**Supplementary Information**

**Functional surface-immobilization of genes using multistep strand displacement lithography**

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**1. Time schedule**

The following table provides a rough estimate for the times required to perform certain steps of the Bephore technique. Several steps contain long incubation times which do not require any action.

|  |  |  |
| --- | --- | --- |
| **Section/Step** | | **Time** |
| 1. Chip fabrication | | Few hours |
| 2. Preparation of genes for immobilization | | 2 h |
| 3. Photolithography | |  |
|  | 3.1 & 3.2 Single step |  |
|  | Bephore mix on chip | 1 h |
|  | Passivation | 2 h |
|  | Oligonucleotides (10 µM) / Genes (100 nM) | 2 h / several hours or overnight |
|  | 3.1 & 3.3 Three steps |  |
|  | Oligonucleotides (10 µM) | ≈1 day |
|  | Genes (100 nM) | ≈2 days |
| 4. PDMS devices | |  |
|  | 4.1 Fabrication of master molds | Few hours |
|  | 4.2 Preparation of the PDMS device | 2 h |
| 5. Compartmentalized gene expression (preparation) | | 1 h |
| 6. Sustained expression in microfluidic devices (preparation) | | 2 h |

**2. Oligonucleotide sequences**

|  |  |
| --- | --- |
| **Name** | **5' Sequence 3'** |
|  |  |
| **a) Bephore** |  |
| PC | CAACCGGCTTTTTPGCCGGTTGATTGATTTAGGAGTAGTGAGCGCGATAGGCTBGAGGC |
| PH | GCCTATCGCGCTCACTACTCCTAAATCAAT |
| DIS | GCCTATCGCGCTCACTACTCCTAAATCAATCAACCGGC |
| FL+DIS | FLGCCTATCGCGCTCACTACTCCTAAATCAATCAACCGGC |
|  |  |
|  |  |
| **b) Primers** | |
| Fwd (Primer) | TGCCACCTGACGTCTAAGAA |
| DIS+TT+S+TT+Fwd | CTATCGCGCTCACTACTCCTAAATCAATCAACCGGCTTSTTTGCCACCTGACGTCTAAGAA |
| Rev (Primer) | ATTACCGCCTTTGAGTGAGC |
| FL+TT+Rev | FLTTATTACCGCCTTTGAGTGAGC |
|  |  |
| B: Biotin dT (“Int Biotin dT”, IDT) | |
| P: Photocleavable spacer (“Int PC Spacer”, IDT) | |
| S: Triethylene glycol spacer (“Spacer 9”, IDT) | |
| FL: ATTO 532 or Alexa Fluor 647 | |

a) DNA strands used as components in Bephore. Modifications are colored in red.

Note: The last six bases of the PC strand (TBGAGGC) do not play an active role and can therefore be replaced by a simple 3’ biotin modification. Sequences were designed and analyzed using the online tool NUPACK (www.nupack.org).

b) Primers for the generation of linearized templates for fluorescent proteins.

DNA strands were purchased from Integrated DNA Technologies Inc. (IDT).

**3. Gene sequence for the fluorescent protein YPet**

Gene coding for the fluorescent protein YPet (used in Figures 6-8 after PCR with modified primers Fwd and Rev):

