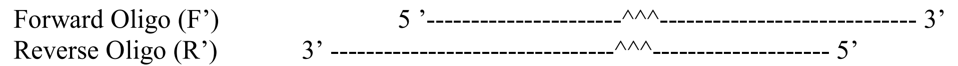
Oligonucleotides

Design of Mutagenic Oligonucleotides

Overview

Length of each oligo should be 35 to 45 bases (or more if needed)
There should be approximately 12 correctly matched bases on either side of the mutation
There should be 6 to 9 bases of 3' overhang on each oligo (illustrated below)



Each end of the oligo should terminate with 1 to 3 'G's and/or 'C's
The GC content of the finished oligo should be approximately 40%
The melting temperature (Tm) should be >= 78° C as given by :

$Tm = 81.5 + 0.41*(\%GC) - 675/N - \%MM$
Where N = total length and %MM = number of mismatched bases (those mutated)

Construction (step by step)

Write the original sequence for area to mutate in the center of a page
Extend the sequence approximately 30 bases in the 3' and 5' directions
- Attempt to terminate each end with some combination of 'G's and 'C's

Count back from each end 6 to 9 bases
- Stop adjacent to a 'G' or 'C'
- If a location was not found, step in form end to next 'G' or 'C' and repeat this step
- Mark both starting and stopping points, on each end, when found

Forward oligo goes from inner 5' mark to outer 3' mark
Reverse oligo goes from outer 5' mark to inner 3' mark

Determine a suitable mutation sequence incorporating a restriction site (addition or deletion) for screening
- Try to overlap the recognition sequence of the restriction enzyme with one or more of the bases changed for the mutant
- Try not to change any more bases than absolutely needed
- Try for 3 or less. Too many can cause priming failure
- If no site can be found, try performing a silent mutation in adjacent area

Write up both forward and reverse (complementary) oligo sequences
- Perform GC percentage and Tm checks
- Triple check the validity of the sequence before ordering
- Do NOT introduce any stray mutations