Chemistry Model-based Method for Estimating DNA Amounts from PCR Amplified Samples

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Background & Objective

Methods in common use for estimating target NA amounts in PCR-processed samples ignore the chemistry occurring each cycle. Rather PCR data is treated as resulting from a growth process, relying either on assumptions related to replication efficiency in cycles up to the first exceeding a (usually low) threshold signal, or on estimated parameters of a fitted logistic function. Our objective: to model the chemical reactions of template replication through enough cycles *beyond the geometric phase* of the PCR process to enable accurately estimating target NA amounts without resorting to special phenomenological models. Our starting point is reference [1].

Extension Reactions

primer p binding to single strand template s

$$p + s \rightleftharpoons h_0 \qquad K_1 = \frac{[h_0]}{[s][p]}$$

polymerase q binding to primer-template duplex h_0

$$q + h_0 \rightleftharpoons r_0 \qquad K_2 = \frac{[r_0]}{[q][h_0]}$$

primer extension in replication complex r_0

$$r_0 + N\,n \rightleftharpoons h_N + q \qquad K_3 = \frac{[h_N][q]}{[r_0][n]^N}$$

amplicon-primer competition for template

$$s + s^* \rightleftharpoons d \qquad K_4 = \frac{[d]}{[s]^2}$$

Approximations & Model Variables

Idealizing approximations:

- all annealed primers extend completely
  $K_3 \approx \infty$
- primer in large excess
  $[p] \simeq [p]_{total} \quad K_1[p]_{total} \gg 1$

Model variables:

- reduced template concentration
  $\mathsf{S} = K_2[s]_T$
- reduced polymerase concentration
  $\mathsf{Q} = K_2[q]_T$
- amplicon-primer competition
  $\mathsf{D} = K_4/[K_2(K_1[p]_T)^2]$

Model Parameters and Dependencies

parameter	meaning	computation mode: full calibration	system calibration	prediction	dependence
X_0	sample target NA amount	fixed	fixed	estimated	sample
$\mathcal{K}$	normalized K_2	estimated	fixed	fixed	chemistry
Q	reduced polymerase conc.	estimated	estimated	estimated	chemistry & sample
D	amplicon-primer competition	estimated	fixed	fixed	chemistry
G	unit conversion factor	estimated	estimated	fixed	system

Model Summary

The measured net profile signal Y_k at cycle k is represented by a general phenomenological model including initial target amount X_0, a system gain parameter G and a product involving cycle efficiencies E_i,

$$Y_k = G X_0 \prod_{i=1}^{k}(1 + E_i).$$

Within each cycle the PCR chemistry model is embodied in a polynomial in replication efficiency $E_k = [r]_k/[s]_{T,k}$, with coefficients functions of model parameters,

$$\mathsf{S}_k^2 E_k^3 - (\mathsf{S}_k^2 + (1 - \mathsf{D} + 2\mathsf{Q})\mathsf{S}_k)E_k^2 + \mathsf{Q}(2\mathsf{S}_k + \mathsf{Q} + 1)E_k - \mathsf{Q}^2 = 0.$$

A recursion links the reduced amplicon concentrations in the cycle sequence,

$$\mathsf{S}_{k+1} = \mathsf{S}_k\,(1 + E_k)\,.$$

S_0 includes sample target amount X_0 and $\mathcal{K}$; $\mathcal{N}$ and V denote Avogadro's number and reaction volume,

$$\mathsf{S}_0 = X_0\mathcal{K}. \qquad \mathcal{K} = K_2/(\mathcal{N}V)$$

Implementation

- amplification detection and NLLS background estimation
- select cycles from profiles having efficiency greater than defined fraction of maximum efficiency (Figure 1)
- assemble data for estimation of parameters common to all profiles by NLLS (Figure 2)

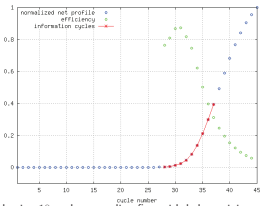

Figure 1. Selecting 10 cycles preceding first with below minimum efficiency

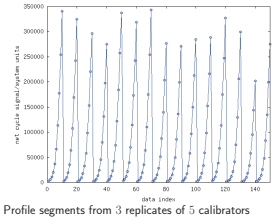

Figure 2. Profile segments from 3 replicates of 5 calibrators

Conclusions

A real model-based analysis of PCR data offers:

- accurate, assumption-free, objective estimates of target NA amount
- estimates of target NA amount uncertainty
- precision as good or better than other methods
- parameters having real, physical significance
- reliable estimate of first cycle efficiency
- useful validity checks
- easy calibration transfer between systems

References

[1] D. Stolovitzky & G. Cecchi, PNAS **93** (1996), 12947-12952.

