**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 Efficiency Calculation**

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

**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**

**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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