n is the number of cycles.

One of the drawbacks of the method, however, is the thermolability of the Klenow fragment of *Escherichia coli* DNA polymerase I used to catalyze the extension

Principle

- The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA.
- Primers(short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase, which the method is named after, are key components to enable selective and repeated amplification.

Principle Cont . . .

- As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified.
- PCR can be extensively modified to perform a wide array of genetic manipulations.

PCR Components

- Almost all PCR applications employ a heat-stable DNA polymerase, such as Taq polymerase (an enzyme originally isolated from the bacterium *Thermus aquaticus*).
- This DNA polymerase enzymatically assembles a new DNA strand from DNA building-blocks, the nucleotides, by using single-stranded DNA as a template and DNA oligonucleotides (also called DNA primers), which are required for initiation of DNA synthesis

PCR Components Cont . . .

- DNA template - that contains the DNA region (target) to amplify
- Two primers - that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strand of the DNA target
- Taq polymerase - with a temperature optimum at around 70 °C
- Deoxynucleoside triphosphates (dNTPs,) - sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups, the building-blocks from which the DNA polymerase synthesizes a new DNA strand

PCR Components Cont . . .

- Buffer solution - providing a suitable chemical environment for optimum activity and stability of the DNA polymerase
- Bivalent cations - magnesium or manganese ions; generally Mg^{2+} is used, but Mn^{2+} can be used for PCR-mediated DNA mutagenesis, as higher Mn^{2+} concentration increases the error rate during DNA synthesis.

Procedure

- The PCR is commonly carried out in a reaction volume of 10–200 µl in small reaction tubes (0.2–0.5 ml volumes) in a thermal cycler.
- The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction.

Denaturation step

- This step is the first regular cycling event and consists of heating the reaction to 94–98 °C for 20–30 seconds. It causes DNA melting of the DNA template by disrupting the hydrogen bonds between complementary bases, yielding single-stranded DNA molecules.

Annealing step

- The reaction temperature is lowered to 50–65 °C for 20–40 seconds allowing annealing of the primers to the single-stranded DNA template.
- This temperature must be low enough to allow for hybridization of the primer to the strand.

Annealing step . . .

