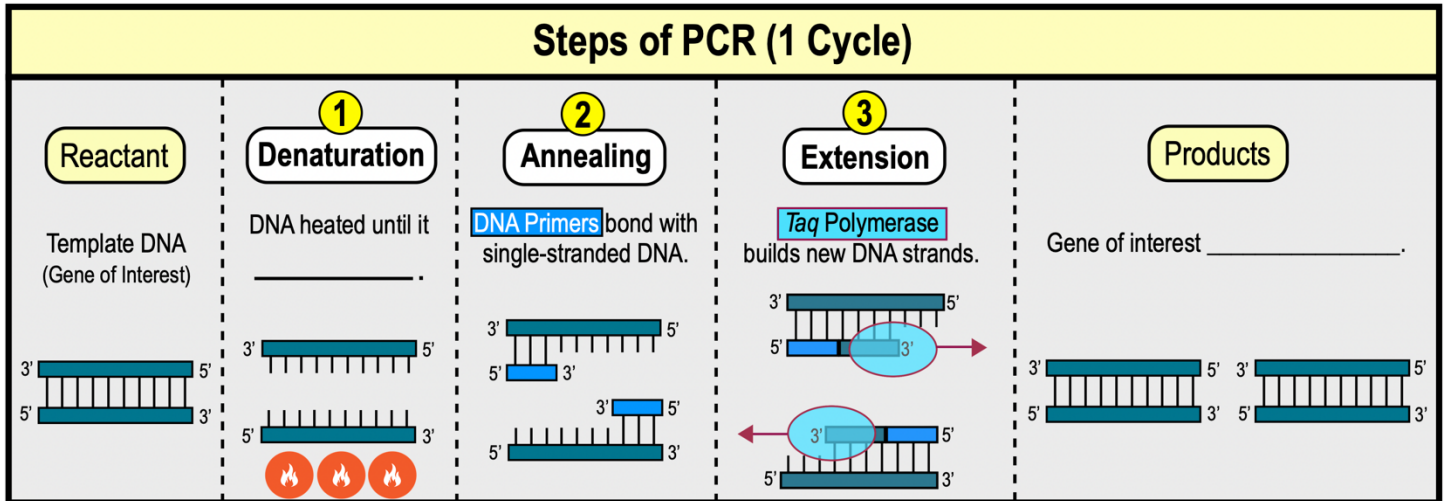


## CONCEPT: THE STEPS OF PCR

• PCR is a cyclical process where each cycle has \_\_\_\_\_ steps:

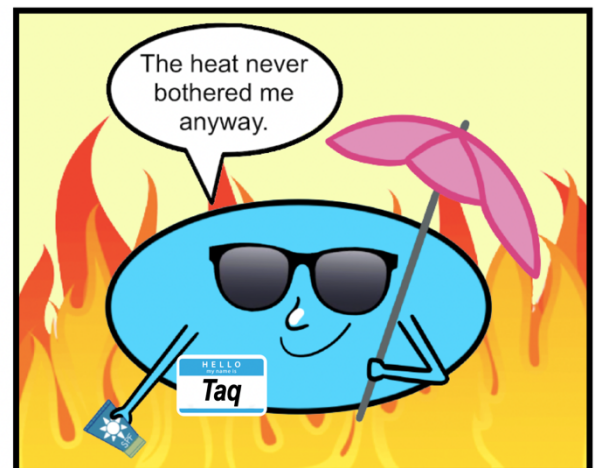
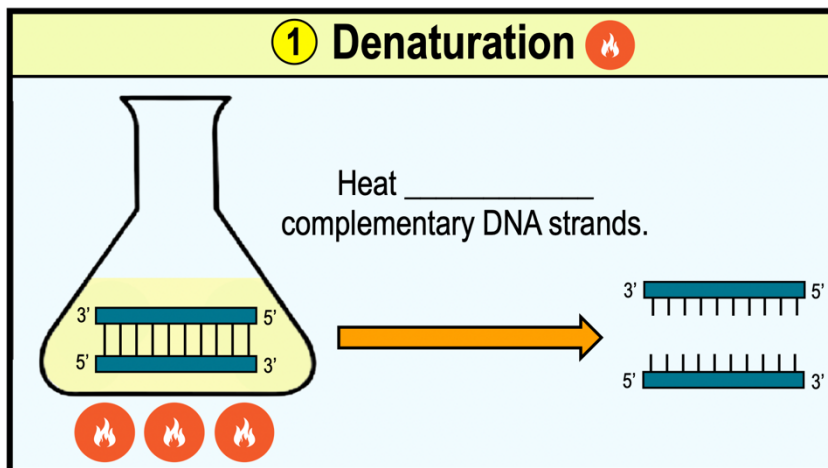
- 1 \_\_\_\_\_ (high temperature) 🔥
- 2 \_\_\_\_\_ (low temperature) ❄️
- 3 \_\_\_\_\_ (moderate temperature) 🌡️

• The steps are repeated in each cycle generating an *exponentially* growing number of DNA molecules.



