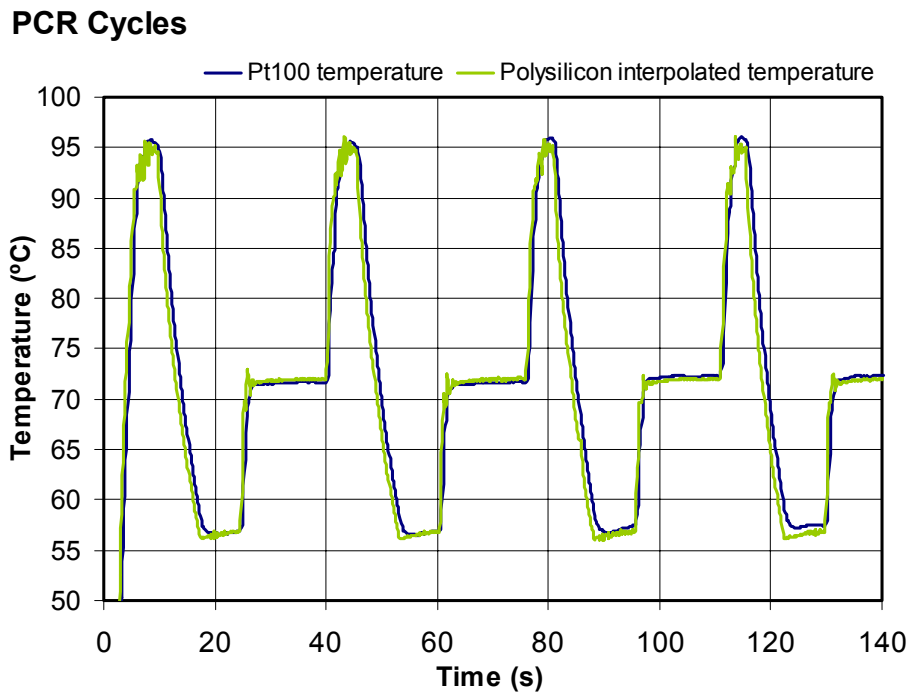


Figure 149 - Pt100 measured and polysilicon resistor interpolated temperatures in dual sensor-actuator PCR-chip mode. As expected, polysilicon resistor measures show a slightly fastest transient response, with the Pt100 lagging behind a constant amount of time due to heat-transfer phenomena.

Functional test

After establishing the correct behavior of active PCR-chips dual sensor-actuator capabilities, a first PCR assay was conducted to functionally ascertain their operation. The assay, in essence, was a replica of that conducted for active PCR-chips actuator validation (see p.247), with a slight variation in the PCR protocol (see Table 22).

Quantity	Reagent
17 µl	milliQ H_2O
2.5 µl	10x $MgCl_2$ Buffer
2.5 µl	10 nM dNTPs
1.25 µl	10 µM sense primer
1.25 µl	10 µM antisense primer
0.2 µl	3.5 U/µl Expand™ High Fidelity System (*Boehringer Mannheim Corp.*)
3.125 µl	20 mg/ml BSA
0.3 µl	200 ng/µl sample DNA

Cycling protocol:

95 ºC - 1 min
95 ºC - 2 s \
64 ºC - 10 s x45
72 ºC - 20 s /
72 ºC - 1 min
4 ºC - ∞

Table 22 - PCR mix and cycling protocols for sensor-actuator functional validation. As in previous experiments, Taq concentration was increased in PCR-chip mixes.

The results shown in Figure 150 evidenced the correct operation of independent active PCR-chips and, again, did not show any telltale differences between rhomboidal and serpentine-like chips efficiency. In the light of these successful results, the only remaining task to conclude the development work here reported was to conduct some titration experiments in order to see if Peltier-driven system optimization parameters were also readily applicable to the new setup.