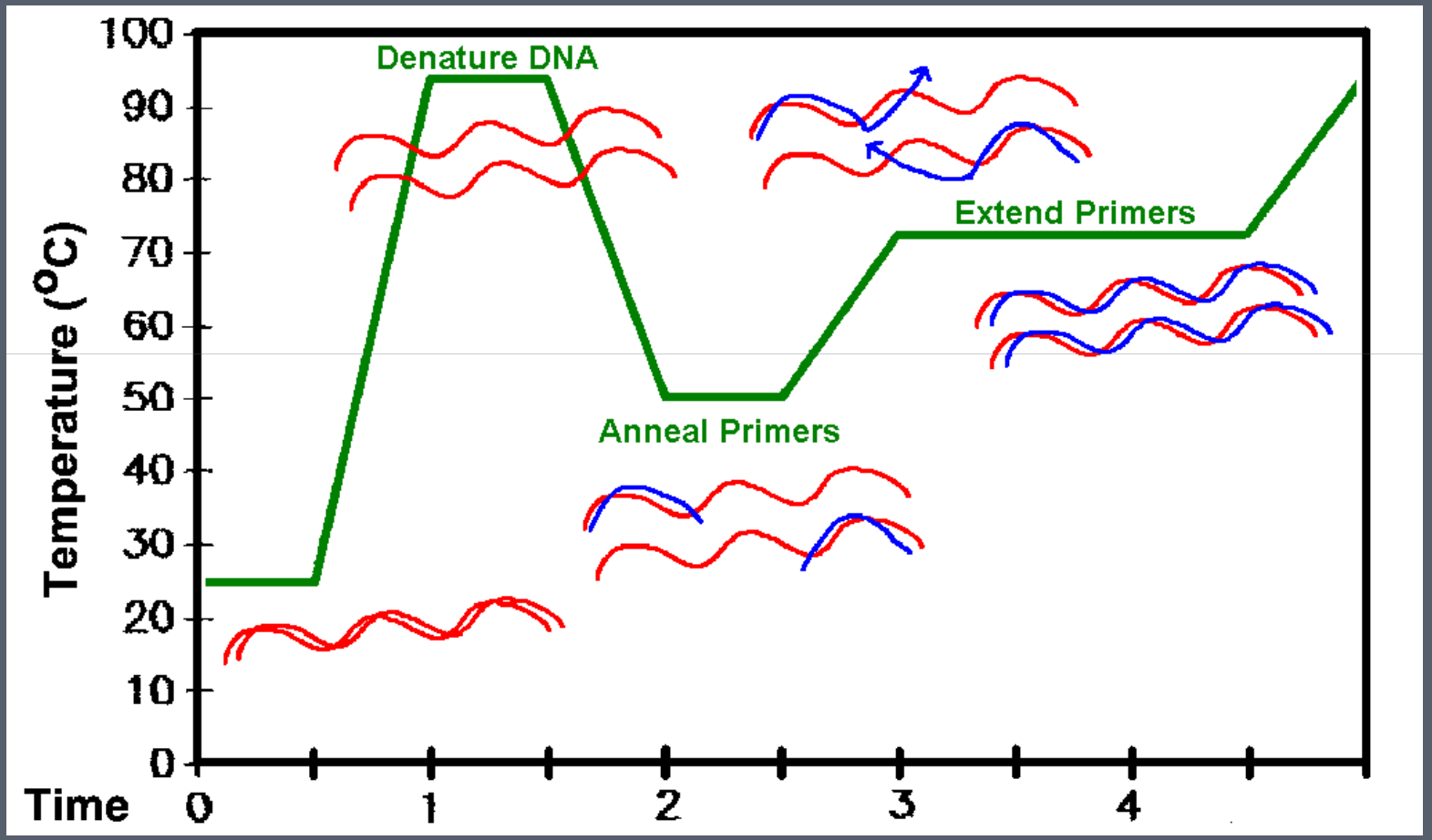
- If the temperature is too low, the primer could bind imperfectly. If it is too high, the primer might not bind.
- Typically the annealing temperature is about 3–5 °C below the Tm of the primers used. Stable DNA–DNA hydrogen bonds are only formed when the primer sequence very closely matches the template sequence.

