

## DNA Sequencing Service Primer Selection Guidelines

1. 18-28 nucleotides in length.
2. 50% G/C content.
3. G & C “clamps” on the 3' and 5' ends (at least a single G or C residue)
4. Primer should be at least 20-30 bases long at 5' of region to be sequenced.
5. Avoid multiple Thymidine residues on 3' and 5' ends.
6. Avoid primers with long runs (more than 3 or 4) of a single base.
7. Avoid primers with tendency to form strong intramolecular base pairs or primer primer dimers.
8. Melting temperature 55-65??.
9. Check primers for specificity in annealing to template. If possible use a computer program to design primers.
10. PRIMERS SHOULD BE 4 $\mu$ M in CONCENTRATION (For average 20 mer, 4 $\mu$ M corresponds to approximately 27 ng/ $\mu$ l) and at least 15 $\mu$ l.