Supplemental Material 5

MAKERGAUL: An innovative MAK2- based model and software for real-time PCR quantification

Christoph André Bultmann and Ralf Weiskirchen

1. Material and Methods

1.1 qPCR data

To test the algorithm, a freely available, comprehensive fluorescence data set [1] of 64 different genes was selected. This set was already used in a large comparative study on the performance for real-time PCR analysis [2]. The fluorescence data of each gene includes three replicates of a dilution series in which the primer pairs were incorporated into PCR with an external standard in dilutions of 150,000, 15,000, 1,500, 150, and 15 copies, respectively.

1.1.1 Excluded data

The data set "AluSq" contains fluorescence data in which competing competimer were added in addition to the specific primers. Since the induced changes in the PCR kinetics can not be compensated by our model, the data set was excluded from our analysis.

1.2 Analysis algorithms

The data of the dilution series were first converted into a readable format (i.e., CSV files) for the MAKERGAUL software (see Supplementary Material 4). Thereafter, the fluorescence values were processed with the MAKERGAUL algorithm. Subsequently, the $Factor_{inhibition}$ was formed from the determined average values using the OpenOffice.org Calc-datasheet "factor_inhibition.ods". These were then taken to conduct a reanalysis with the MAKERGAUL_C algorithm. The only different between both algorithms (i.e., MAKERGAUL and MAKERGAUL_C) is that MAKERGAUL_C uses a fixed value for $Factor_{inhibition}$ that is not calculated with a Downhill-Simplex-method. For re-examination of each dilution series, the mean was used as described above for $Factor_{inhibition}$ in MAKERGAUL_C.

1.3 Evaluation

The DNA values calculated by both methods were copied into the enclosed Excel sheet "analysis_dilution_series.xls" given in [1]. To ensure comparability between the different algorithms, the calculation of all technical parameters were essentially done as outlined in [2].
The following parameters were compared with for algorithms:

1.3.1 Bias

The highest measured DNA value was set to 1, all descending values were normalized to this value. The distance between the highest and lowest value should ideally be 10,000, all deviations display a positive or negative bias.

1.3.1.1 Absolute bias

For comparing the bias of our algorithms with the methods presented in [2] by using the Friedman-test, we had to transform the values for bias into an unidirectional

sortable parameter. Therefore we build an absolute bias as the absolute value of the difference between 10,000 and the value for bias.
This means the ideal value is 0, any higher represents an absolute bias.

1.3.2 Precision (also called reproducibility)

All DNA data were log transformed. Then the average variance between the triplicates of each dilution series was determined. This value provides information on the reproducibility of a measured value in repetitions. A small value indicates a high precision.

1.3.3 Linearity

This parameter describes the deviation of the logarithmic mean values of the triplicates of each dilution from the regression line of the expected values. As for the precision, a value of 0 represents the ideal state for this parameter.

1.3.4 Resolution

The resolution is defined as the n-fold change of a measured value that will be detected by the method in a 5% confidence interval.

1.3.5 Changed variance

The variance of the values for the log transformed triplicates obtained for each algorithm were compared with the enclosed values that were determined by Cq method that represents the gold standard [3]. A value of 1 means unchanged variance. Values less than 1 represent an improvement, while values larger than 1 represent a deterioration to the reference analysis.

