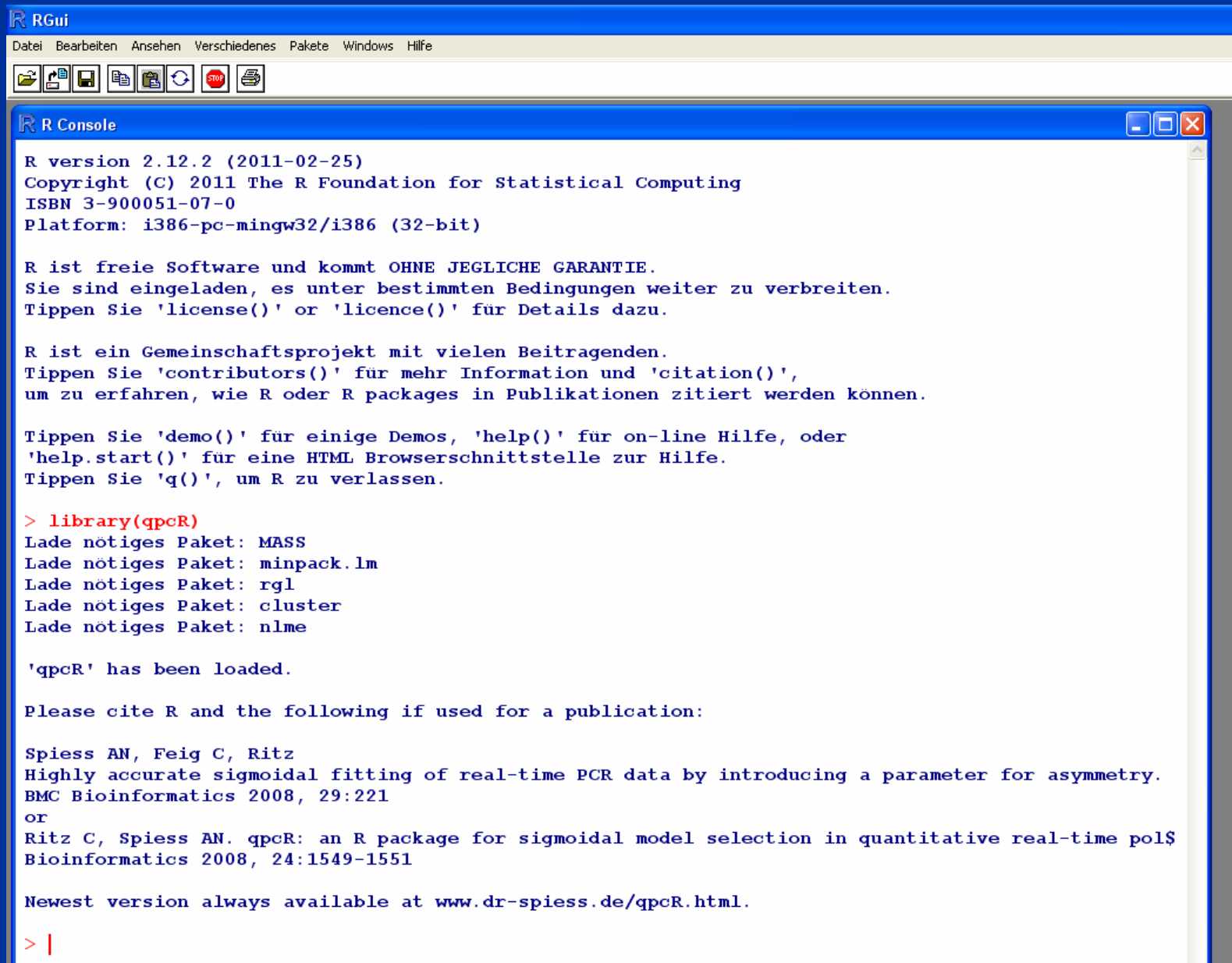


Evaluation and applicability of advanced/exotic qPCR quantification strategies and their implementation

Andrej-Nikolai Spiess

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Department of Andrology

qpcR: An R package for real-time PCR analysis



The screenshot shows the RGui application window. The title bar reads 'RGui'. The menu bar includes 'Datei', 'Bearbeiten', 'Ansehen', 'Verschiedenes', 'Pakete', 'Windows', and 'Hilfe'. The toolbar contains icons for file operations and execution. The main window is titled 'R Console' and displays the following text:

```
R version 2.12.2 (2011-02-25)
Copyright (C) 2011 The R Foundation for Statistical Computing
ISBN 3-900051-07-0
Plattform: i386-pc-mingw32/i386 (32-bit)

R ist freie Software und kommt OHNE JEGLICHE GARANTIE.
Sie sind eingeladen, es unter bestimmten Bedingungen weiter zu verbreiten.
Tippen Sie 'license()' or 'licence()' für Details dazu.

R ist ein Gemeinschaftsprojekt mit vielen Beitragenden.
Tippen Sie 'contributors()' für mehr Information und 'citation()',
um zu erfahren, wie R oder R packages in Publikationen zitiert werden können.

Tippen Sie 'demo()' für einige Demos, 'help()' für on-line Hilfe, oder
'help.start()' für eine HTML Browserschnittstelle zur Hilfe.
Tippen Sie 'q()', um R zu verlassen.

> library(qpcR)
Lade nötiges Paket: MASS
Lade nötiges Paket: minpack.lm
Lade nötiges Paket: rgl
Lade nötiges Paket: cluster
Lade nötiges Paket: nlme

'qpcR' has been loaded.

Please cite R and the following if used for a publication:

Spiess AN, Feig C, Ritz
Highly accurate sigmoidal fitting of real-time PCR data by introducing a parameter for asymmetry.
BMC Bioinformatics 2008, 29:221
or
Ritz C, Spiess AN. qpcR: an R package for sigmoidal model selection in quantitative real-time PCR.
Bioinformatics 2008, 24:1549-1551

Newest version always available at www.dr-spiess.de/qpcR.html.

> |
```

Advantages/Disadvantages of R

Pro's

- very flexible
- implementation of new methods is fast
- code inspection: what is happening with my data?
- fast algorithms (many functions call C routines)
- customized plots and functions
- can import many different data formats (tab, csv, Excel etc)

Con's

- steep learning curve

```
dat <- read.delim(...)
```

```
X <- matrix(rnorm(1000), nrow = 100)
```

```
mod <- lm(y ~ x)
```

```
barplot(X, breaks = 100)
```

- no „click and calc“
- basic knowledge of program structures is feasible

But...

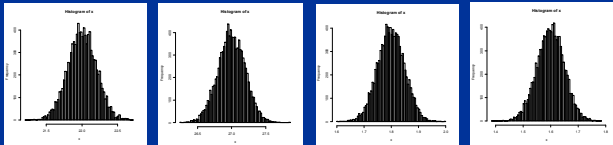
qpcR has simplified functions for the user:

```
res <- pcrfit(dat, 1, 2, 15)
```

propagate: different approaches to qPCR error analysis

E1 = 1.72; 1.75; 1.82; 1.77
 ct1 = 22.5; 22.8; 22.7; 23.1
 E2 = 1.88; 1.85; 1.83; 1.84
 ct2 = 28.4; 28.2; 28.7; 28.1