|  |
| --- |
| **Fwd Primer**– T7 promoter – RiboJ - RBS (BBa\_B0034) - YPet – Terminator (ECK120033737) - **Rev Primer** |
| **tgccacctgacgtctaagaa**accattattatcatgacattaacctataaaaataggcgtatcacgaggcagaatttcagataaaaaaaatccttagctttcgctaaggatgatttctggaattcgagtaagcccctctagaggaccacgcatcgtgatgcctatgcgcggtagtcccaccttgtccactagaatggaagattggcacgtatcaagactttggagtagtaccataacgccgtaatacgactcactatagggtagcgcagcgctcaacgggtgtgcttcccgttctgatgagtccgtgaggacgaaagcgcctctacaaataattttgtttaatcatgagaaagaggagaaaactagatgtctaaaggtgaagaactgtttacgggtgtcgtgccgattctggtcgagttggacggcgacgtgaacggtcacaaattcagcgtgagcggcgagggcgagggtgacgcgacgtacggtaagctgactctgaagctgctgtgcaccacgggtaaattgccggttccgtggccgaccctggtcacgacgctgggttatggtgtacaatgttttgcacgctatccggaccacatgaaacagcacgatttcttcaagagcgcgatgccggaaggctatgttcaggaacgtaccatctttttcaaagatgatggtaattacaaaacccgcgcagaagtgaagttcgagggtgacaccctggtgaaccgtattgagctgaagggtattgacttcaaggaagatggcaatattctgggtcacaaactggagtacaactataacagccataacgtctacatcaccgcggataagcaaaaaaatggtatcaaagcaaatttcaagattcgccacaacatcgaagatggcggcgtgcaactggccgatcattatcagcagaataccccaatcggtgacggtccggtgctgttgccggataaccactacctgagctatcaaagcgcgttgttcaaagacccgaatgaaaaacgtgaccacatggttctgctggaatttctgaccgctgcgggcatcactgaaggcatgaatgaactgtacaagacgcgtggtggcggcggttcgatgagcaagactatcgttttgtccgtcggcgaggctacccgtaccttgaccgaaattcaatccaccgcggaccgtcaaatttttgaggaaaaagtcggtcctctggtgggtcgtctgcgtctgaccgcgagcctgcgccagaacggtgccaaaacggcataccgtgttaatctgaaactggatcaggccgacgttgtggacagcggtctgccgaaagtccgctacacccaggtgtggagccacgatgtgacgatcgttgcgaatagcaccgaagcgagccgcaagagcctgtacgacctgaccaagagcctggtggcaacgtcccaagttgaagatctggttgttaacctggtgccgctgggtcgttaaagcatgccggaggaaacacagaaaaaagcccgcacctgacagtgcgggctttttttttcgaccaaaggggtgcatactagtagcggccgctgcagtccggcaaaaaagggcaaggtgtcaccaccctgccctttttctttaaaaccgaaaagattacttcgcgttatgcaggcttcctcgctcactgactcgctgcgctcggtcgttcggctgcggcgagcggtatca**gctcactcaaaggcggtaat** |

**4. PCR**

PCR kit: Phusion High-Fidelity PCR Master Mix with HF Buffer (New England Biolabs)

Reaction setup: 720 µL total volume (aliquoted to 12×60 µL), 500 nM primer, 100 ng plasmid DNA

Extension rate: 25 s/kbp

Tanneal=63°C (calculated with NEB Tm Calculator - tmcalculator.neb.com - for primers Fwd and Rev)

PCR program for the amplification of the gene in supplementary section 3:

|  |  |  |  |
| --- | --- | --- | --- |
| **1 cycle** | |  |  |
|  | 98°C | 30 s |  |
|  |  |  |  |
| **4 cycles** | |  |  |
|  | 98°C | 5 s |  |
| ramp | 66°C -1°C / cycle | 30 s |  |
|  | 72°C | Extension time |  |
|  |  |  |  |
| **26 cycles** | |  |  |
|  | 98°C | 5 s |  |
|  | 63°C | 30 s |  |
|  | 72°C | Extension time |  |
|  |  |  |  |
| **1 cycle** | |  |  |
|  | 72°C | 5 min |  |
|  |  |  |  |
| **1 cycle** | |  |  |
|  | 4°C | Forever |  |

**Purification**

Spin column purification kit: Promega Wizard SV Gel and PCR Clean-Up System

Note: To fit the entire PCR reaction volume into a single column, we split the volume in two and performed the membrane binding step twice. Also, we eluted twice with 50 µL of nuclease-free water to retrieve as much DNA as possible.

**Concentration measurement**

To estimate the concentration of the PCR product after purification, we measured the concentration (ng/µL) of dsDNA in a NanoPhotometer (Implen) and then calculated the molar concentration (average mass per basepair ≈650 g/mol).

**Quality control (optional)**

Success of the PCR and purity of the sample after purification can be assessed *via* agarose gel electrophoresis. Prior to immobilization, expression of the gene from the PCR product can be checked in a test tube, followed by an appropriate analysis method (*e.g.* by fluorescence spectroscopy in the case of a fluorescent protein).

