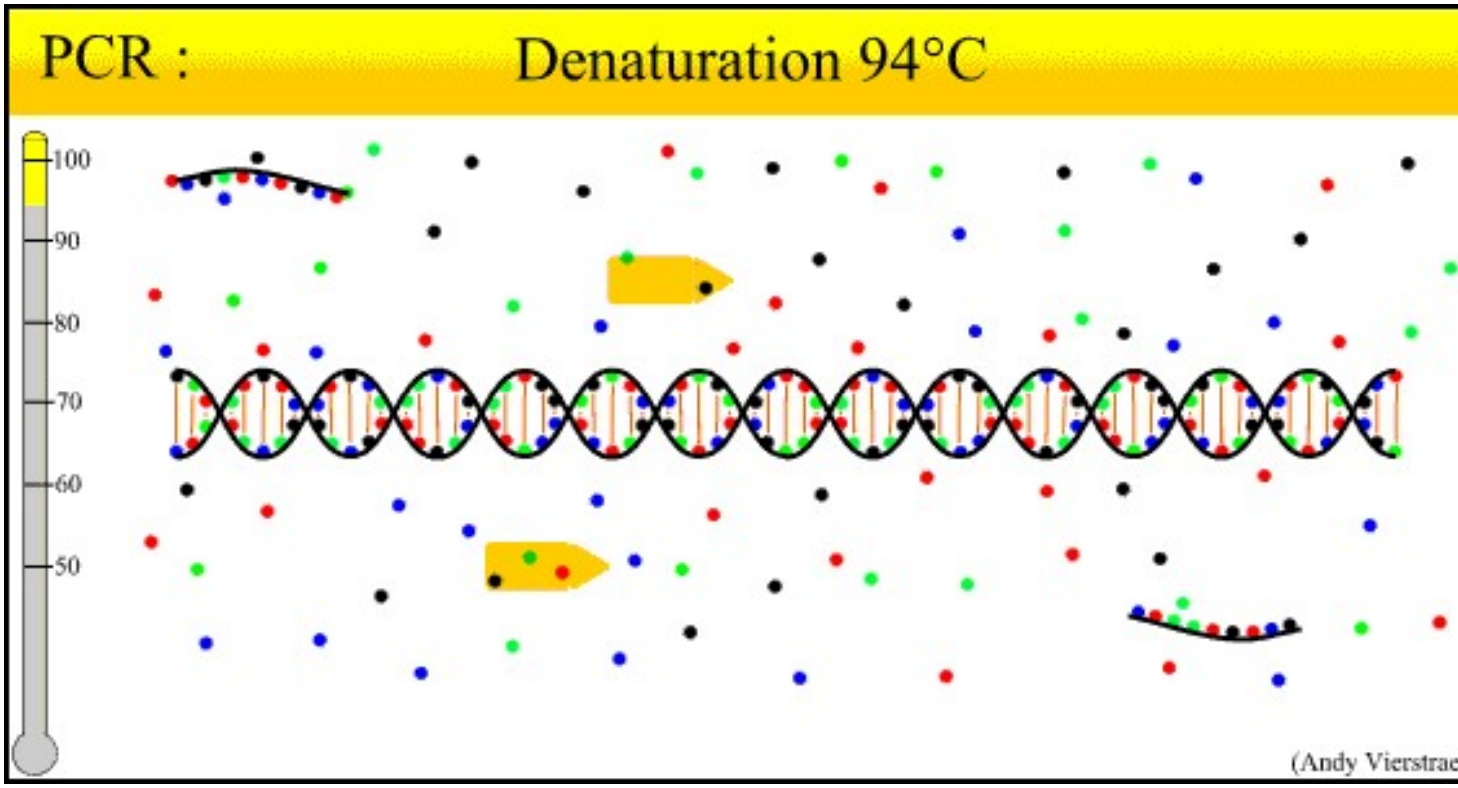
A closer look at PCR

--PROCESS

By Nanjing_NFLS

PCR



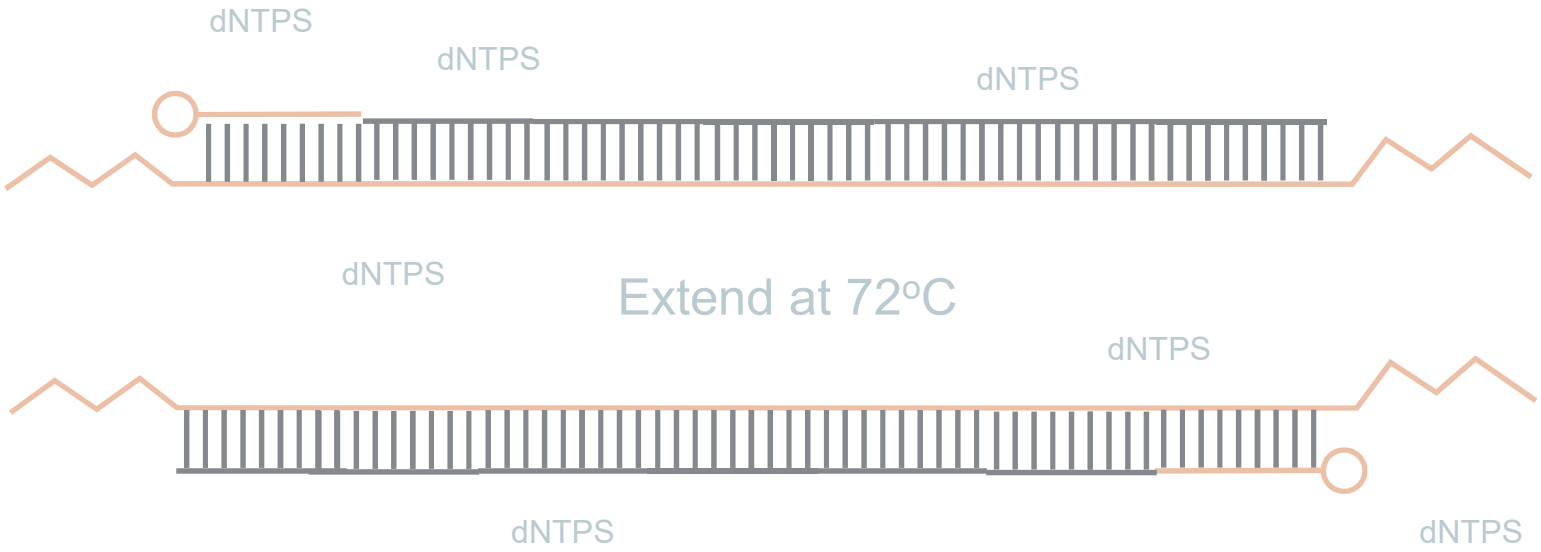
1 . Heat causes DNA strands to separate

Denaturation of DNA at 94^{o}C

