Full-Process-Kinetics PCR analysis: a holistic model to PCR data interpretation.

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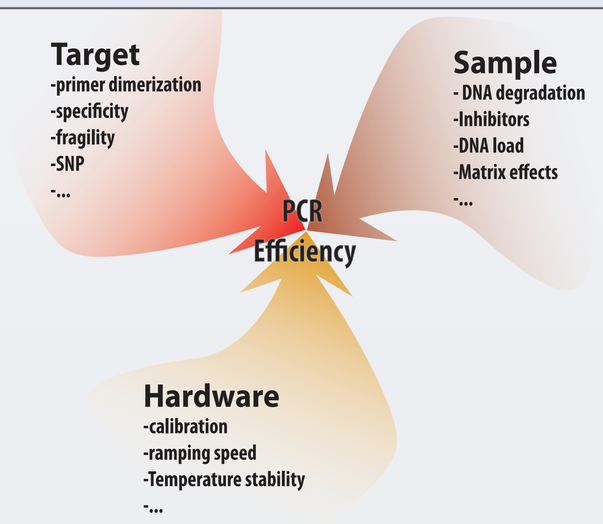
Current analysis methods: Adequate performance when efficiency is comparable.

Challenge: Small differences in efficiency between reactions => considerable quantification errors.

Solution: Estimation of initial reaction efficiency.

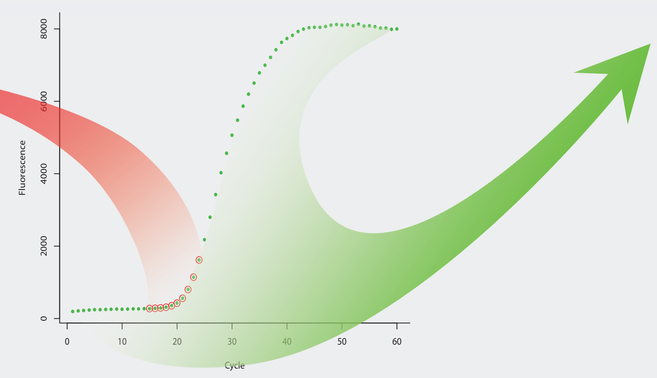
Consequences: Classical assays demand additional analysis and costs.

Remedy: Single reaction efficiency calculation.



Current Methodology in Single Reaction Effiency Calculation

- limited to a window of application
- assumes constant efficiency
- "exponential phase" may be hard to define



Full Proces Kinetics-PCR (FPK-PCR)

- analyzes full range of observed values
- uses a variable efficiency model
- kinetically more realistic
- yields additional information
- better reproducability

FPK-PCR Procedure

A) Cycle efficiencies are calculated & their double log is regressed against the respective fluorescence values

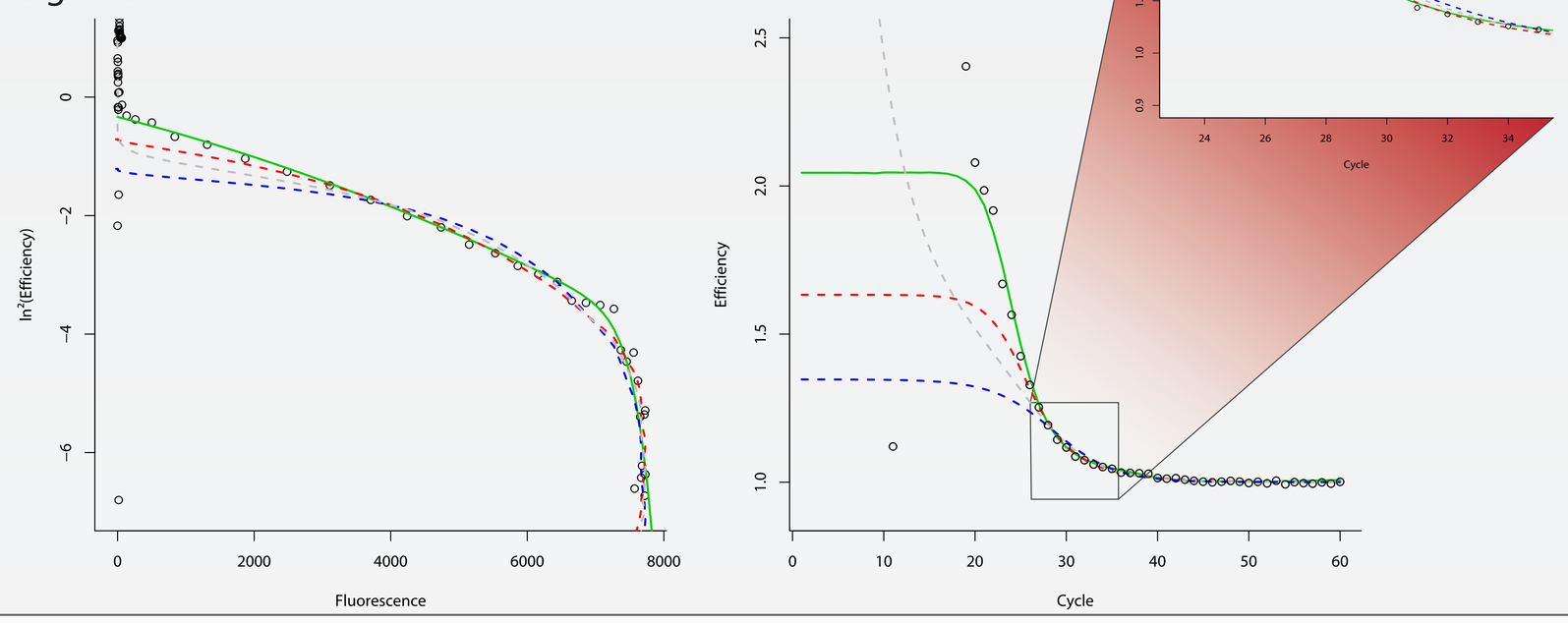
B) A bilinear model is fitted to the transformed cycle efficiencies, revealing changes in efficiency throughout the reaction.

C) Fluorescence values are reconstructed, creating quality control over the efficiency estimates.

Results

- ***Improved maximal efficiency*** estimation
- FPK-PCR ***explains fluorescence increases*** better than other models
- ***Precision and accuracy*** are comparable to the golden standard
- Results allow a more ***in-depth analysis*** of real time PCR data
- FPK-PCR allows ***reliable*** detection of ***inhibited reactions***

Full Process kinetics-PCR
Sigmoid Model
Four Parameter Logistic Model
Five Parameter Logistic Model



Conclusion

- FPK-PCR model is a ***kinetically more realistic*** approach to analyzing real time PCR data, readily giving access to internal efficiency behavior.
- Reconstruction of the ***entire chain of cycle efficiencies*** reveals amplification steps underlying fluorescence increases.
- The FPK-PCR implements a ***global efficiency model***: a window of application is not needed.
- The FPK-PCR approach makes single reaction analysis a ***powerful tool*** for widespread application.

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