**5. Lithography masks**

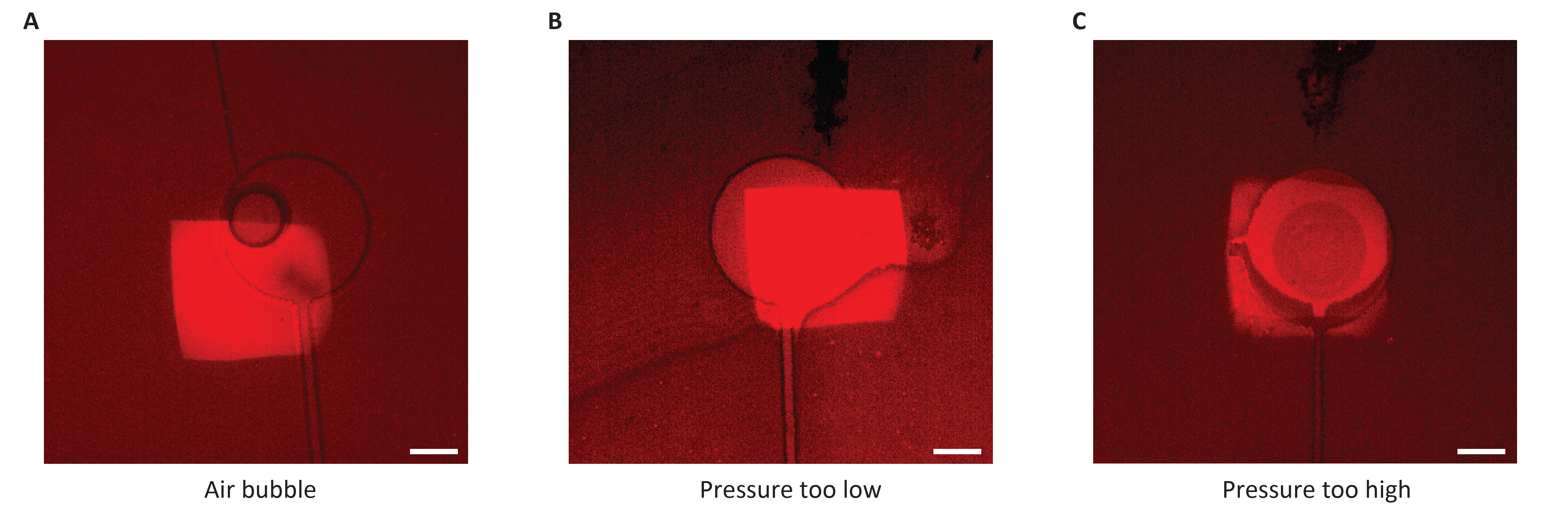
The supplementary file “SI\_Lithography\_masks.pdf” shows exemplary mask designs (drawn with Adobe Illustrator) for photolithography. These masks were used to create master molds for PDMS devices (top) and for mask projection lithography on Bephore chips (bottom). Masks were ordered as printed photolithography masks with a resolution of 64,000 dpi and with a size of 180 mm × 240 mm. In the printed mask, white structures in the design appeared transparent.

Top left: Compartments with a diameter and spacing of 300 µm and a channel of 20 µm width.

Top right: Compartments with a diameter and spacing of 300 µm. A channel of 20 µm width and 300 µm length connects them to a 30 µm wide feeding channel. Inlet and outlet of 1.5 mm diameter allow for the connection to a pressure controller or a syringe pump.

Bottom: Masks used in projection lithography on Bephore chips (Figures 5-7). The leftmost crosses can be used as alignment marks on the mask holder by cutting out the central region with a scalpel and gluing it to the holder (Figure 2B).

**6. Compartmentalization - troubleshooting**



**Figure S1: Fluorescence images of early attempts to align compartments and DNA brushes (protocol section 5, Figure 6).**