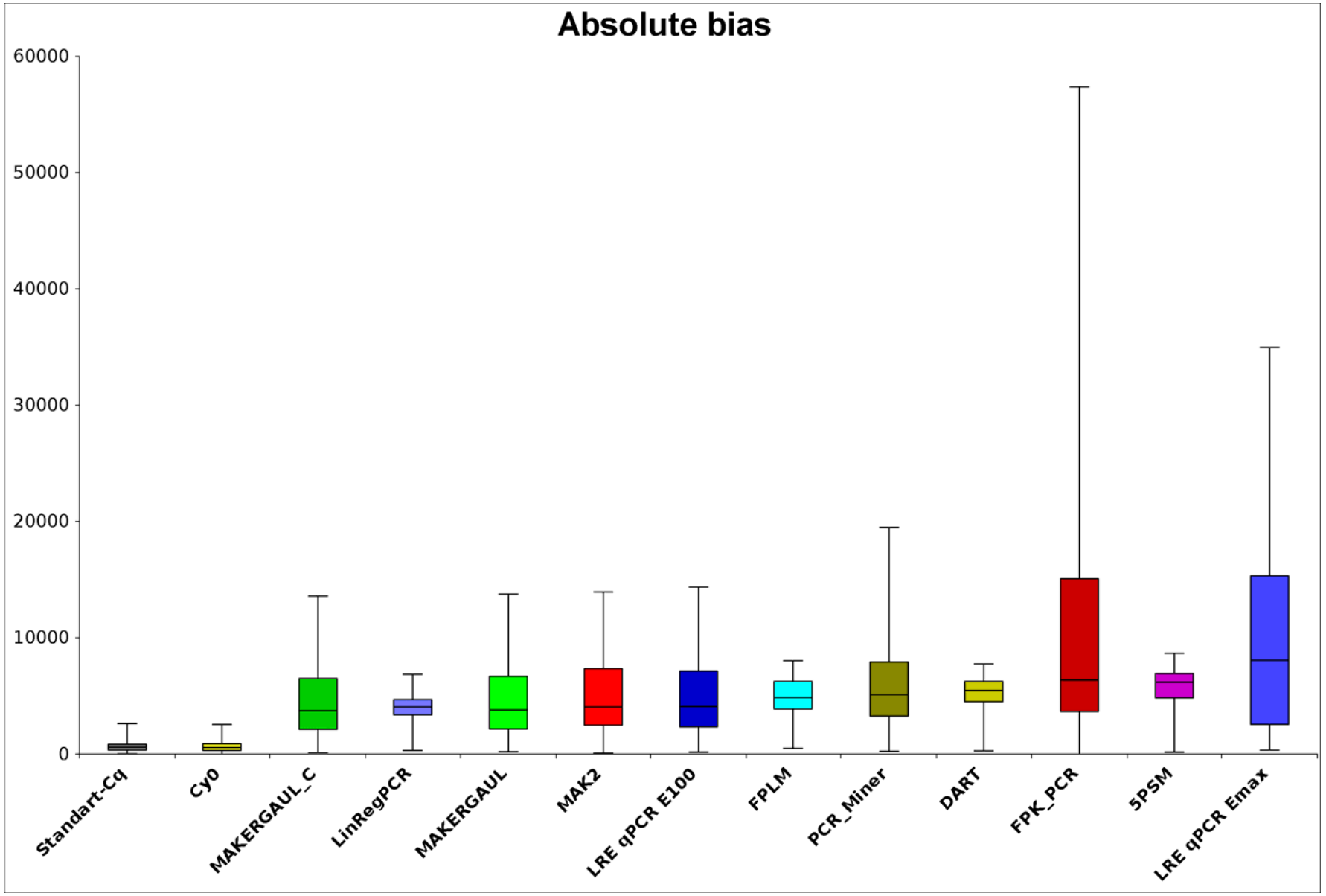
1.3.6 Friedman-test

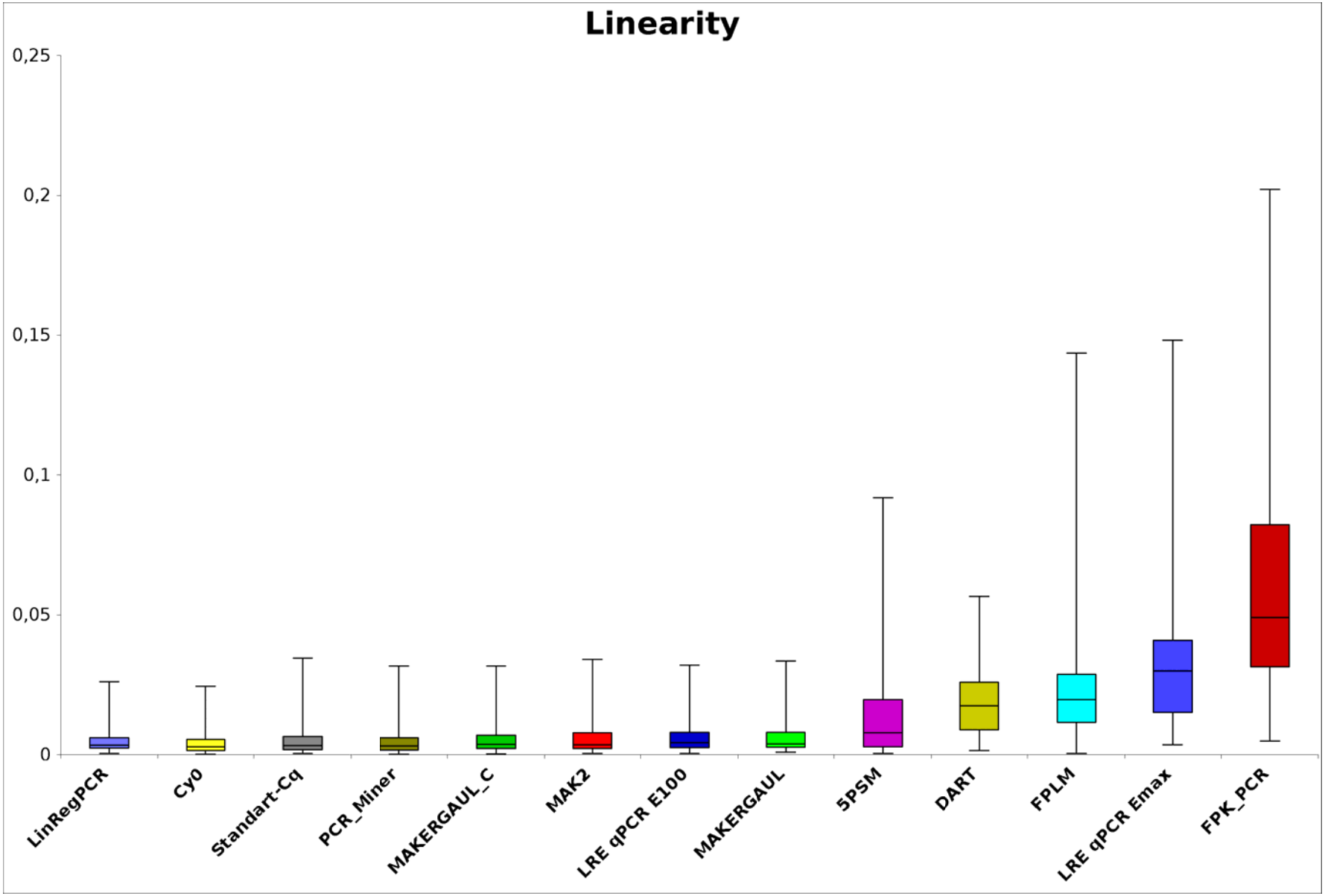
For finding a significant difference between the performance parameters of the tested algorithms (See the main Article), we used the Friedman-test. It is a non-parametric test for independent groups of dependent samples without the need of normal distribution [4]. To test the data we used the unpaired groups (the different genes) as columns, the paired samples (the results by different analysis algorithms) as rows of a single table for the every examined performance-parameter (absolute bias, precision, linearity, changed variance and resolution). If there was a difference between rows (methods), the null hypothesis (no difference between methods) was rejected and multiple comparisons between rows where applied to determine the methods (or groups of methods) which differed from the other.
The described analysis was performed by a program named ‘Friedman test: non-parametric 2-way ANOVA’ [5], basing on the methods described in [6]. The resulting prepared values and the resulting data can be found in supplemental material 4.

