PCR Optimization: Primer Design

- Generally, primers should be 18 – 30 nt in length. This provides for practical annealing temperatures.
- Primers should avoid stretches of polybase sequences (e.g. poly dG) or repeating motifs; these can hybridize inappropriately on the template, or generate complex structures within the primer.
- Aim for a GC content of 40 to 60%.
- If possible, the 3′ end of the primer should end in GC bases (GC clamp) to enhance annealing of the end which will be extended.
- Inverted repeat sequences should be avoided to prevent formation of secondary structure in the primer, which may prevent hybridization to template.
- Minimize primer complementarity to prevent hybridization between primers (primer dimers).
- Primer pairs should have similar Tm values (within 2°C).
- When adding sequences to the 5′ end of the primer to create a restriction site, it is important to include a few extra bases on the 5′ end (4-6 bases) to serve as a clamp to keep the 5′ ends from separating during digestion.