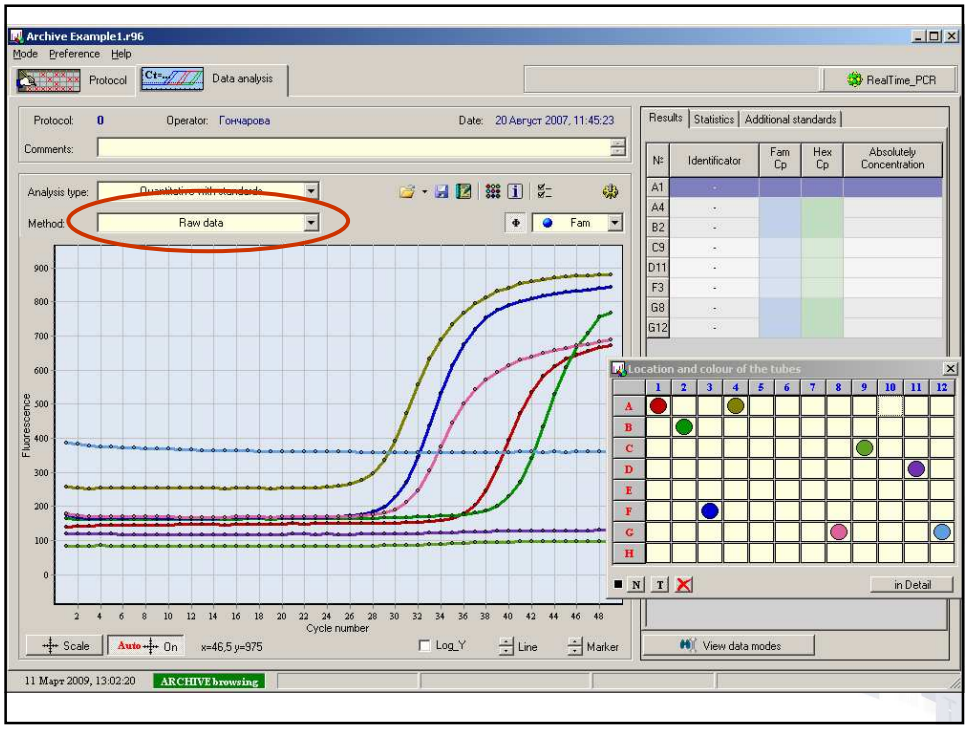
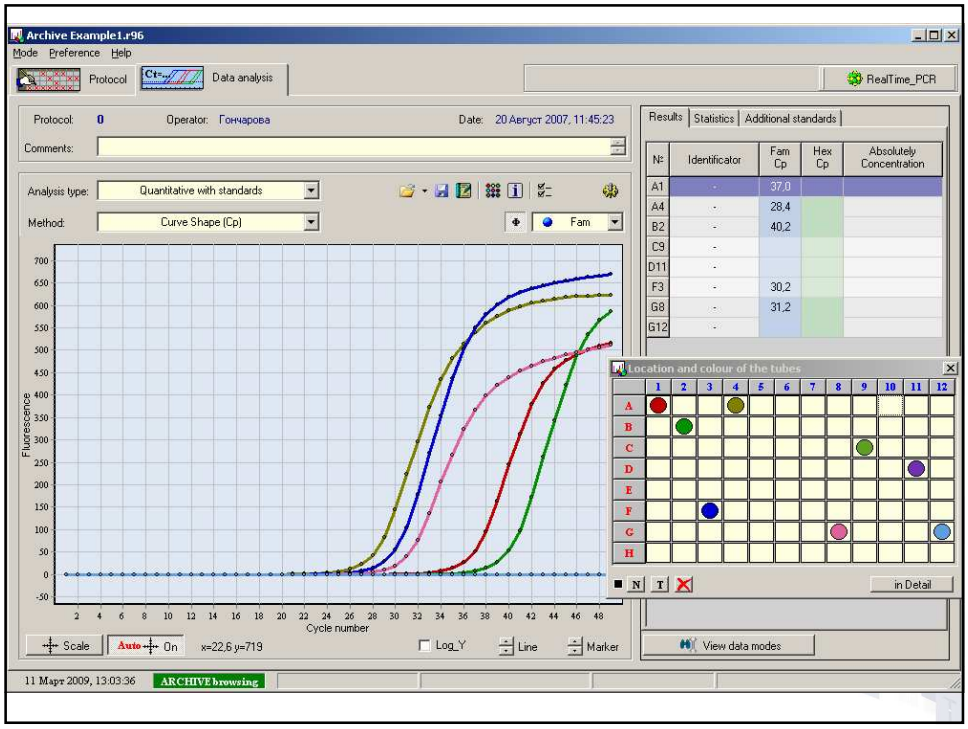
DNA-TECHNOLOGY
research & production
The Delta-TF Method for Real-Time PCR Data Standardization
Denis Rebrikov
DNA-Technology JSC
Moscow, Russia
denis@dna-technology.ru
4th International qPCR Symposium,
9-13th March 2009, Freising, Germany