2. Primers bind to DNA sequence

Primers anneal at 55°C-60°C

3. DNA polymerase bind to dsDNA and extends primer



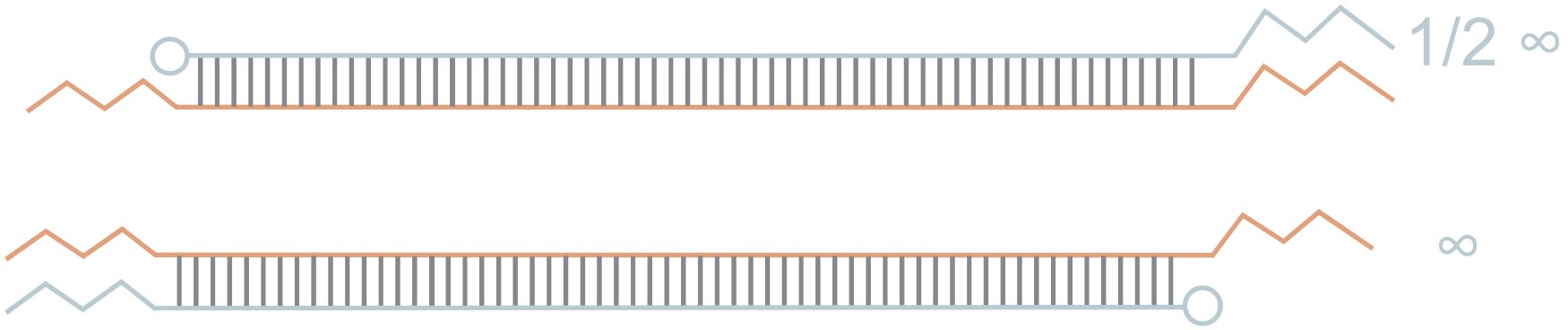
extending speed for rTaq： 1min/kb

Now DNA is replicated.

