Appendix B. Robertson and Phillips
Primer Design Exercise

Currently Monsanto owns the patent on glyphosate, which is commonly known as Roundup®. It is the most popular herbicide used today because it kills a broad spectrum of weeds and is easily broken down into non-toxic compounds. The catch is that Monsanto also owns the patent on the gene that confers resistance to glyphosate, which they have transformed into several crops such as corn and soybean to make them "Round-up Ready", or resistant to glyphosate. Many researchers are trying to find novel genes that will also confer resistance to glyphosate for both evolutionary and economic reasons. Recently, a Chinese group found a bacterium, *Pseudomonas putida* strain 4G-1, which is naturally resistant to glyphosate[1]. They have cloned the novel gene, *aroA*, that is significantly different in sequence from the previous *AroA* gene, and are hopeful that it will be another source of glyphosate resistance.

The *AroA* gene encodes the enzyme 3-phosphoshikimate 1-carboxyvinyltransferase, which plays a key role in the biosynthesis of aromatic amino acids. Glyphosate works by disrupting this enzyme and thus the biosynthesis of aromatic amino acids. Resistance is found by mutating the *AroA* gene such that glyphosate cannot bind to the resulting protein.

You have just been hired to select strains of *Pseudomonas putida* with the new *aroA* gene to provide a new glyphosate resistant cultivar. Your first challenge will be to create primer pairs (forward and reverse) that will amplify a portion of the *aroA* gene that contains the underlined region (see sequence below).

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

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).

b. What is the size of your target DNA? (Note: each line contains 70 nucleotide bases)

[1] Sun, Y. et al. 2005. Novel AroA with high tolerance to glyphosate, encoded by a gene of *Pseudomonas putida* 4G-1 isolated from an extremely polluted environment in China. *Applied and Environmental Microbiology*. 71 (8): 4771-4776

>gi|51587624|emb|AJ812018.1| Pseudomonas putida aroA gene for 3-phosphoshikimate 1-carboxyvinyltransferase

5′-

GATCATAAAACATGCTTGTATAAAGGATGCTGCCATGTTCCGTGAACTGGAAGCGAACAATCTTGCGGTA
TATCAGAAAAAGCCAAAGCTGATTGCAGTGCTTCTTCAGCGTAATGCTCAGTTAAAAGCGAAGGTTGTTC
AGGAGGATGAGTTCGAAAAGTCGGTAAGGCGTTTGTTGAACTTTGGTCATACATTGGGGCATGCCATCGA
AAATGAATATGCGTTGATGCATGGCCATGCGGTTGCTATAGGAATGACATACGCGTGTCATATTTCTGAG
CAATTGTCTGGATTCAAACAAACAAATCGCGTGGTAGAAGTGTTGGAACAATATGGGTTACCGACTTATA
TGGCATTCGATAGGGAAAAGGCTTTTAATCTGTTGAAAATGGACAAGAAGCGTGAAAAAAAGGAAATGAA
CTATGTGTTGCTGGAAAAA**GTAGGGAAGGGAGTGGTGAAGAG**<u>**TATTCCACTGGTTCAATTAGAAAAAATC**</u>
<u>**ATTCAAGCATTACCAAAGTGAAAGTAACAATACAGCCCGGAGATCTGACTGGAATTATCCAGTCACCCGC**</u>
<u>**TTCAAAAAGTTCGATGCAGCGAGCTTGTGCTGCTGCACTGGTTGCAAAAGGAATAAGTGAGATCATTAAT**</u>
<u>**CCCGGTCATAGCAATGATGATAAAGCTGCCAGGGATATTGTAAGCCGGCTTGGTGCCAGGCTTGAAGATC**</u>
<u>**AGCCTGATGGTTCTTTGCAGATAA**</u>CAAGTGAAGGCGTAAAACCTGTCGCTCCTTTTATTGACTGCGGTGA
ATCTGGTTTAAGTATCCGGATGTTTACTCCGATTGTTGCGTTGAGTAAAGAAGAGGTGACGATCAAAGGA
TCTGGAAGCCTTGTTACAAGACCAATGGATTTCTTTGATGAAATTCTTCCGCATCTCGGTGTAAAAGTTA
AATCTAACCAGGGTAAATTGCCTCTCGTTATACAGGGGCCATTGAAACCAGCAGACGTTACGGTTGATGG
GTCCTTAAGCTCTCAGTTCCTTACAGGTTTGTTGCTTGCATATGCGGCCGCAGATGCAAGCGATGTTGCG
ATAAAAGTAACGAATCTCAAAAGCCGTCCGTATATCGATCTTACACTGGATGTGATGAAGCGGTTTGGTT
TGAAGACTCCCGAGAATCGAAACTATGAAGAGTTTTATTTCAAAGCCGGGAATGTATATGATGAAACGAA
AATGCAACGATACACCGTAGAAGGCGACTGGAGCGGTGGTGCTTTTTTACTGGTAGCGGGGGCTATTGCC
GGGCCGATCACGGTAAGAGGTTTGGATATAGCTTCGACGCAGGCTGATAAAGCGATCGTTCAGGCTTTGA
TGAGTGCGAACGCAGGTATTGCGATTGATGCAAAAGAGATCAAACTTCATCCTGCTGATCTCAATGCATT
TGAATTTGATGCTACTGATTGCCCGGATCTTTTTCCGCCATTGGTTGCTTTGGCGTCTTATTGCAAAGGA
GAAACAAAGATCAAAGGCGTAAGCAGGCTGGCGCATAAAGAAAGTGACAGAGGATTGACGCTGCAGGACG
AGTTCGGGAAAATGGGTGTTGAAATCCACCTTGAGGGAGATCTGATGCGCGTGATCGGAGGGAAAGGCGT
AAAAGGAGCTGAAGTTAGTTCAAGGCACGATCATCGCATTGCGATGGCTTGCGCGGTGGCTGCTTTAAAA
GCTGTGGGTGAAACAACCATCGAACATGCAGAAGCGGTGAATAAATCCTACCCGGATTTTTACAGCGATC
TTAAACAACTTGGCGGTGTTGTATCTTTAAACCATCAATTTAATTTCTCATGAATAGCTTCGGCCGCATC
TTCAGGGTGCATATTTTTGGCGAATCACATGGTGAATCAGTAGGCATCGTTATTGATGGTTGTCCTGCTG
GTCTGTCATTGTCCGAAGAAGATC-3′

2. For each primer you designed, use the website and the guidelines on the instruction sheet to determine whether they meet the basic primer requirements. Record the Tm, length, molecular weight, and possibility of secondary structures or primer dimers and use the statistics to qualify your decision to use or not use the primers for a PCR reaction.

3. To determine the specificity of your primer pair to the *aroA* sequence, run a nucleotide BLAST by following the directions on the instruction page.

a. What does the E-value indicate? What is another way to determine homology between two sequences?

b. Write down the organism and E-value score from the two highest matches for each primer sequence. Did you get back the sequence you put in?

c. How many nucleotides aligned between your sequences and the first match for each?

4. Now that you have some experience designing primers and an idea about what makes a good primer, check the CamV35S primers used to determine the presence of a transgene in your food products.

Sense (forward) primer: 5'GCT CCT ACA AAT GCC ATC A3'

Antisense (reverse) primer: 5'GAT AGT GGG ATT GTG GGT CA3'

a. Provide a short summary about the primer pairs including characteristics such as melting temperature, secondary structures, and specificity using this worksheet as a guide.

b. Do you think these primers are a good choice to amplify the 35S promoter from your food product? Consider the specificity of your primer pairs to transgenic ingredients as well as other ingredients that may be in your products.