Archive Example1.r96
Mode Preference Help
Protocol
Data analysis
RealTime_PCR
Protocol: 0
Operator: Гончарова
Date: 20 Август 2007, 11:45:23
Comments:
Analysis type:
Method:
Raw data
Fam
Results
Statistics
Additional standards
№
Identificator
Fam Cp
Hex Cp
Absolutely Concentration
A1
A4
B2
C9
D11
F3
G8
G12
Location and colour of the tubes
Fluorescence
Cycle number
Scale
Auto On
x=46,5 y=975
Log_Y
Line
Marker
View data modes
in Detail
11 Март 2009, 13:02:20
ARCHIVE browsing



PCR formula

DNA-TECHNOLOGY research & production

$$N_n = N_0 2^n$$

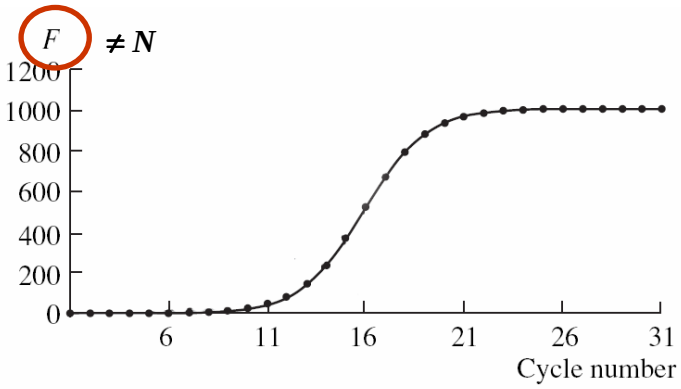
PCR efficiency ≠ 2

$$\downarrow$$

$$N_n = N_0 E^n$$

4

What Cycler detects in qPCR?



