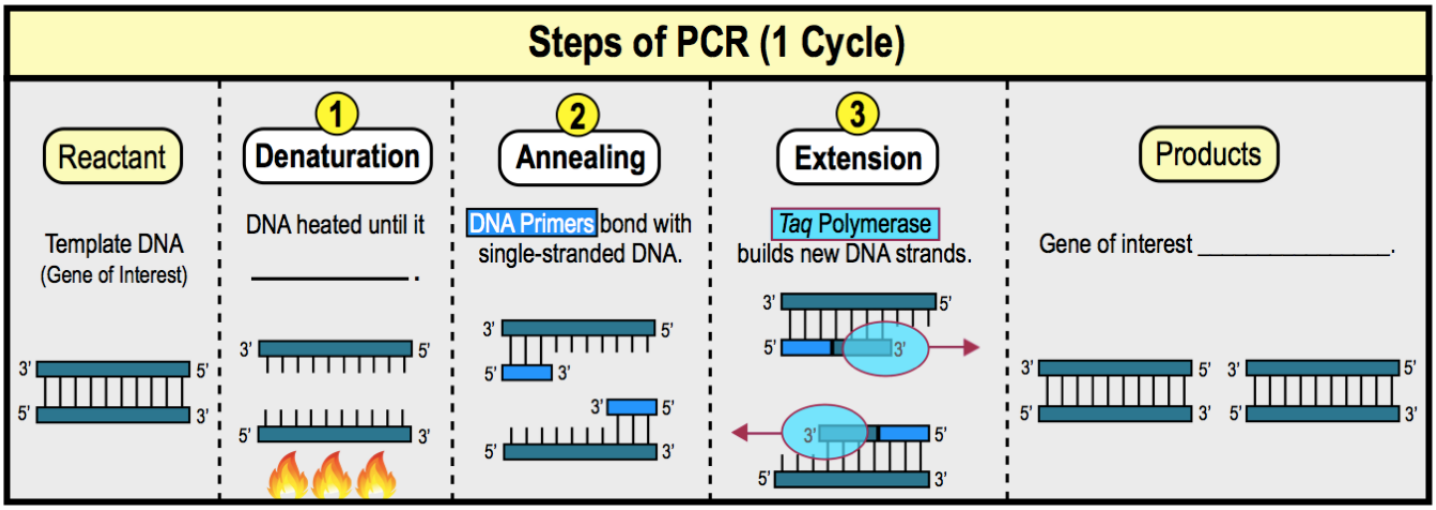
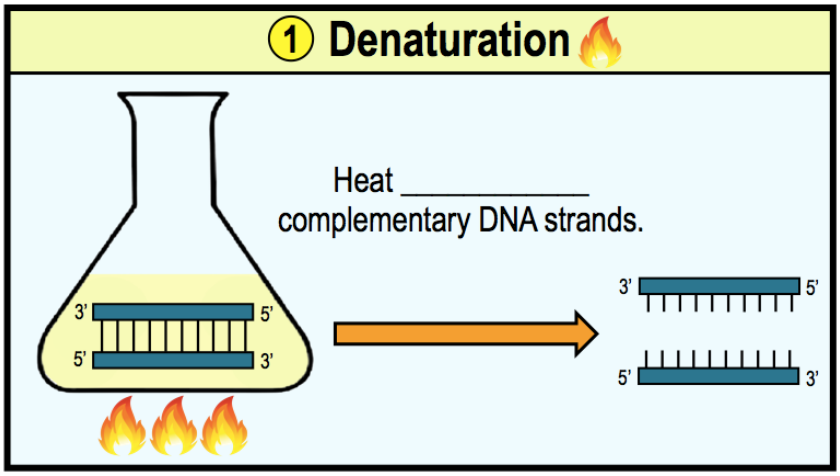
CONCEPT: THE STEPS OF PCR

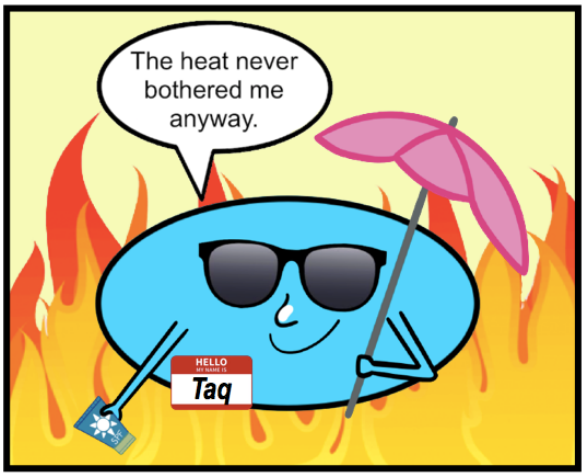
- *PCR* is a cyclical process where each cycle has _____ steps:
  1. _____ (high temperature)
  2. _____ (low temperature)
  3. _____ (moderate temperature)
- The steps are repeated in each cycle generating an *exponentially* growing number of DNA molecules.



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.





