
4.6. CHARACTERIZATION OF SURFACE PASSIVATION LAYERS

As it has been previously discussed (see p.102), most studies on PCR-chips hinted at an intrinsic PCR-inhibitor nature in bare silicon. However, only one research group ([Shoffner1996]) had carried out detailed studies of the behavior of PCR in the presence of typical silicon-technology layers, and their results were not conclusive, all the more so since they were mainly conducted on PCR-chips, in which other factors could be at work simultaneously. The experiments conducted by *Shoffner's* team and others ([Shoffner1996], [Cheng1996a], [Taylor1997]) also suggested that, disregarding surface silanization, silicon oxide layers were the most PCR-friendly of standard silicon-technology layers, and that part of the inhibition observed might be due to adsorption problems derived from the huge increase in surface-to-volume ratio of PCR-chips. Hence, in the light of previously experienced uncertainties with temperature control factors and with the knowledge of the multifaceted nature of PCR optimization, it was decided to first carry out a series of off-chip experiments to separately assess inhibition problems and then proceed, with the hindsight of the acquired inhibition knowledge, to gauge adsorption phenomena on PCR-chips.

4.6.1. INHIBITION EXPERIMENTS

The aim of inhibition experimentation was to independently assess the level of PCR inhibition introduced by each micro-fabrication material. Hence, experiments were carried out using conventional thermocycler instrumentation, fungibles and protocols.

Methodology

Materials

Silicon and layer-material fragments and powder were obtained by physically smashing available silicon wafers from past technological setup fabrication batches. In this way, powder and small fragments were obtained from:

- 7740-glass (Pyrex)
- SiO₂ passivated silicon
- Polysilicon passivated silicon bonded to 7740-glass
- SiO₂ passivated silicon bonded to 7740-glass

Eppendorf tubes that were to be used in the experiments were first weigh-calibrated and then filled with either 0.0025 g of material powder or ~1 mm² fragments. To accommodate possible physical impediments the fragments might originate on PCR kinetics, positive control tubes were also filled with 1 mm² fragments of eppendorf material cut from an unused tube. After filling, all eppendorfs were sterilized under UV irradiation in a laminar flow chamber for 30 min prior to mix elaboration.

PCR methodology

PCR amplification experiments were carried out with ~200 bp IS200 *Salmonella typhymurium* fragments cloned into a pGEM®-T vector (see *Materials and Methods*, p.319). Amplifications were conducted on a CETUS DNA Thermal-cycler (*Perkin Elmer*) using standard laboratory techniques (see also *Materials and Methods*, p.322) for mix preparation and PCR protocols.

The PCR mix and protocol were as follows:

Quantity	Reagent	Cycling protocol:
17 µl	milliQ H ₂ O	
2.5 µl	10x MgCl ₂ Buffer	95 °C - 2 min
2.5 µl	10 nM dNTPs	95 °C - 1 min \
1.25 µl	10 µM sense primer	61 °C - 1 min x30
1.25 µl	10 µM antisense primer	72 °C - 2 min /
0.2 µl	3.5 U/µl Expand™ High Fidelity System (<i>Boehringer Mannheim Corp.</i>)	72 °C - 7 min
1 µl	40-70 ng/µl sample DNA	4 °C - ∞

Table 4 - PCR protocols for DNA adsorption experiments.

Two 250 µl master mixes (see *Materials and Methods*, p.322) were prepared and distributed among 10 different eppendorf tubes each, eight containing fragments and powder of the different materials, one for positive control with an eppendorf tube fragment and one for negative control.

Gel analysis

All the PCR products were analyzed by gel electrophoresis in double-combed 3% agarose gel using a MiniSub® Cell GT (*BioRad*) and a 200/2.0

power supply (*BioRad*) and controlled with a ϕ X174 DNA/Hae-III ladder (*Promega*, see *Materials and Methods*, p.325 for details).

