Custom Sequencing Primer Design Guidelines

1. Thermal cycling Conditions: Denaturing step heats the reaction mix to 96°C. The annealing step cools the reaction mix to 55°C, and the polymerization step extends at 60°C. Primers must have annealing temperatures of 56°C or higher.

2. Primer length should be at least 20- to 25-mers. GC content should be 50% or more.  3. Primers should be designed with a tightly binding 3' end.  4. When designing a primer, do not pick a region that is closer than 50 bases to the region of interest.  5. Primers for PCR reactions tend to work fine for automated sequencing.