Low Energy Extreme PCR: developing an efficient, low cost thermocycling engine

Justin Buckland, Tom Jellicoe
TTP plc

Introduction

The conditions required for “Extreme” PCR are well established, with amplification in less than one minute without loss of sensitivity or specificity using a capillary tube moved rapidly between two water baths to achieve fast temperature changes [1]. However the development of efficient and convenient thermocycling instrumentation remains a significant challenge. A microfluidic serpentine channel with different temperature zones is a promising implementation [2] but still suffers from large surface area and long channel length. Here we present an energy efficient low heat capacity thermal cycling mechanism capable of heating and cooling at 100°C/s with passive cooling and no fluid motion required during the thermal cycling process [3].

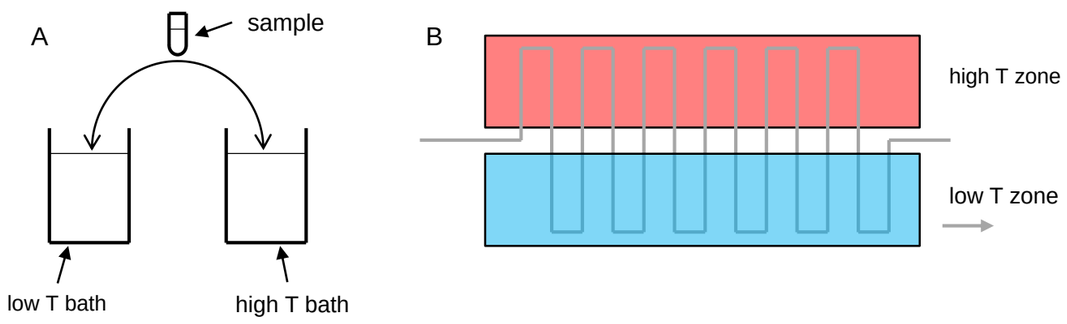

Figure 1: Previous approaches - rapid thermal cycling by moving the sample.
A: rapid motion of a capillary tube between water baths;
B: microfluidic channel in contact with a heater block with temperature zones.

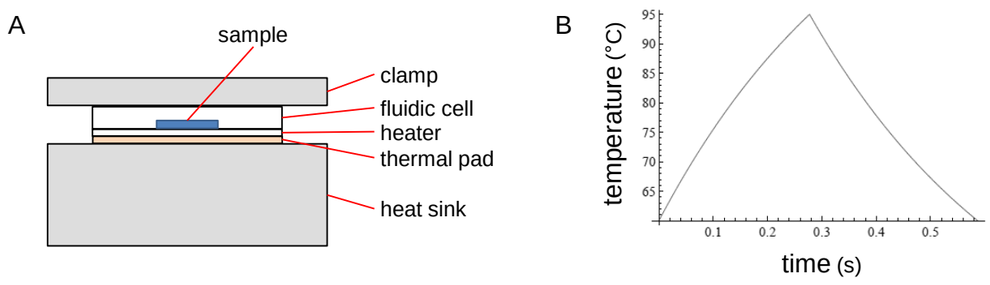

Figure 2: This work – rapid thermal cycling with stationary sample. A: key components are the fluidic cell, heater and heat sink. The sample thickness is 100µm to ensure rapid thermal equilibration (thermal diffusion time constant ≈ 70ms). B: calculated variation of sample temperature over time for minimum cycle time showing equal heating and cooling rates.

Thermal design

Our design objective was to carry out thermal cycling with cycle times down to 1-2 seconds, so that the PCR amplification speed is limited by the extension rate of the enzyme (approximately 100 bp/s) rather than by instrumentation. The thermal design principles are summarised below:

1. **Rapid thermal diffusion:** approximately 100ms from heater to sample to allow rapid thermal equilibration. This requires a fluidic channel height of 100µm and a thinner sealing layer between the sample and heater.

2. **Low heat capacity:** to minimise the time and heat flow required for temperature changes. Preferably, the heat capacity of the sample itself is larger than that of the heater and fluidic cell that contains the sample.

3. **Rapid temperature sensing:** any delay between heater output and temperature sensor response can cause control problems. We use the resistance of the heater itself in the sensing mechanism, minimising delay.

4. **Temperature uniformity across the sample area:** this can be achieved by increasing heat output in edge zones.

5. **Passive cooling:** by allowing heat to flow continuously through a thermal resistance from the sample into a heat sink near ambient temperature. While this approach may seem inefficient, it is effective when combined with low heat capacity and rapid amplification as this limits the total energy required.