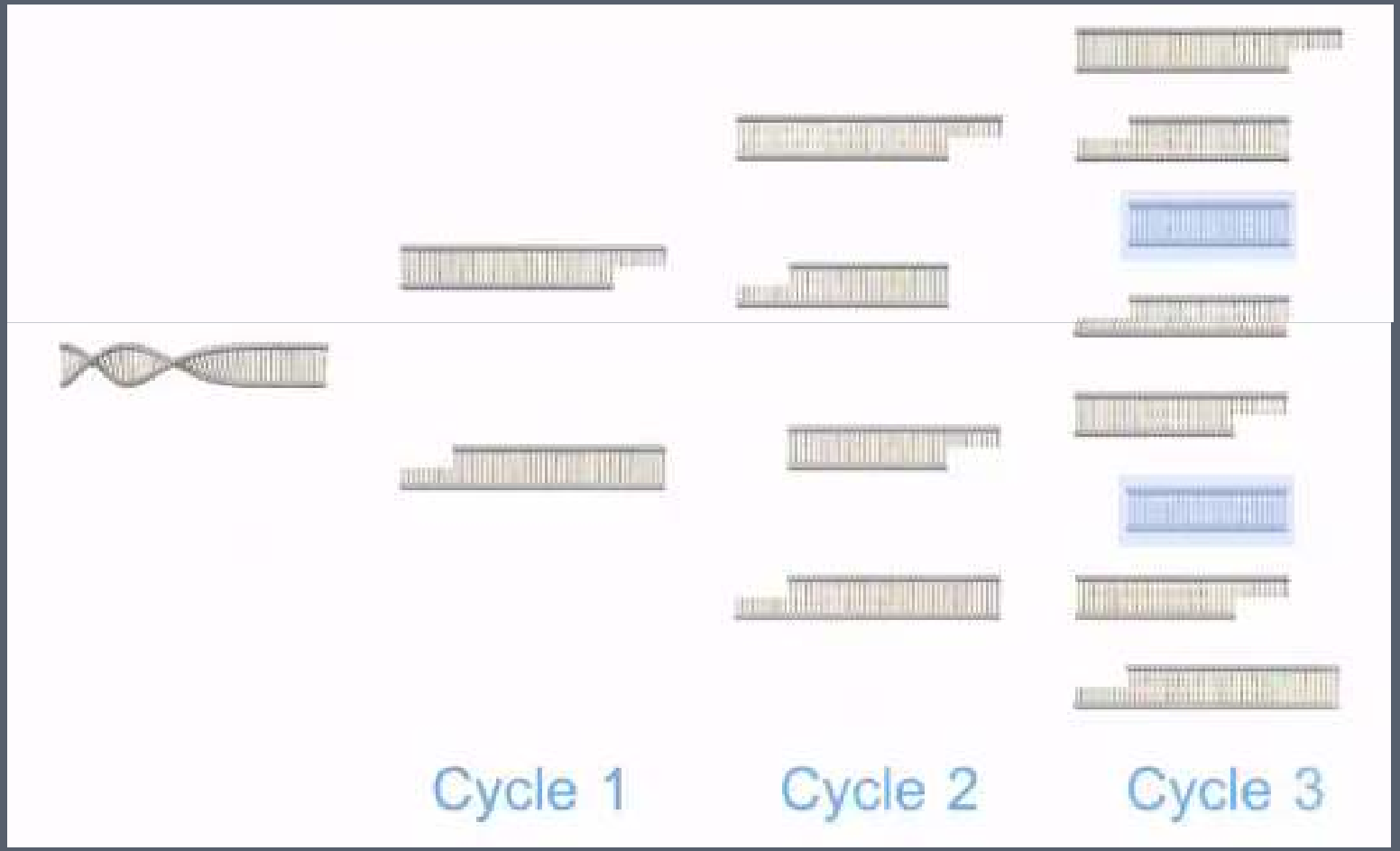
Extension/elongation step

- The temperature at this step depends on the DNA polymerase used; Taq polymerase has its optimum activity temperature at 75–80 °C, and commonly a temperature of 72 °C is used with this enzyme.

Extension/elongation step . . .

- At this step the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding dNTPs that are complementary to the template in 5′ to 3′ direction, condensing the 5′-phosphate group of the dNTPs with the 3′-hydroxyl group at the end of the nascent (extending) DNA strand.
- The extension time depends both on the DNA polymerase used and on the length of the DNA fragment to amplify.


Denature DNA
Anneal Primers
Extend Primers
Temperature (°C)
100
90
80
70
60
50
40
30
20
10
0
Time
0
1
2
3
4


Cycle 1
Cycle 2
Cycle 3

Final hold

- This step at 4–15 °C for an indefinite time may be employed for short-term storage of the reaction.

Polymerase chain reaction (PCR)

