INTRODUCTION AND TYPES OF PCR (THERMAL CYCLERS)

Introduction:

PCR stands for polymerase chain reaction and it is the key mechanism of thermal cyclers(PCR cyclers). This Technique was invented by Kary Mullis in 1985 while working as a chemist at Cetus Corporation(a biotechnology firm in Emeryville, California).

In 1993, Michael Smith and Kary Mullis was awarded by Nobel prize in Chemistry. The contribution of each scientist was crucial for molecular Biology research and development . Michael Smith was awarded for his fundamental contributions to the establishment of oligonucleotide-based, site-directed mutagenesis and its development for protein studies and Kary Mullis for PCR technique invention.

The journey of modern PCR technique begins in 1976 with the isolation of Taq polymerase enzyme from the thermophilic bacterium *Thermus aquaticus* offered the development of modern PCR technique. *Thermus aquaticus* was identified from Yellowstone National Park in Montana, USA. The discovery of Taq polymerase boost the molecular research in life science field. Taq polymerase increased the efficiency of PCR cyclers (thermal cyclers) because it was thrmostable DNA polymerase and that was capable of repeat PCR cycling without the need to add fresh DNA polymerase after each cycle. Taq polymerase is still in use.

PCR steps :

Steps	Temperature
Initial Denaturation	94°C
Denaturation	94°C
Primer Annealing**	T_m (melting temperature) – (5-10°C)**
Extension	72°C
Final Extension	72°C

Remark: (Primer Annealing**) The actual annealing temperature gradient should start with temperature 5–10 °C lower than annealing temperature. The variability in primer annealing temperature greatly affected by GC content and length of DNA stretch.

Basic components of PCR:

Distilled water	**Forward primer**
PCR buffer	Reverse primer
Taq polymerase	$MgCl_2$
Mixed dNTPs	Template

- **Online Tools for Primer design:** https://www.ncbi.nlm.nih.gov/tools/primer-blast/
  https://tmcalculator.neb.com/#!/main
  https://www.genscript.com/tools/pcr-primers-designer

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Different types of PCR techniques :

Traditional PCR	Multiplex PCR	Methylation- specific PCR	Colony PCR
Conventional PCR	Nested –seminested PCR	In-silico PCR	Dial-out PCR
Qualitative PCR	RT-PCR	Inter sequence PCR	Digital PCR
Semiquantative PCR	Touchdown PCR	Ligation-mediated PCR	Standard PCR
Quantitative PCR	Inverse PCR	Miniprimer PCR	
Solid phase PCR	Allele specific PCR	Suicide PCR	
Assembly PCR	Arbitrary PCR	Nano particle PCR	
Thermal asymmetric interplaced PCR	Asymmetric PCR	Core sample PCR	
Overlap-extension PCR	Hot start PCR	Degenerate PCR	

Applications of PCR :

Gene cloning	Molecular archaeology
Gene therapy	Molecular ecology
Site- directed mutagenesis	Molecular epidemiology
Bioinformatics	DNA fingerprinting
Gene expression studies	Genetic matching
Drug discovery	Prenatal diagnosis
Mutation screening	Detection of pathogens (Virus, Bacteria etc.)
Genotyping	
Molecular classification of organisms	

Key statements:

a. PCR helps in amplification of RNA/DNA molecules up to millions of copies of the same.
b. PCR mimics the mechanism of natural Replication of DNA.
c. *Both Taq polymerase* from *Thermus aquaticus* and *Pfu* from *Pyrococcus furiosus* are thermostable DNA polymerase.
d. The product (DNA/RNA) of PCR is called amplicon.

PCR formula:

$N = N_0 \times 2^n$ (1) Where N is molecules of amplicons to be generated

N_0 is the template DNA molecules

n = Numbers of PCR cycles

Statement: A 200 µl of polymerase are chain reaction has 100 template DNA molecules and the reaction was performed for 10 cycles. [GATE 2011]

Q.1. How many molecules of amplicons will be generated?

A. 1.024×10^4
B. 1.024×10^5
C. 1.024×10^4
D. 1.024×10^5

Solution: Use equation no. 1 (as mentioned above)

$N = N_0 X 2^n$

Here N_0 = 100 template DNA molecule

n = 10 cycles

$N = 100 \times 2^{10}$

$= 100 \times 1024$

$= 1.024 \times 10^5$ amplicons (answer)

Q.2. How many molecules of amplicons will be present in 0.1µl of reaction?

Solution: From previous calculation 200 µl mixture after reaction has 1.024×10^5 amplicons

200 µl…….. 1.024×10^5 amplicons

1 µl…….. 1.024×10^5 amplicons/ 200

0.1 µl…….. 1.024×10^5 amplicons/ 200 * 0.1 = 51.2 amplicons (answer)

References:

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Remarks: For academic purpose only (revision notes for Biotech students)