A temperature-dependent computational model of the PCR reaction

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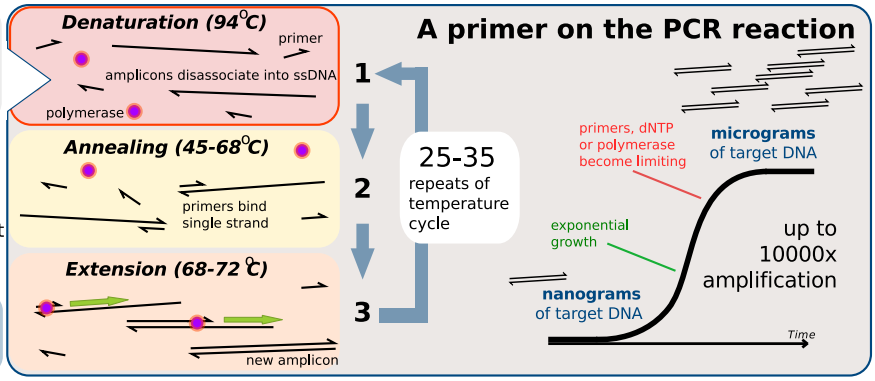
EPSRC Engineering and Physical Sciences Research Council

Introduction

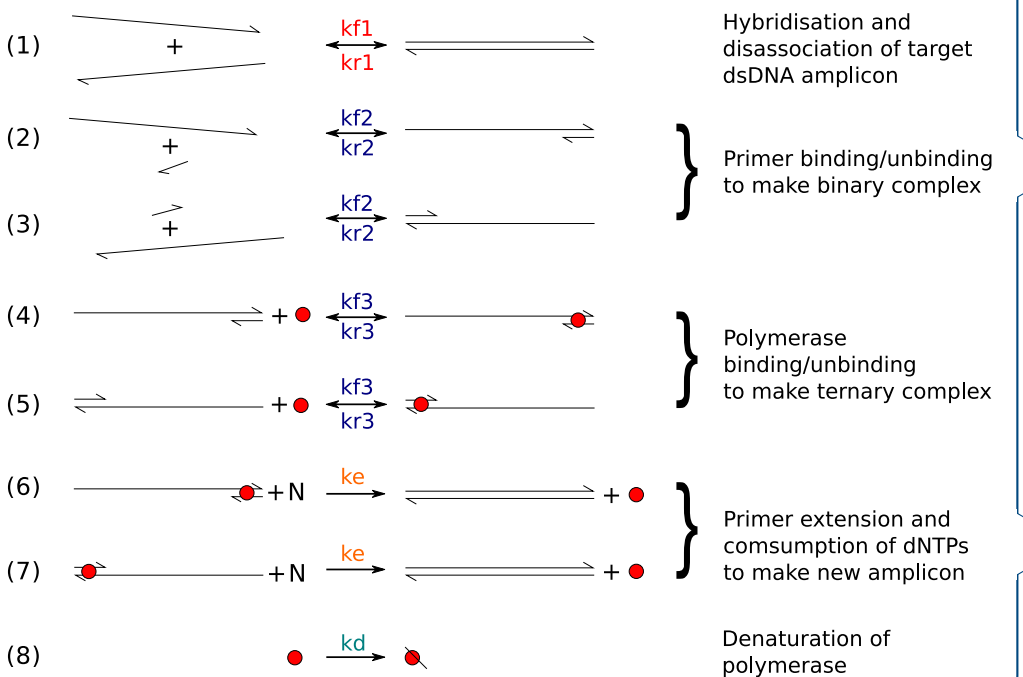
Famous for it's temperature cycle and now widely used in molecular biology, the polymerase chain reaction (PCR) allows for the selective amplification of a target DNA sequence from a mixed sample.

In PCR, the target DNA amplification achieved is dependent on a **complex interplay of operating conditions** and several theoretical models capturing the biophysics of PCR have been proposed [1-3] to better understand how operating parameters effect yield. However, these PCR models often assume that the chemistry of the system changes dependent on the current temperature phase (unrealistic), and they enforce a fixed temperature cycle to escape the problem of T-dependent rate constants.

In this work, we implement a new model of PCR that is abstract, yet thermodynamically consistent. The reaction rate constants are properly T-dependent and the temperature cycle can be set without restrictions.

