#ret

0.1 | **Questions**

Describe what is happening during each cycle of the PCR:

1. *Denaturation at approximately 95°C*
    1. Denaturation splits the DNA, creating single-strands which act as 'templates.'
2. *Annealing at approximately 55°C*
    1. Annealing allows the primers to bind to their respective sequences on the earlier created 'templates.'
3. *Extension at approximately 72°C*
    1. During the Extension phase, Taq polymerase creates new strands of DNA by extending the primers.

In one or two sentences for each, explain why the following mistakes would lead to a failed PCR reaction (assume 30 cycles of the typical denaturation, annealing, and extension temperature sequence unless otherwise noted):

1. *A human DNA polymerase was used rather than Taq DNA polymerase.* 1. Taq DNA polymerase was isolated from temperature-tolerant bacteria, and thus, it is thermostable. Human DNA polymerase is not, and would be nonfunctional under the temperatures used in PCR.
2. *Nucleotides were left out of the reaction.*
    1. Nucleotides are the building blocks of DNA. Without them, the DNA could not be synthesized.
3. *The denaturation phase temperature was set to 55°C.*
    1. A temperature of 55°C is not sufficient to denature the DNA strands. A temperature of ~95°C is needed.
4. *The extension phase temperature was set to 4°C.*
    1. Without a temperature of ~72°C, Taq polymerase won't extend the primers. Being sourced from bacteria used to very high temperatures, it is probably used to said temperatures.

Luke set up his first PCR reaction recently.

> After Luke's teacher ran his sample through the correct program on the thermal cycler, she analyzed the results. Strangely, she noticed that most of Luke's PCR product was **single-stranded rather than double-stranded DNA**, and that his **total yield of PCR product was lower than expected** (but he still had more material after thermocycling than before). Luke said he got distracted by a classmate while setting up the PCR, and might have left out one ingredient. **What do you think Luke left out of his PCR reaction and why? Your explanation should be linked to the strange results that the teacher noticed.***

Luke most likely left out either his forward primers or reverse primers. This absence would lead to only one strand being a source of replication, as only one strand would have the primers. Thus, many single strands would form as the source of replication would not be able to be replicated – only its partner strand would be. Hence, Luke would end up with mostly single stranded DNA, and less

ingrdients: - Taq: nope - primers: withought primers, no replication - template DNA: no, otherwise would have nothing. Actully, could just use primers? - nucleotides: cant be cus more material

- buffer?