Electronic Supplementary Material (ESI) for Lab on a Chip

Solid-phase PCR in a picowell array for immobilizing and arraying 100,000 PCR products to a microscope slide

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SUPPLEMENTARY INFORMATION

Content

1) Preparation of template DNA

a) PCR reaction setup

For amplifying three different templates with the same PCR reaction mix, DNA sequences contain at both ends a generic primer sequence for amplification: 5' - CTG AGC GGG CTG GCA AGG C - 3' attached to the 5' end and 5' - CTG ATG GC G CGA GGG AGG C - 3' attached to the 3' end of each template. A synthetic ssDNA 100 bp template comprises a 58 bp sequence of the plasmid pTYB1 flanked by the generic primer sequences. Template sequences in length of 346 bp and 1513 bp are generated by PCR using bipartite primers containing a *pTYB1-specific amplification sequence* at the 3' end and generic primer sequences at the 5' end. The PCR reaction mix for generating the 346 bp dsDNA contained 0.3 U HotStarTaq Plus DNA polymerase, 1 × reaction buffer, 0.2 mM dNTPs, forward bipartite primer 5' - CTG AGC GGG CTG GCA AGG C *CAA GTC GTG AAG TGC CAG* - 3' and reverse bipartite primer 5' - GCC TCC CTC GCG CCA TCA G *AGA AAG ATC TCT GGC CTC AAT* - 3' and 1×10^6 copies of pTYP1. Two-step PCR is performed on a RotorGene 2000 real-time cycler (Qiagen, Hilden, Germany) by 5 min activation at 95 °C followed by 50 cycles of 94 °C for 30 sec and 60 °C for 60 sec. The 1513 bp dsDNA template is produced in the same way but using the reverse bipartite primer 5' - GCC TCC CTC GCG CCA TCA G *CCG GAT ATA GTT CCT CCT TTC A* - 3' and an annealing and extension times of 120 sec instead of 60 sec.

b) Analysis of template DNA

PCR products are purified using a DNA purification kit (Roche, Mannheim, Germany) according to its manual. Purified DNA was quantified by NanoVue (VWR, Bruchsal, Germany) and serially diluted in 0.2 × TE buffer supplemented with 10 ng/µL salmon sperm DNA. Real-time PCR is performed to verify that template DNA is amplified using primers being complementary to the generic primer sequences. The PCR reaction mix for amplifying all three template DNA sequences contained 0.2 U HotStarTaq Plus DNA polymerase, 1 × reaction buffer, 0.2 mM dNTPs, 0.3 µM forward primer 5' - CTG AGC GGG CTG GCA – 3', 0.3 µM reverse primer 5' - GCC TCC CTC GCG CCA TCA G - 3' and 0.2 µM of the TaqMan probe 5' - 6-FAM - ACG TGT AAT GCG ACC CAT GAG TTG G - DDQ-1 - 3'. The reaction mix is aliquot in four tubes, subsequently adding 10^3, 10^4, and 10^5 copies of respective template DNA in a certain length. PCR grade water is inserted to a final reaction volume of 30 µL. For every template DNA, to one tube only PCR grade water is added as no-template control. From each 30 µL reaction mix three 10 µL are aliquot into 100 µL reaction tubes and stored on ice until processed in the RotorGene 6000 real-time cycler. Hot-start polymerase is activated 5 minutes at 95 °C, cycling is performed by alternating 55 times between 94 °C for 30 seconds and 60 °C for 120 seconds for the templates 346 and 1513 bp, and 60 °C for 45 seconds for the 100 bp template. Figure S1 show logarithmic plots of real-time PCR for the 100 bp (a), 346 bp (b), and 1513 (c) template DNA.

