#ret

1 | Questions

1.0.1 | Describe what is happening during each cycle of the PCR:

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

1. 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): :CUSTOM$_{ID}$: 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
   (a) *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.
   (b) *Nucleotides were left out of the reaction.*
      i. Nucleotides are the building blocks of DNA. Without them, the DNA could not be synthesized.
   (c) *The denaturation phase temperature was set to 55°C.*
      i. A temperature of 55°C is not sufficient to denature the DNA strands. A temperature of ~95°C is needed.
   (d) *The extension phase temperature was set to 4°C.*
      i. Without a temperature of ~72°C, Taq polymerase won't extend the primers. Being sourced from bacteria used to very high temperatures, the Taq polymerase most likely functions best under said temperatures.

1.0.2 | 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 product than expected.