

Methods of Comparing Digital PCR Experiments

Michał Burdukiewicz¹, Piotr Sobczyk², Paweł Mackiewicz¹, Stefan Rödiger³

¹University of Wrocław, Department of Genomics, Poland

²Wrocław University of Technology, Institute of Mathematics and Computer Science, Poland

³Faculty of Natural Sciences, Brandenburg University of Technology Cottbus–Senftenberg, Germany

Introduction

The outcome of digital PCR (dPCR) experiments are mean copies per partition (λ). Results are derived from the measured data, an ordered (in one or two dimensions) sequence of positive partitions. The usual analysis involves assumption the template molecules are Poisson distributed among partitions. On this premise, already proposed approaches, based on the confidence intervals (Dube et al., 2008) or uncertainty quantification (Bhat et al., 2009), allow a comparison of experiments.

GLM testing scheme

Generalized Linear Models (GLM) are linear models for data, where the response variables may have non-normal distributions (as binomially distributed positive partitions in digital PCR experiments). We propose a model reflecting relationships in results of digital PCR as given by:

$$\log Y = \beta^T X \tag{1}$$

where Y are counts, X are experiments names (categorical data) and β are linear model coefficients for every experiment. Moreover, $\exp \beta = \lambda$. Estimated means copies per partitions obtained from the model are compared each other using multiple t-test (Bretz et al., 2010). The linear model above can be used only when the concentration of template molecules in samples is small (positive partitions contain very rarely more than 1 template particle). Higher concentrations basically requires binomial regression.

Multiple testing scheme

The dPCR experimentes are compared pairwise using the uniformly most powerful (UMP) ratio test (Fay, 2010). Furthermore, computed p-values are adjusted using Benjamini Hochberg correction (Benjamini and Hochberg, 1995) to control family-wise error rate.

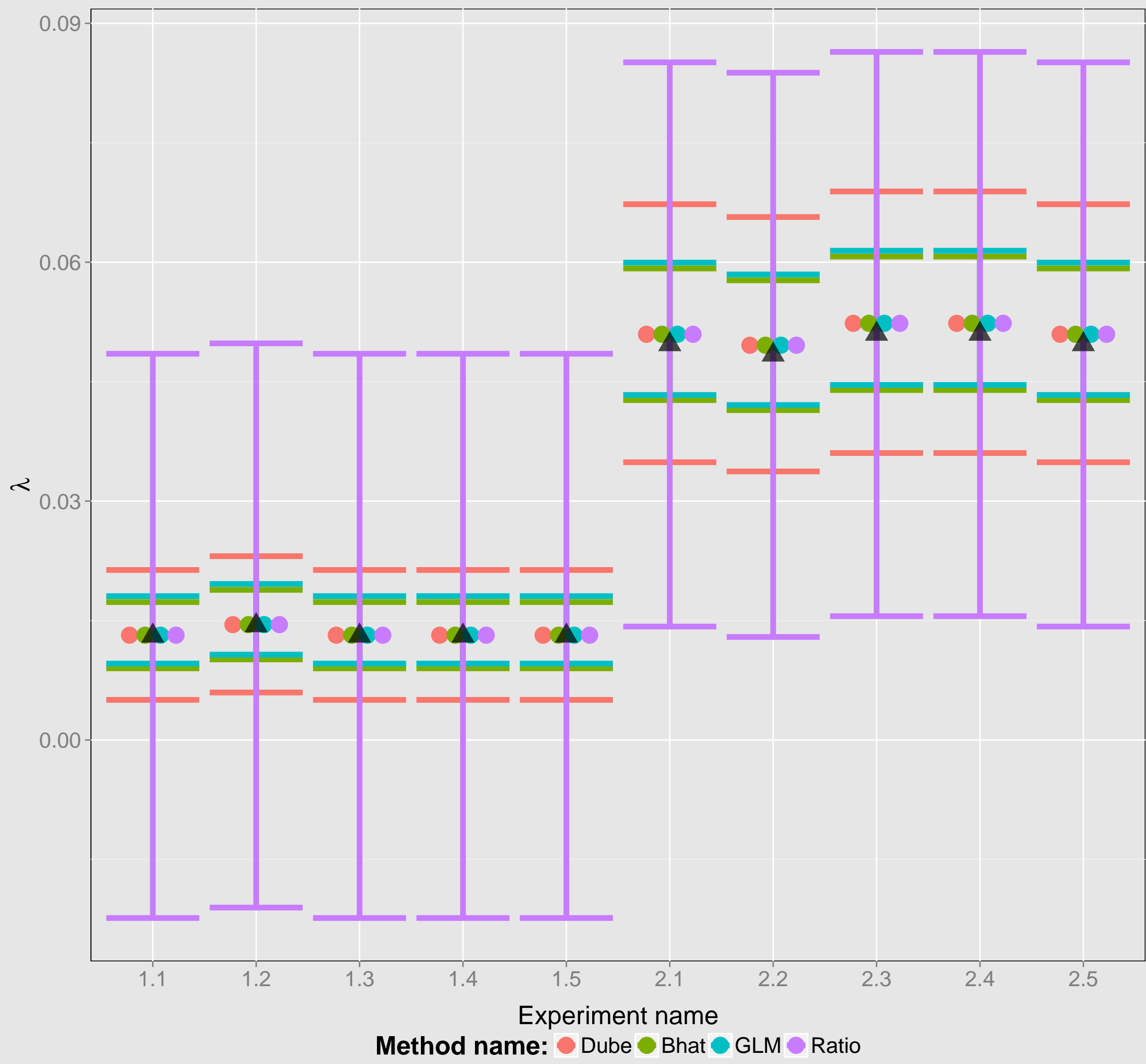
The UMP ratio test has following null-hypothesis:

