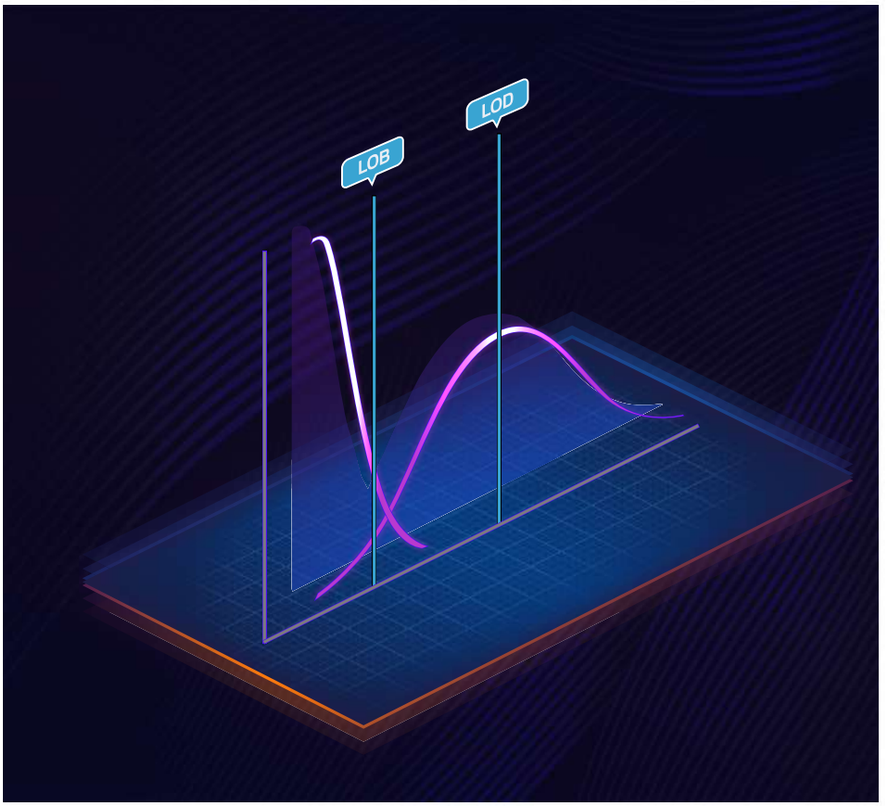
STILLA

How to characterize the Limit of Blank for digital PCR?



Why calculate a LOB in digital PCR?

In digital PCR (dPCR) false-positive events can arise from several sources of molecular biology noise. Determining a false positive cutoff when quantifying nucleic acids is therefore critical to the robustness of a dPCR assay. To define this cutoff, detection thresholds are commonly set above the Limit of Blank (LOB) value that must first be determined for each target during assay calibration.

We recommend the application of this LOB decision tree as a good practice before any new assay, particularly when a target is expected in low concentrations.

Furthermore, the experimental LOB value is necessary both to determine if a reaction is positive or negative and to calculate the theoretical Limit of Detection (LOD).

Note that once a LOB has been defined for an assay, it does not need to be redefined for the assay as long as the subsequent experiments are performed in the same experimental and environmental conditions.

Let's start with some definitions

Limit of Blank (LOB) for a given target: The highest apparent number of positive droplets expected to be found with more than 95% probability, when replicates of a sample containing no target are tested[1]. Setting the detection threshold above the LOB value ensures a false positive rate of less than 5% for this target.

Target: DNA or RNA sequence to be amplified and quantified in a dPCR assay. There could be multiple targets on the same nucleic acid template (e.g. cancer mutations or copy number variants in patient DNA, and foreign sequences in DNA of a genetically modified organism) or one or multiple targets on a mixture of templates (pathogen, viral detection, etc.).

Template: Input DNA for dPCR assays, or input RNA converted to complementary DNA (cDNA) for RT-dPCR assays.

No Template Control (NTC): The control used to monitor contamination and primer-dimer formation that increases the risk of obtaining a false positive event. For this control reaction, simply leave out the nucleic acid template[2].