Thankyou

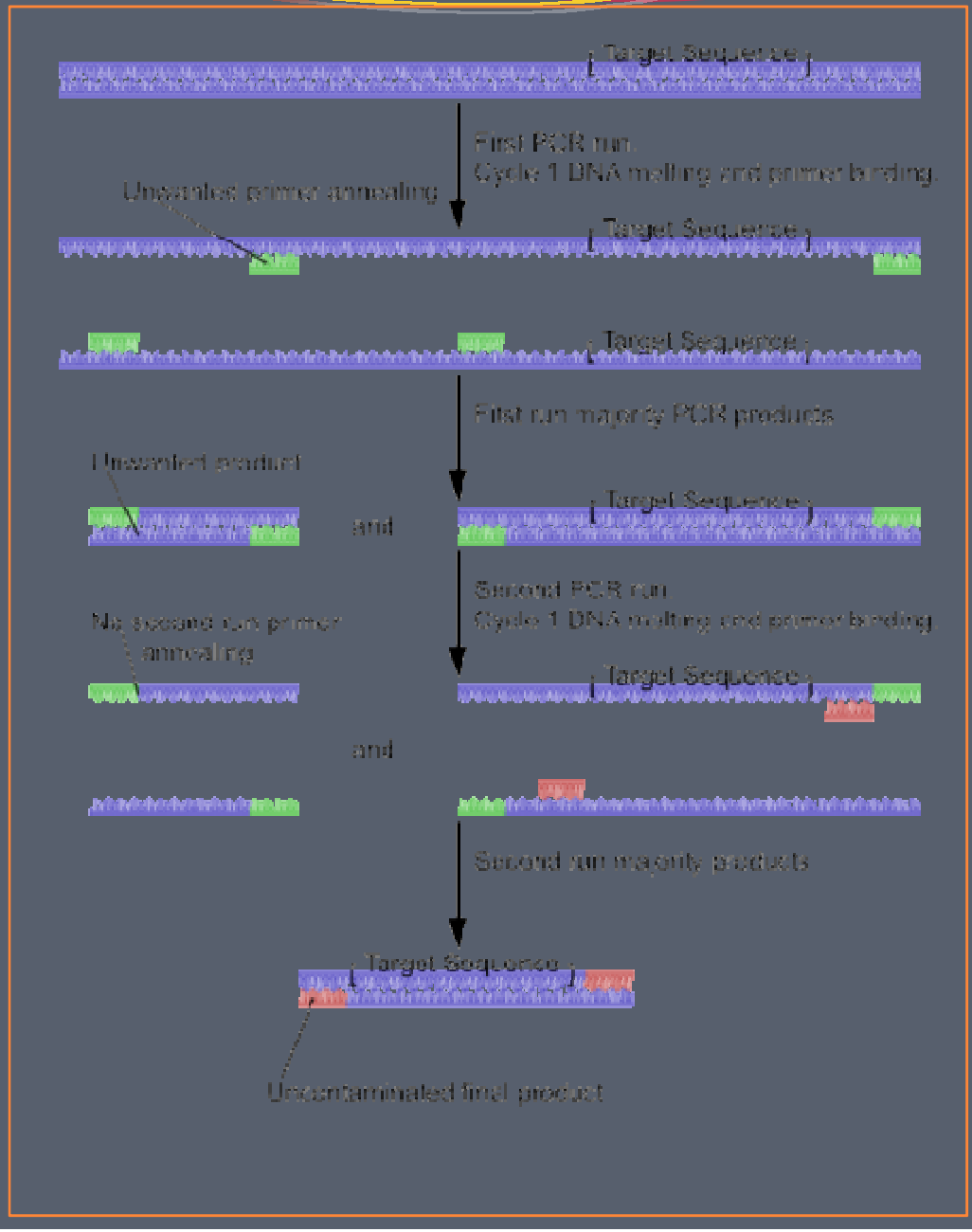
Types of PCR

- Nested PCR
- Multiplex PCR
- Inverse PCR
- RT-PCR
- Real Time PCR
- Touch Down/Step down PCR
-

Nested PCR

- Nested PCR is used to increase the specificity of DNA amplification. Two sets of primers are used in two successive reactions.
- In the first PCR, one pair of primers is used to generate DNA products, which may contain target DNA.
- The products from the first PCR are then used as template in a second PCR, using another primers whose binding sites are located (nested) within the first set, thus increasing specificity.

- Nested PCR is often more successful in specifically amplifying long DNA products than conventional PCR.



Multiplex PCR

- Multiplex-PCR uses several pairs of primers annealing to different target sequences.
- This permits the simultaneous analysis of multiple targets in a single sample.
- For example, in testing for genetic mutations, six or more amplifications might be combined.
- Multiplex PCR has also been used for analysis of microsatellites and SNPs.

