

PCR Primer Design Guidelines

Length ▶ 18 to 30 nucleotides

GC Content ▶ 40% to 60%

Annealing Temperature ▶ For annealing temperature, use 5°C to 10°C below melting temperature (T_m).
Example T_m calculation:
$$T_m (^{\circ}\text{C}) = 2(\#A + \#T) + 4(\#G + \#C)$$
▶ Generally, a T_m between 55°C and 80°C will yield the best results.

Sequence ▶ Avoid polybase sequences (3 or more) Gs and Cs at the 3' end.
▶ Avoid mismatches at the 3' end.
▶ Avoid complementary sequences within the primer.
▶ Avoid primer–dimer formation by complementarity at 3' ends of primer pairs.