Primer Designing

Primer

An elementary book for teaching children to read

A segment of DNA or RNA that is complementary to a given DNA sequence and that is needed to initiate replication by DNA polymerase

Why should we design primer?

Stephen Andrew Bustin
British scientist
Professor of molecular sciences

“For PCR the primers are what the tires are for a car”

Amplicon

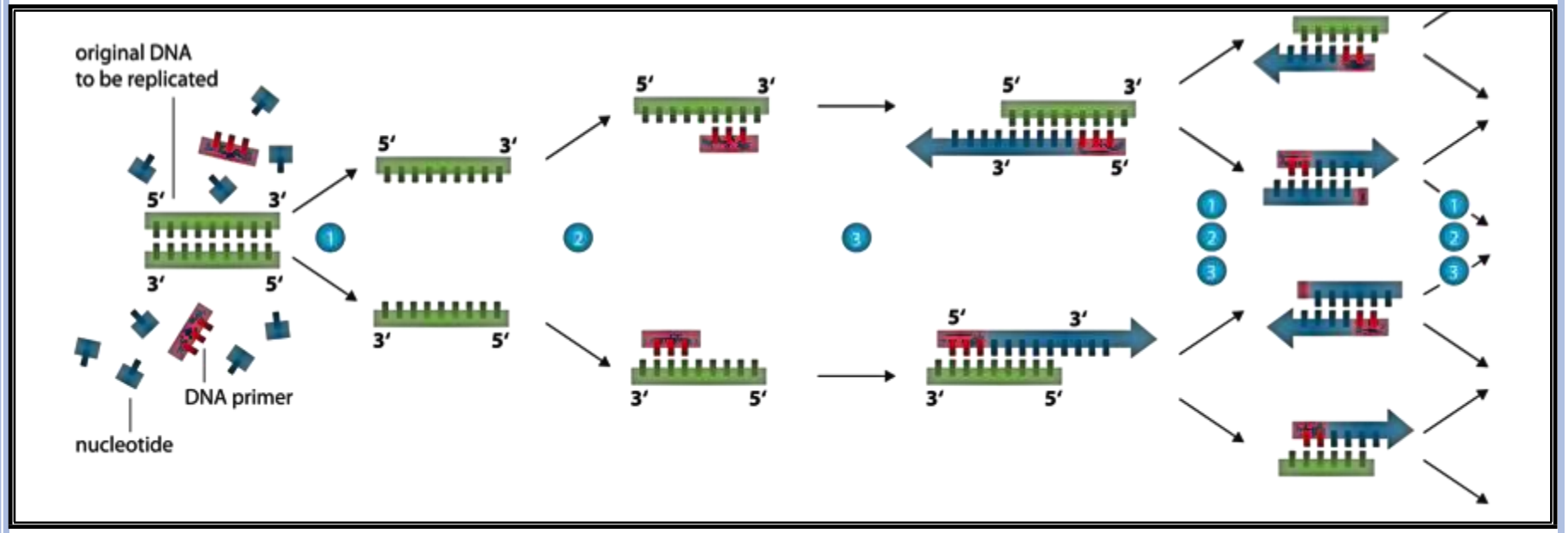
Any PCR amplification product

OR

A segment of DNA to be amplified (e.g., in PCR)

OR

Any fragment of replicating DNA produced by n atural or artificial amplification events


original DNA
to be replicated
5′
3′
3′
5′
DNA primer
nucleotide
1
2
3

General rules for primer design

1. Primer length

Primer length determines the specificity and significantly affect its annealing to the template

- **Too short- low specificity, resulting in non-specific amplification**
- **Too long- decrease the template-binding efficiency**

❖ **Optimal primer length**

- **18-30**

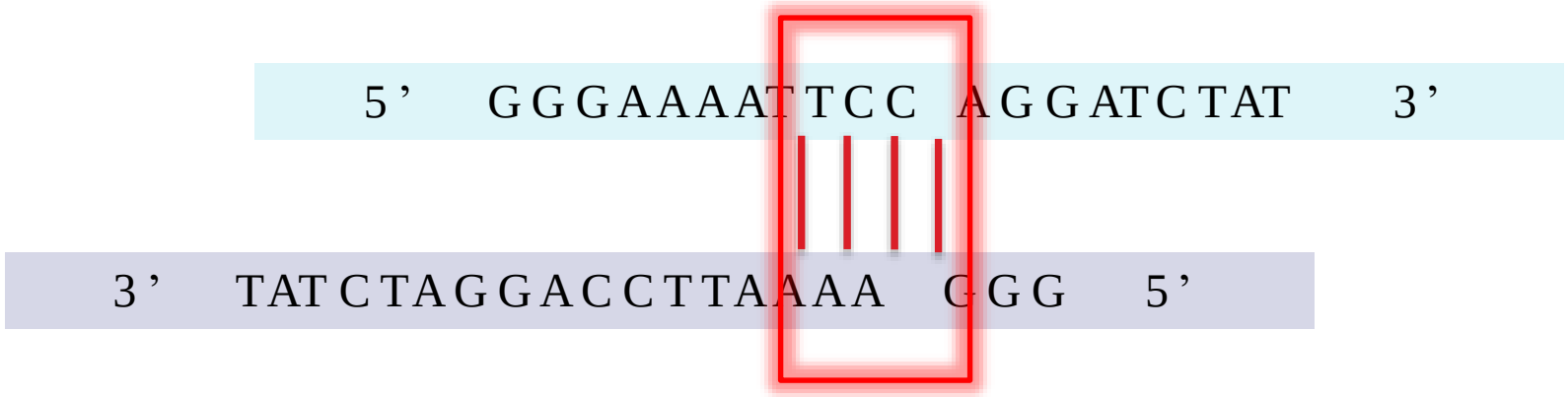
2. Melting temperature (Tm)