E1^{ct1}
E2^{ct2}

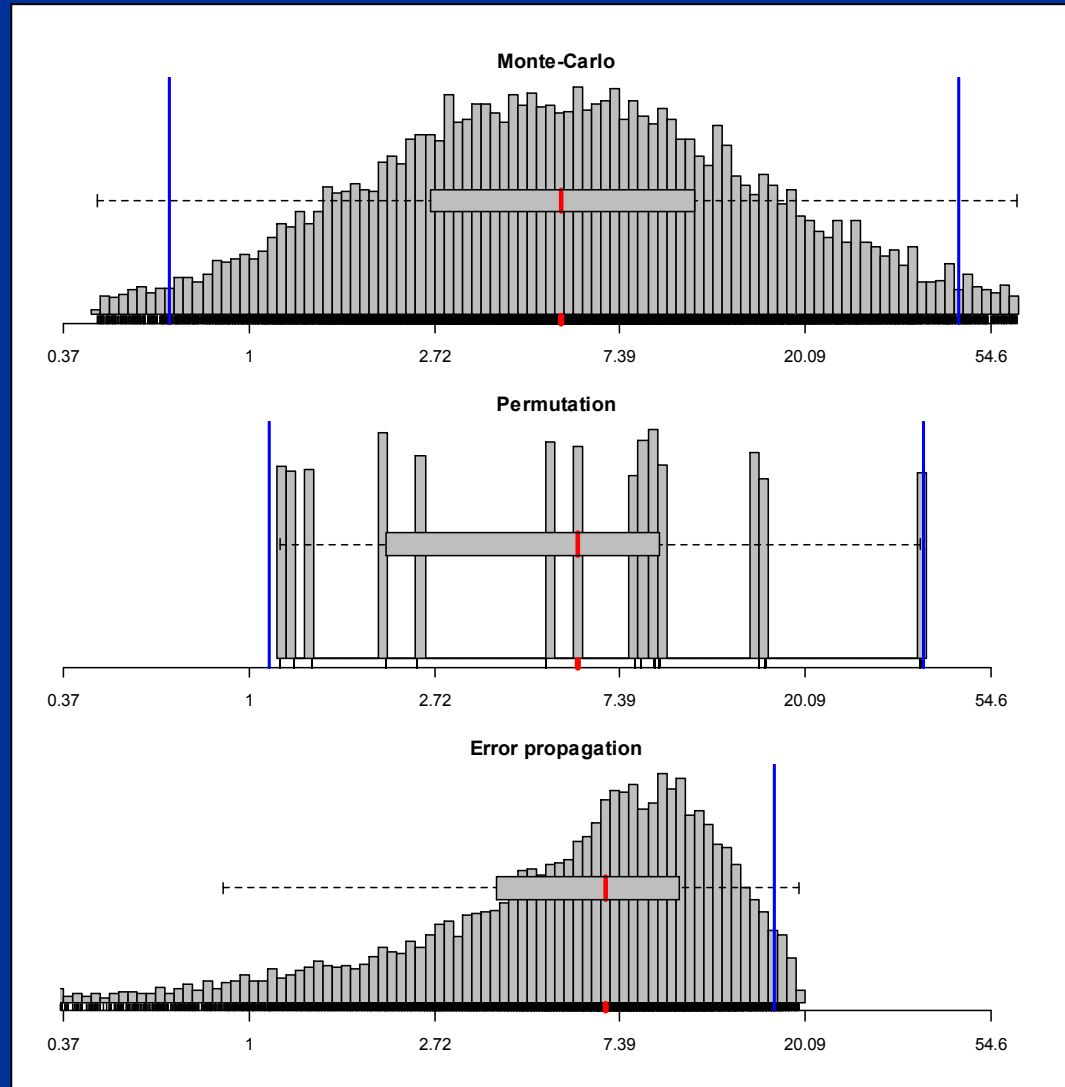


E11^{ct11}
E21^{ct21}

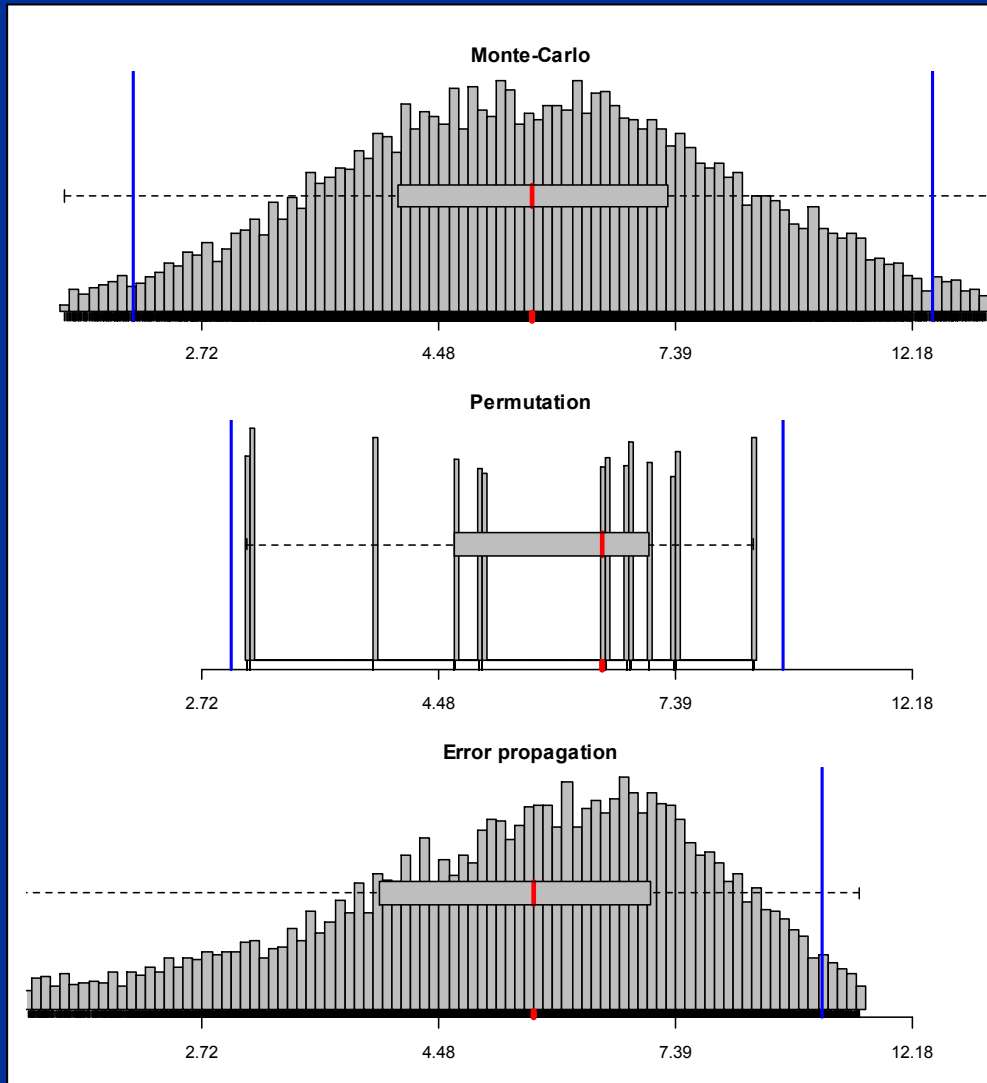
E12^{ct12}
E22^{ct22}

E1_n^{ct1_n}
E2_m^{ct2_m}

$$\sigma_Y^2 = \sum_i \left(\frac{\partial f}{\partial X_i} \right)^2 \sigma_{X_i}^2 + \sum_{i \neq j} \sum_{j \neq i} \frac{\partial f}{\partial X_i} \frac{\partial f}{\partial X_j} \sigma_{X_i X_j}$$



propagate: averaging the efficiencies over the replicates improves confidence intervals!



...or keep efficiency variance low:

