PART II - SYSTEM DESIGN AND EXPERIMENTATION

4. PASSIVE PCR-CHIPS

4.1. WORK-PLAN

4.1.1. EVALUATION OF SERPENTINE-LIKE PCR-CHIPS

After the basic technological development of PCR-chips, concerning purely technological issues (like passivation, sealing and etching procedures) and design and set-up ones (methacrilate devices, design optimization, etc.), a first batch of serpentine-like PCR-chips was ready for experimental validation. The following is an outline of the final technological specifications of these devices, the experimental set-up for functional assays and their experimental validation.

Technology

The basic technological process for serpentine-like PCR-chips, after all the technological and design hurdles described in the earlier section had been addressed, is summarized in Figure 61.

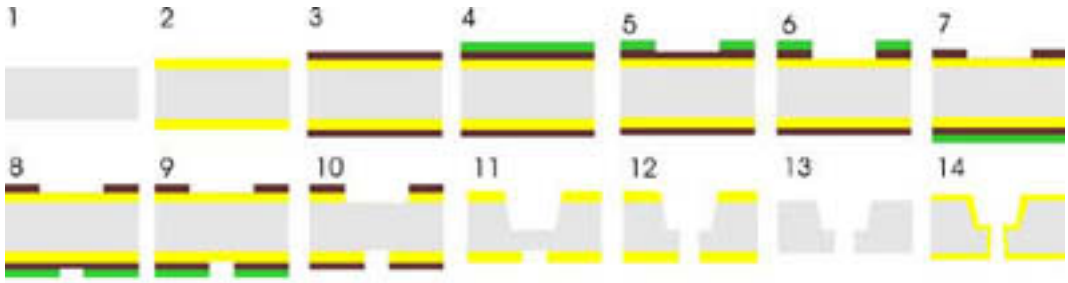

Figure 61 - Complete technological process for serpentine-like PCR-chips. Note: sizes are not up to scale.

Basically, a 1 µm silicon dioxide layer (2) is grown by wet thermal oxidation onto a 300 µm double-side polished P-type silicon wafer (1). Afterwards, a 1800 Å silicon nitride layer is deposited by LPCVD (3) and patterned on the wafer front-side with a negative photo-curable resist (4, 5) to open a window with RIE (6) for TMAH etching of silicon in order to create the PCR reservoirs. After removal of resist remnants (7), the Si_3N_4 window is transferred by wet HF etching to the SiO_2 layer (8). A similar process is then repeated at the backside (8, 9, 10) to define TMAH etch windows for access holes. Finally, a two-step deep silicon TMAH-etching process is undertaken, creating first the PCR chamber (11) and opening then the access holes (12).

To ensure a homogeneous passivation layer, the remaining oxide is washed away by a HF attack (13) and a new 380 Å oxide layer is grown over the entire wafer by dry oxidation techniques (14). Finally (not shown), a 1 mm SD-2 (*Hoya*) glass wafer is anodically bonded to the front-side surface, creating the complete PCR-chips (see Figure 60, p.133). For a detailed discussion on the complete technological process of passive PCR-chips (a slightly different version of the one depicted here), see *Materials and Methods*, p.279.

Experimental set-up and results

Experimental set-up

Functional PCR studies were carried out at the BioPAT service of the Hospital de Barcelona with the assistance of *Nadina Erill*. To ensure accurate temperature cycles, experiments were carried out on a HP2400 thermocycler (*Perkin-Elmer*) with 24 reaction wells. The 25 µl serpentine-like chips, consisting in five interconnected 50x1 mm^2 channels etched down to 100 µm, were effectively loaded (between 95-100% capacity) by capillary effect after positioning a pipette tip on top of an access hole while leaving the other port open, using a custom devised methacrilate appliance (see p.128). After filling, the chips were sealed with Arcare 7759 adhesive tape (*Adhesives Research*, [Taylor1997]), and placed (silicon facing down for better heat conduction) directly onto the thermocycler PCR-wells, which had been previously overloaded with mineral oil (see Figure 62). A control eppendorf tube containing the same PCR mix as the chip was also inserted into the thermocycler wells, side to side with the chip.

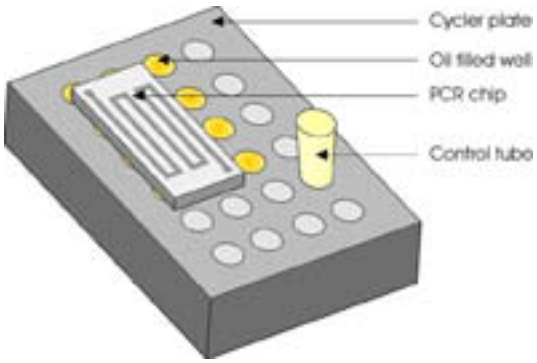

Figure 62 - Schematic representation of the experimental setup for serpentine-like PCR-chips.

