

CONCEPT: THE STEPS OF PCR

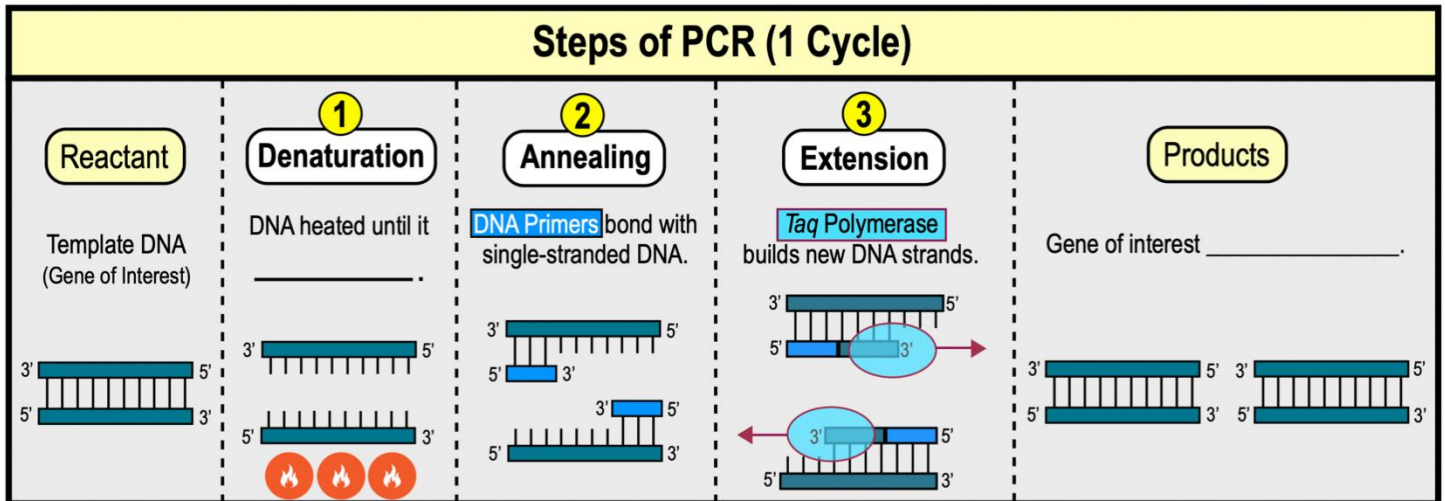
- PCR is a cyclical process where each cycle has _____ steps:

① _____ (high temperature) 🔥

② _____ (low temperature) ❄️

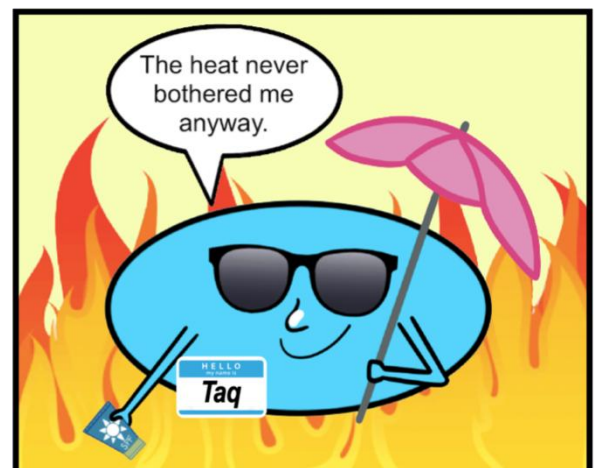
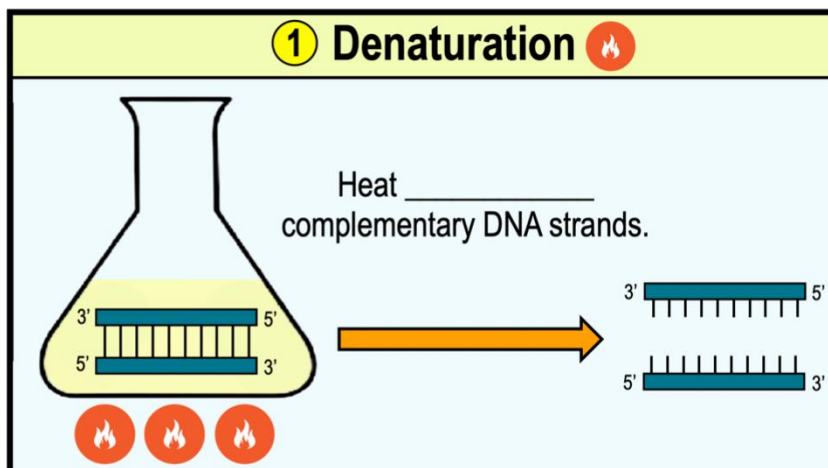
③ _____ (moderate temperature) 🌡️