0.2% error in E

0.02% error in ct

2.5% 0.048 0.045 -0.049

97.5% 0.626 0.652 0.395

0.02% error in E

0.2% error in ct

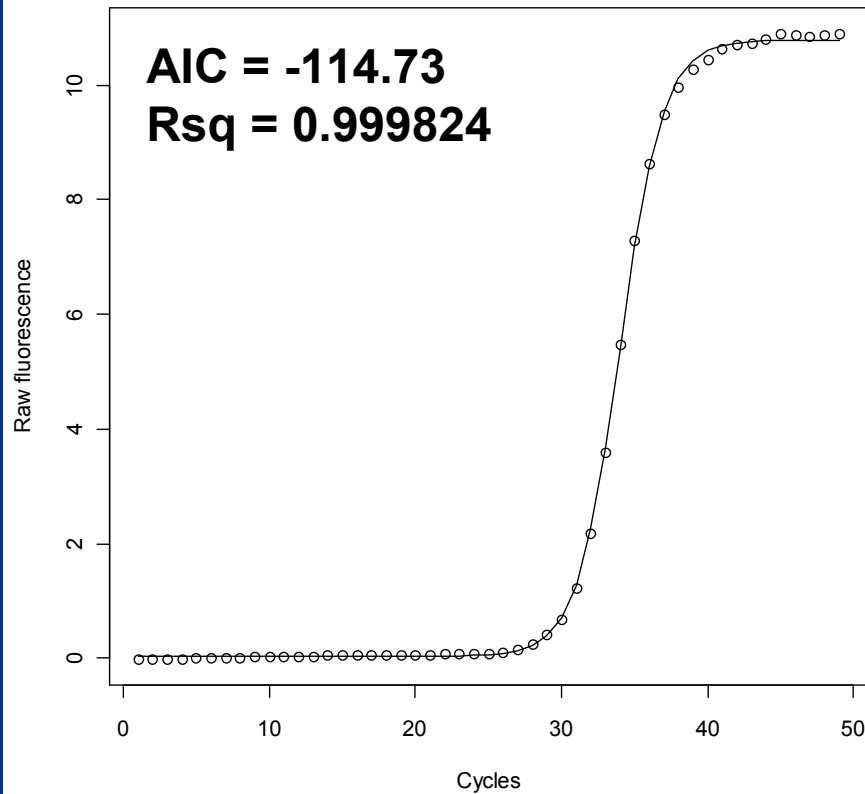
2.5% 0.078 0.077 0.036

97.5% 0.369 0.380 0.307

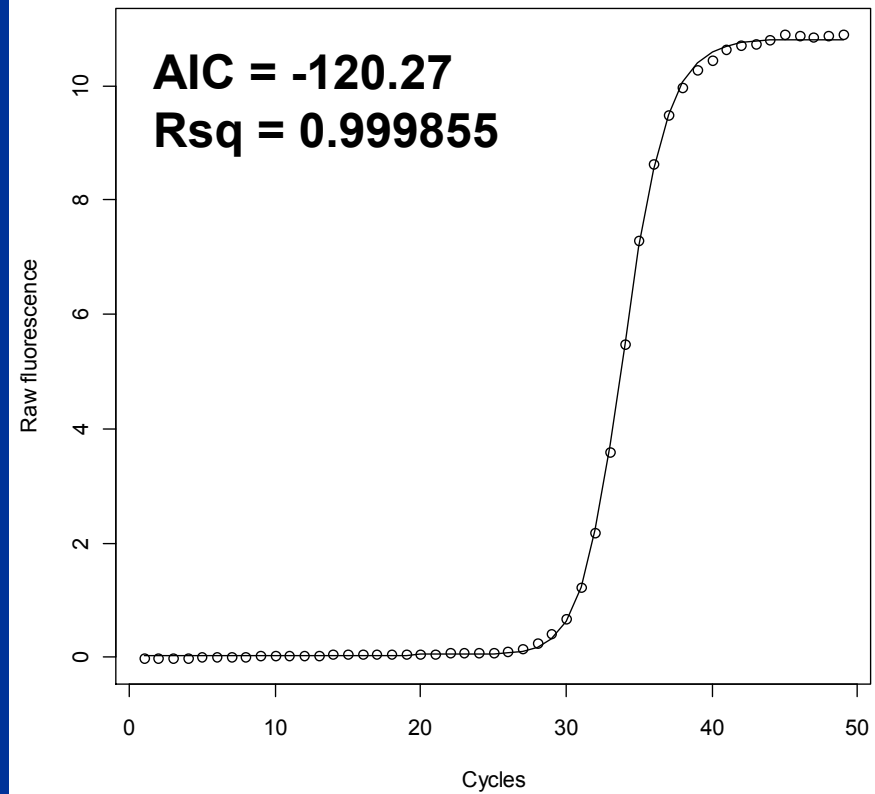
One way to get good efficiency estimates: 5-, 6-, 7-parameter sigmoidal models (1)

$$f(x) = c + \frac{d - c}{1 + \exp(b(x - e))}$$

$$f(x) = c + \frac{d - c}{(1 + \exp(b(x - e)))^f}$$



Rutledge et al. (2004)

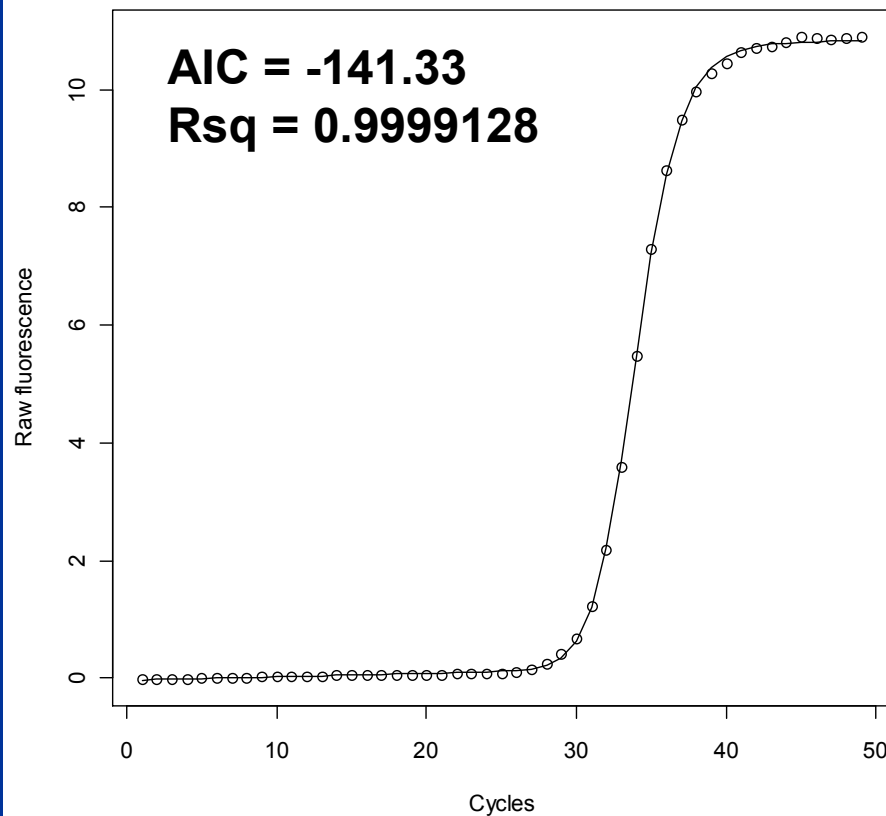


Spiess et al. (2008)

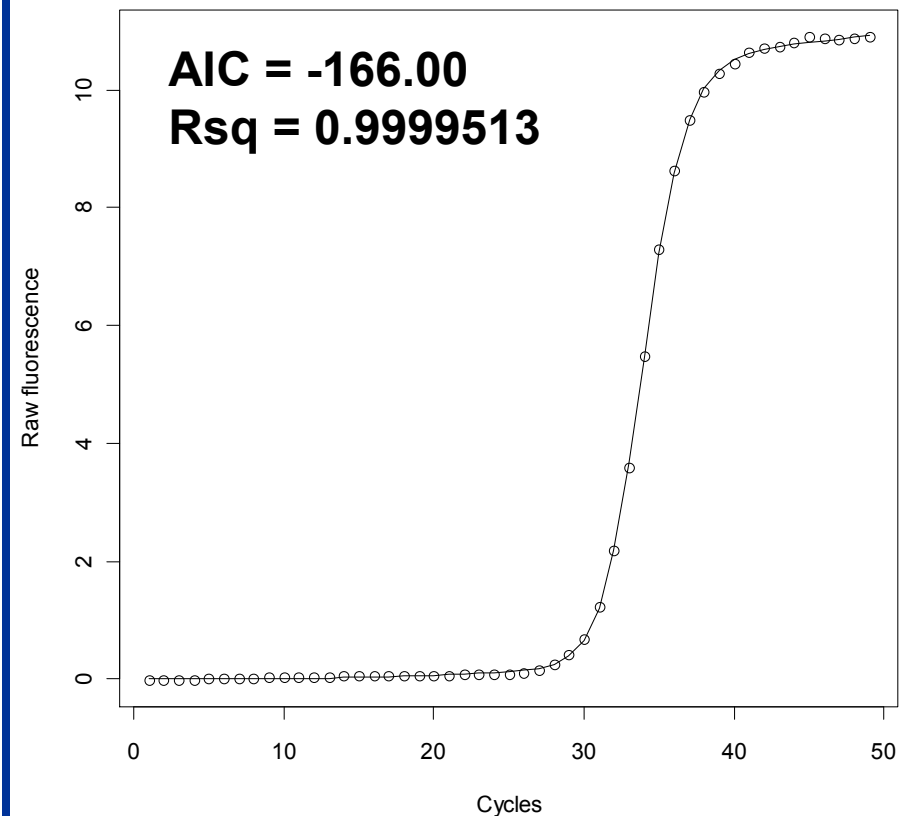
One way to get good efficiency estimates: 5-, 6-, 7-parameter sigmoidal models (2)

$$f(x) = c + k \cdot x + \frac{d - c}{(1 + \exp(b(x - e)))} f$$

$$f(x) = c + k1 \cdot x + k2 \cdot x^2 + \frac{d - c}{(1 + \exp(b(x - e)))} f$$



qpcR 1.3 - 4

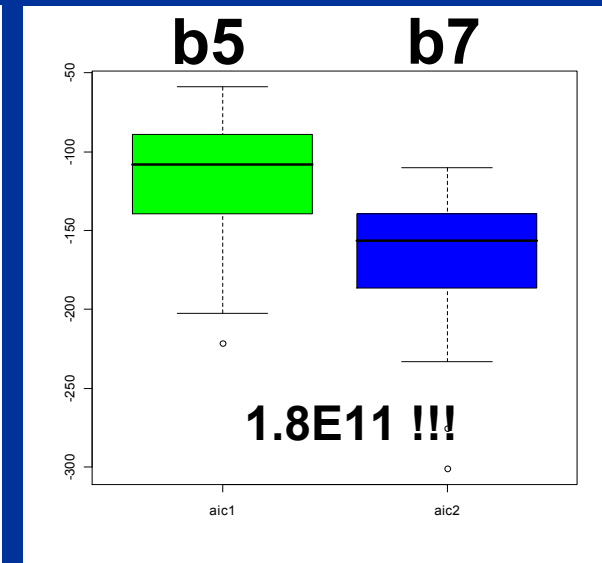
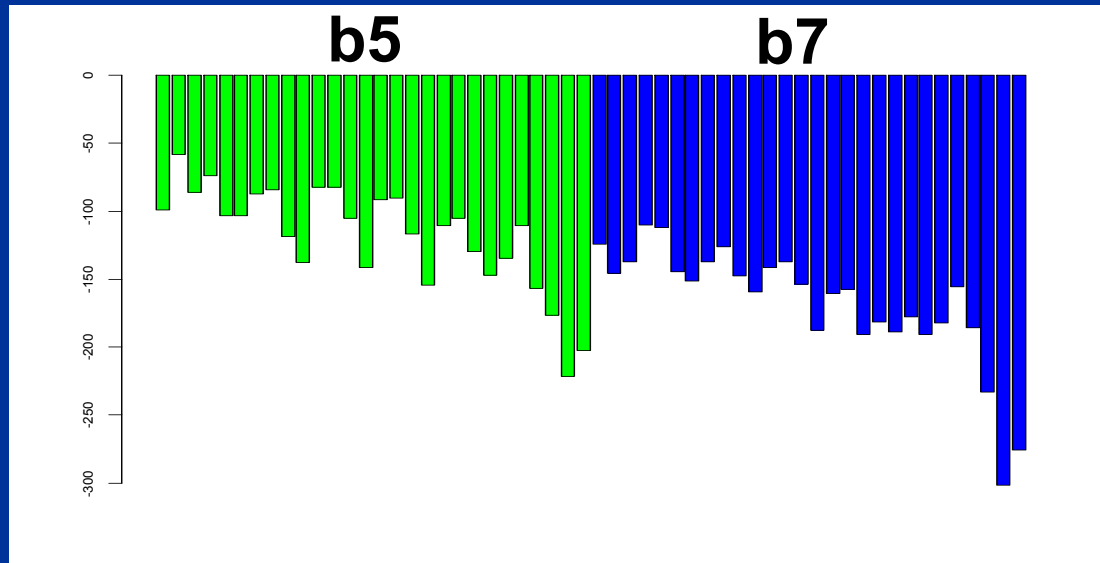


