MICROMON SANGER SEQUENCING

PRIMER DESIGN FACTORS

Similar to PCR applications, the following factors are important in obtaining a good primer for successful DNA sequencing:

Primer length
18-24 nucleotides long to ensure effective hybridisation

GC Content
30-80% (ideally 50%)

Melting Temperature
50-65^{o}C If the primer has a Tm below 50 ^{o}C , the annealing temperature will need to be lowered accordingly $Tm = 4(G + C) + 2(A + T)º C$

Single Nucleotide Runs
Avoid runs of a single nucleotide, especially runs of four or more dGs

Primer-Dimers
Avoid sequences that can lead to the primer annealing to itself because this depletes the available primer in the reaction and can result in a very large peak at the start of the read, quenching the basecall data

Hairpin & Secondary Structures
Avoid sequences that allow the primer to fold and form hairpin or secondary structures eg. Palindromes

Secondary Hybridisation Site
Ensure that the primer does not bind to any other site on the target template

Degenerate Primers
Not recommended for DNA sequencing

G or C Clamp at 3' End
Recommended to have a G or C at the 3' end to act as a clamp, as this end binds most strongly to the template

Purity
Standard, desalted oligonucleotides are satisfactory for DNA sequencing. While purified oligos will work better, that level of purity is not essential.

Concentration

The commercial primer stock must be diluted to a working concentration prior to use. We recommend that a small aliquot of the stock is diluted to 3-5 µM (3-5 pmoles/µl) in Milli-Q ultrapure water (not TE buffer).

PRIMER DESIGN SOFTWARE

Most comprehensive, molecular biology software packages include a primer design application. We recommend BENCHLING which is a free software that contains the primer design functionality.