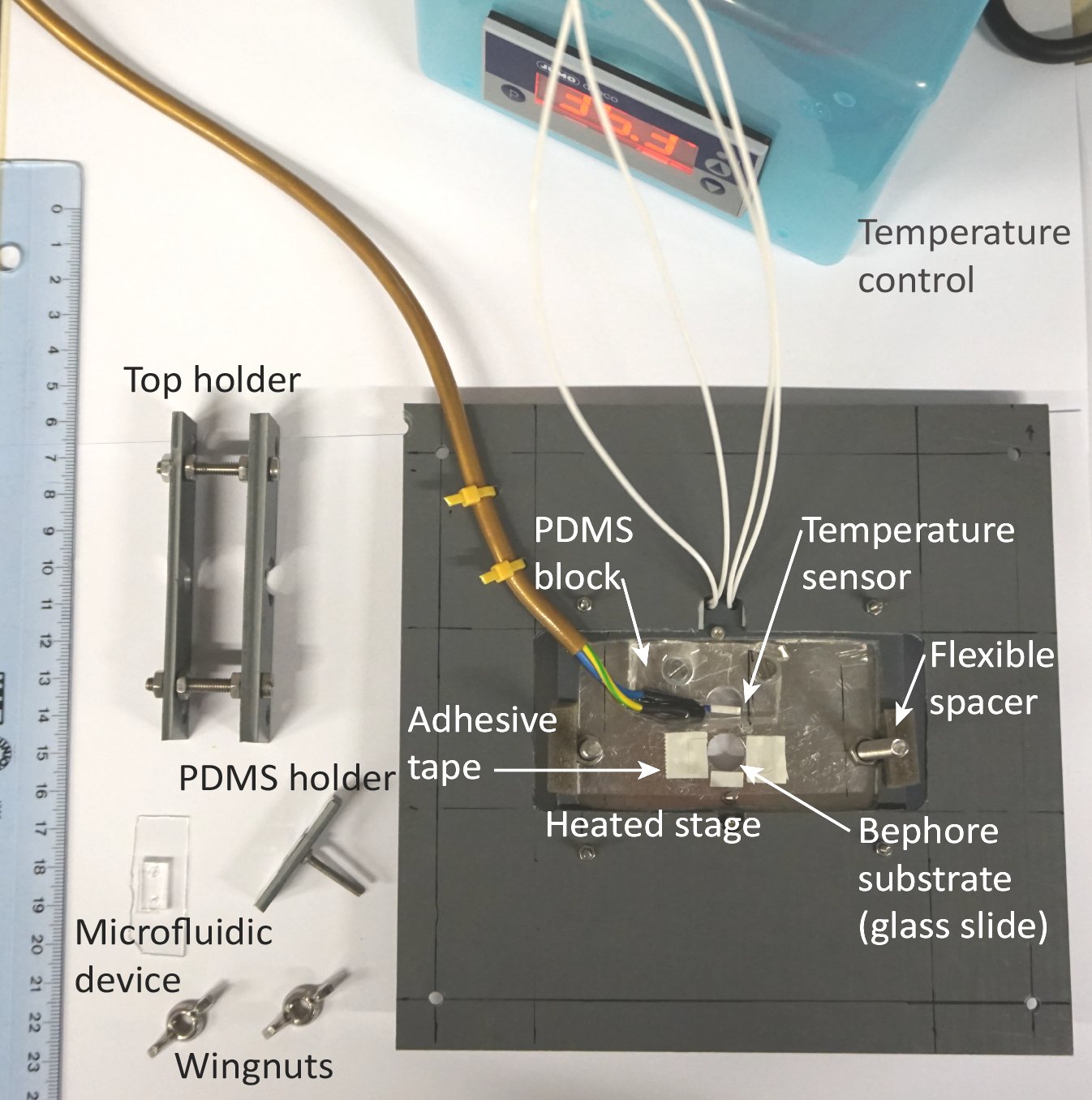
**A.** Air bubble caught in a chamber. Try to avoid the formation of air bubbles in the cell-free gene expression system by mixing its components *gently*. Also, plasma-clean the PDMS *right before* using it, rendering it hydrophilic in order to ensure easy wetting of the compartments without the formation of an air bubble inside the chamber.

**B.** If the PDMS compartments are not pressed tightly onto the Bephore chip, a liquid film remains between chip and PDMS.

**C.** If the applied pressure is too high, the compartment ceiling is pressed onto the brush (dark, circular region). Furthermore, high pressure can shift the compartment after its first contact with the chip and scrape parts of the brush off the chip.

To get alignment and pressure right, start with a test run using a chip without DNA brush and first practice the alignment of a chamber and a mark (*e.g.* a scratch) on the chip. Second, use a chip with DNA brush, align chamber and brush and then stepwise increase the pressure, imaging chamber and brush in between steps to see the phenomena shown in Figure S1B&C.

**7. Temperature-controlled stage for the microfluidic setup**

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**Figure S2: Parts of the sample holder, the PDMS device, the temperature-controlled microscope stage and the Bephore glass slide (ruler unit: cm).**

The heated stage consists of a 4 mm thick aluminum plate (central region), which can be mounted onto a motorized microscope stage *via* a larger PVC adapter plate (dark grey). In the center, the Bephore glass slide is glued to the aluminum plate *via* double-sided adhesive tape, with a hole in the aluminum allowing for the observation of the Bephore slide in an inverted microscope, *e.g.* using a 4× objective with a large working distance).

Two pieces of foam material (*e.g.* from a cover slip box) act as compressible spacer between the stage and the top holder.

Heating was provided by a temperature controller (JUMO di eco, LCD display visible at the top), which was connected to two 1.25 W adhesive heating mats (12 V, 25 mm × 50 mm, RS Pro) glued to the backside of the aluminum (only their white cables are visible). A Pt100 temperature sensor (RS Pro) was attached to a cable (colored in gold) and embedded in a block of PDMS. The sensor was then placed on a second hole in the aluminum plate and connected to the temperature controller. This setup ensures that the temperature sensor is in a similar environment as the compartmentalized gene brushes.

CAUTION: Danger to life! The assembly of an electronic device must only be carried out by an expert.