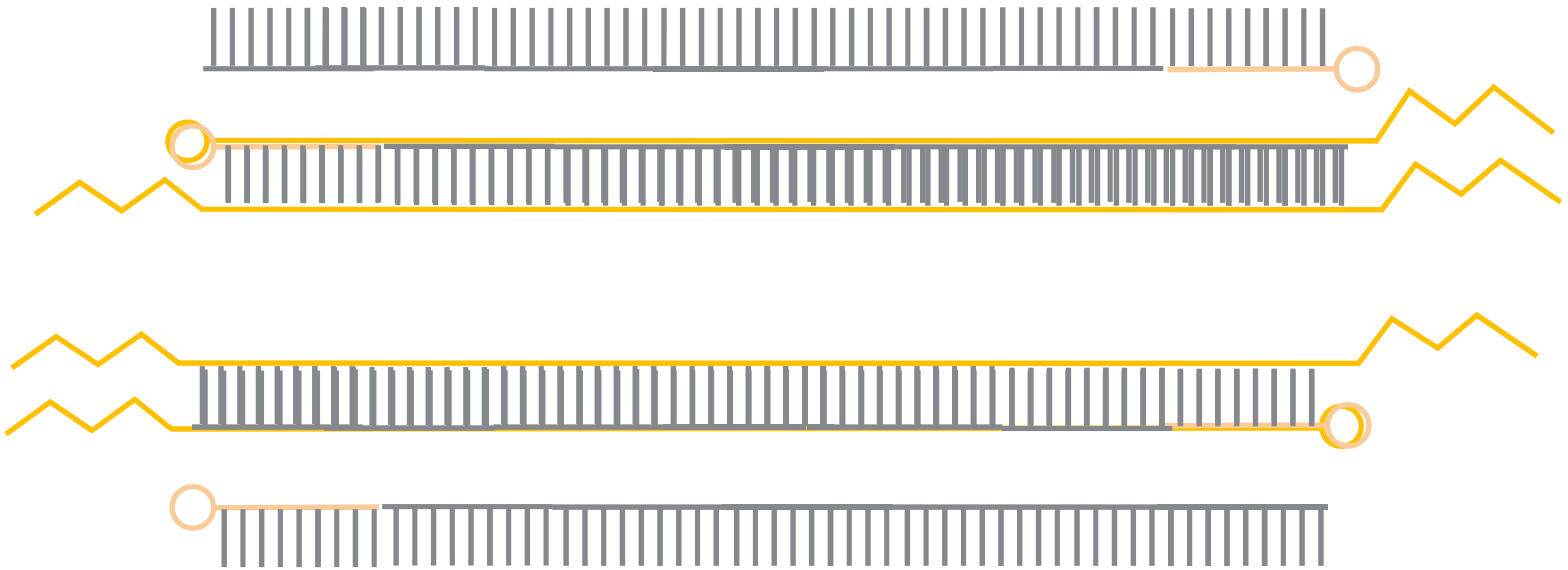
cycle 1

cycle 1

Type	Number
∞	2
1/2 ∞	2
X	0



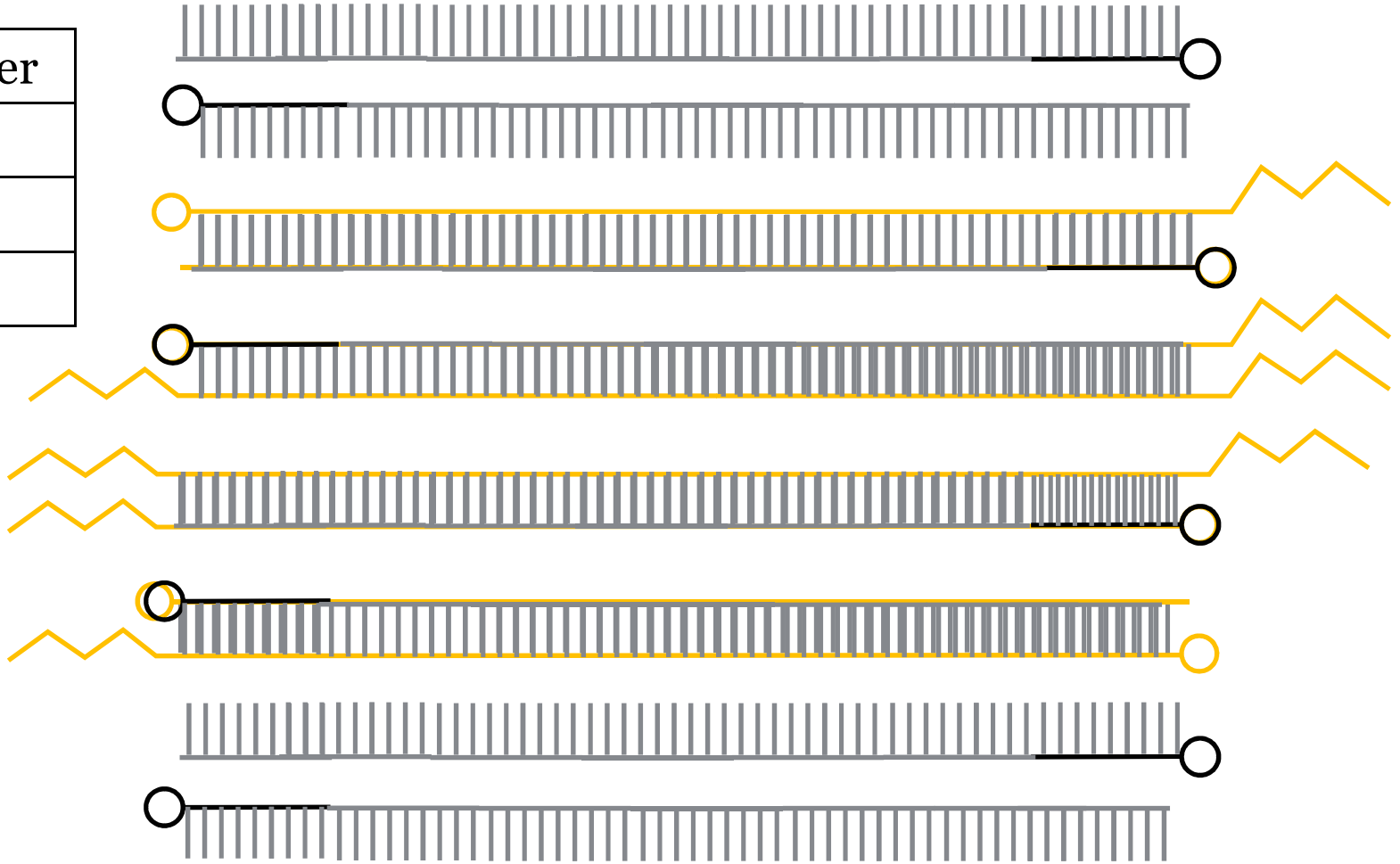