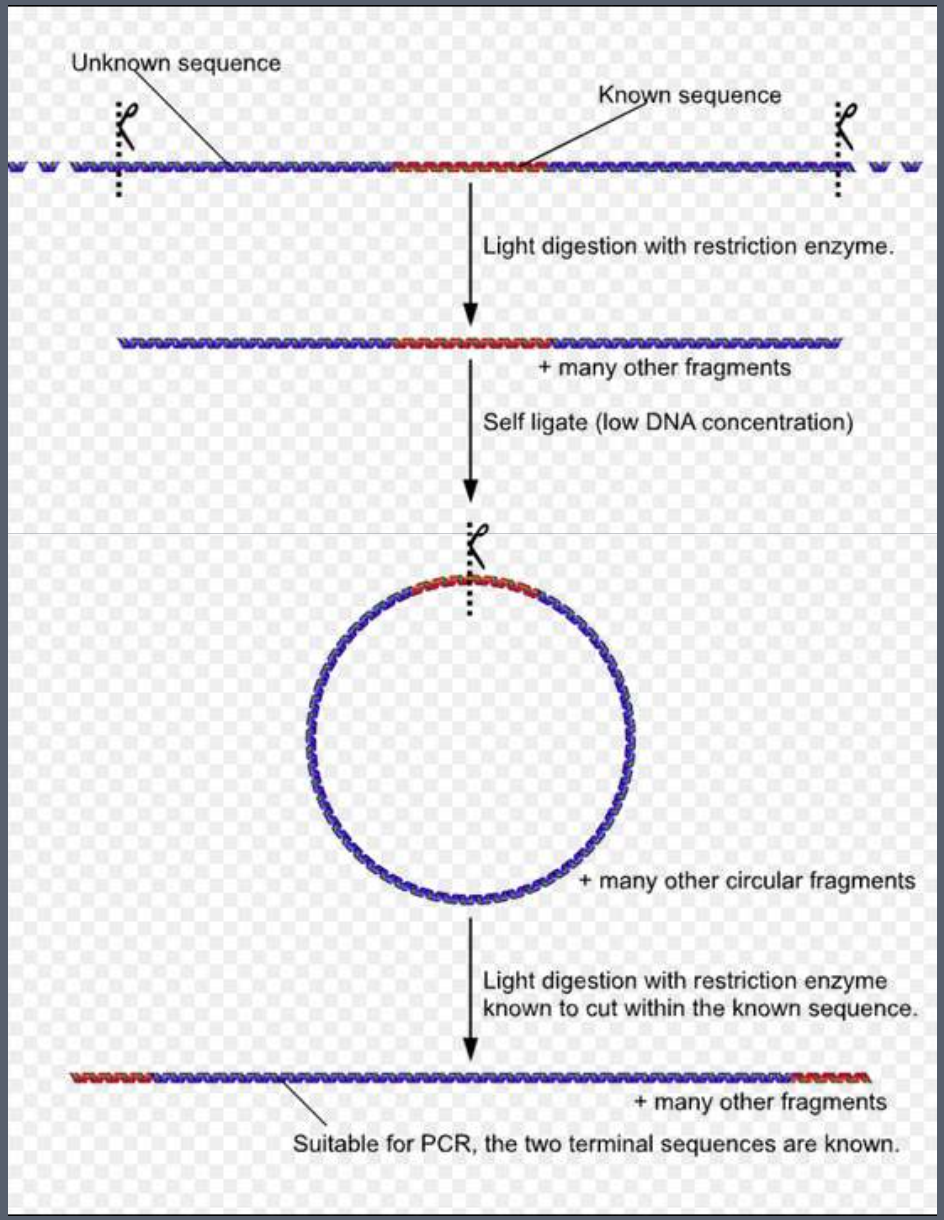
Touch Down PCR

- It is used to reduce non specific binding by changing annealing temperature above and below that allow greater specificity of primer binding and more efficient amplification.

