General guidelines for primer design

If you would like to use a different tool for designing primers, please take into account the following information regarding amplicon length and melting temperature for dPCR and qPCR QC primers.

	dPCR primers	qPCR QC primers
Amplicon length	120-160 bp	80-120 bp
Melting temperature	~ 60°C	~ 60°C

Note: The qPCR QC primer pair must be different from the enrichment dPCR primer pair and placed within 2 kb distance from it. The qPCR QC and dPCR amplicons must not overlap.

The risk of false-negative enrichment prediction increases if the validation qPCR assay is placed further away from the dPCR assay.

The following guidelines apply to both dPCR and qPCR QC primer pairs:

- Avoid primer pairs with more than 2°C difference in melting temperature between forward and reverse primer.
- Avoid placing primers in low complexity regions.
- Primers need to be specific. Avoid primer pairs that amplify sequences not related to the target sequence.
- Follow the general recommendations for PCR primer design: avoid self-complementarity, stable secondary structures, hairpins etc.