cycle 2



Type	Number
∞	2
1/2 ∞	4
X	2

cycle 3

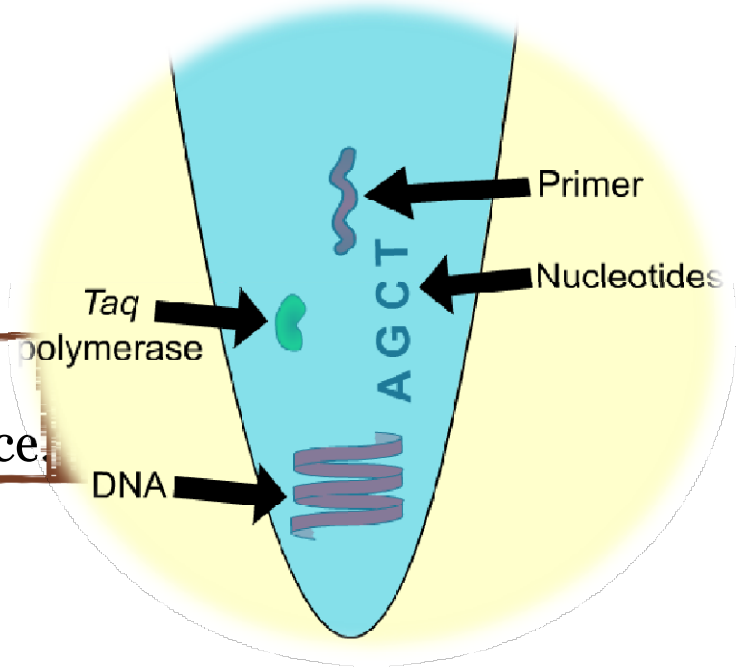
e	Number
	2
∞	6
	8