Inverse PCR

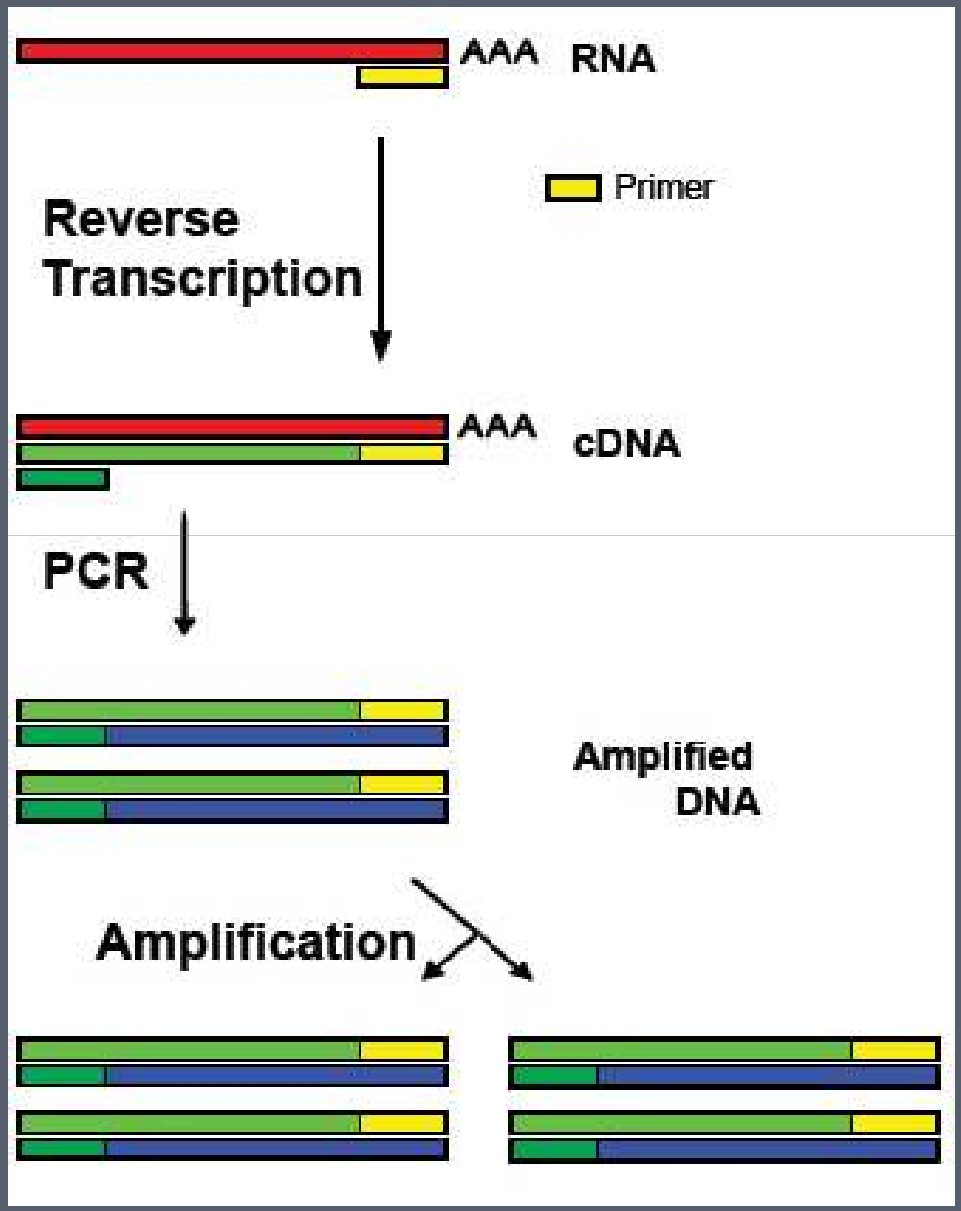
- Inverse polymerase chain reaction (Inverse PCR) is a variant of the polymerase chain reaction that is used to amplify DNA with only one known sequence.
- One limitation of conventional PCR is that it requires primers complementary to both termini of the target DNA.

- Inverse PCR is especially useful for the determination of insert locations.
- For example, various retro viruses and transposons randomly integrate into genomic DNA.
- To identify the sites where they have entered, the known, "internal" viral or transposon sequences can be used to design primers that will amplify a small portion of the flanking, "external" genomic DNA.
- The amplified product can then be sequenced and compared with DNA databases to locate the sequence which has been disrupted.


Unknown sequence
Known sequence
Light digestion with restriction enzyme.
+ many other fragments
Self ligate (low DNA concentration)
+ many other circular fragments
Light digestion with restriction enzyme
known to cut within the known sequence.
+ many other fragments
Suitable for PCR, the two terminal sequences are known.

