

Detection of defective PCR samples with module Outlier of Kineret software

www.labonnet.com mail to: tzachi.bar@labonnet.com



Introduction

For proper quantification with real-time PCR, compared samples should have similar kinetics. *Outlier* is a computational tool implemented within *Kineret* software that analyses real-time PCR data and verifies the fulfilment of this prerequisite by detecting PCR samples with defective kinetics. By disabling such samples from the analysis, *Outlier* improves the overall results.

Methodology

To identify defective PCR:

1. Characterize the kinetics of each amplification curve by several parameters.
2. Define a reference set consisting of well performing samples, usually the standard curve samples.
3. Self-asses the reference set for PCRs with outlying kinetics.
4. Test the kinetics of each unknown PCR against the reference set.
5. Use replicates at a given experimental level (e.g. PCR replicates, RT replicates) to detect PCRs with outlying CT.
6. Intersect the results of kinetics outlier detection and CT outlier detection.

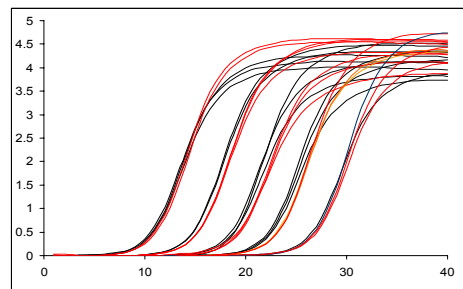
Performance evidence in controlled experiment

2 Standard curves with 15 PCRs each.

Black – uninhibited PCR, serve as reference set

Red – Inhibited PCR

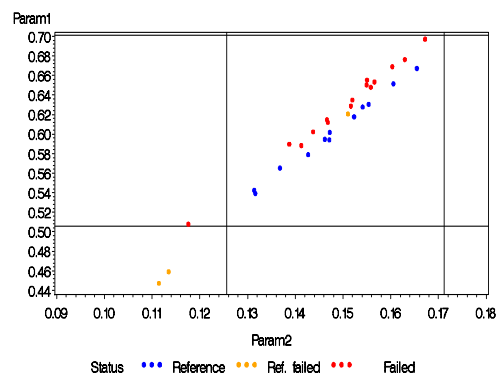
Yellow – Outlying samples in reference set



Spatial distribution of amplification kinetics described by two parameters:

The vertical and horizontal lines within the plotted area delimit the acceptance range calculated for either of the two parameters.

Only the combination of parameters detects small differences in kinetics.



Kineret vs. other methods for kinetics validation

Percentage of detected kinetics outliers in 5 sets with increasing levels of inhibition (by competimers).

When the effect of inhibition on CT becomes significant (t-test, $\alpha=0.05$) many of the inhibited samples were detected by Kineret.

The strength of Kineret to detect inhibited PCR is in strong contrast to low performance of recently published methods by Bar et al. (2003) and Charvoneva et al. (2006).

	% of outliers detected in Set of inhibited PCR					
	No inhibition	1	2	4	8	16
Competimers [%]						
Kineret	14	67	33	27	80	100
Bar et al. (1)	7	27	33	13	7	13
Chervoneva et al. (3)	0	7	0	14	7	0
Average deviation of CT	0	-0.066	0.030	0.152	0.347	0.469*

Reference

1. Bar, T., Stihlberg, A., Muszta, A. and Kubista, M. (2003) *Nucleic Acids Res*, **31**, E105.
2. Ramakers, C., Ruijter, J.M., Deprez, R.H. and Moorman, A.F. (2003) *Neurosci Lett*, **339**, 62-66.
3. Chervoneva, I., Hyslop, T., Iglewicz, B., Johns, L., Wolfe, H.R., Schulz, S., Leong, E. and Waldman, S. (2006) *Anal Biochem*, **348**, 198-208. Epub 2005 Nov 2017.