

## What results should I expect from an oligonucleotide hybridization?

Three different amine-C12-modified oligonucleotide probes were coupled to carboxylated microsphere sets according to our standard Oligonucleotide Coupling protocol. For each 18 nucleotide probe, 0.2 nanomoles were coupled to 5 million microspheres on two separate occasions by three different individuals between August 2001 and July 2002. The functional coupling efficiency was assessed by direct hybridization to 0 to 200 femtomoles of the biotinylated reverse complementary oligonucleotide target. Hybridization was performed at 52-55°C according to our standard Oligonucleotide Hybridization protocol. The net median fluorescent intensity values obtained were very consistent between coupling episodes with an average CV of 15%.

