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Strategies to Minimize Variability Between Individual qPCR Reactions

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Abstract

Precise pipetting is paramount for generating precise and reliable qPCR results. Despite meticulous pipetting, significant variability can arise in qPCR, even between technical replicates. While some variability is expected in samples with low target concentrations, it is concerning in samples with high target concentrations. To maintain data integrity, it is generally recommended to discard data with a discrepancy of more than 0.2-0.5 Ct values between technical replicates, in particular when the Ct values are below 30 [Anonymous, 2019].
This protocol provides approaches to reduce this variability through an optimized pipetting strategy. Central to this strategy is the use of pipetting schemes and built-in redundancies in the workflow keeping track of individual pipetting steps.

Attachments

PDF

02_setting_qPCR_assa...

677KB

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Protocol references

Anonymous, 2019. Tips, Tricks & Best Practices, The Ultimate qPCR Assay Design Guide. Bio-Rad - Bulletin 6894 Ver B | 19-2076 NASD

Bustin S.A., et al. (2009) The MIQE guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clin Chem 55, 611–622. PMID: 19246619. doi: 10.1373/clinchem.2008.112797.

Taylor S.C. et al. (2019) The Ultimate qPCR Experiment: Producing Publication Quality, Reproducible Data the First Time. Trends Biotechnol., 37, 761-774. PMID: 30654913. doi: 10.1016/j.tibtech.2018.12.002.