Appendix C. Robertson and Phillips

An excerpt from a student response to question 1 on the Primer Design Exercise.

Question:
1. Given the sequence on the following page, choose forward and reverse primers that will amplify the underlined portion of the *aroA* gene.

CTATGTGTTGCTGGAAAAAGTAGGGAAGGGAGTGGTGAAGAGTATTCCACTGGTTCAATTAGAAAAAATC
ATTCAAGCATTACCAAAGTGAAAGTAACAATACAGCCCGGAGATCTGACTGGAATTATCCAGTCACCCGC
TTCAAAAAGTTCGATGCAGCGAGCTTGTGCTGCTGCACTGGTTGCAAAAGGAATAAGTGAGATCATTAAT
CCCGGTCATAGCAATGATGATAAAGCTGCCAGGGATATTGTAAGCCGGCTTGGTGCCAGGCTTGAAGATC
AGCCTGATGGTTCTTTGCAGATAACAAGTGAAGGCGTAAAACCTGTCGCTCCTTTTATTGACTGCGGTGA

a. Underline the primer sequence on the following page. Then write out your primers below and indicate the 5' and 3' ends. Remember that the 3' or reverse primer is the reverse complement of the template (think about which direction DNA extends).

Correct Response:

The student chose the highlighted sequence as forward and reverse primer binding sites. Correct forward (sense) and reverse (antisense) primers written in standard notation (5' → 3') should be:

Forward: 5'-GGAAGGGAGTGGTGAAGAG-3'
Should match the sequence given 5'→3'
Reverse: 5'- CTGCAAAGAACCATCAGGC-3'
Should be complement of sequence given written in the reverse orientation (5'→ 3')

Incorrect Student Response:

Forward: 3'-CCTTCCCTCACCACTTCT-5'
Written as complement of the given sequence in the wrong orientation
Reverse: 5'- GCCTGATGGTTCTTTGCAG-3'
Written as a match of the given sequence, written in the wrong orientation

The way the student designed the forward and reverse primers showed incorrect primer orientation and DNA complementarity. These errors would result in DNA extension in the opposite direction, and therefore, no amplification of the target DNA. These types of errors were commonly seen in student responses and reflect misconceptions about DNA replication, complementarity and primer annealing.