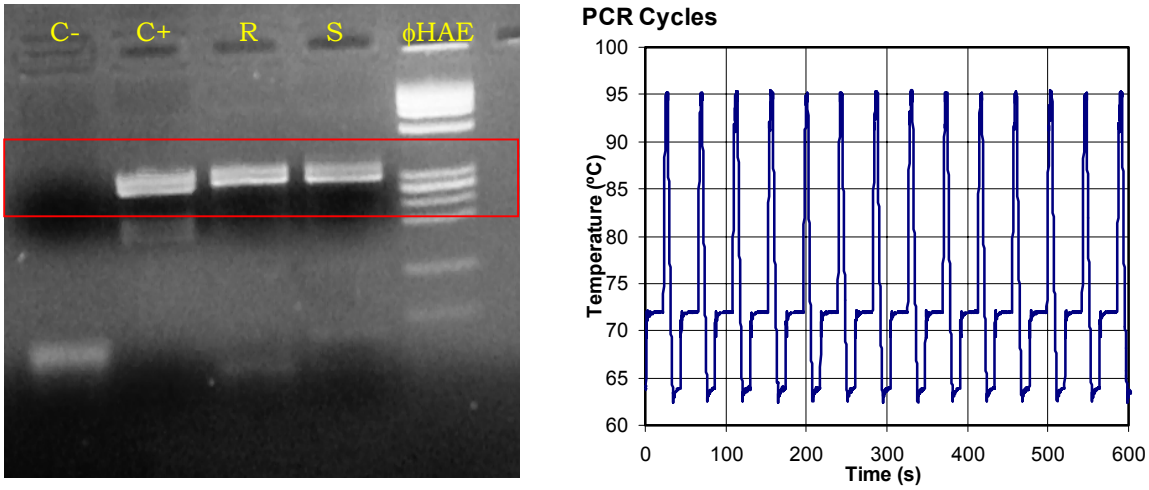


Figure 150 - Results for the initial functional test with independent active PCR-chips (3% agarose gel; 20 µl per well; 5 µl ϕHAE). No significant difference between rhomboidal (R) and serpentine (S) chips can be observed. The positive control efficiency seems to be a bit more robust, but, at the same time, chip results do not reveal any revealing signs of secondary structure formation, while positive control results do. This might indicate a more astringent temperature control in PCR-chips (most acutely at 64 °C), which could also be the cause of the slightly lesser efficiency shown in these systems. Average heating rate: 11.3 °C/s. Average cooling rate: 5.6 °C/s. Mean chip analysis time: 34:27 min.

Titration experiments

Different titration experiments (increasing BSA concentration, leveling out Taq concentrations, etc.) were conducted with independent active PCR-chips and are summarized in this last assay, in which most of the previously assessed optimization parameters (see p.200-212) were checked out. A 200 µl master mix was elaborated following the now standard method (see Table 23a) and distributed among eight different eppendorfs (two 25 µl control tubes and five 30 µl different chip experiments). The PCR cycling protocol was again fine-tuned to provide fast-PCR results (see Table

23b), and a modified version of it was used to test PCR on ultra-fast conditions (see Table 23c).

Quantity	Reagent
17 µl	milliQ H_2O
2.5 µl	10x $MgCl_2$ Buffer
2.5 µl	10 nM dNTPs
1.25 µl	10 µM sense primer
1.25 µl	10 µM antisense primer
0.2 µl	3.5 U/µl Expand™ High Fidelity System (*Boehringer Mannheim Corp.*)
3.125 µl	20 mg/ml BSA
0.3 µl	200 ng/µl sample DNA

Fast cycling protocol:

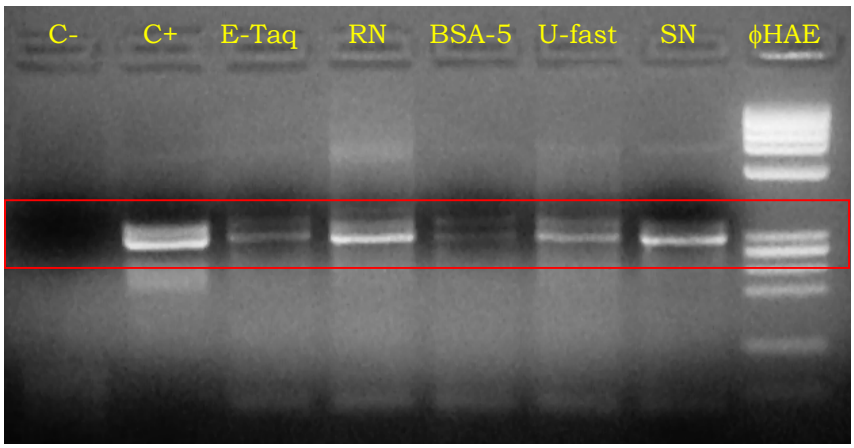
95.5 °C - 1 min
95.5 °C - 3 s \
64 °C - 7 s x45
72 °C - 17 s /
72 °C - 1 min
4 °C - ∞

Ultra-fast cycling protocol:

95.5 °C - 1:30 min
95.5 °C - 1 s \
64 °C - 2 s x45
72 °C - 10 s /
72 °C - 30 s
4 °C - ∞

Table 23 - PCR mix and cycling protocols for the last titration assay.

Titration results (see Figure 151) were very similar to those previously obtained with the Peltier-driven system.