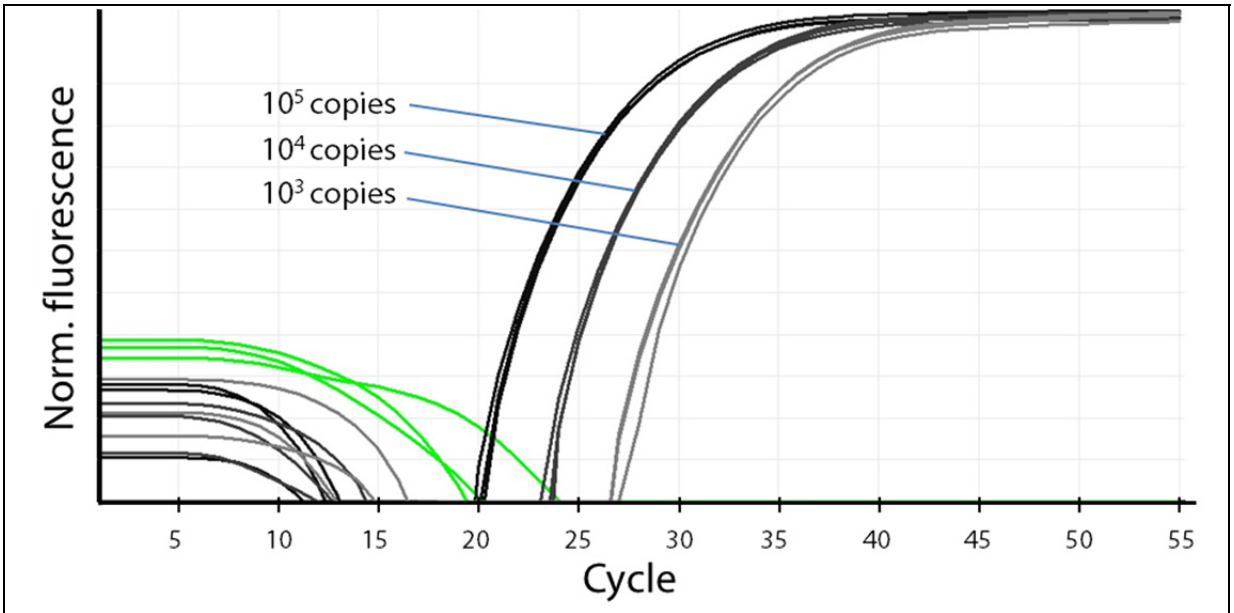

Figure S1 a: Real-time PCR data from the amplification of the 100 bp template using generic primer sequences.

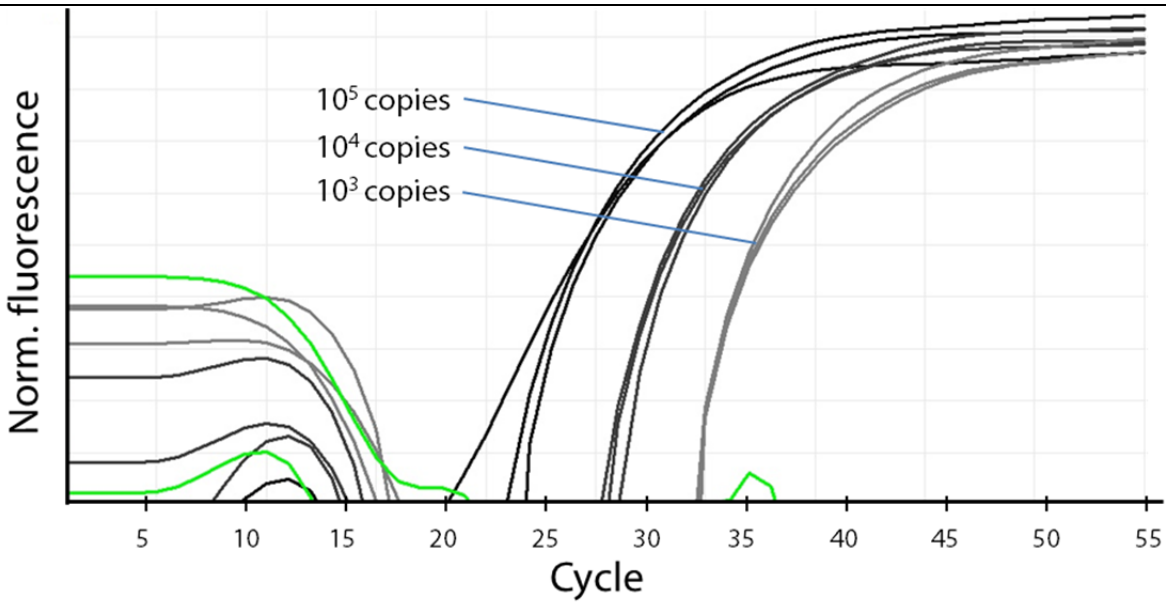

Figure S1 b: Real-time PCR data from the amplification of the 346 bp template using generic primer sequences.