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



1) Denaturation

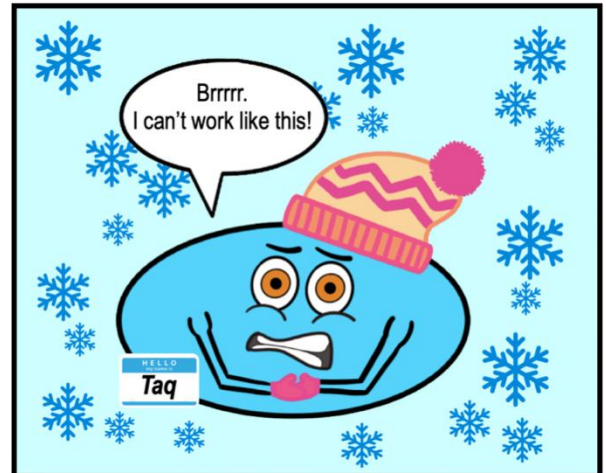
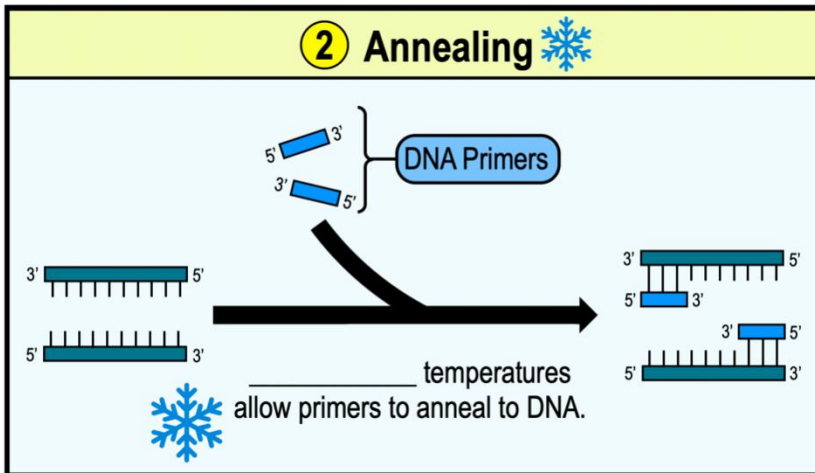
- The first step of a PCR cycle is to *heat denature* the double-stranded DNA to its single-stranded form.
- Temperature of the PCR mixture is _____ to 95°C to break H-bonds between complementary base pairs.
- _____ **polymerase**: special *thermo-stable* DNA polymerase that doesn't denature at high 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

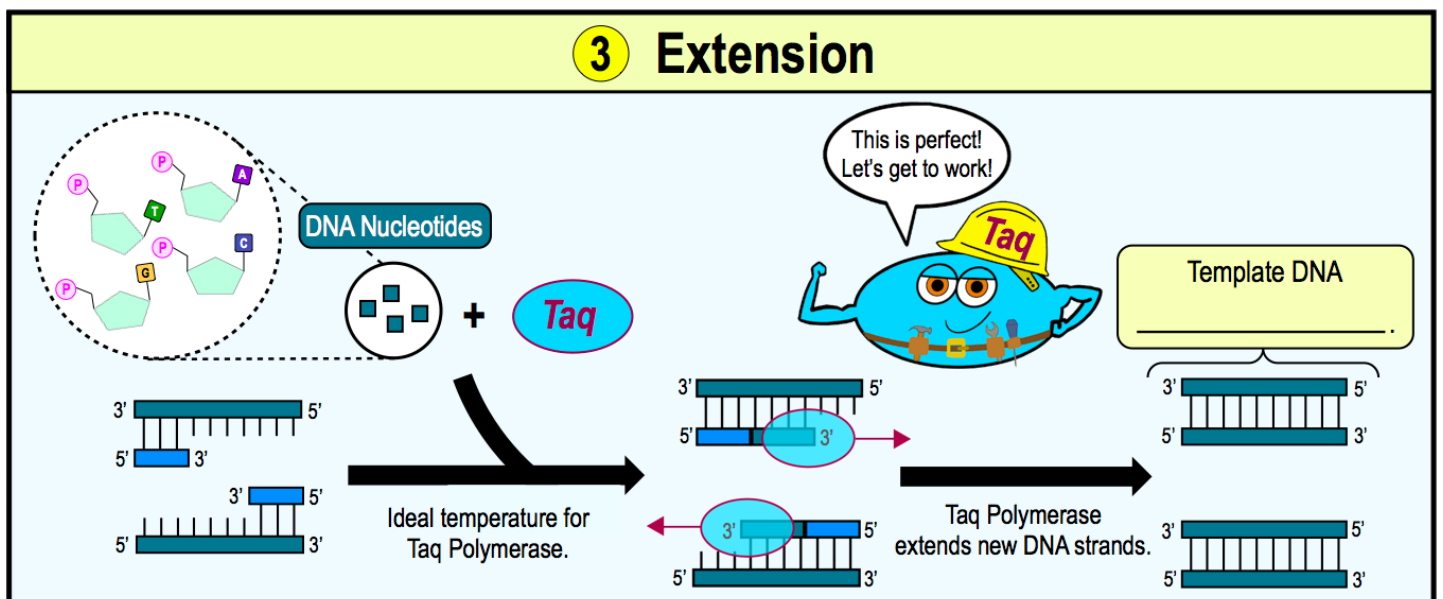
2) Annealing

- The second step of PCR is *annealing* of the DNA primers 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°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.