- The positive strand follows some basic requirements for fluorogenic allele-discriminating probes. These basic requirements include: i) Keep G-C content in the 30-80% range. ii) Avoid repeats of an identical nucleotide. This is especially true for guanine, where runs of four or more Gs should be avoided. iii) Select the strand that gives the probe more Cs than Gs, and do not put Gs on the 5' end. iv) The position of the polymorphic site (mismatch) should be approximately in the middle of the sequence.

- Make the negative strand as short as possible, without being shorter than the PCR primer, i.e. its T_m should be close to but not lower than that of the PCR primer.
- Select positive strand that is 0-3 nucleotides longer than the negative strand, calculate its T_m as well as the T_m of the duplex between the positive strand and the single mismatched target (using software like T_m *Utility*). Chose the positive strand with the highest Tm possible than the single-mismatched duplex.
- Before submitting for synthesis, the two negative strands differ only in one nucleotide can be synthesized as degenerated oligonucleotides. For example, the negative strand of the wild-type probe and the mutant probe is 5'-TATTGCTATTACCTTAACCCAG-Dabcyl-3', and 5'-TATTGCTATTGCCTTAACCCAG-Dabcyl-3', respectively. These two strands differ only in one nucleotide at the underlined position. They can be replaced with a single oligonucleotide of 5'-TATTGCTATTRCCTTAACCCAG-Dabcyl-3' accordingly.