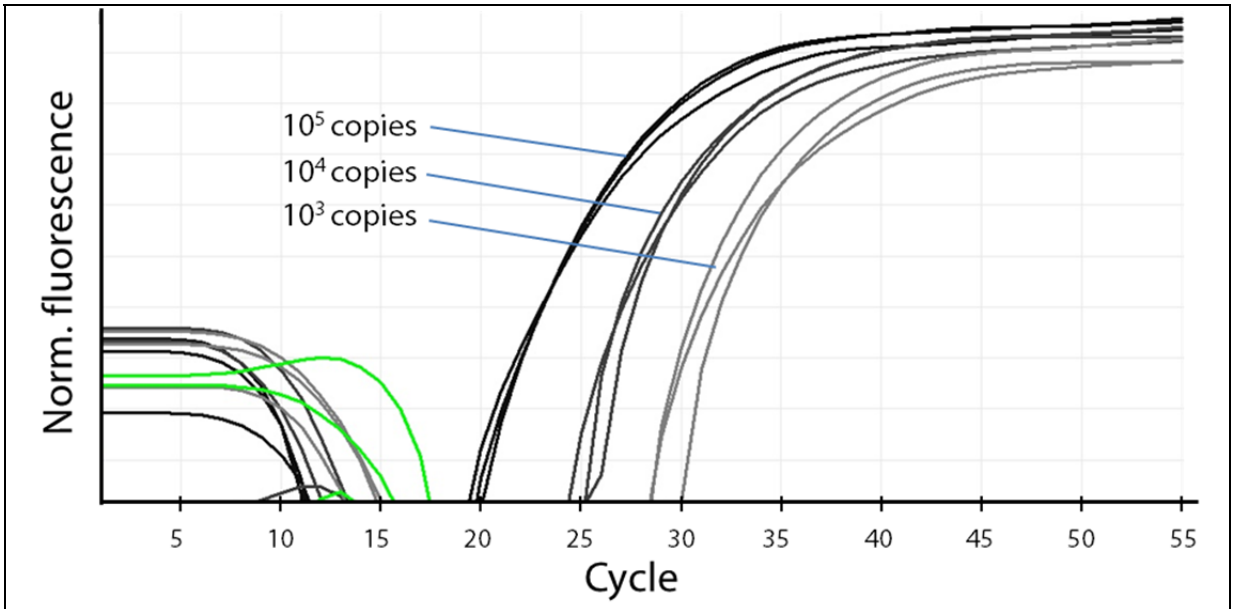

Figure S1 c: Real-time PCR data from the amplification of the 1513 bp template using generic primer sequences.

2) Determination of temperature profile

The PCR thermoprofile within the cycling cartridge is determined by a temperature data logger (177-T4, Testo, Lenzkirch, Germany) clamped in between the glass-PDMS slide (Figure 2, C) and the P (Figure 2, A) of an assembled cycling cartridge. To realize a 5 minutes activation for the polymerase at 95 °C, temperatures at the slide cycler are set to 105 °C for 2' 58", 103 °C for 40", 102 °C for 40", 101 °C for 1' 30", 99 °C for 1', 54 °C for 1', 59 °C for 1' 30", the last two steps realizing an initial 105 seconds of annealing and extension time at 60 °C. 50 temperature cycles alternating between 30 seconds at 95 °C and 90 seconds at 60 °C are realized by temperature steps of 105 °C for 55", 102 °C for 20", 54 °C for 50" and 58 °C for 1' (Figure 3) (' stands for minutes, " for seconds). After cycling, cartridges were kept at 8 °C for 0.5 h $\leq$ t $\leq$ 12 h in the slide cycler until disassembly.
Thermal energy for performing PCR within the array must be transmitted from the heating block surface of the slide cycler thorough the cycling cartridge (Figure 1, B) and further on into the filled and sealed picowell array (Figure 1, A) and vice versa overcoming thermal transfer resistances between adjacent surfaces and material specific thermal resistances. To compensate the resulting thermal delay, we empirically determined the required thermal profile of the

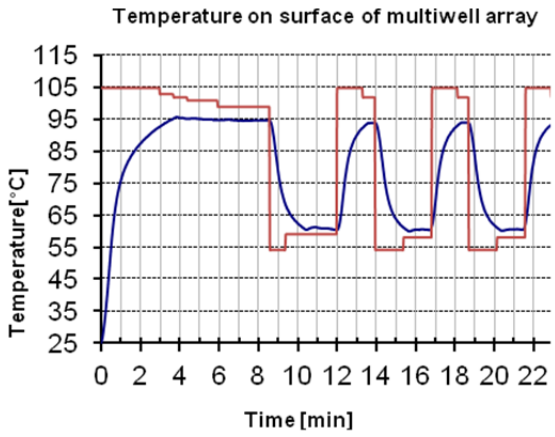

Figure S2. Thermoprofile in cycling cartridge. For running a PCR reaction with the wells of the array, the correct temperature profile of the assembled cycling cartridge is determined on the surface of the array. The red curve shows the set profile on the slide cycler

slide cycler for measuring a typical PCR temperature profile at the surface of the picowell array. Figure S2 shows the temperature profile set on the slide cycler (red curve) and the measured temperature profile on the surface of the array (blue curve). The heating and cooling rates are longer than those for conventional PCR, because the whole cycling cartridge requires extended times to reach steady-state temperatures.

