How to Design Primers for PCR and quantitative real time PCR (qPCR) Tips for primer design: Keep the melting temperatures (Tm) 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 Tm 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 Tm 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.