

#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
3. Extension at approximately 72°C

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.
2. Nucleotides were left out of the reaction.
3. The denaturation phase temperature was set to 55°C.
4. The extension phase temperature was set to 4°C.

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.