qpcR 1.3 - 4

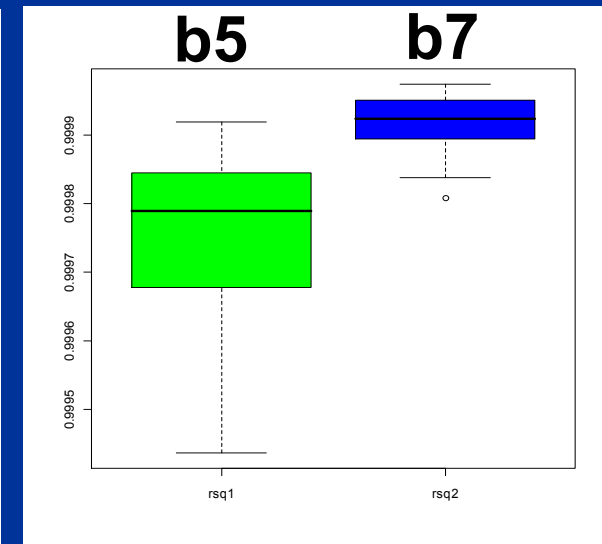
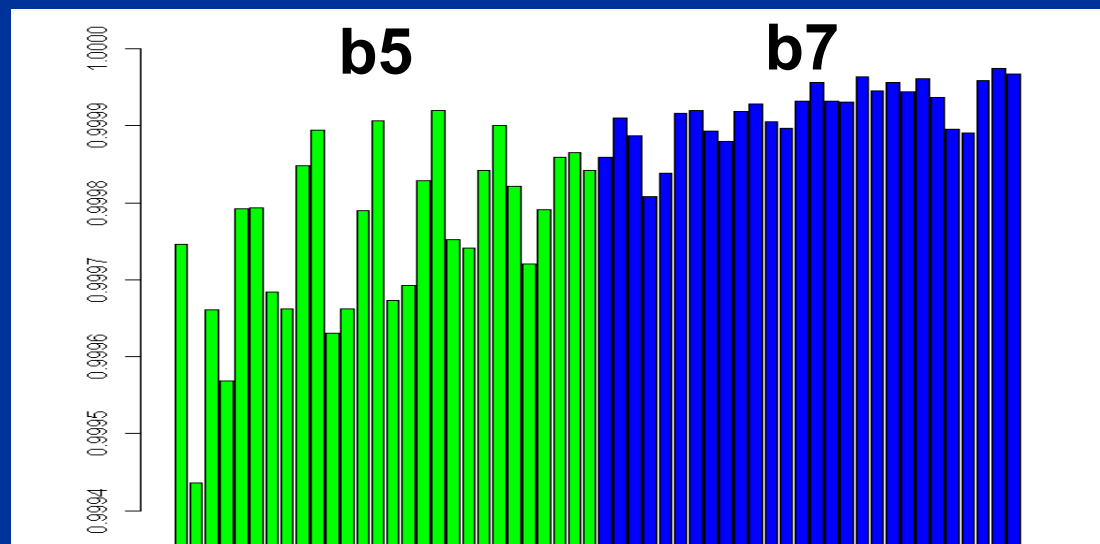
R^2 is inadequate for measuring the sigmoidal model's goodness-of-fit

Spiess & Neumeier, BMC Bioinf (2010)

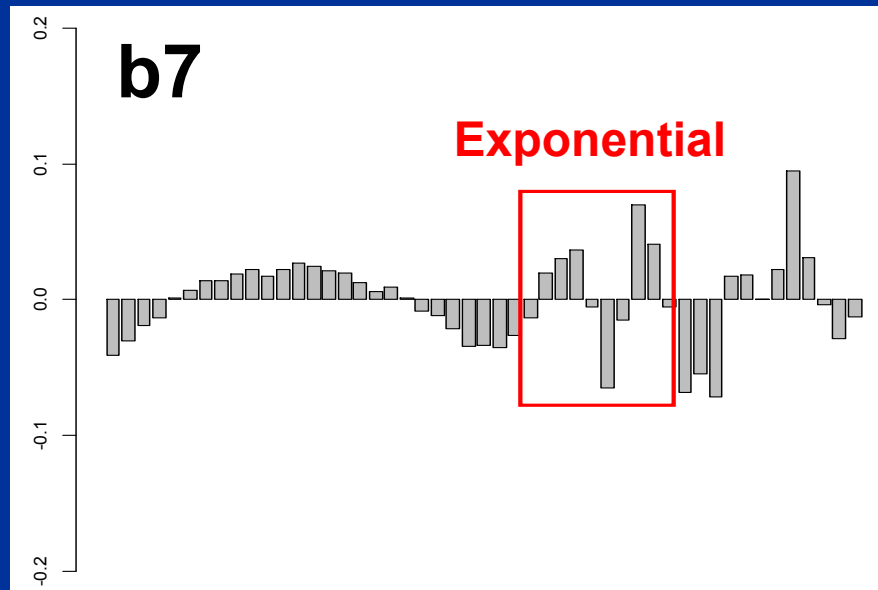
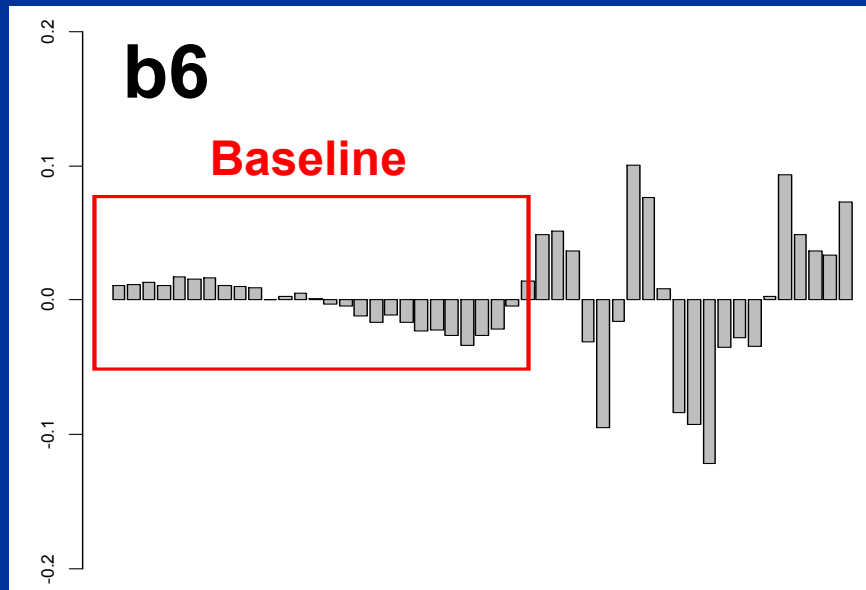
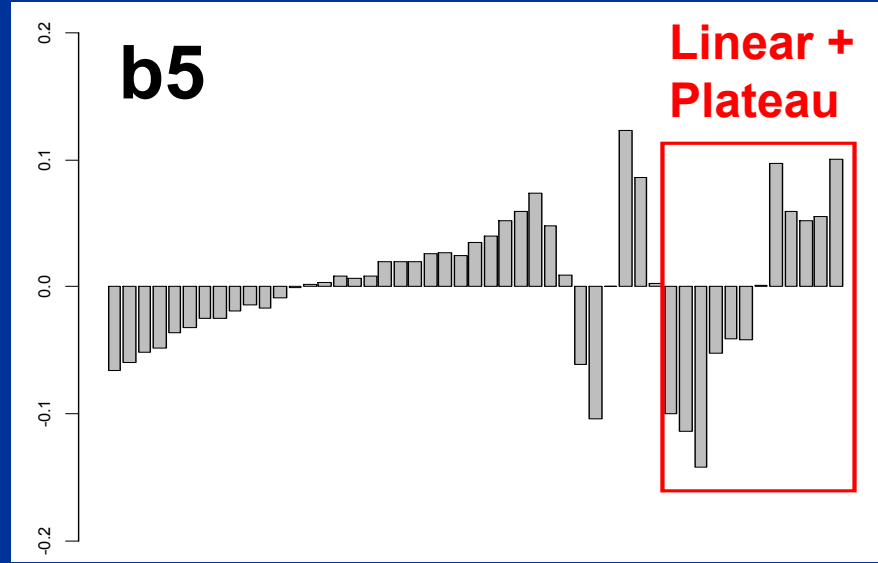
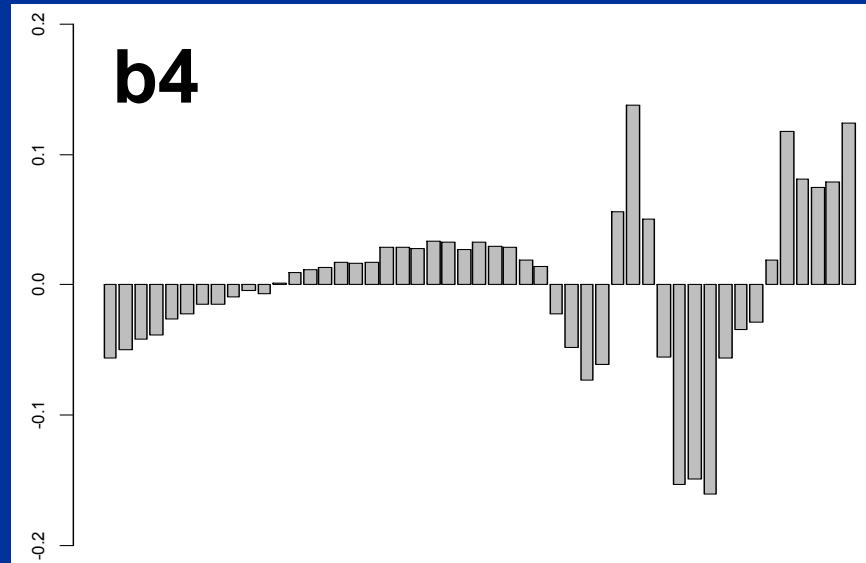
AIC



