Using Primer Express®, follow recommendations from ABI for Taqman® primers and probes. I like to use Primer Express for probe design then adjust the primers for additional criteria.

Probe Design
1. Keep the G–C content in the range of 30–80%.
2. Avoid runs of an identical nucleotide, especially guanine. Avoid 3 or more Gs in a row. (Lots of guanine decreases yield. 5’–G next to dye specifically quenches dye.
3. No G on the 5’end of the probe.
4. Using PrimerExpress™ software, the T_m should be 10°C greater than the primer T_m.
5. Probe should have more Cs than Gs. If not, select the reverse strand probe, if using Tamra–quenched probes. This is not necessary for MGB probes.
6. Place middle of the probe over polymorphism for SNP analysis or over exon–exon junction site. Probe will tolerate mismatch on termini better than mismatch in middle. For allelic discrimination, can drop probe temp a little bit.
7. For multiplexing, adjust probe length so that probes have the same T_m.

Primers
1. Amplicon should be 50–150bp but can use larger if necessary.
   Slightly larger amplicons (100–200bp work well for Sybr® Green).
2. Primer to probe distance should not exceed 100 bp. Put probe closest to primer that sits on the same strand as the probe.
3. Choose primers with a maximum of 2 G/C bases on the 3’ terminus. Avoid G/C on the 3’ terminus if possible.
4. Keep the G–C content 30–80%.
5. Avoid runs of an identical nucleotide.
6. Place both primers as close as possible to the probe without overlapping the probe.
7. Try to design primers with a T_m close to 60°C.
8. For best results, only use 500bp of your sequence of interest in Primer Express™.

Other primer design criteria:
1. After selecting potential primer/probe sets in Primer Express™, you can use another primer design program (such as Oligo™ or Primer3) to check for potential hairpins and primer dimer formation.
2. Choose primers that:
   a. do not form hairpins.
   b. do not form more than 2 bonds at the 3’ terminus.
   c. do not form stable homo- or hetero-dimers (choose primers that form a maximum of 5 bonds over the length of the primer with a maximum of 3 bases in a row).
   d. do not have a G or C on the 3’end if possible. Avoid primers with 2 or more G/Cs at 3’end.
   e. have roughly equal numbers of G, C, A, & T.
   f. are at least 18bp long.
   g. do not contain repeat elements or stretch of the same base.

3. Choose primers in an appropriate section of the gene. e.g., if you are using poly dT-primed cDNA, you will probably want to design primers and probe near the 3’end of the gene.