|  |  |
| --- | --- |
| Amplicon size | 100bp-250bp |
| Primer size | 18bp-25bp |
| Probe size | 20bp-25bp (No G base at the 5’ end) |
| Melting temperature (Tm) of the primers | 60°C-64°C, within 2°C of each primer |
| Melting temperature (Tm) of the probe | 66°C to 70°C (6°C to 8°C higher than the Tm of the primers) |
| GC content  | 35-65% |
| max Poly-G | 3 |
| GC clamp | 0 |
| Hairpin structures and dimers | Checked using OligoAnalyzerTM tool in IDT <https://www.idtdna.com/pages/tools/oligoanalyzer>  |