Assay	Taq U/µl	DNA ng/µl
E-Taq	0.028	2.4
RN	0.05	2.4
BSA-5	0.028	2.4
U-fast	0.05	4.8
SN	0.05	2.4
C+	0.028	2.4

Figure 151 - Slab gel (3% agarose; 15 µl per well; 5 µl ϕHAE) results of the final titration assay. E-Taq stands for chip experiment with equal (respect to positive control) Taq concentration, while RN and SN refer to normal (doubled Taq) mixes for rhomboidal (R) and serpentine-like (S) chips. BSA-5 is the chip experiment with double BSA concentration (5 µg/µl) and U-fast is the ultra-fast PCR assay with doubled Taq and template DNA concentrations.

On the one hand, at equal Taq concentrations and a typical (2.5 µg/µl) BSA concentration, the chip gave a smaller yield (even though it did amplify) than the positive control, and this could not be meliorated (indeed it was worsened) by the use of higher BSA concentrations (5 µg/µl). On the other hand, increased Taq concentrations (roughly doubled: 0.05 U/µl instead of 0.028 U/µl) produced similar results for chip and positive control

amplifications and, again, a slightly better amplification on the positive control was counterbalanced by a lesser degree of secondary structure amplicates in chip assays. Finally, positive results were demonstrated for ultra-fast PCR operation (20 minute PCR) with active PCR-chips and, even though these results were clearly poorer than those of normal operation (ultra-fast chip mix had doubled Taq *and* template DNA concentrations), they still produced enough amplicate contrast to be of use in experiments where only positive (qualitative instead of quantitative) PCR is required (see p.92, [Belgrader2000]).

Concerning cycling performance (see Figure 152), active PCR-chips improved forced ambient Peltier-driven chips by at least 5 min in ~30 min experiments (a ~15% improvement). Heating rates were roughly doubled 5→10 °C/s and the 5 °C/s cooling rate of Peltier systems was maintained. Therefore, robust analysis times lay in the whereabouts of 30 min, halving the required time (about 1 h) in the fastest conventional thermocyclers. In addition, 20 min assays yielded contrastable positive results, showing that reasonable ultra-fast PCR techniques could be implemented in chip systems.

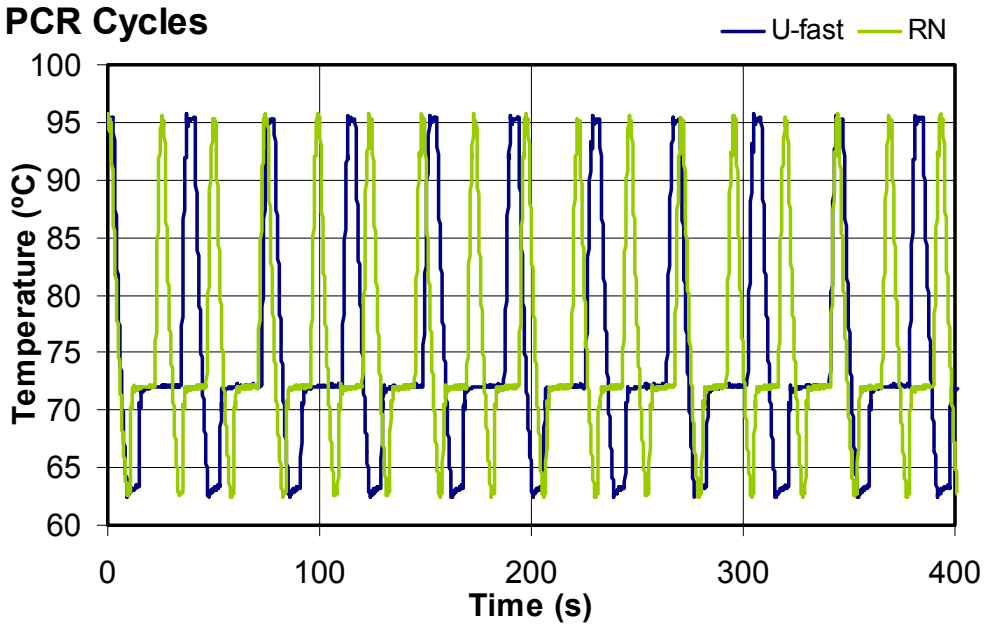


Figure 152 - Thermal cycling for ultra-fast (U-fast) and normal (RN) protocols. Average time for standard chip analysis: 30:28 min. Ultra-fast analysis time: 20:19 min. Positive control thermocycler analysis time: 56:43 min. Average heating rate: 10.73 °C/s. Average cooling rate: 5.6 °C/s.