Acknowledgements

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FOCUS Diagnostics

Comparison of chemistry model method with threshold method *

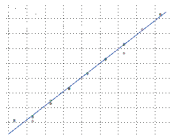

Figure 3. Chemistry model analysis of CMV validation plot

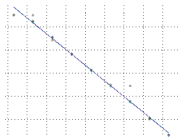

Figure 4. Threshold method analysis of CMV calibration plot

CMV true X_0	chemistry model: est X_0	%error	%CV	E_1	threshold method: est X_0	%error	%CV	efficiency
200 000 000	199 841 706	−0.1	1.2	0.926	278 136 085	39.1	3.5	0.961
20 000 000	19 331 065	−3.3	1.0	0.925	24 441 198	22.2	4.6	0.961
2 000 000	1 503 930	−24.8	46.2	0.905	1 598 590	−20.1	59.2	0.961
200 000	186 896	−6.6	6.0	0.921	198 072	−1.0	8.3	0.961
20 000	20 121	0.6	2.8	0.923	20 541	2.7	3.8	0.961
2 000	2 053	2.7	6.6	0.923	1 922	−3.9	5.3	0.961
200	220	10.1	24.2	0.921	186	−7.2	24.0	0.961
20	21	5.9	27.2	0.912	14	−29.2	36.7	0.961
2	14	587.5	8.1	0.896	6	210.5	2.7	0.961

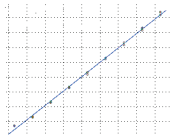

Figure 5. Chemistry model analysis of EBV validation plot

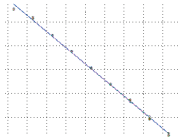

Figure 6. Threshold method analysis of EBV calibration plot

EBV true X_0	chemistry model: est X_0	%error	%CV	E_1	threshold method: est X_0	%error	%CV	efficiency
200 000 000	208 863 846	4.4	23.5	0.916	240 491 477	20.2	16.1	0.973
20 000 000	18 837 207	−5.8	19.1	0.925	23 109 357	15.5	16.3	0.973
2 000 000	1 836 084	−8.2	22.5	0.926	1 915 625	−4.2	13.0	0.973
200 000	184 923	−7.5	9.5	0.925	187 195	−6.4	8.2	0.973
20 000	19 295	−3.5	15.0	0.925	18 295	−8.5	9.3	0.973
2 000	2 014	0.7	9.4	0.923	1 678	−16.1	3.0	0.973
200	204	2.0	8.1	0.927	173	−13.6	6.0	0.973
20	21	4.9	8.1	0.922	15	−27.2	11.6	0.973
2	5	162.4	5.0	0.926	4	93.7	10.7	0.973

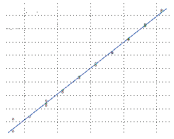

Figure 7. Chemistry model analysis of FLU validation plot

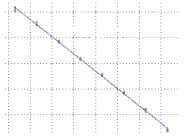

Figure 8. Threshold method analysis of FLU calibration plot

FLU true X_0	chemistry model: est X_0	%error	%CV	E_1	threshold method: est X_0	%error	%CV	efficiency
200 000 000	219 835 414	9.9	14.1	0.926	273 152 958	36.6	15.0	0.984
20 000 000	18 158 971	−9.2	14.5	0.922	18 816 832	−5.9	19.2	0.984
2 000 000	1 638 426	−18.1	9.3	0.930	1 789 355	−10.5	12.4	0.984
200 000	157 962	−21.0	6.5	0.931	158 050	−21.0	9.5	0.984
20 000	22 789	14.0	20.1	0.931	20 050	0.2	11.9	0.984
2 000	2 347	17.4	10.4	0.931	1 967	−1.6	10.8	0.984
200	211	5.7	18.8	0.936	182	−9.2	17.3	0.984
20	27	33.2	41.0	0.945	27	33.2	30.8	0.984
2	2.4	20.0	—	0.928	n.d.	—	—	—
0.2	0.9	361.0	111.0	0.883	n.d.	—	—	—

* samples processed on the 3M Integrated Cycler®