GEN-FISH
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OpenArray Assay Development

If possible, always use Primer Express to design OpenArray (OA) assays. The software can be downloaded remotely, and we do have a registration code, although there may be a limit to the number of times it can be used. If Primer Express is not available for assay development, please send the primers and probes to Jonathon LeBlanc or someone else who has the software, so that we can get an accurate melting temperature (Tm) of the probe with the MGB moiety. Probe Tm calculators that do not account for the MGB moiety give much lower values and cannot be used for OA assay development.

The below guidelines are directly from Thermo Fisher's website and a presentation that was given at the University of Windsor when the system was first installed.

Primer Development

- Ideal length is 20bp; however anywhere from 9-45 will work
- No more than 4 Gs in a row
- Tm of 58°C–60°C
- GC% content between 30–80%

Probe Development

- Should be 13–18bp
- No G on the 5' end of the probe (it acts as a pseudo-quencher)
- No more than 2 C and/or Gs within the last 5 nucleotides of the 3' end
- No more than 4 Gs in a row
- Tm of 68°C–70°C
- GC% content between 30–80%

General guidelines

- The amplicon should be between 50–150bp in length
- The primer Tms should be 8°C–10°C lower than that of the probe
- The amplicon should span an intron-exon boundary, if possible, to prevent amplification of genomic DNA