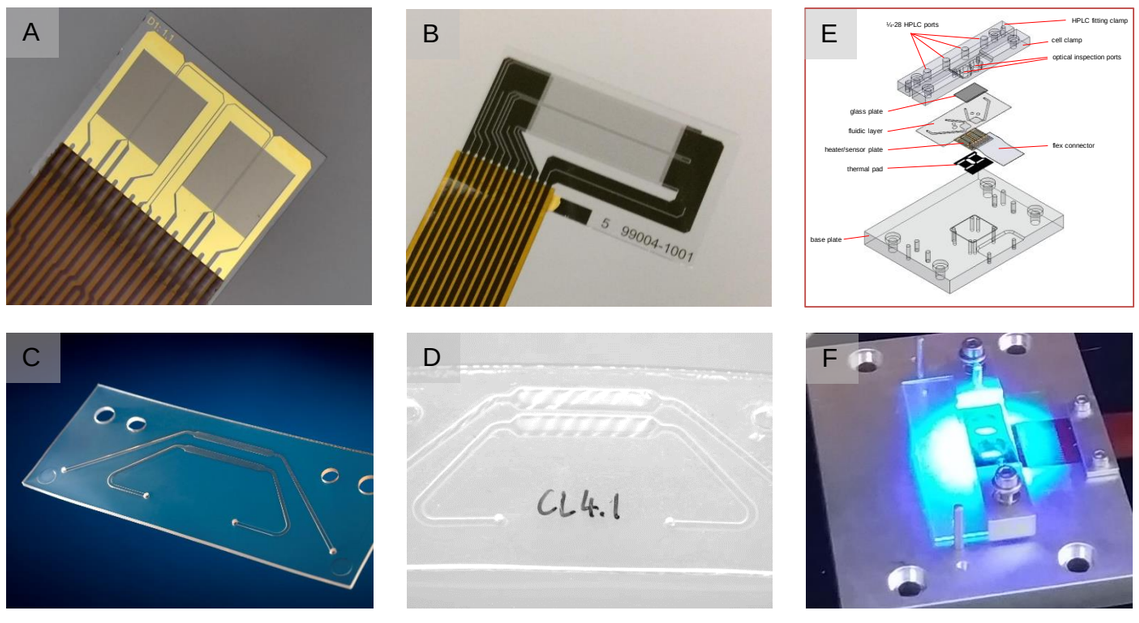

Figure 3: Heater and fluidic cell components. A: Thin film heater V1 with square heater regions; B: thin film heater V2 with rectangular heater regions and guard heaters to minimise edge effects; C: channels in polypropylene layer used to form fluidic cell; D: fluidic cell with polypropylene sealing layer; E, F: assembly of fluidic cell, heater, base plate (heat sink) and clamp.

Results

Temperature uniformity simulations (COMSOL) indicate that the temperature uniformity of ±0.25°C can be achieved across the fluidic cell. This is achieved by a combination of guard heaters and higher heat output zones at the perimeter of the heated area. Heater temperature measurements (obtained by monitoring the heater resistance) confirm the high ramp rate (~100°C/s) and demonstrate avoidance of overshoot.

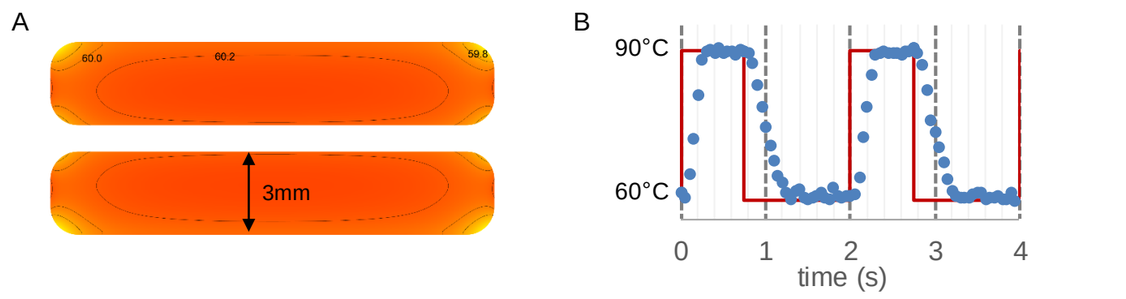

Figure 4: Heater uniformity and response time. A: simulation of heater uniformity across the area of the fluid cell (target: 60°C, heat sink: 30°C); B: measured heater temperature variation for 2s cycling (750ms high, 1250ms low)

PCR thermocycling tests used two sets of reagents, “Standard” (Fast SYBR Green Master Mix) and “Fast” (HemoKlenTaq), as described in the table below. Fluorescence imaging was used to monitor dsDNA during amplification, and tests with progressively shorter cycle times were carried out to determine the minimum viable cycle time for each case:

- Standard reagents: 5s cycle time (see Figure 5)
- Fast reagents: 2s cycle time (not shown).

