GENE-π

MEMO - How to calculate the limit of blank (LOB)

For automated computation, see the statistical tools available on www.gene-pi.com

a) Definition: the limit of blank LOB with a confidence level $(1-\alpha)$ is defined (in number of partitions in a well) as the maximum number of positive partitions expected in a well with a probability of 1-α in a sample containing no target sequence.

In other words, this is the maximum number of false positives that are plausible with a $1-\alpha$ probability (typically 95% for $\alpha = 5\%$).

b) Calculation method:

1) Make R negative control replicates without any target sequence (take $R \geq 30$ wells, knowing that Stilla recommends $R = 36$ wells, obtained with 3 racks of 3 chips).

   It is recommended to perform these replicates under the same experimental conditions as the test experiments, in particular with a quantity of nucleic acids which is representative of that of the tests (if this is not constant, then take the most unfavorable case with a large quantity of nucleic acids, for example between 1000 and 10000 copies / µl).

2) Count the number $x(i)$ of "false positive" partitions observed in the well of each replicate i

3) Calculate the mean μ and the standard deviation σ of the $x(i)$

4) Calculate the corrected mean: $\mu_{corr} = \mu + 1.696\ \sigma/\sqrt{R}$

5) The LOB with 95% confidence level is determined as follows (calculation based on Normal Law approximation and Chernoff's inequality):

μ_{corr}	$LOB(95\%)$ in number of positive partitions in well
$0 < \mu_{corr} \leq 0.180$	2
$0.180 < \mu_{corr} \leq 0.477$	3
$0.477 < \mu_{corr} \leq 0.863$	4
$0.863 < \mu_{corr} \leq 1.314$	5
$1.314 < \mu_{corr} \leq 1.813$	6
$1.813 < \mu_{corr} \leq 2.348$	7
$2.348 < \mu_{corr} \leq 2.913$	8
$2.913 < \mu_{corr} \leq 3.503$	9
$3.503 < \mu_{corr} \leq 4.115$	10

Special case: if $\mu = 0$ (i.e. false positives are never present) then $LOB(95\%) = 0$