

How to Design Primers for PCR and quantitative real time PCR (qPCR) Tips for primer design: Keep the melting temperatures ( $T_m$ ) of each primer pair within 2°C of one another. Cytosine and guanine have stronger binding affinity than adenine and thymine and repeats of more than 4 G or C can bind to many places in the genome with high affinity. A GC content between 35% and 65% without long stretches (> 4 bases) of the same nucleotide will ensure enough sequence complexity for optimal primer specificity. Amplicons between 70–140 base pairs are generally long enough to allow the design of two efficient primers and a probe (if a TaqMan-based assay is desired) for qPCR assays. Having a similar  $T_m$  between primers ensures that the forward and reverse primers will be bound to their complementary DNA strands at the same time, reducing the chance that the primer with the highest  $T_m$  will bind to nonspecific DNA sequences. If these repeats are at the 3' end of the primer, DNA polymerase can extend amplicons in off-target locations, which can ultimately decrease the PCR efficiency.