### 1) Denaturation

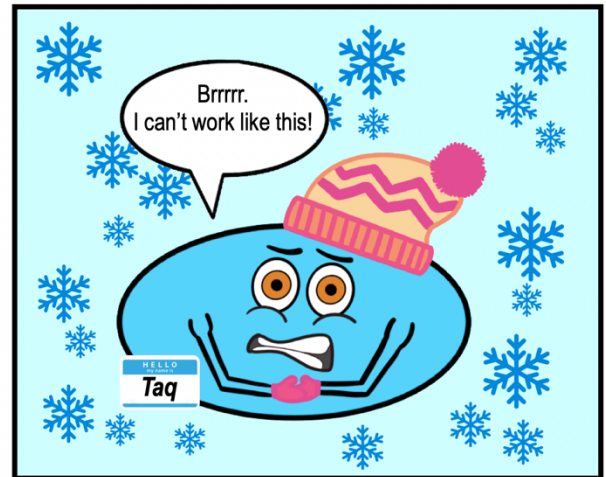
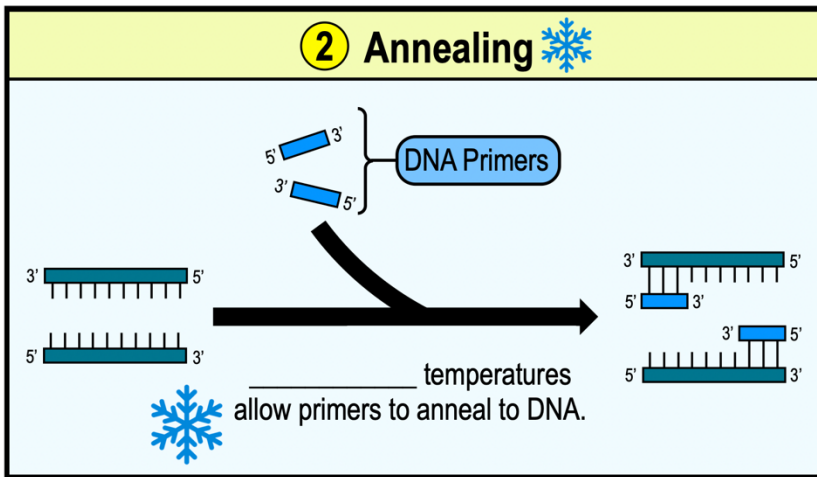
- The first step of a PCR cycle is to *heat denature* the double-stranded DNA to its single-stranded form.
- Temperature of the PCR mixture is \_\_\_\_\_ to 95°C to break H-bonds between complementary base pairs.
- \_\_\_\_\_ **polymerase**: special *thermo-stable* DNA polymerase that doesn't denature at high temperatures used in PCR.
  - Can withstand extremely \_\_\_\_\_ temperatures & rapid temperature \_\_\_\_\_.
  - However, it does not synthesize DNA unless it is at an *ideal* temperature.