$$H_0 : \frac{\lambda_1}{\lambda_2} = 1 \tag{2}$$

The Wilson's confidence intervals (Brown et al., 2001) are calculated independently for every dPCR experiment. The Dunn - Šidák correction ensures control of the family-wise error rate.

Model evaluation

Two approaches above were compared in a simulation approach.



Simulations

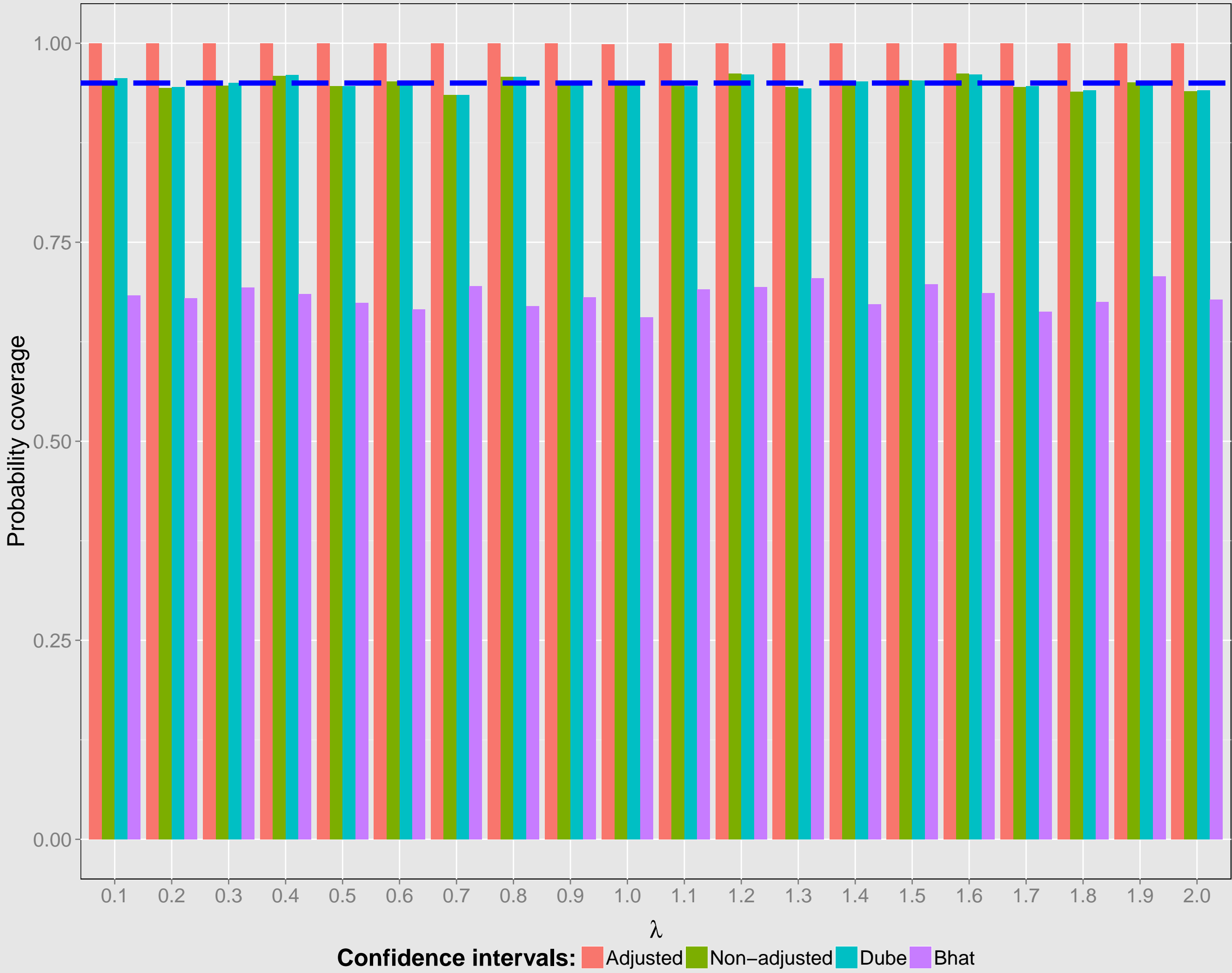
All in-silico experiments were performed using newest method of simulating digital PCR data (Jacobs et al., 2014).

Evaluation

stuff stuff stuff

Probability coverage of confidence intervals

Covarge probability is the proportion of the time that the interval contains the true value of λ .



The dashed blue line marks 0.95 border.

Summary

Availiity

dpcR web server:
dpcR R package:
<http://cran.r-project.org/web/packages/dpcR/>

Bibliography

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