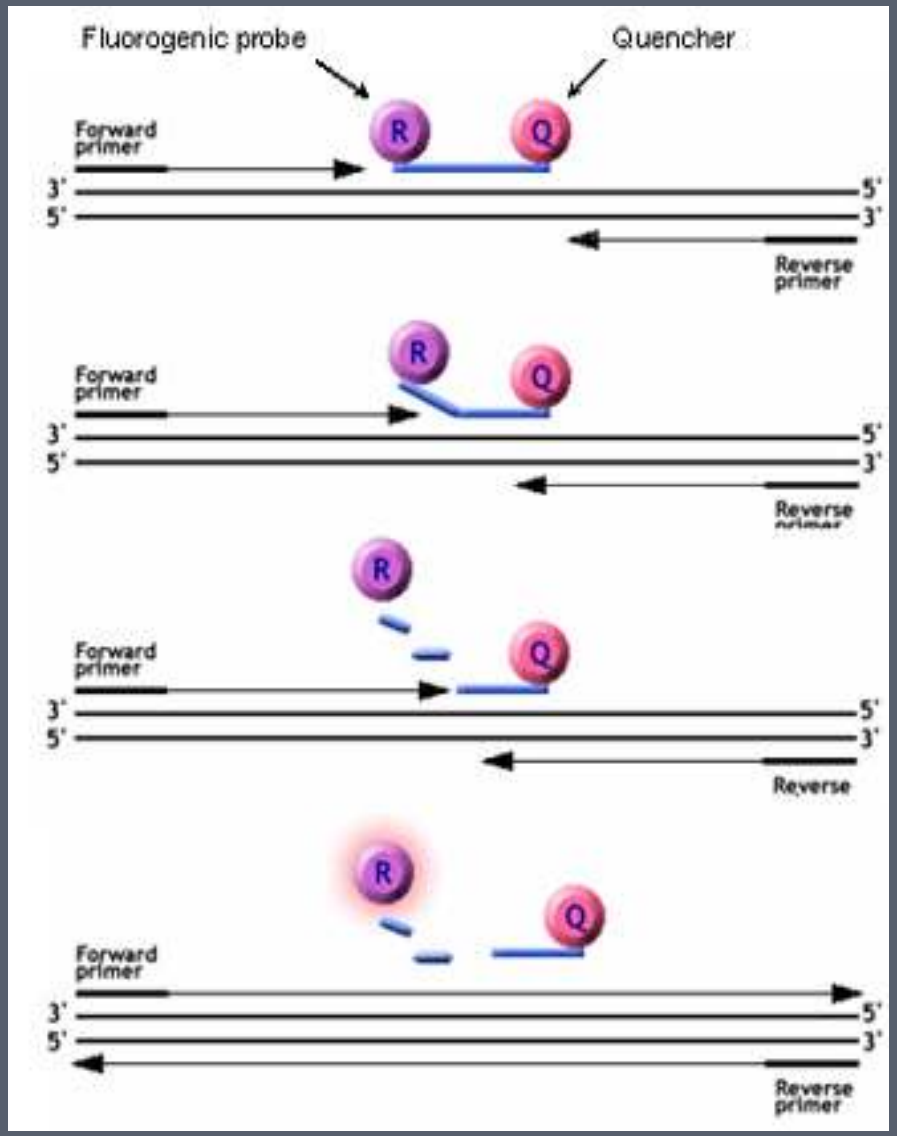
RT-PCR

- It used to amplify m-RNA
- It uses reverse transcriptase enzyme to convert mRNA to cDNA.
- Then cDNA is amplify by Taq Polymerase using normal PCR process.
- It is used to diagnosis of RNA virus, mRNA expression.