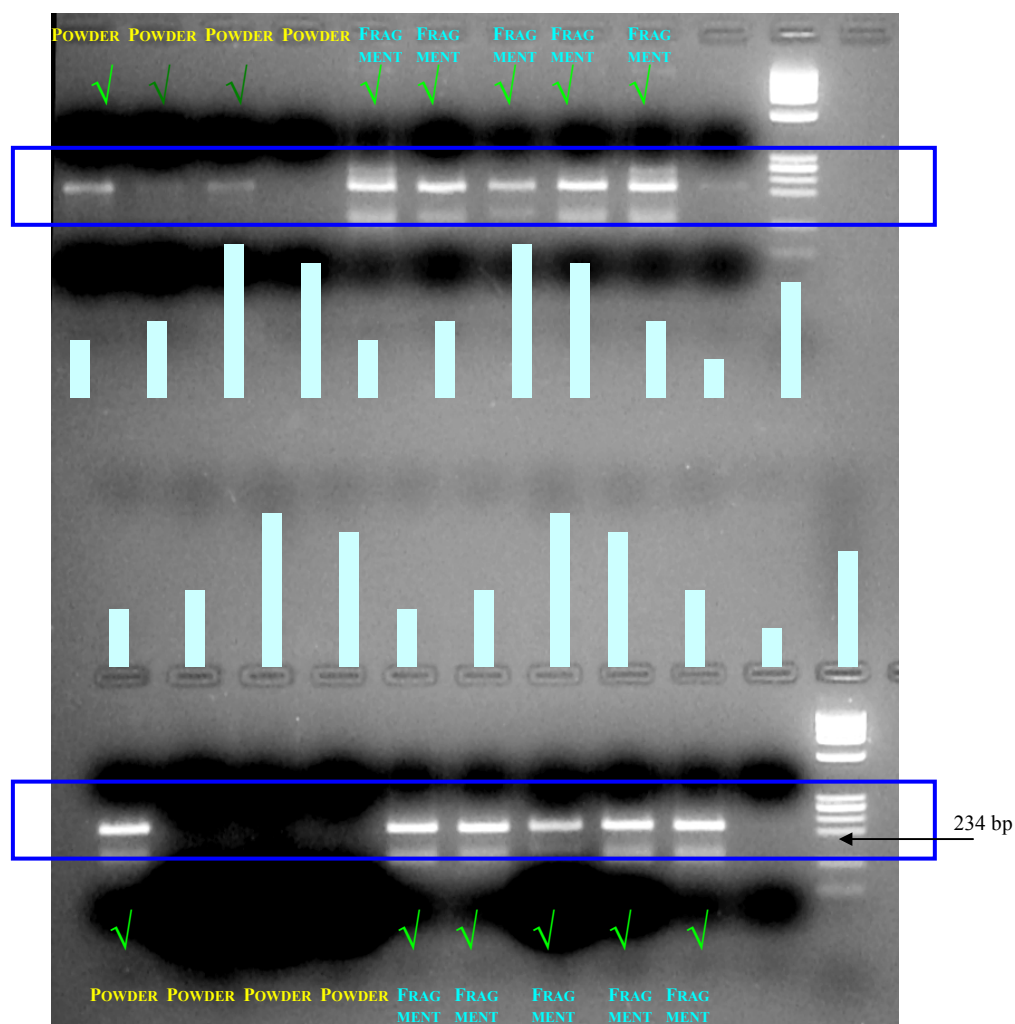


Figure 100 - Slab gel image of PCR inhibition experiments. The two horizontal lanes (blue boxes) correspond to the two independent PCR master mixes.

Results

These inhibition experiments were conducted thrice, under similar circumstances and showing similar results. Displayed in Figure 100 are the results for the last, and most methodological, of these experiments.

As it can be clearly observed in Figure 100, powder-containing eppendorf tubes yielded very poor or negligible results with all the assayed materials except 7740-glass. On the other hand, fragment experiments did not reveal a substantial inhibition with any of the assayed materials.

A first-glimpse conclusion from the above displayed experimental data could be that, in powder, almost all materials present high PCR-inhibition properties. Nevertheless, this conclusion does not take into account that all powder assays, due to the own nature of powder generation, display a far greater amount of silicon (which is the common substrate) than of any of the passivation layers. Moreover, the use of powder often led to unreliable recover of PCR products, thus throwing a veil onto powder-based experiments. Nevertheless, it seems clear that the use of powder, which yields a greater silicon surface reaction area, positively increases inhibition in PCR. That holds true for all silicon-based powders, but it is not the case of 7740-glass. Since the major constituent of 7740-glass is silicon oxide (mainly doped with boron impurities), it was not an offshoot conclusion to derive that silicon dioxide presented low, if any, inhibitory effects on PCR, whilst bare silicon clearly inhibited PCR.

On the other hand, results from fragment-carrying tubes indicated that inhibition of PCR was not an inherent and strong property of silicon (which displayed no inhibition in fragment experiments), but rather a phenomenon arising from an increased surface-to-volume ratio, a fact that was consistent with the prevailing hypotheses on the inhibitory effects of silicon dioxide, but which had not been previously assessed for bare silicon.

Hence, combining both kinds of results, it was inferred that silicon dioxide was probably the most PCR-friendly layer, with other passivation materials probably presenting a gradual level of inhibitory effects, and bare silicon being the strongest inhibitor. This was in accordance with previous literature reports [Shoffner1996], but the mainly diverging conclusion of these inhibition experiments was that the inhibitory properties of the different assayed materials were a consequence of adsorption phenomena caused from an increased surface-to-volume ratio, rather than from other standard chemical properties (ion release or the like) of the own materials. Therefore, it was decided to use silicon dioxide as the main passivation material (with a slight concession to polysilicon for compatibility with electrophoresis chip processes) and to carry out experiments to determine the predicted adsorption phenomena of silicon dioxide when used in PCR-chips.