Melting Temperature (T_m) is the temperature at which one half of the DNA duplex will dissociate to become single stranded

- **Optimal melting temprature**
  - **55°C - 65°C**
- **Temparature difference**
  - **Not more than 5°C**

3. PRIMER DIMER

Primer dimer consists of primer molecules (forward and reverse primer) that have attached to each other because of strings of complementary bases in the primers



4. GC CONTENT

- **More GC- primer fail to bind with target sequence**
- **More AT- unstable primer**

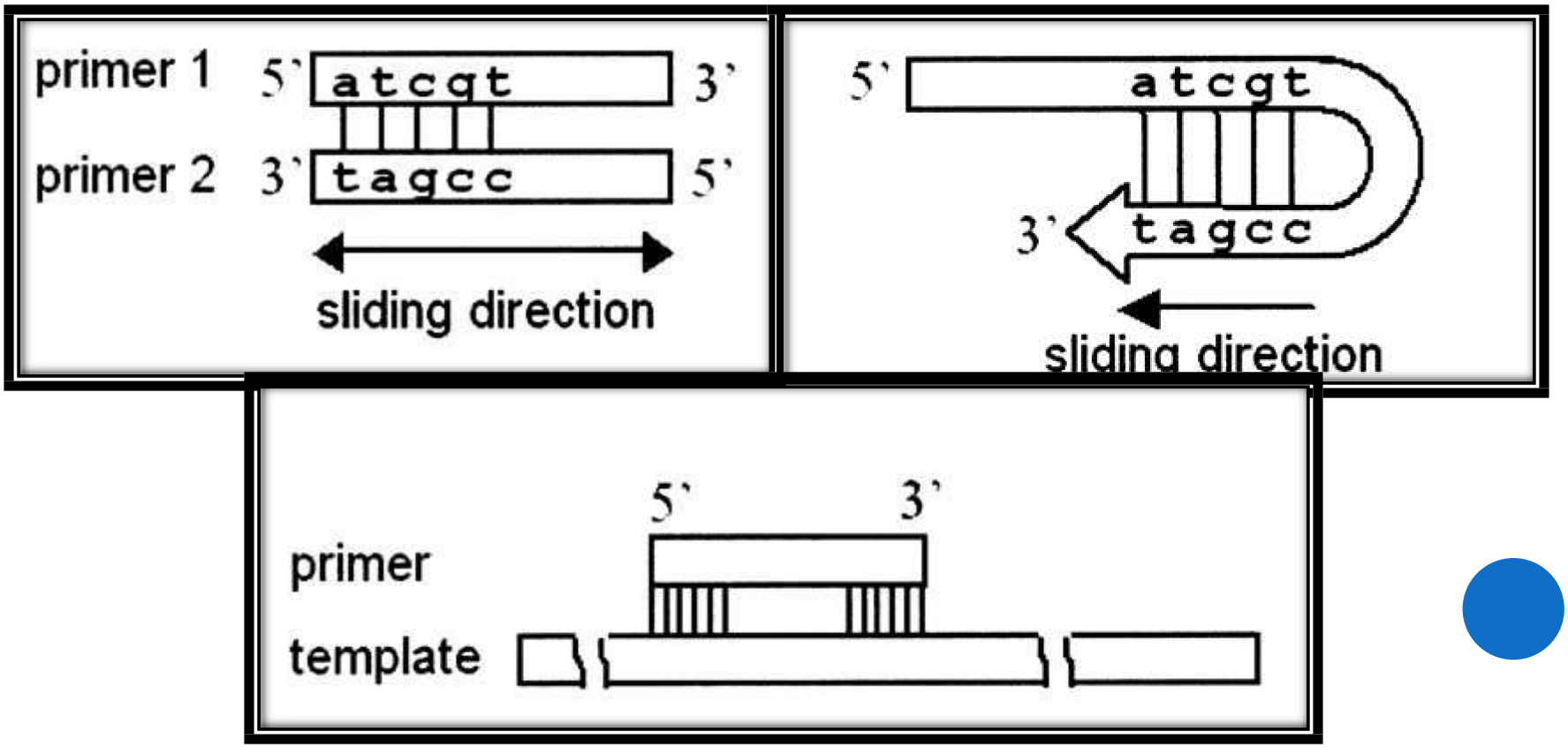
❖ **Optimal GC content**

- **40%-60%**

RULES FOR PRIMER DESIGN

5. Runs and repeat

Runs	5’	GGGAAAATTCCAGGATCTAT 3’
Repeat	3’	TATCTAGAGAGATATAGGG 5’



6. Distance between primers (Product size)

- **Minimum- 150 bp**
- **Maximum- 10 kbp**

- **More than 10 kbp- Desired product may not be obtained**

General rules for primer design

7. Secondary structures