

## ***Oligonucleotide Synthesis***

### **PCR Primer Design**

1. PCR primers should be designed to amplify a region containing the SNP of interest.
2. PCR primers should not be labeled.
3. Amplicon size is not restricted.

### **OLA Probe Design**

1. OLA probes should be synthesized for all sequence variants and should be from the same DNA strand (per target sequence).
2. OLA probes should be matched for melting temperature at 51-56°C.
3. OLA probes should extend out to and include the SNP as the 3' nucleotide.
4. Use Tag IT™ oligo design software to select an appropriate TAG sequence.
5. The OLA probe is synthesized with the TAG sequence incorporated at the 5' end.
6. The reporter probe should have a melting temperature of 51-56°C.
7. The reporter should begin with the nucleotide immediately downstream from the SNP as the 5' nucleotide.
8. The reporter probe must be modified with phosphate at the 5' end and with biotin at the 3' end.