## CONCEPT: THE STEPS OF PCR

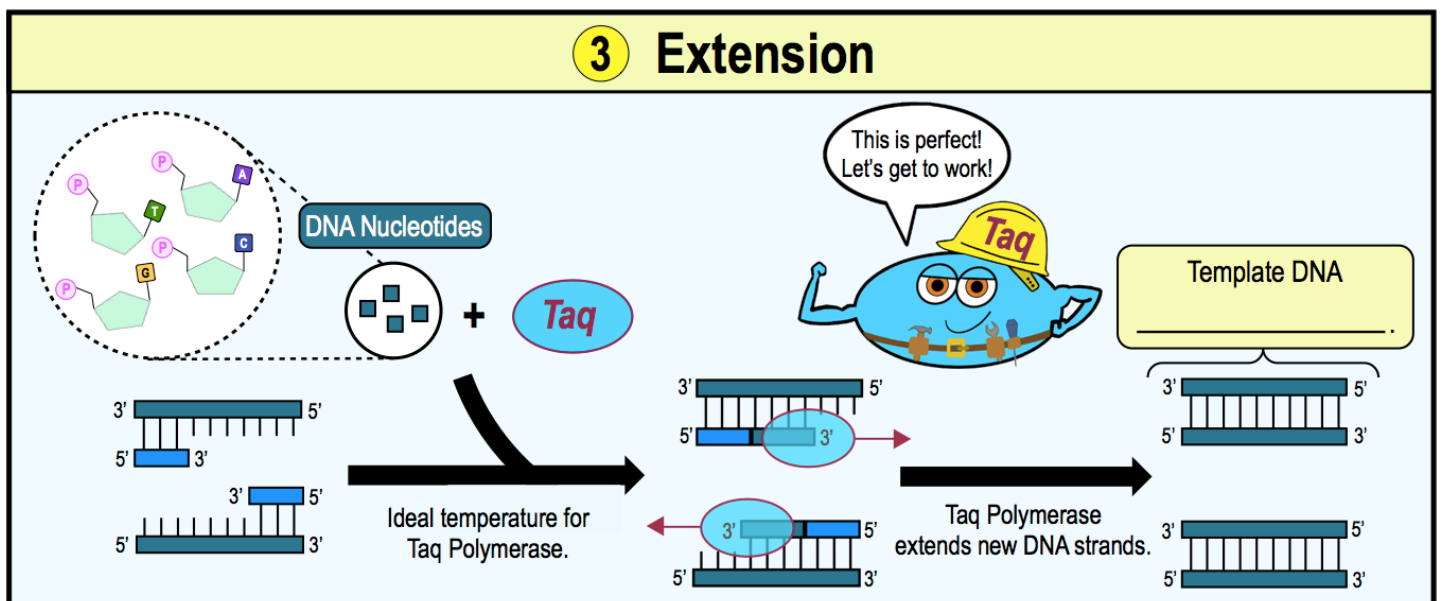
### 2) Annealing

- The second step of PCR is *annealing* of the DNA \_\_\_\_\_ to the heat-denatured single-stranded DNA.
- Temperature is *lowered* to  $\sim 55^{\circ}\text{C}$  so \_\_\_\_\_ base-pairing between primers & DNA can form.
  - *Taq polymerase* remains *inactive* because the temperature is too \_\_\_\_\_ for it to synthesize DNA.



### 3) Extension

- The final step of a PCR is the *extension* of the new DNA strand by the *thermo-stable* \_\_\_\_\_ **polymerase**.
  - The temperature is changed to  $72^{\circ}\text{C}$  which is the *ideal* temperature for activity of the **polymerase**.
  - Deoxyribonucleotides used to *extend* primers on each strand of DNA making 2 \_\_\_\_\_ copies.



### **CONCEPT: THE STEPS OF PCR**

**PRACTICE:** Which of the following correctly lists the steps in order for one cycle of the polymerase chain reaction (PCR)?

- a) Denature DNA; Add DNA polymerase; Anneal primers; Add dNTPs; Extend primers.
- b) Anneal primers; Denature DNA; Extend primers.
- c) Extend primers; Anneal primers; Denature DNA.
- d) Denature DNA; Anneal primers; Extend primers utilizing a thermostable DNA polymerase.

**PRACTICE:** Why is a DNA polymerase from a thermophilic bacterium used in PCR?

- a) The enzyme makes DNA that is extremely similar to human DNA.
- b) It is cheaper to obtain from live microorganisms than producing the enzyme in a lab.
- c) This thermophile's DNA polymerase does not require primers to begin DNA synthesis.
- d) This thermophile's DNA polymerase can withstand high temperatures that denature most proteins.

**PRACTICE:** PCR is known for its power of amplifying a target DNA sequence at a high speed. Each cycle can double the number of DNA molecules (target sequence). Which of the following is CORRECT regarding PCR?

- a) In order to make 10 copies of the DNA, you need at least 5 cycles of PCR.
- b) Helicase is required in order to separate the two strands in PCR.
- c) Dideoxynucleotides are used in PCR.
- d) DNA primers are needed in PCR.
- e) All of the above.

**PRACTICE:** If you start with one double-stranded DNA molecule and you perform SIX cycles of PCR, how many double-stranded copies of the DNA will you have?

- a) 6.
- b) 8.
- c) 16.
- d) 32.
- e) 64.