Guidelines for Primer Design

Modified by: VGO 10-31-16

1. Specificity

Primer length should be 17-25 nucleotides.

Be sure to choose a primer whose sequence is in your desired template. Also be sure that there is only one binding site for your primer. You can use BLAST primer tool to check primer specificity in available genomes, or different sequence software to search as well.

The primer should match the template exactly.

Near the 3' end an exact match is essential, especially the last 8 bases. When designing a primer from a sequence obtained from the DNA Sequencing Facility remember that sequence data beyond 500 bases is more likely to have errors than the first 50-500 bases. Unless you have sequence information from the opposite strand or overlapping data from another sequence, be conservative and choose your primer in the safer region, preceding base 500. Degenerate primers are not recommended.

2. Estimated Melting Temperature (Tm)

Primers for cycle sequencing should have a Tm of 50-70C, with the best at 55-65C.

The melting temperature of your primers will vary depending on the conditions used in the PCR. Some PCR enzymes (such as Q5 and SYBRgreen) have online tools already preset with the specific conditions of their PCR reactions. Otherwise, you can use IDT primer check tool to estimate a melting temperature.

If the Tm of your primer is on the low side, please consider redesigning a longer primer. When the Tm is too low, the primer may anneal incorrectly or not at all. A high Tm can be OK if there are no long strings (>3) of Gs or Cs that can bind quickly, often incorrectly, and very tightly. Your G+C content should be approximately 50%.

Be aware that primer design software packages calculate Tms based on some theoretical model that does not always yield actual experimental Tms. Base stacking and nearest neighbor models give the most accurate theoretical Tms. However, we have found that two fairly simple equations can give useful results.

1. 1. The McConaughy equation (Biochemistry 8: 3289-3295, 1969) modified for cycle sequencing:

   Tm = 60 + 41(G + C)/L - 500/L where L = length of primer

2. The Wallace equation (Nucleic Acids Research 6: 3543-3557, 1979):

   Td = 2(A + T) + 4(G + C) (This is actually dissociation temperature.)

Remember that all calculated Tms are only estimates. They are meant only as starting points and do not guarantee success. We recommend that you avoid the extremes and choose a Tm between 55-65?C, if possible.

The Tm of the 5' end should be similar to the Tm of the 3' end.

A quick way to determine the Tm at each end of the primer is to count the number of A/T bases and C/G bases within 6 nucleotides of each end. Choose the primer with the most similar numbers. This will help ensure that the primer anneals flat with the template strand.

3. Primer Sequence

Avoid primers that can form hairpin loops or primer-dimers. You can check for primer dimers (both homodimers and heterodimers) on the IDT website primer check tool. Also avoid stretches of more than 2 identical bases (especially C or G), particularly at the 3' end. This can cause slippage or

mismatch during annealing, resulting in a bulge in the primer/template hybrid which could prevent the polymerase from priming.

Many primer design programs are currently available from our computing facility to assist you with primer design and evaluation. If you have strict limitations on the location (cloning or mutant making) you will typically design them manually. If you're checking for presence absence of a gene, you can use a software.

Before you order primers, always have at least two lab members check and initial/date them.