3) Evaluation of acquired images

Positive signals from the copy reaction are counted by analyzing acquired 16-bit tiff images with the free software ImageJ version 1.44o using following macro:

```
run("Smooth");
run("Convolve...", "text1=[-1 -1 -1 -1 -1\n-1 -1 -1 -1 -1\n-1 -1 24 -1 -1\n-1 -1 -1 -1 -1\n-1 -1 -1 -1 -1\n] normalize");
setAutoThreshold("Default dark");
setThreshold(10000, 65535);
run("Convert to Mask");
run("Fill Holes");
run("Analyze Particles...", "size=36-75 circularity=0.50-1.00 show=Outlines display clear include summarize");
```

Acquired scans from copy reactions are analyzed with the macro as described. Figure S3 shows modifications to images and stands as example for the evaluation macro.

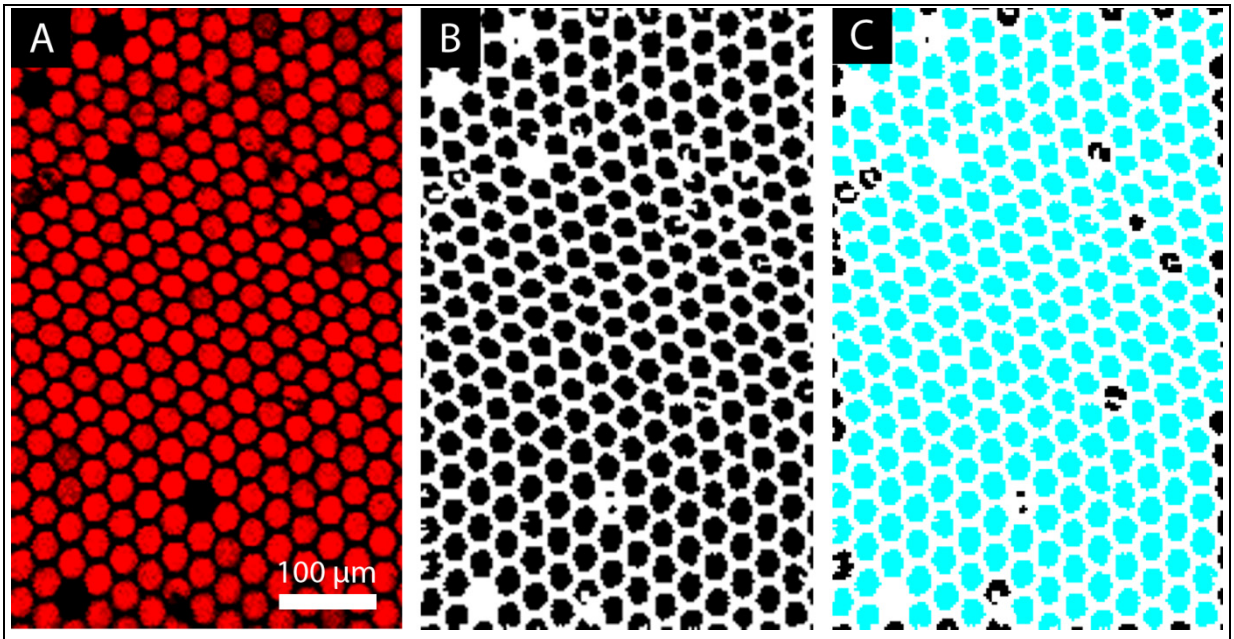

Figure S3. Image processing for counting of positive spots. (A) Native scan of a target array in the Cy5 channel. (B) Pixels with intensities between 10.000 and 65535 are defined as black for creating a binary image. (C) A cluster of pixels is defined as positive (blue) and therefore counted, if pixels form a black area in the range of 0.5 - 1.1 times the theoretically expected area featuring a circularity of 0.6 – 1.0.