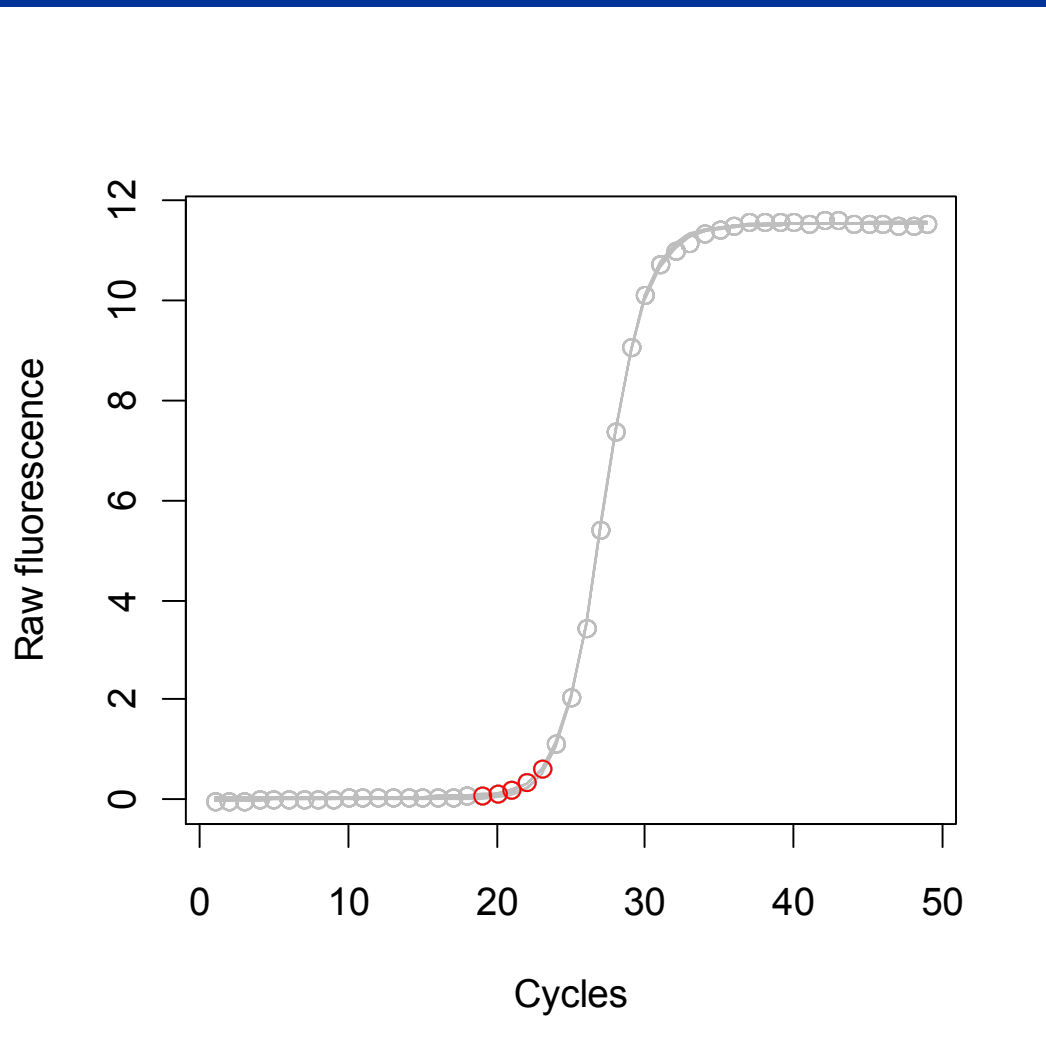
R^2



One way to get good efficiency estimates: 5-, 6-, 7-parameter sigmoidal models (3)



expcomp: automatic comparison of the models in the crucial exponential region



model	RMSE
"expGrowth"	0.002663578
"b6"	0.01679089
"b7"	0.01769595
"l6"	0.01815866
"l7"	0.01876456
"b4"	0.02969948
"b5"	0.0414908
"l5"	0.04742501
"b3"	0.05412748
"l4"	0.0812357
"l3"	0.1196964

Implementing the mechanistic **mak2** model from Boggy et al. (2010)

OPEN ACCESS Freely available online

PLoS one

A Mechanistic Model of PCR for Accurate Quantification of Quantitative PCR Data

Gregory J. Boggy*, Peter J. Woolf

Department of Chemical Engineering, University of Michigan, Ann Arbor, Michigan, United States of America

$$\frac{dP}{dt} = -k_{aH} * P * S + k_{dH} * PS$$

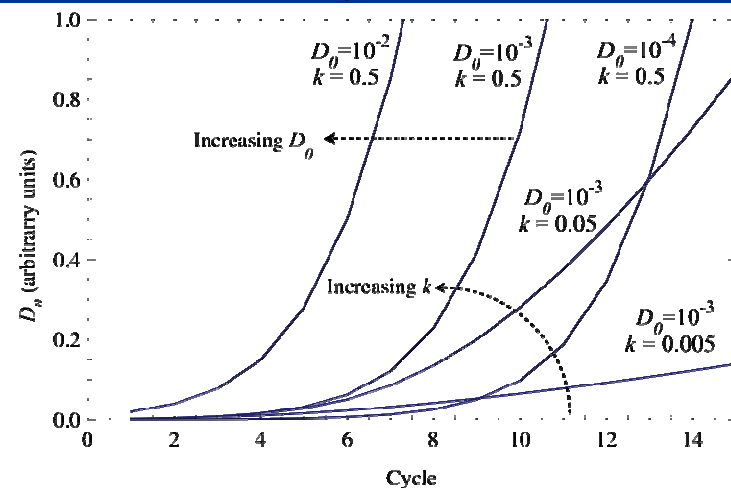
$$\frac{dS}{dt} = -k_{aH} * P * S + k_{dH} * PS - k_{aD} S^2$$

$$\frac{dPS}{dt} = 0 = -k_{aE} * E * PS + k_{dE} * PSE + k_{aH} * P * S - k_{dH} * PS$$