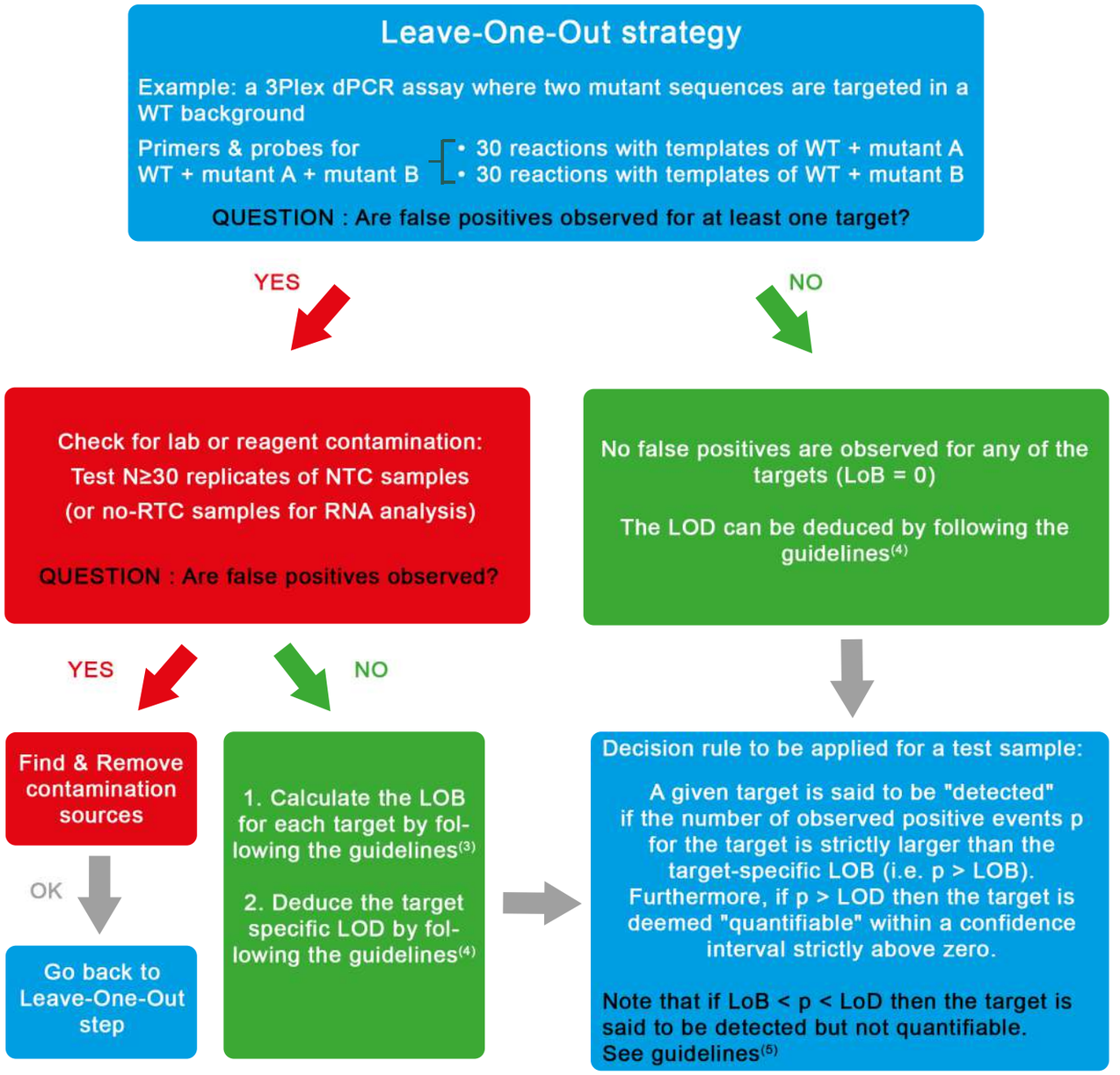
No Reverse Transcriptase Control (–RT or no-RT): The control recommended to monitor genomic DNA contamination when the target sample is cDNA. For this control reaction, simply leave out the reverse transcriptase[2].

Limit of Detection (LOD): The lowest target concentration that can be detected with more than 95% probability, ensuring a false negative rate of less than 5%. The theoretical LOD is calculated from the measured LOB, whereas the experimental LOD is determined from the analysis of positive control replicates containing low concentrations of a target[1].

The LOB Decision Tree

The first step of the LOB decision tree is a Leave-One-Out strategy.

For each target sequence, perform N≥30 replicate reactions in which the target template is excluded, and report the number of false positive events observed in each replicate:



References

1. Armbruster & Pry 2008: “Limit of Blank, Limit of Detection and Limit of Quantitation”
2. IDT, “qPCR terminology - what does it mean?”, https://www.idtdna.com/pages/education/decoded/article/qpcr-terminology-what-does-it-mean-
3. LOB calculation: https://www.gene-pi.com/wp-content/uploads/2018/03/Memo_LOB_calculation_method.pdf
4. LOD determination: https://www.gene-pi.com/wp-content/uploads/2019/03/NaicaProduct_CrystalDigitalPCR_LOB-LOD-CNV_Statistics.pdf
5. Calculator “Stilla_dPCR-SapphireChip_LOB-LOD_ReplicateStrategy” and its protocol of use "S20190129-PRO-1.0"

Technical Note Highlights

- The LOB sets the false positive cutoff for detecting nucleic acids with digital PCR.
- The experimentally defined LOB for a target is necessary both to determine if a reaction is positive for the target and to calculate the theoretical LOD of the target.
- Before any new assay, our Decision Tree should be followed to characterize the LOB for each target, particularly when a target is expected in low concentrations.
- A target is said to be "detected" if the number of observed positive events is strictly higher than the target-specific LOB.
- A target is deemed "quantifiable" if the number of observed positive events is strictly higher than the target-specific LOD.

To learn more about digital PCR, please visit Stilla Technologies' Learning Center at www.gene-pi.com

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