PRIMER DESIGN FOR PCR AMPLIFICATION AND SEQUENCING

(*Phil's Rules)*

1. Average primer length (region homologous to template): 20 bp
2. G+C content should be as close to 50% as possible
3. G and C residues should be fairly evenly distributed throughout primer (not all in one clump)
4. The 3′-most end of the primer should conform to the following pattern where possible:

   .....SSW

   where S indicates a G or C residue and W indicates an A or T residue
5. The 5′-most nucleotide pair should be "SS....."
6. Primers should be examined to guard against "primer dimers"; i.e., individual primers or sets of primers should not be able to anneal to one another at their 3′ ends
7. Restriction enzyme recognition sites may incorporated at the 5′ end of the primer
8. Mismatches may be introduced into primers, but should not occur within about 9 bases of the 3′ end
9. **Double check that primers match the target sequence and that they have been written in the 5′-3′ direction**