$$\frac{dPSE}{dt} = 0 = -k_{cat} * PSE + k_{aE} * PS - k_{dE} * PSE$$

$$\frac{dD}{dt} = k_{cat} * PSE + \frac{1}{2} k_{aD} S^2$$

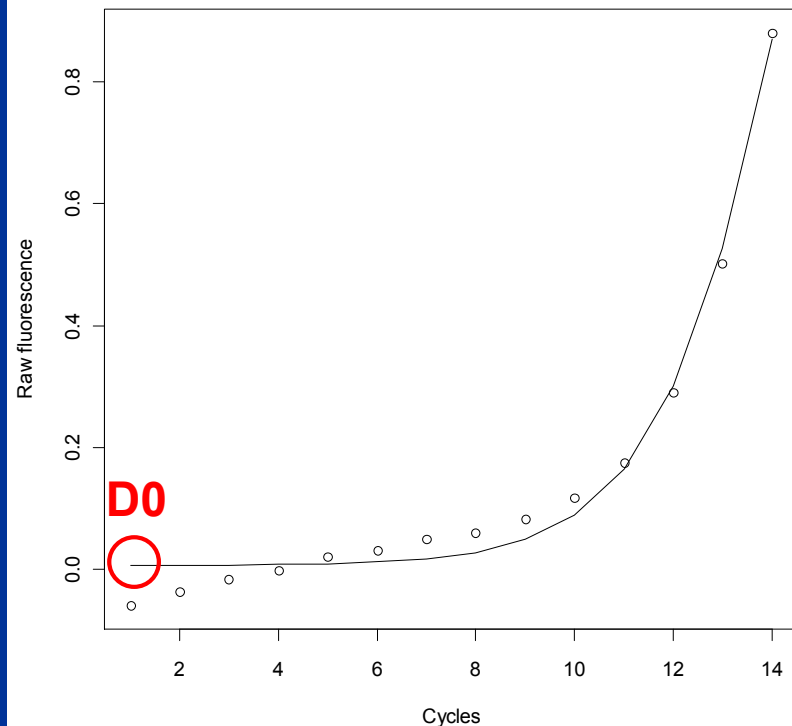
$$D_n = D_{n-1} + k \ln \left(1 + \frac{D_{n-1}}{k} \right)$$



Implementing the mechanistic **mak2** model from Boggy et al. (2010) ...and **mak3**

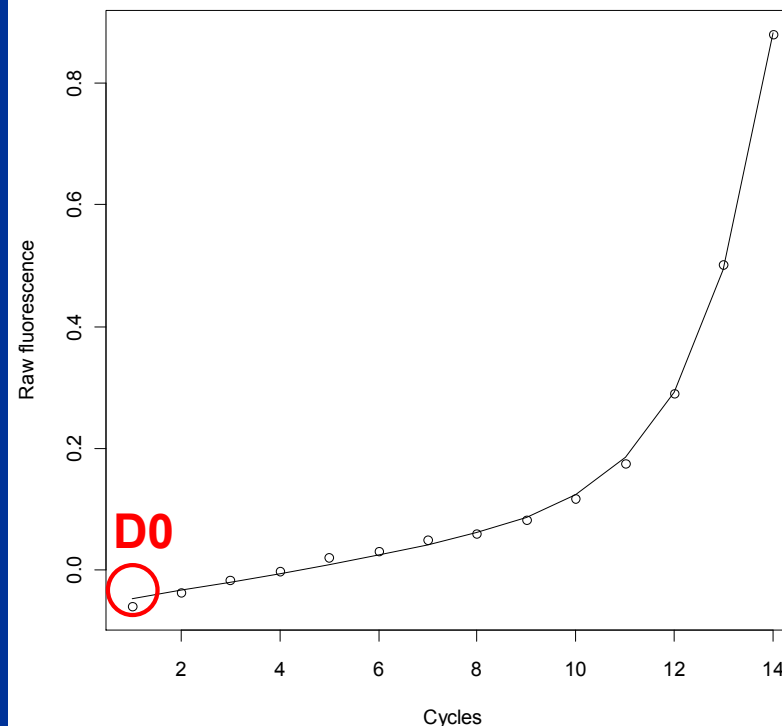
mak2

$$D_n = D_{n-1} + k \ln \left(1 + \frac{D_{n-1}}{k} \right)$$



mak3

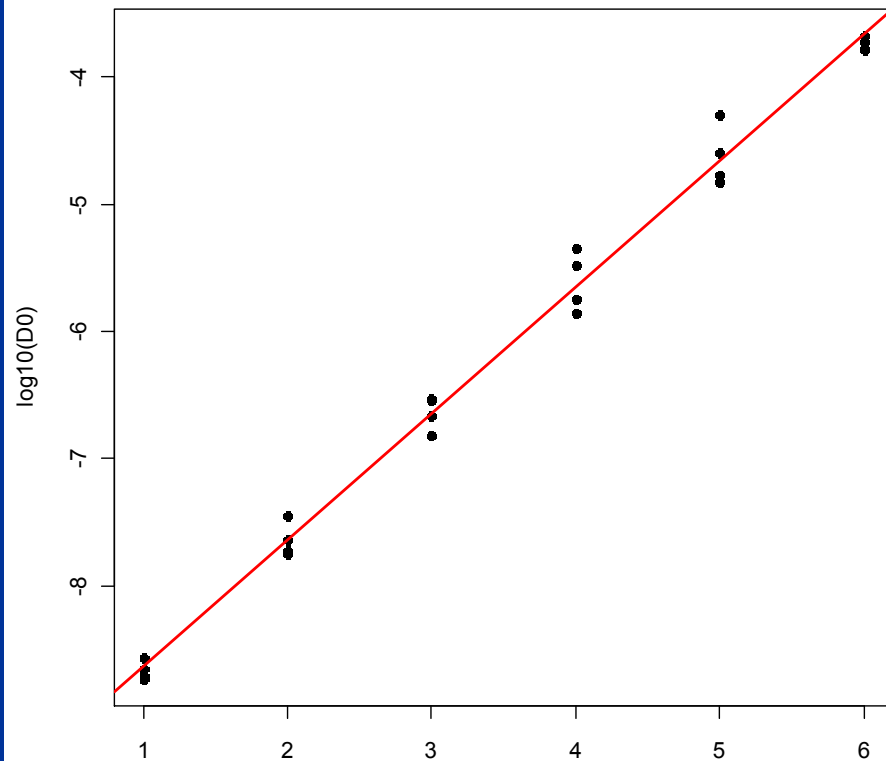
$$D_n = D_{n-1} + k \ln \left(1 + \frac{D_{n-1}}{k} \right) + a * D_n$$