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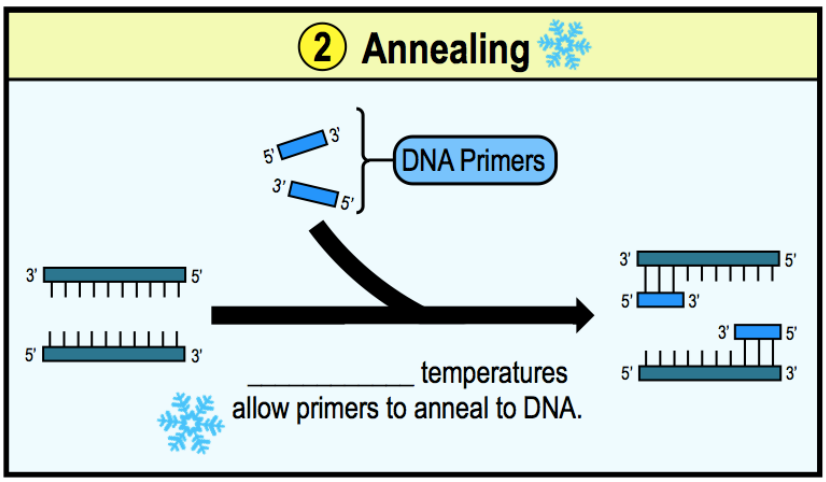
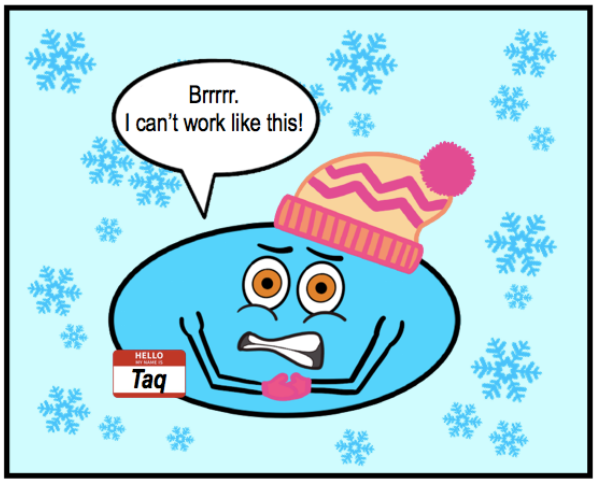
2) Annealing

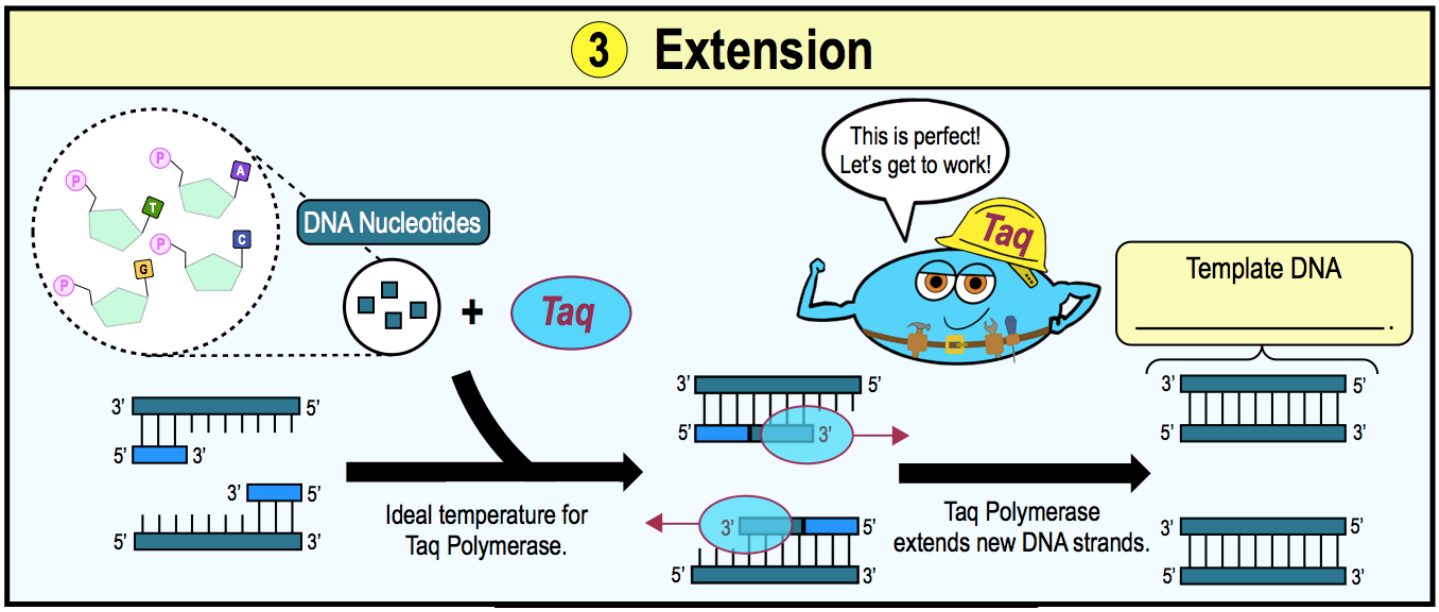
- The second step of PCR is *annealing* of the DNA _____ to the heat-denatured single-stranded DNA.
- Temperature is *lowered* to ~55°C so _____ base-pairing between primers & DNA can form.
  - □ *Taq polymerase* remains *inactive* because the temperature is too _____ for it to synthesize DNA.





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.



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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.