What tools are used for PCR reactions?

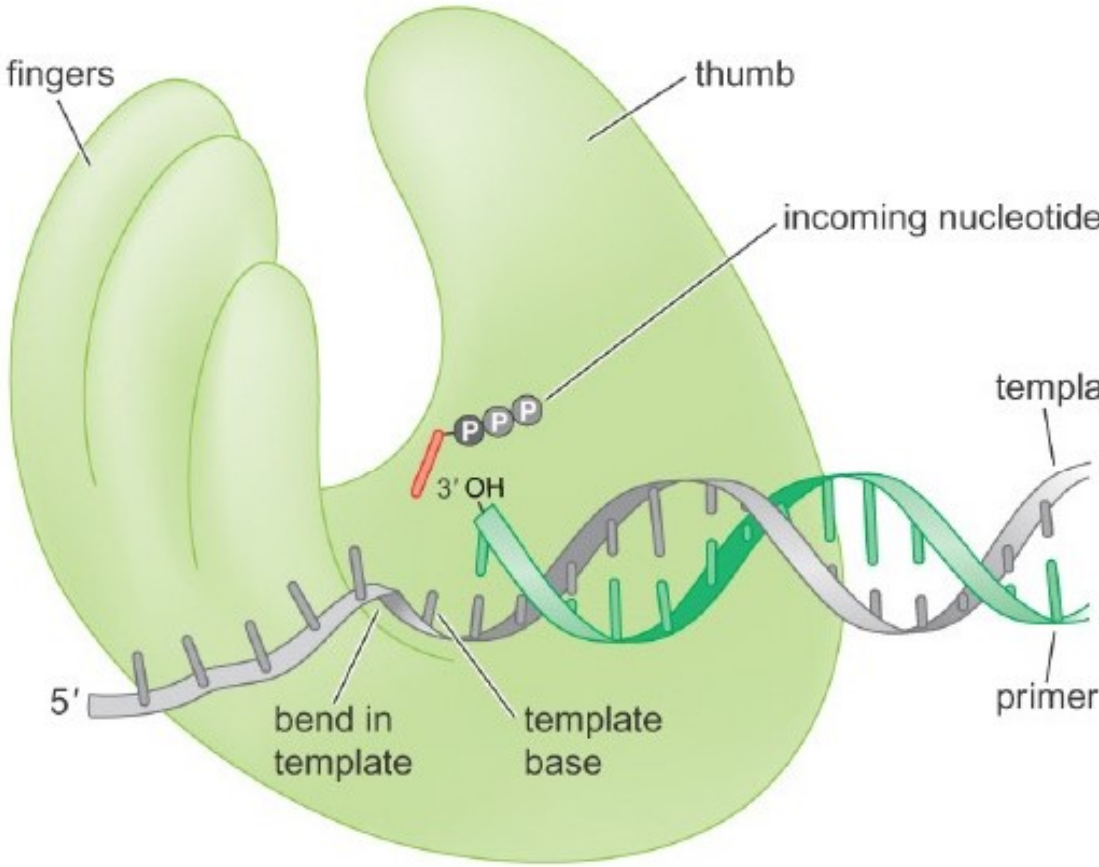
- Systems (mix) required to complete a PCR reaction