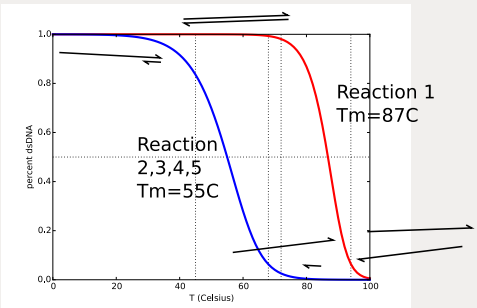


PCR reaction kinetic model

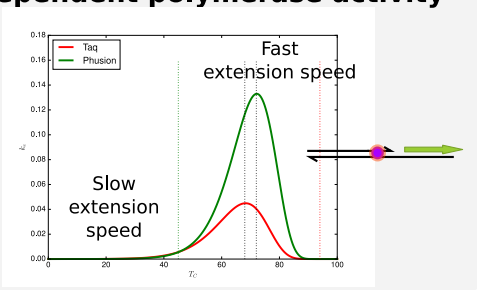


DNA duplex melting

Temperature-dependent kinetic rates for reversible reactions 1-5 are determined by their melting curves. We assume a simple 2-part model for DNA hybridisation.

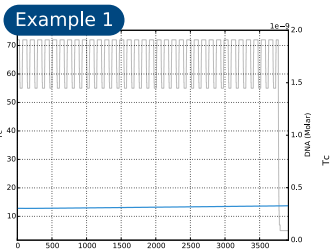


T-dependent polymerase activity



Simulation examples

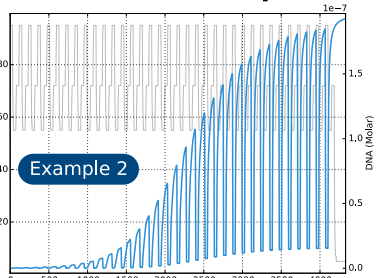
Example 1



72C-55C-72C for 35 cycles

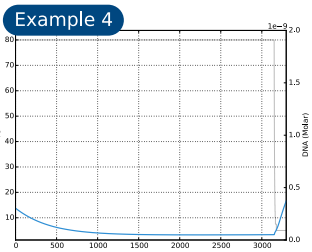
No amplification because denaturation temperature 72C is too low to make amplicon DNA single stranded

Example 2



95C-55C-72C for 35 cycles
Successful PCR reaction
640x increase of amplicon

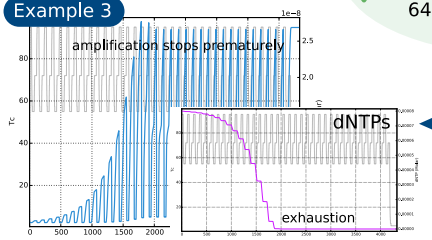
Example 4



Constant 80C temperature

Minor/no amplification because constant high temperature denatures target DNA and primers cannot bind

Example 3



But, if dNTPs are present at 10x less concentration, then they limit the reaction.
Amplification is only 89x

Initial reaction mix concentrations for examples:

-10ng target DNA amplicon
-0.2mM of each dNTP
-200nM of each primer
-20nM polymerase
-50uL reaction mix

Summary & outlook

The PCR kinetic model we have presented here:

- Reproduces the characteristic PCR amplification curve
- Can produce a **rich set of outputs** in response to the user changing the temperature cycle and initial concs.

Useful in its present state for educational purposes, we immediately plan to further augment the model with:

- Primer non-specific binding when annealing temperature is too low.
- A multi-step extension reaction (Reactions 6 and 7)
- In the long term, reaction kinetics based on DNA *sequence* leading to a more predictive, quantitative model of PCR able to interface with PCR experiments.

Check developments at:
http://virtual-pcr.ico2s.org/

References

[1] Mehra, S. & Hu, W-S. (2005). A Kinetic Model of Quantitative Real-Time Polymerase Chain Reaction. Biotechnol Bioeng, 91(7), 848-860
[2] Gevertz, J. L. et al. (2005). Mathematical model of real-time PCR kinetics. Biotechnol Bioeng, 92(3), 346-355
[3] Cobbs, G. (2012). Stepwise kinetic equilibrium models of quantitative polymerase chain reaction. BMC Bioinformatics, 13:203

Funded by EPSRC Grant EP/N031962/1
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