5

qPCR formula

$$N_n = N_0 2^n$$

$$\downarrow$$

$$N_n = N_0 E^n \qquad F = \alpha N$$

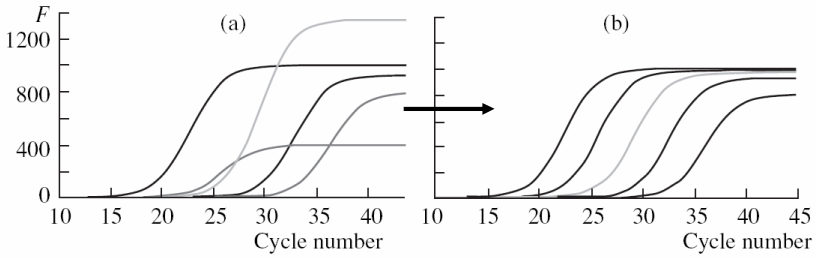
$$\downarrow$$

$$F_n = \alpha N_0 E^n$$

6

α normalization

$$F_n = \alpha N_0 E^n$$



7

qPCR formula

$$N_n = N_0 2^n$$

$$\downarrow$$

$$N_n = N_0 E^n \qquad F = \alpha N$$

$$\downarrow$$

$$F_n = \alpha N_0 E^n$$

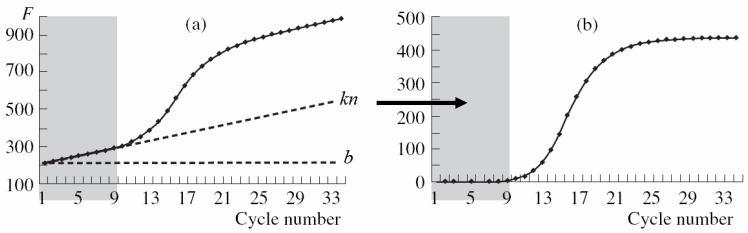
$$\downarrow$$

$$F_n = \alpha N_0 E^n + \text{background}$$

8

Background subtraction

$$F_n = \alpha N_0 E^n + b + kn$$



9

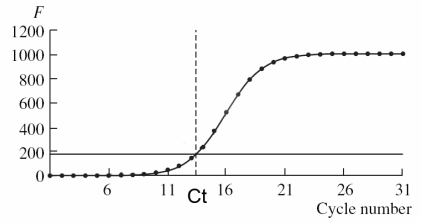
α, *b* and *kn* depends on experiment and tube

- Tubes parameters (esp. transparency, cleanness)
- Optical scheme heterogeneity
- Block temperature heterogeneity
- Electronics noises
- Instrument settings
- Pipetting errors
- etc

To decrease the effect of these factors on the qPCR results, some curve analysis algorithms can be applied

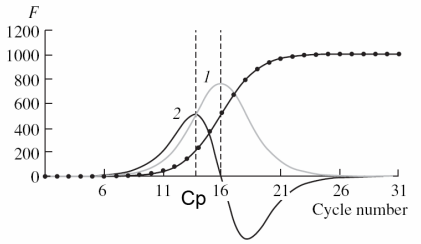
10

Curve-comparison methods



→ Ct

(threshold based)



→ Cp

(curve shape based)

11

Ct vs Cp

Ct and Cp has a different stability to «nonbiological» factors (artifacts):

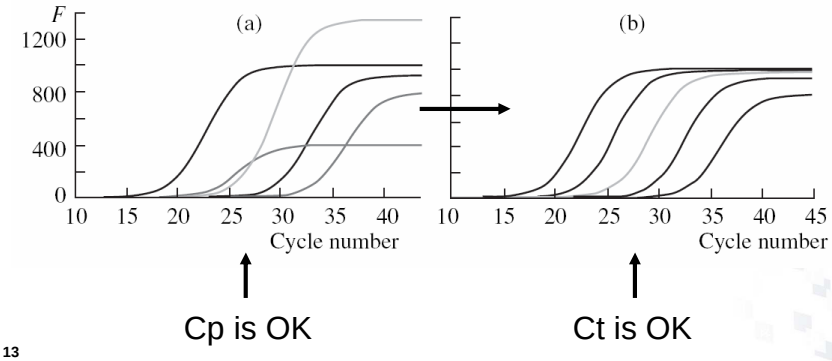
Cp better in case of:

- **scale disproportion (α)**
- **background fluorescence variations (*b* + *kn*)**

12

α normalization

$$F_n = \alpha N_0 E^n$$



Cp is OK

Ct is OK

13

Ct vs Cp

Ct and Cp has a different stability to «nonbiological» factors (artifacts):

Cp better in case of:

- **scale disproportion (α)**
- **background fluorescence variations (*b* + *kn*)**

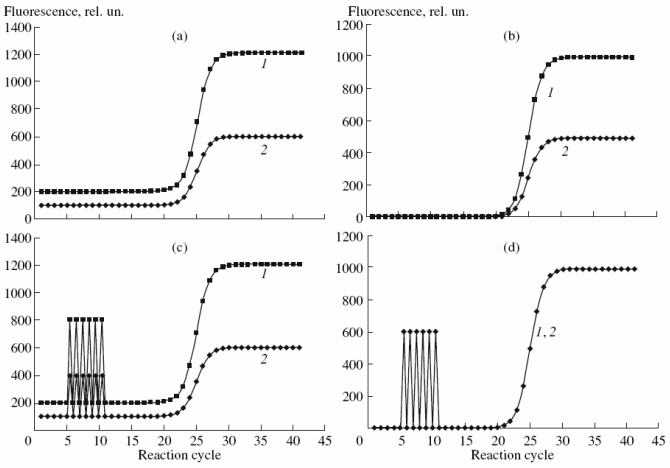
Ct better in case of:

- **weak fluorescent signal**
- **nonexponential amplification**

Is it possible to combine the advantages of both Ct and Cp methods?