buffer: chemical conditions that provide the reaction
dNTPs: Materials providing DNA polymerization
Template: As a template for aggregation
DNA polymerase: complete the polymerization reaction
Primer: Leads the polymerase to replicate a specific sequence
Water: make-up volume



Tool 1: DNA polymerase

- The most critical tool for PCR
- Identifiable primer-template aggregates that extend along the 3' direction of the primer
- Existence of fidelity: they have different error correction capabilities, some are more likely to introduce errors



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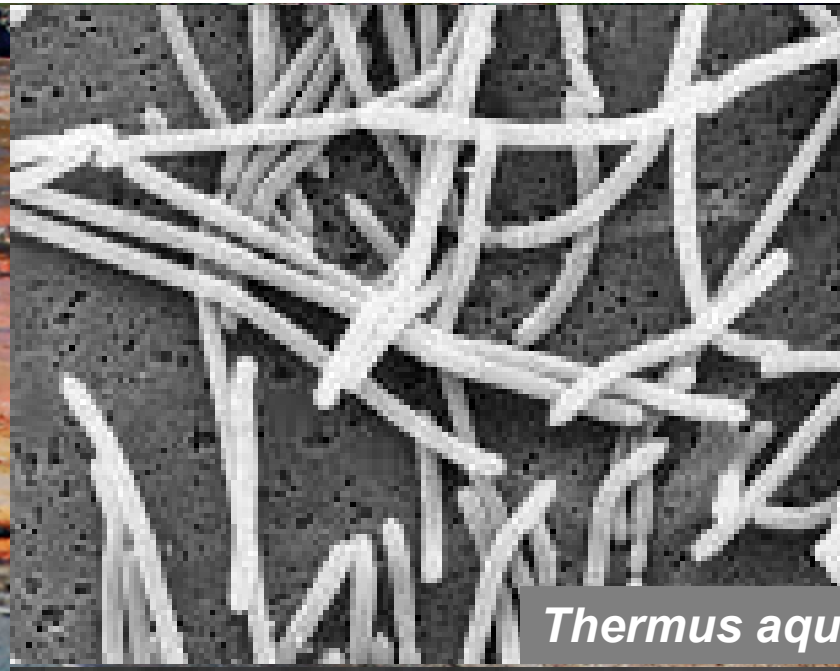
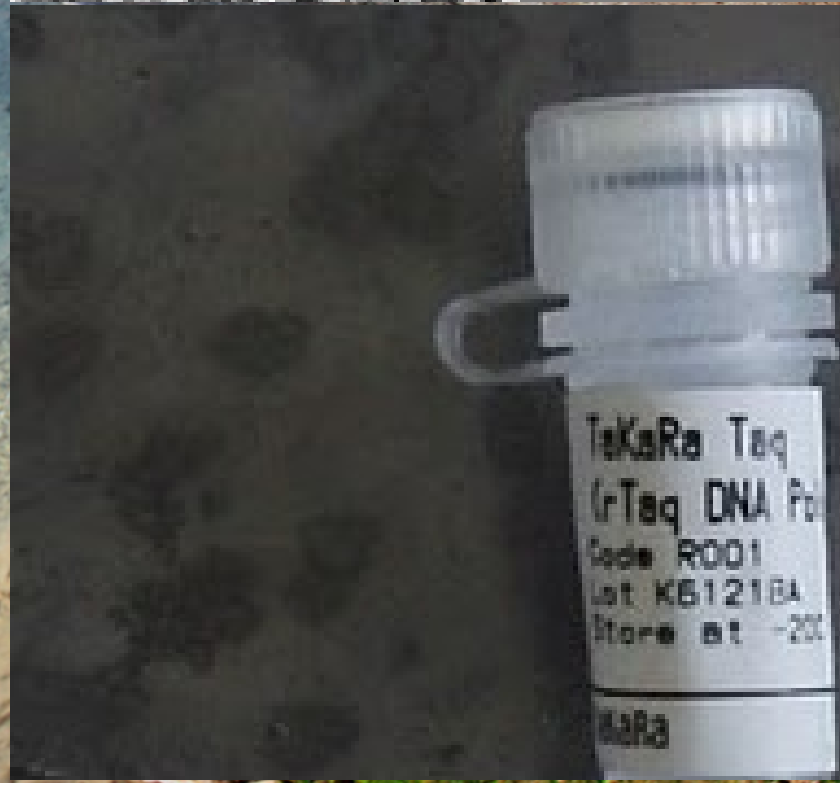
Taq Polymerase