After completing the reaction, the PCR product was extracted from the chip by syringe low pressure pumping at one port and active suction of the liquid bubble that emerged at the other port with a pipette. About 18-20 µl of PCR product were recovered on average, meaning that the extraction yield was around 80%. Although many recoveries were far more successful (around 90% of the inserted sample), the procedure could not achieve a higher mean efficiency due to the difficulty at predicting the emergence behavior of liquid bubbles, which sometimes formed small jets due to decompression, leading to poor recoveries. Extracted PCR products were then analyzed by conventional slab-gel electrophoresis.

The temperature cycles for PCR were as follows:

Time	Temperature	Function
5 min	94 °C	Initial denaturation
0.5 min	94 °C	Denaturation
0.5 min	62 °C	Annealing
0.5 min	72 °C	Extension
7 min	72 °C	Final extension
∞	4 °C	Storage cooling

Table 1 - Temperature cycles for PCR.

And the PCR mix for 25 µl is detailed below:

Quantity	Reagent
17 µl	milliQ H_2O
2.5 µl	PCR buffer (Mg^{++})
2 µl	10 mM dNTP solution
1 µl	Up primer
1 µl	Down primer
10 µl	3.5 U/µl Taq Expanol polymerase
1 µl	DNA*, **

Table 2 - PCR mix (for 25 µl)

Results

After repeated negative amplification results (see Figure 63a) in assays with the ~500 bp haemochromatosis gene exon#2 genomic DNA aliquots (*), it was decided to use the concentrated PCR product of previous experiments in re-amplification assays of a 200 bp region of the haemochromatosis gene

exon#2 (**). The PCR was carried out using the same described protocols and effective amplification was observed on the chip (see Figure 63b).

Evaluation of serpentine-like PCR-chips results

Although encouraging, the above results for serpentine-like PCR-chips did not yield high efficiency (only re-amplification could be accomplished) nor excessive reproducibility and, in addition, the use of a commercial thermocycler did not allow evaluation of the intrinsic benefits of PCR-chips (speed, better specificity, etc. [see p.97]). As it is prone to happen in such a multidisciplinary assay, the reasons for lack of efficiency could come from too many sources. For instance, it was not known if serpentine-like structures imposed any kinetic restrictions on PCR or, for that matter, if the use of adjuvants (and which and in what proportion) could improve results. It was also unknown whether the acrylic sealing tape, although thus reported [Taylor1997], was truly PCR friendly, nor if the lack of efficiency came from temperature gradients (meaning lower temperatures than desired) from the PCR-wells to the surface of the PCR block onto which the chip lay.

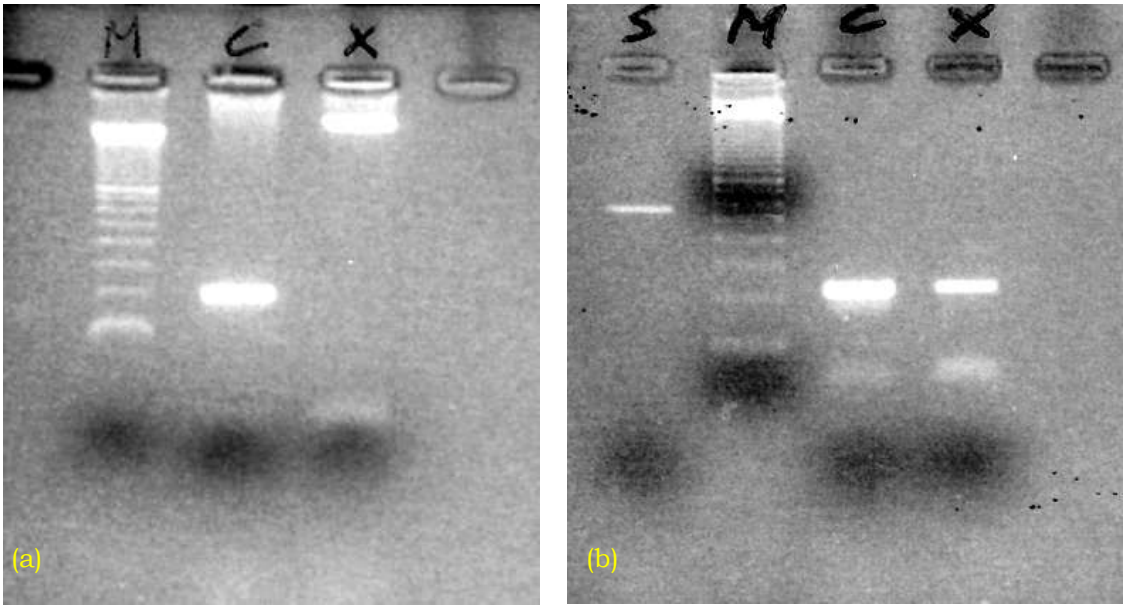

Figure 63 - Agarose gels corresponding to (a) unsuccessful PCR genomic DNA amplification and (b) successful re-amplification of PCR-products. The M lane corresponds to a DNA ladder, while the C and X lanes corresponds to control (C) and chip (X) PCR products. In (b), the S lane corresponds to the original 500 bp PCR-product, whilst X and C correspond to the re-amplified PCR-products.