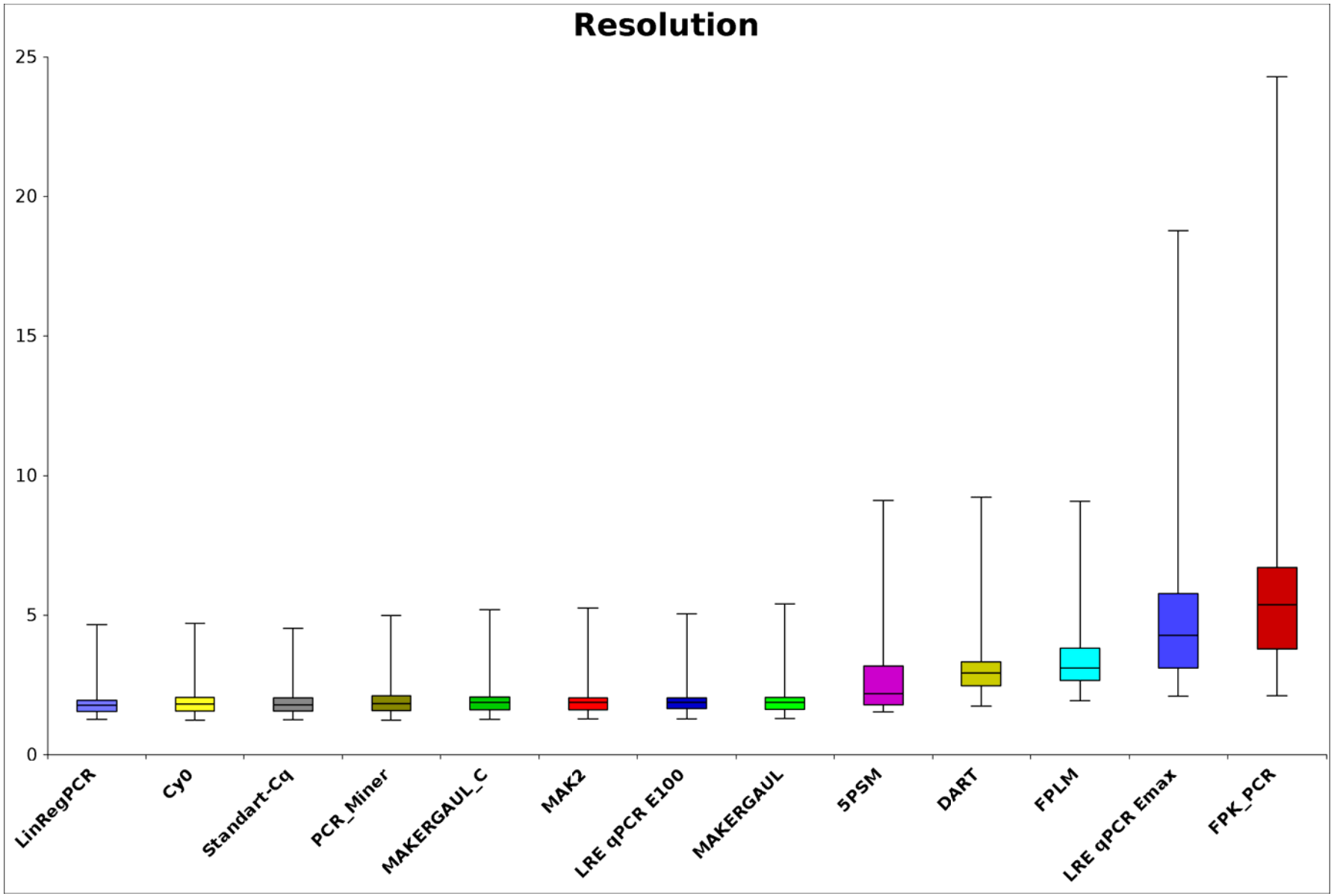
2. Results and illustrations

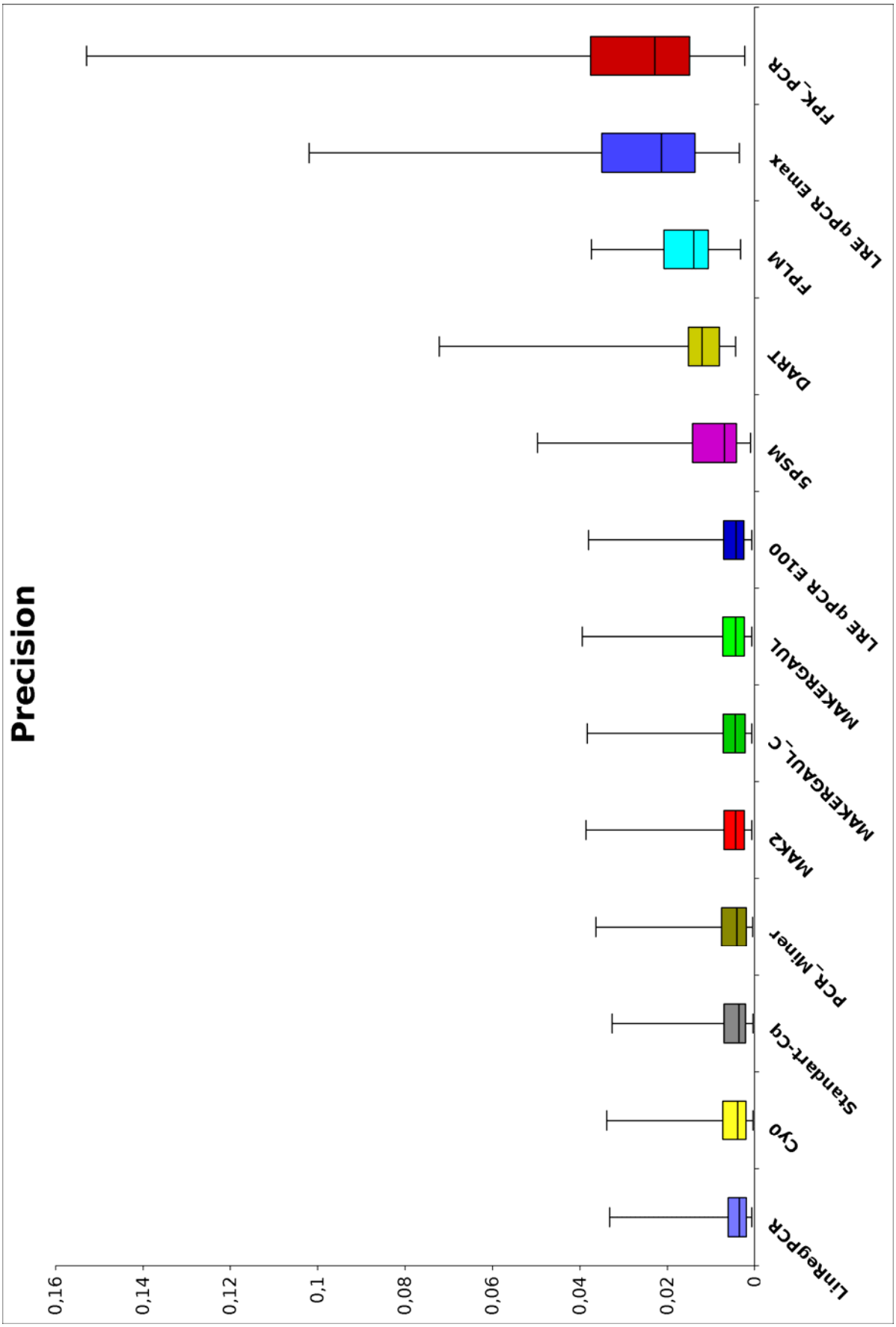
The core results and the discussion about their implications can be found in the main article. Here we present four additional diagrams which illustrate the technical parameters of the compared methods, their range and differences for the 63 enclosed dilution series.

Diagrams 1-4: Comparison of absolute bias, precision, linearity and resolution between MAKERGAUL, MAKERGAUL_C, 5PSM [8], Cy0 [9], CAmpER: DART [10], CAmpER: FPLM [11], FPK-PCR [12], LinRegPCR [13], LRE-qPCR [14] with Emax and with E100 [2], MAK2 [15], and PCR-Miner [16]. The data is presented as box-and-whiskers-plot. Whiskers are representing maximum and minimum value, the box includes the values between upper and lower quartile. The band inside shows the median value. The methods are sorted by the mean ranking from best to worst as it was computed with [5].


Absolute bias
60000
50000
40000
30000
20000
10000
0
Standart-Cq
Cy0
MAKERGAUL_C
LinRegPCR
MAKERGAUL
MAK2
LRE qPCR E100
FPLM
PCR_Miner
DART
FPK_PCR
5PSM
LRE qPCR Emax


Linearity
0,25
0,2
0,15
0,1
0,05
0
LinRegPCR
Cy0
Standart-Cq
PCR_Miner
MAKERGAUL_C
MAK2
LRE qPCR E100
MAKERGAUL
5PSM
DART
FPLM
LRE qPCR Emax
FPK_PCR


Resolution
25
20
15
10
5
0
LinRegPCR
Cy0
Standart-Cq
PCR_Miner
MAKERGAUL_C
MAK2
LRE qPCR E100
MAKERGAUL
5PSM
DART
FPLM
LRE qPCR Emax
FPK_PCR



The graphs in these diagrams were generated using the Gnumeric Spreadsheet [17].

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