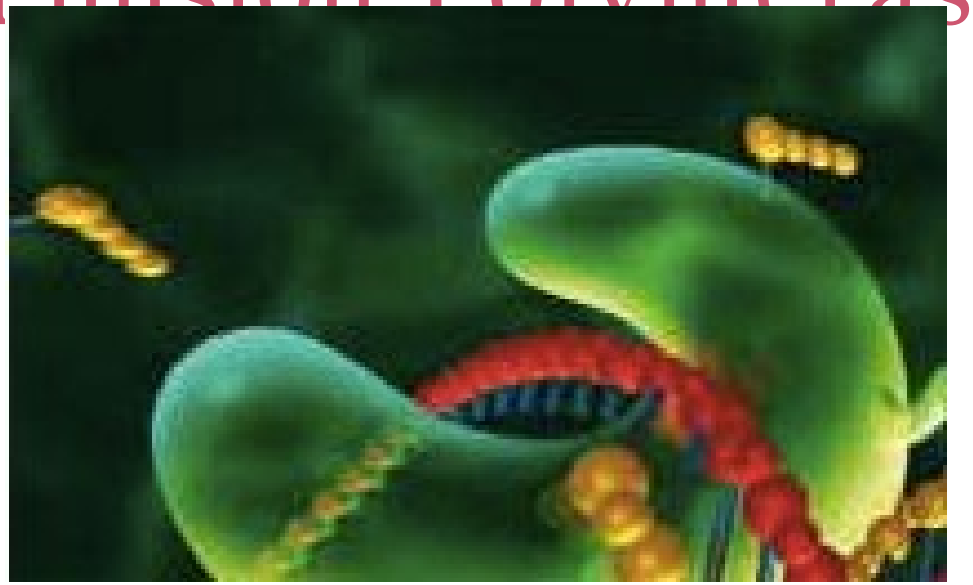
- Can withstand temperatures up to 94° C
- Poor fidelity of rTaq, relatively high fidelity of ex-Taq