Performance of **mak2/mak3** in dilution curve analysis

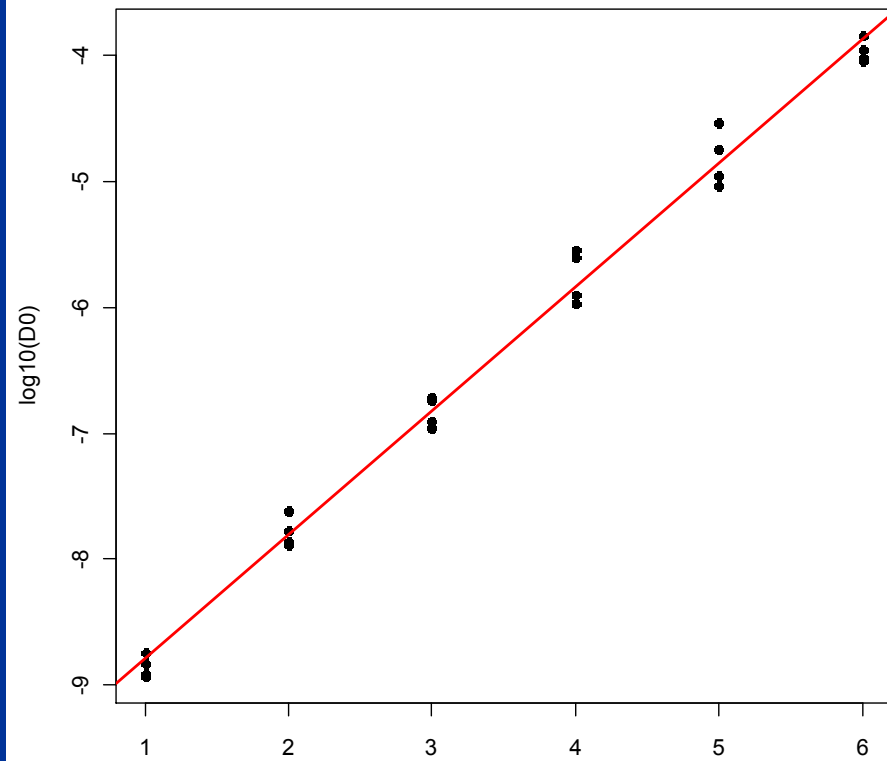
mak2

R²: 0.99263



mak3

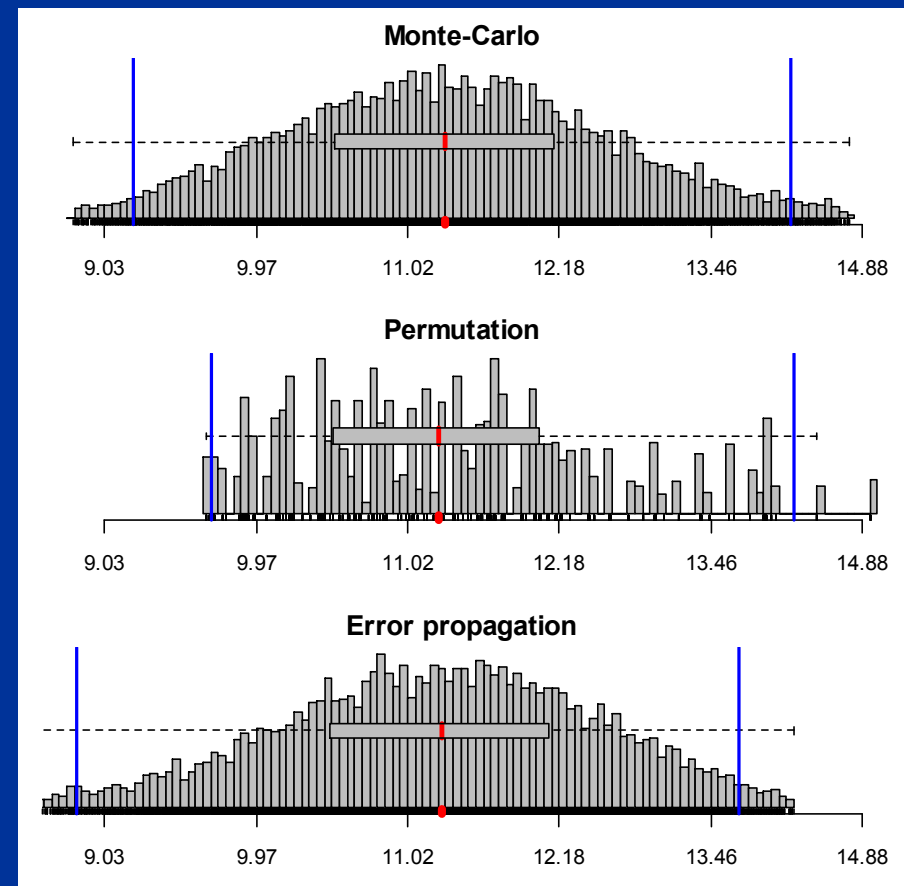
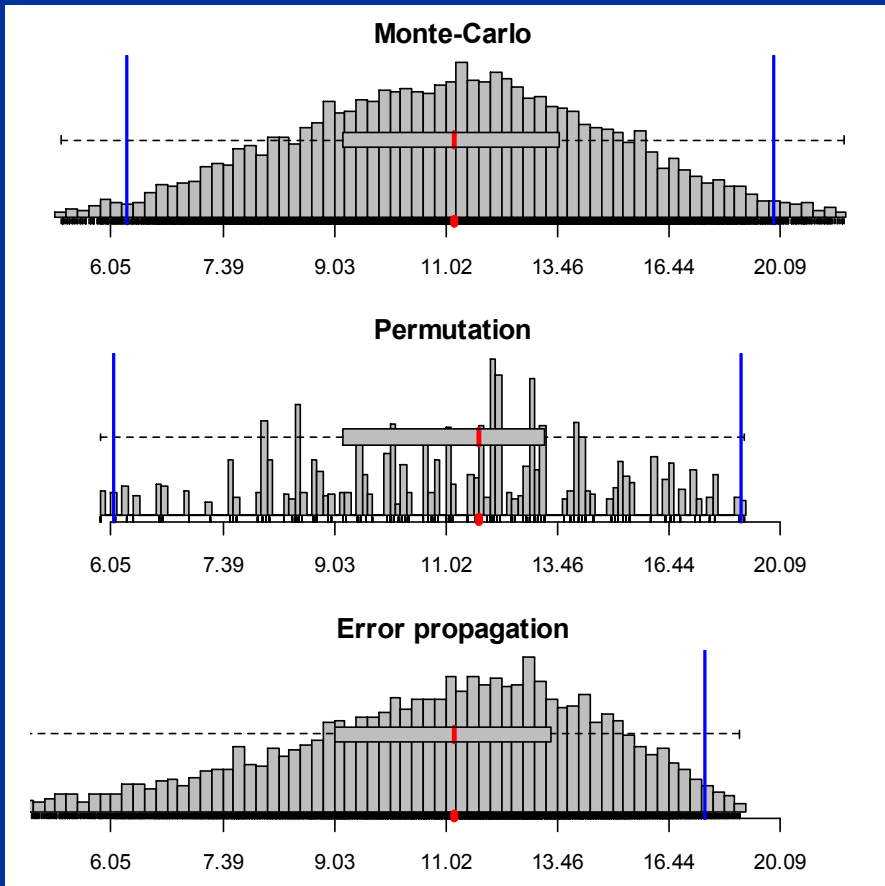
R²: 0.99251



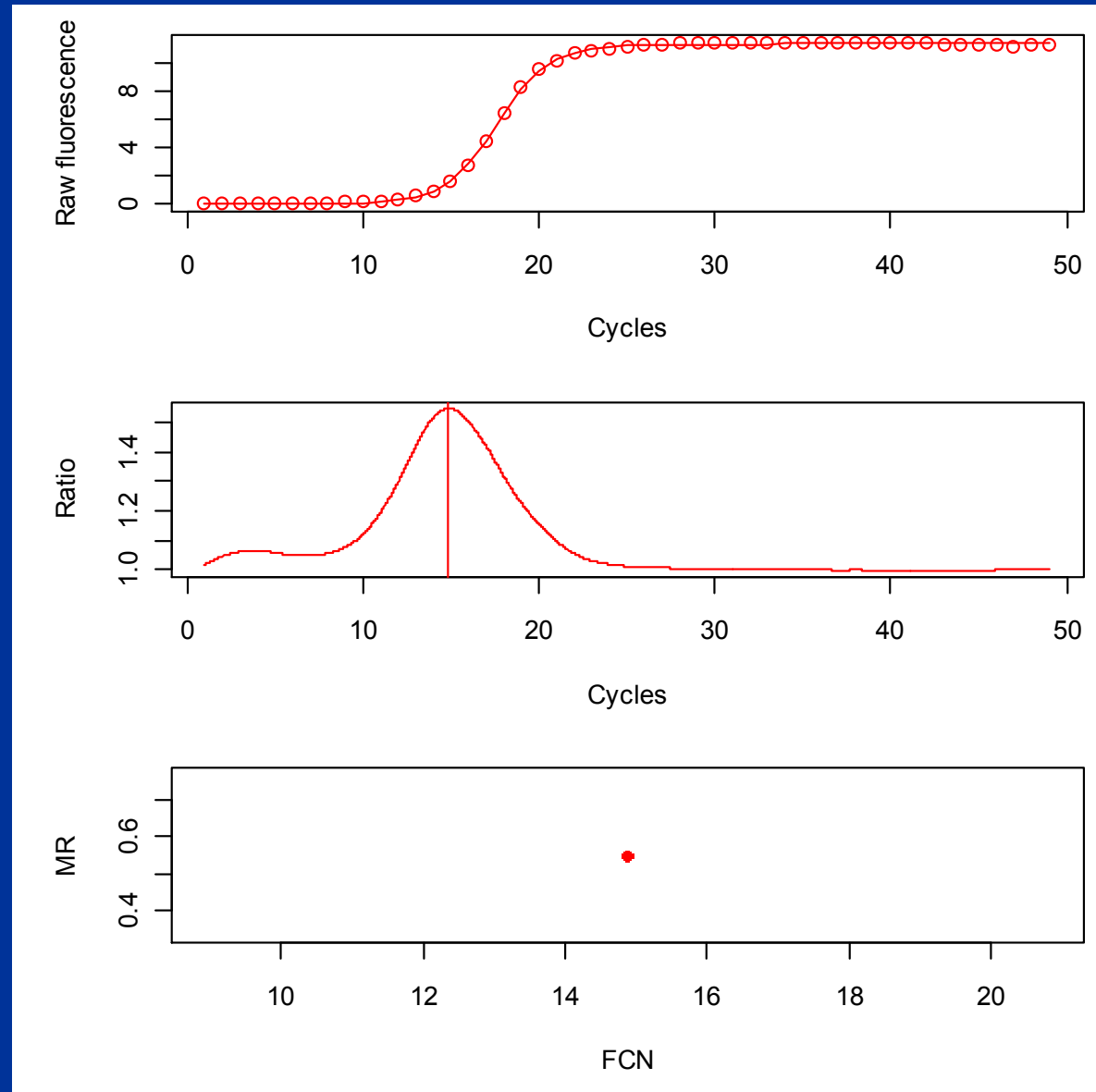
mak2/mak3 offer ratio calculation without threshold cycles and efficiencies! (But slightly different results...)

