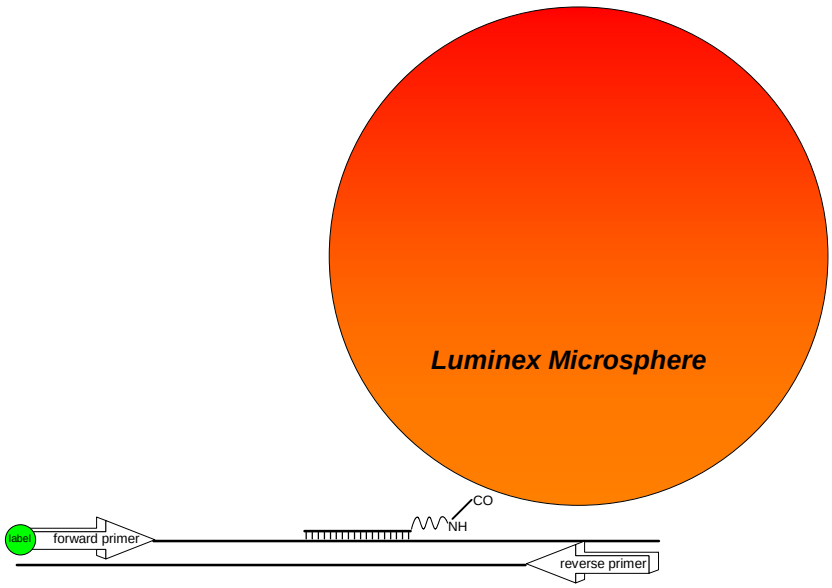
Recommendations for Probe/Primer Design for Direct Hybridization

Probe Design Strategy

1. All probes should be exactly the same length per target sequence (using TMAC hybridization buffer).
2. For detection of point mutations, we typically use probes between 18 and 24 nucleotides in length. 20 nucleotides is a good starting point.
3. Point mutations (or SNPs) should be positioned at the center of the probe sequence (i.e., position 10 or 11 for a 20 nucleotide probe). Multiple polymorphisms should be equally spaced throughout the probe sequence.
4. Probes should be synthesized for all sequence variants (all mutant and wild type sequences) and should be from the same DNA strand (per target sequence).
5. For unrelated sequences, probes may be lengthened. Better sensitivity may be achieved with longer probes (50 or 70 nucleotides).
6. Probes must have a primary amino group for coupling to the carboxyl group on the microsphere. We suggest synthesizing the oligonucleotide with a 5' amine-spacer (See 7.).
7. Probes must have a spacer between the reacting amine and the hybridizing sequence. We recommend synthesizing capture probes with 5' Amino Modifier C12 or 5' Uni-Link Amino Modifier.

PCR Primer Design For Direct Hybridization Format

1. Primers should be designed to yield amplicons in the 100 to 300 basepair range.
2. One PCR primer must be labeled with a 5'-biotin (for streptavidin-phycoerythrin reporter) or 5'-Cy3.
3. The labeled strand of the amplicon must be complementary to the capture probes on the microspheres.

Luminex ***Direct Capture of a Labeled PCR Product***