

## Sanger Sequencing: Tips for primer design

Sequencing primers should adhere to the following specifications in order to increase the likelihood of success:

- A length of 18-25 bases
- %GC content between 40 and 60%
- A  $T_m$  (melting temperature) between 50 and 60°C
- No secondary priming sites
- No dimerization capability
- No significant hairpins (> 3 bp)

If you are using sequencing data to design primers, choose an area about 40-60 bases upstream of the target sequence.