Primer Checklist

By VGO 10-31-16

Follow this checklist before ordering your primers.

I) Regular primers:

- Check the orientation (5’ to 3’ and in the correct strand)

- Check that there is a single binding site in genome/template for each primer (Product size must be within ability of the polymerase)

- Check the length and Tm (Between 17-25 nt and Tm between 65°C and 75°C, within 5°C of each other.)

- Check for dimers and hairpins (Can be checked on IDT olygo analyzer)

II) qRT-PCR primers

Additionally to above requirements:

- Product should be between 75-200 bp.

III) Mutant making (SOE-ing PCR) primers
(From “Making a Mutant in *Listeria monocytogenes*” SOP)

1. Make sure that primers are in the correct orientation 5' to 3', and correspond to the correct strand.

2. Make sure the tail of B (or C) is the reverse complement of C (or B).

3. Check that the restriction sites are correct and have the right number of bases as "clamp". Check that there are no restriction sites for these enzymes that will form the mutant allele (inside the region to be excised is acceptable).

4. Check that 3 out of the last five or six bases at the 3' end are G or C.

5. Check that there are no significant hairpins.

6. The melting temperature must be higher than 52°C.

7. The B and C primers must be 'in frame'. The codons and their amino acids must be written out for both primers, from the start codon upstream of the B primer, to the stop codon downstream of the C primer, which will give the mutant allele.

8. All known regulatory elements from the Broad institute website and from PubMed publications, must be marked on the map.

9. Recalculate the distances from the 5' ends of A and B, and from C to D, to confirm that they are within 5 bp of each other in size.

10. Check that the primers as written for ordering are identical to the primers as outlined on the gene map. Also make sure that the A and D primers include their restriction sites and clamps, and that the "tail" is included in the B or C Soeing primer.