Primer Design Rules

1. Primer Length: Optimal length for the primers should be between 19-21 base pairs (bp). This length is optimum for specificity and primer binding.

2. Primer Melting Temperature: Melting Temperature of the primers (T_m) is the temperature at which one half of the DNA will dissociate to become single stranded. Primers with melting temperatures in the range of 58-61 give the best results. Primers with melting temperatures above 65^{o}C have a tendency for secondary annealing. The T_m depends on the GC content of the primer. The higher the GC content, the higher the melting temperature (more hydrogen bons).[1]

3. Primer Annealing Temperature (T_a)**:** Annealing temperature of the primes is the temperature at which the primers will bind to your DNA.

Higher annealing temperature will produce an insufficient primer-template.

Lower annealing temperature will lead to non-specific binding of the primers to the DNA and base pair mismatches. Annealing temperature must be tested with a temperature gradient PCR using different temperatures.

4. GC Content: The GC content of the primers should be around 45-55%. CG content is the number of C's and G's in the primer as a percentage of the total bases.

5. GC Clamp: The presence of C or G bases in the last five bases from the 3' end of primers (GC clamp) ensures a specific binding at the 3' end due to the stronger bonds (more hydrogen bonds) between G and C bases. More than 3 C's or G's should be avoided in the last 5 bases at the 3' end of the primer.

6. Repeats: A repeat is a nucleotide occurring consecutively and should be avoided because they can cause mispriming.

For example, GCGCGCGCGCGCG. Try avoiding more than 4 nucleotides repeated in a primer.

7. Avoid Cross Homology: Primers should not amplify other genes in your DNA. We use tools such as BLAST: Basic Local Alignment Search Tool[2]

You must BLAST the templates against the known gene database and the software give you a result. It will recognise regions with significant cross homologies in each template.

Parameters for Primer Pair Design

8. Amplicon Length: The product length after the amplification must be around 700-800b long.

9. Product Position: Strive for amplification near 3' end.

Notes:

1. Tm Calculator- NEB Tm Calculator
2. BLAST: Basic Local Alignment Search Tool- Nucleotide BLAST: Search nucleotide databases using a nucleotide query (nih.gov)