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Primer Design

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Lab-4

Why are primers important?

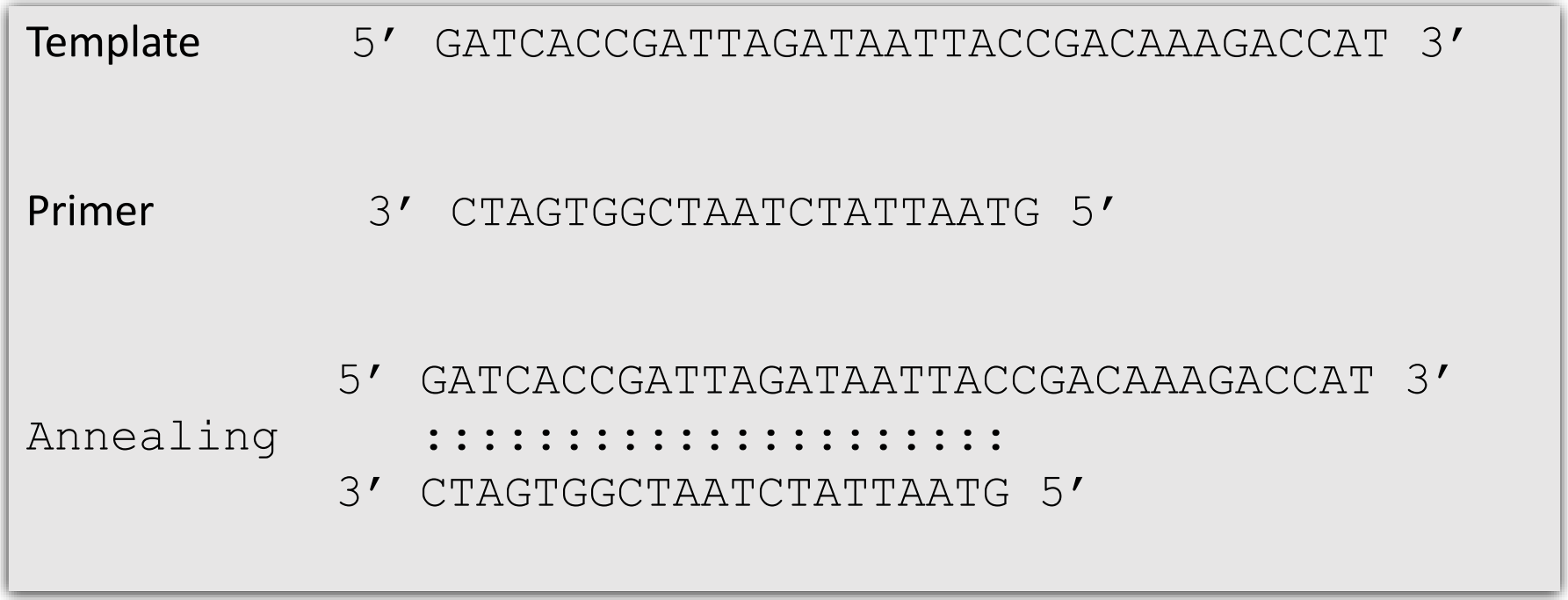
- Primer is one of the PCR or DNA sequencing reagents.
- Good primer design: PCR works great and the result is great.
- Bad primer design: PCR works terribly and the result is rubbish.

PCR reagents

- PCR buffer
- dNTP Mix
- Taq DNA polymerase
- **Primer**
- Template
- DDW

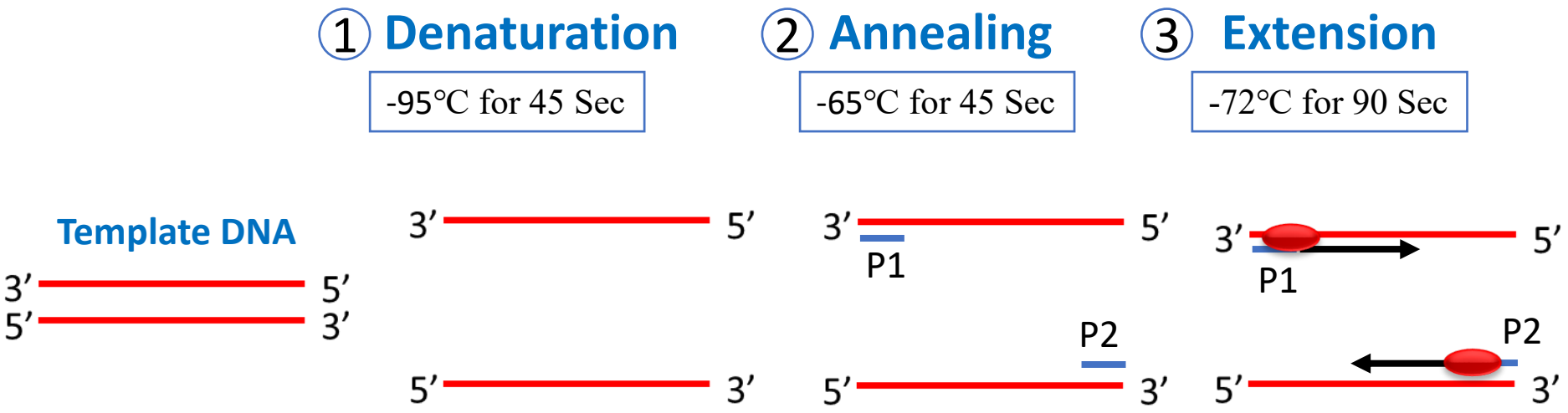
What is Primer?