wer Geyser Basin of Yellowstone National Park

Phusion Polymerase

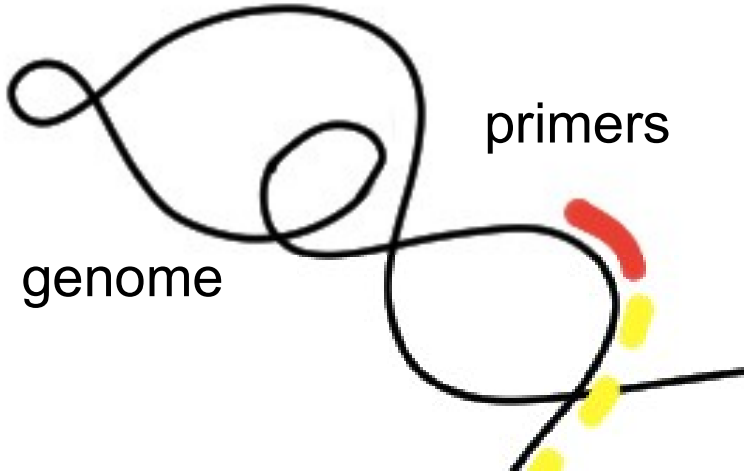


- Extracted from hot spring ancient bacteria, can withstand high temperature of 98° C

- Better fidelity, faster extension, many high fidelity derivatives available

Tool 2: Primers

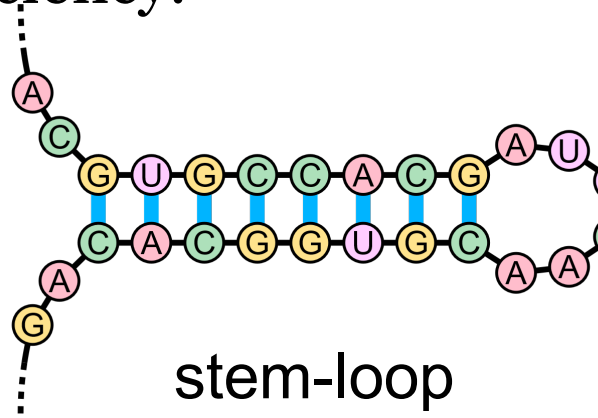
Definitions:
15-30 nucleotide single-stranded DNA
Can be combined with target fragments
Polymerase recognizes sites of primers and begins polymerization



Role:
Leads the polymerase to polymerize the desired fragment. The prime
determines the boundary at which the DNA is polymerized
Primer design directly determines the quality of the PCR reaction

imer specificity -
w well the guidelines match the
mplate

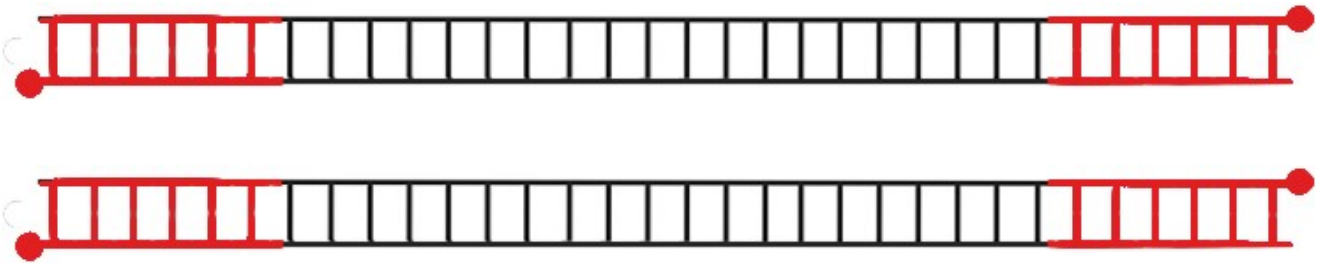
- Rarely binds to non-target fragments or pairs with each other, and rarely forms a stem-loop structure by itself, which binds well to the target template.
- Large amounts of target fragments can be obtained; primers with low specificity can introduce instabilities that affect PCR efficiency.



Primer characteristics: 5' end manipulability

- After a pair of primers, a new sequence is introduced on either side of the target DNA sequence by the primers. This section of the sequence can be an enzymatic site, or other sequences that match other assembly methods

- Manipulation of the 5' end of the primer allows arbitrary modification of the DNA sequence by PCR.



Thanks