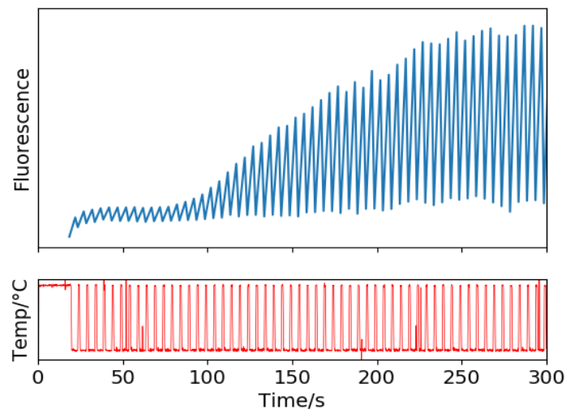

Figure 5: PCR amplification with “standard” reagents. A 20s hot-start is followed by 5s thermal cycles (1s at 95°C, 4s at 60°C).

Thermocycling conditions			
Phase	Temp (°C)	Duration (s)	
		Standard	Fast
Denature	95	1	0.75
Anneal + extend	60	4	1.25
Cycle time		5	2

Standard reagents	
Reagent	Vol (µl)
2x Fast SYBR Green Master Mix (Life Technologies)	50
hGAPDH assay (Tataa Biocenter)	4
Positive control cDNA (Tataa Biocenter)	10
water	36
Fast reagents	
5x HemoKlenTaq buffer (New England Biolabs)	20
HemoKlenTaq (New England Biolabs)	27.2
1ml/gl BSA (Sigma)	25
Positive control cDNA (Tataa Biocenter)	10
dNTPs, 25mM each (New England Biolabs)	0.8
hPPIA assay (Tataa Biocenter)	16
1/100 dilution SYBR Green I dye (Thermo)	1

Product concepts

- Fast PCR in the lab: digital or multi-sample with rapid time-to-answer
- True point-of-care PCR diagnostics: battery-powered, hand-held
- Potential for multiplexed and digital PCR with diffusion-limited partitioning enabled by short amplification time
- Polypropylene fluid cell consumable compatible with high volume manufacture using embossing or moulding combined with lamination processes

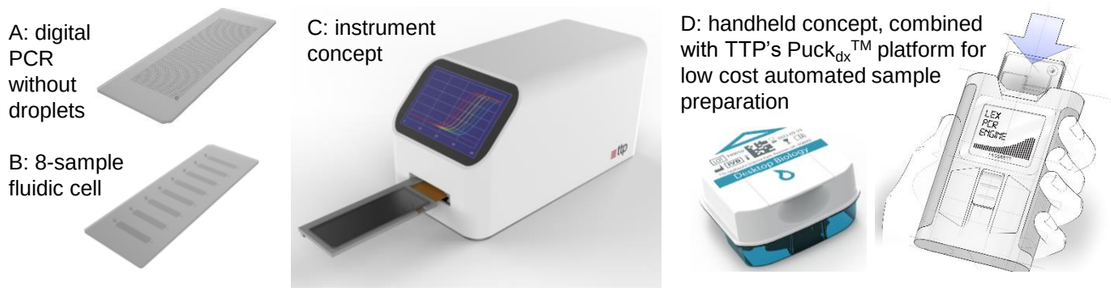

Figure 6: Product concepts for consumables and instruments. Fluidic cells for digital PCR (A) or multiple samples (B); instrument (C); automated sample processing consumable, combined with ultra-fast PCR (D).

References

1. Farrar, J.S. and Wittwer, C.T. (2015) Extreme PCR: Efficient and Specific DNA Amplification in 15–60 Seconds. Clinical Chemistry, 61:1 145–153.
2. Trauba, J.M. and Wittwer, C.T. (2017) Microfluidic Extreme PCR: <1 Minute DNA Amplification in a Thin Film Disposable. J. Biomedical Science and Engineering, 10, 219-231.
3. UK Patent Specification, application number 1812192.1 (2018).

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Contact

email: justin.buckland@ttp.com tel: +44 1763 262626
TTP plc, Melbourn Science Park, Melbourn, SG8 6EE, UK www.ttp.com