Primer: is a short synthetic oligonucleotide DNA sequence (18 – 30 bps) that binds to its complementary region on template DNA & provides starting site for DNA polymerase. Usually, two primers (forward & reverse primers) are used. It is used in many molecular techniques from PCR to DNA sequencing.



Very brief PCR reminder

PCR is a molecular technique to amplify large quantities of a specific DNA sequence. It includes 3 steps.



Good primer's traits

1. Primer length determines the specificity and significantly affects its annealing to the template.

A. Too Short: low specificity, resulting in non-specific amplification.

B. Too Long: decrease the template-binding efficiency at normal annealing temperature due to the higher probability of forming secondary structures such as **hairpins**.

Good primer's traits

2. Base composition: usually average G+C content around 50-60% will give you the right melting/annealing temperature for ordinary PCR reactions and appropriate hybridization stability.

- GC increases the primer efficiency due to the stronger hydrogen bonding of GC.

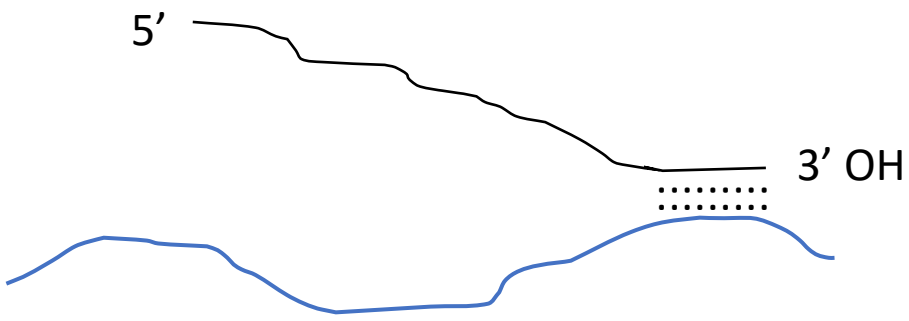
```
3′  ATGCCGTGCTACCTGATACGCGCATTTTAT
    || || |||||||||||||||| || || |||| |||| |||| || ||||||||
5′  TACGGCACGATGGACTATGCG 3′
```

Good primer's traits

3. Max 3' end stability: it is critical that the stability at the 3' end be high.

Basics of primer design

- Primer melting temperature (T_m): is the most important factor in determining The optimal PCR annealing temperature (T_m). It is important that all primers used in a reaction have similar T_m so that annealing is at a similar temperature.
- Melting temperature (T_m) between 50-70 is preferred.



Melting temperature (T_m) calculation

DNA has stability that depends on the sequence of the double helix. Heat can be used to disrupt this duplex. higher G + C content DNA has a higher T_m than lower G + C content DNA.

A simple, generic formula for calculating the T_m is:

$$T_m = 4(G+C) + 2(A+T)° C$$

Example: Find T_m of the following sequence:

GTGCTCGCCAAACATGAAAT

$$T_m = 4\ (4 + 5) + 2\ (7 + 4)$$

$$T_m = 4\ (9) + 2\ (11) = 58° C$$

Software for primer designing

1. Primer 3
2. PCR primer designer
3. The primer generator
4. Primer Quest
5. Raw primer
6. PRIMO

Avoidance and prevention of secondary structure

- 5’- and 3’- ends of a primer should not be complementary, otherwise partially double-stranded internal **hairpin** structure will be formed at 30℃ & amplified.
- Forward primer should not be complementary to reverse primer, otherwise primer-dimers will be formed.
- To avoid secondary structures, primers should contain 50% G + C