$$\frac{E1^{ct1}}{E2^{ct2}}$$

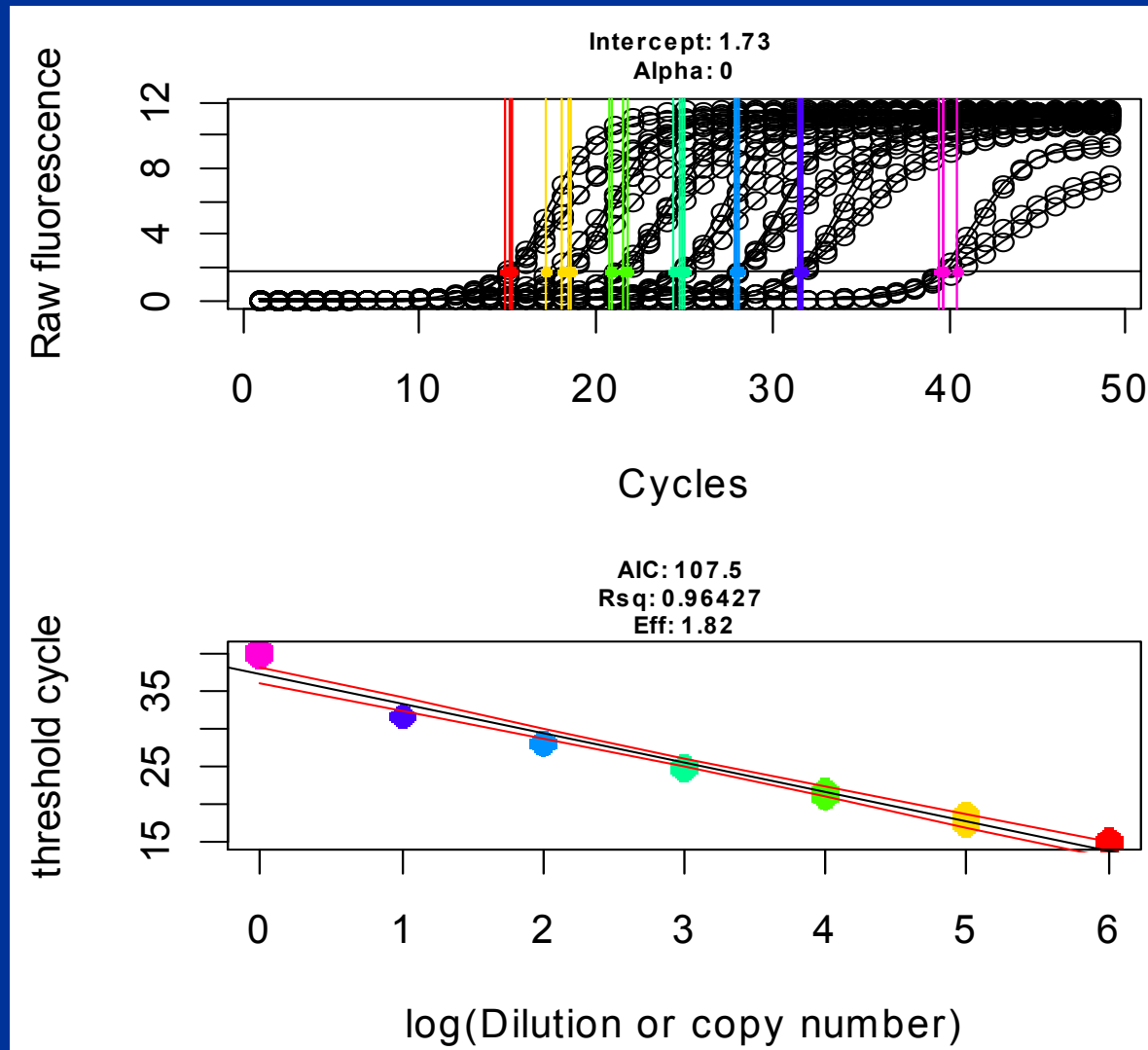
$$\frac{D0_1}{D0_2}$$



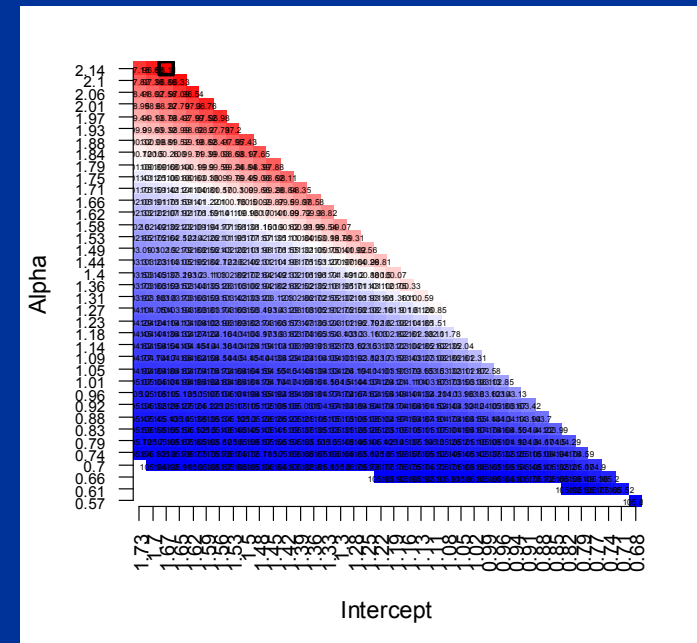
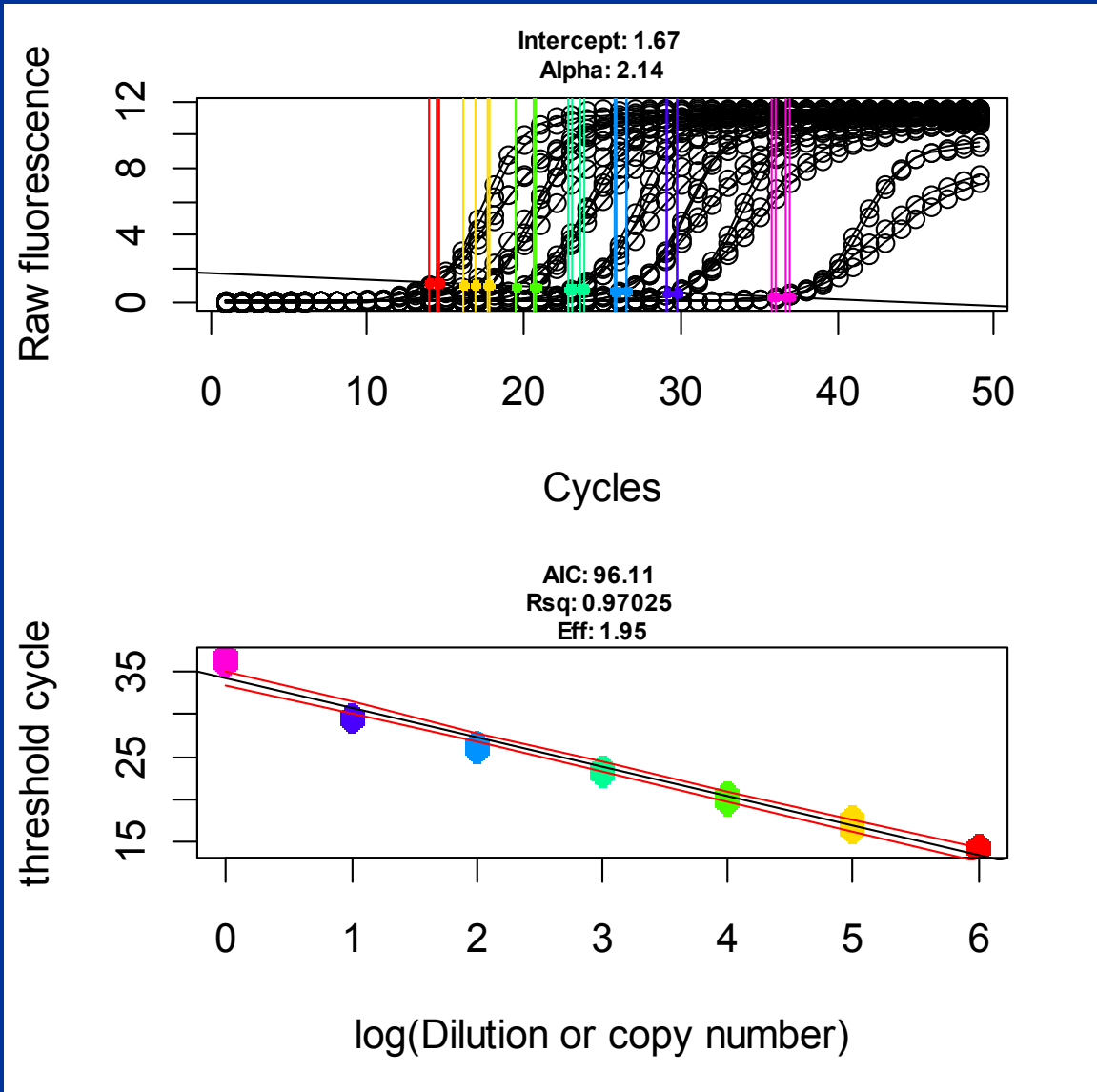
maxRatio: model-free cubic spline fitting of qPCR data (Shain et al., 2008)



The tilted threshold line: Improvement over its horizontal counterpart ? (1)



The tilted threshold line: Improvement over its horizontal counterpart ? (2)



Acknowledgements

Error Propagation:

Prof. Nathalie Neumeyer, Department of Mathematics, Hamburg

Prof. Wang, National Institute for Standards and Technology, USA

6- and 7-parameter models:

Prof. Joel Tellinghuisen, Purdue University, TA, USA

mak2 and mak3 models:

Gregory Boggy, University of Michigan, USA

THANKS FOR LISTENING!