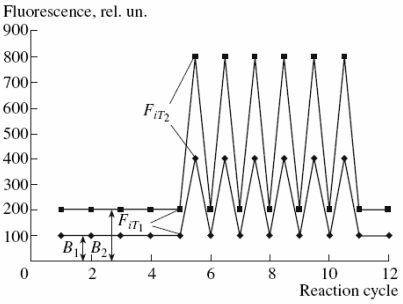
14

Delta-TF method



15

Delta-TF normalizing coefficient

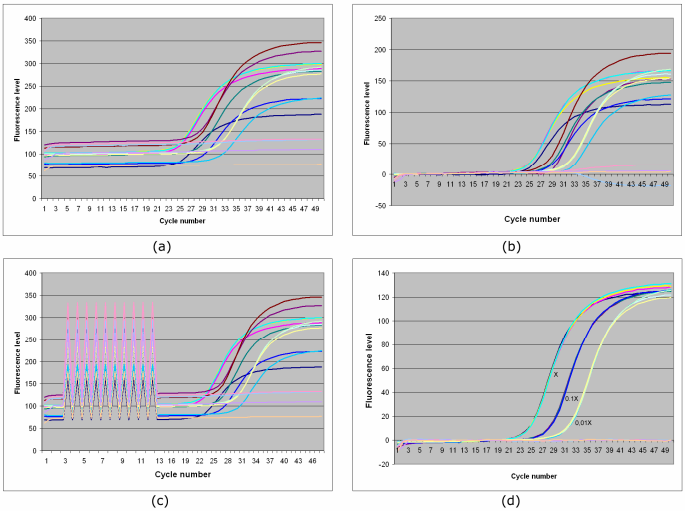


$$K_i = \frac{1}{F_i(T_2) - F_i(T_1)}$$

$$F_{i(n)\text{norm}} = K_i(F_{i,n} - B_i)$$

16

Example



17

Cv for different approaches

DNA concentration, un.	No. reaction	C_t	C_p	delta-TF and C_t
X	1	24.9	26	23.6
	2	24.2	26.1	23.5
	3	24.2	26	23.5
	4	24.3	26.1	23.6
	Std	0.34	0.06	0.06
	Mean	24.4	26.05	23.55
	C_v, %	1.4	0.2	0.2
$0.1X$	1	27.6	29.5	27.1
	2	27.4	29.6	27.1
	3	28	29.6	27.4
	4	28.3	29.8	27.4
	Std	0.40	0.13	0.17
	Mean	27.825	29.625	27.25
	C_v, %	1.4	0.4	0.6
$0.01X$	1	31.8	33.3	30.7
	2	31.2	33.2	30.7
	3	31.3	33.3	30.7
	4	31	33	30.5
	Std	0.34	0.14	0.10
	Mean	31.325	33.2	30.65
	C_v, %	1.1	0.4	0.3
	Mean C_v, %	1.3	0.4	0.4

Note: *Std*, standard deviation; C_v, coefficient of variation. C_t and C_p in relative units.

18

How to use

For DNA-Technology PCR Cyclers (96 and 384) algorithm integrated into the software

BIO-RAD's iQ, Stratagene's Mx and some other Cyclers gives you the ROW data in a standard format (i.e. Excel)

19

Acknowledgements

This work was supported by:
- EU FP6 "DIAGNOSIS" (project #037212)
- Russian Federal Health and Innovation Agency (project #02.522.12.2001)

Thanks for your attention!

20