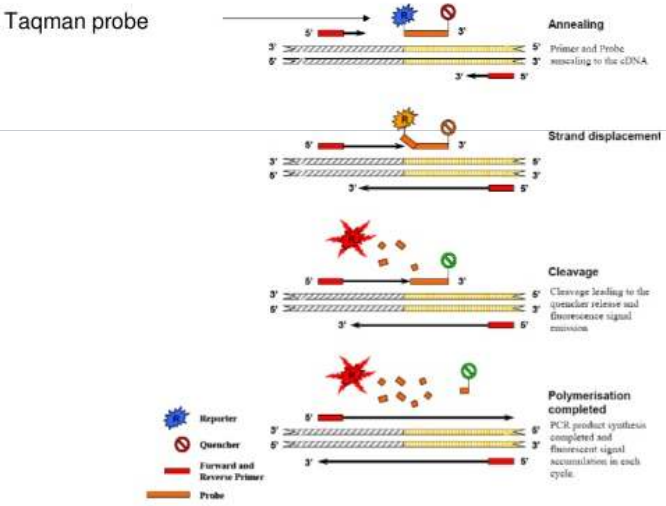

AAA
RNA
Primer
Reverse
Transcription
AAA
cDNA
PCR
Amplified
DNA
Amplification

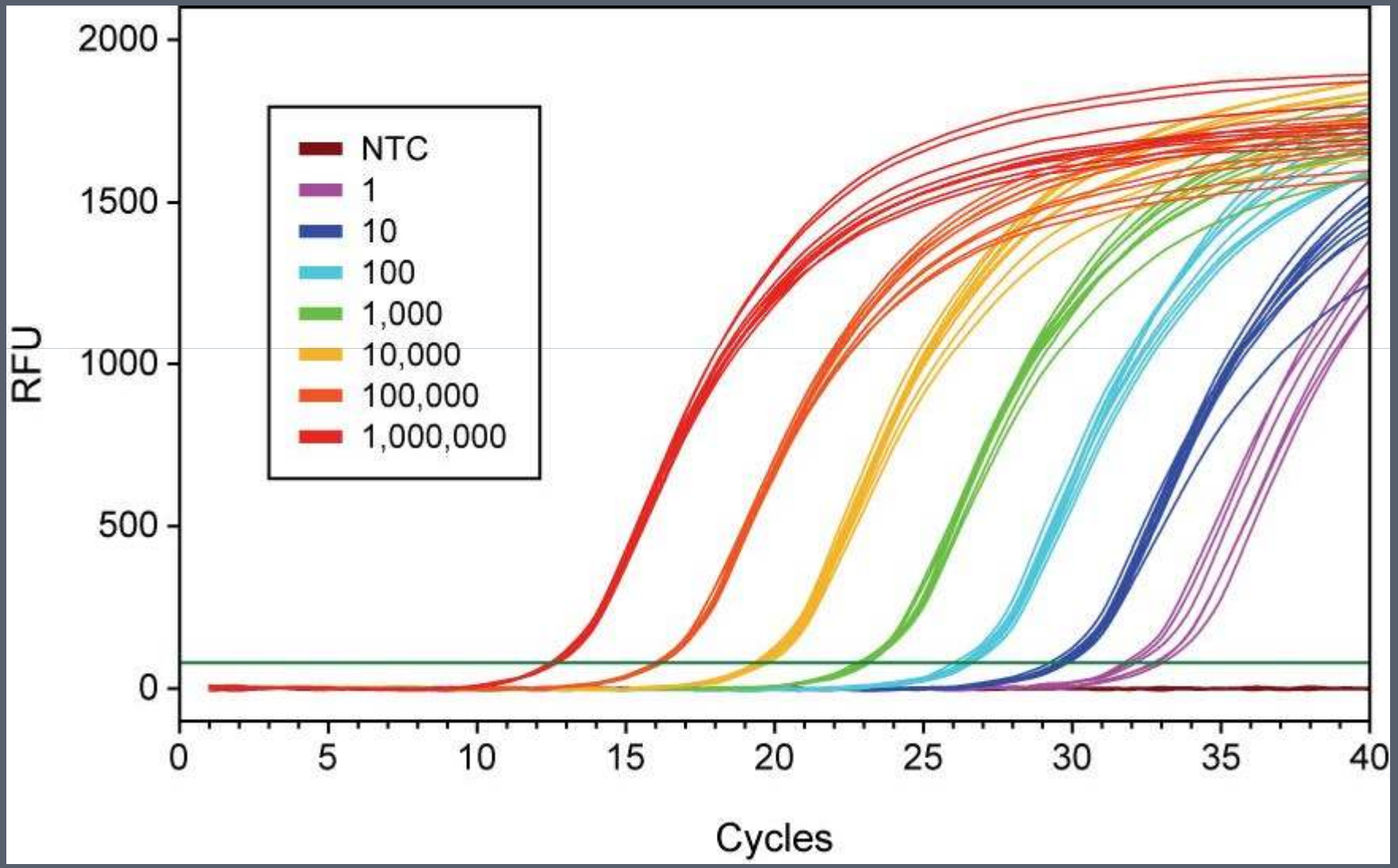
Real time PCR

- A real-time polymerase chain reaction or quantitative polymerase chain reaction is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR), which is used to amplify and simultaneously detect or quantify a targeted DNA molecule.



Quantitative real-time-PCR




2000
1500
1000
500
0
RFU
NTC
1
10
100
1,000
10,000
100,000
1,000,000
0
5
10
15
20
25
30
35
40
Cycles

Thankyou