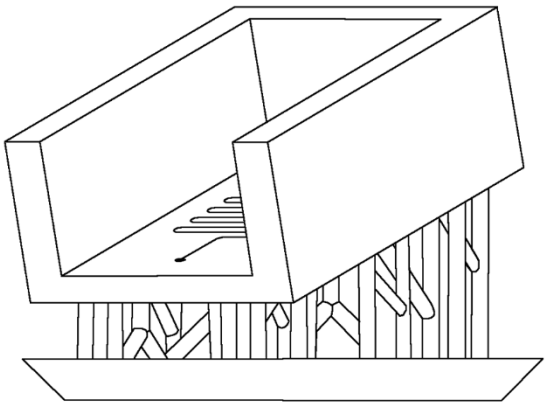
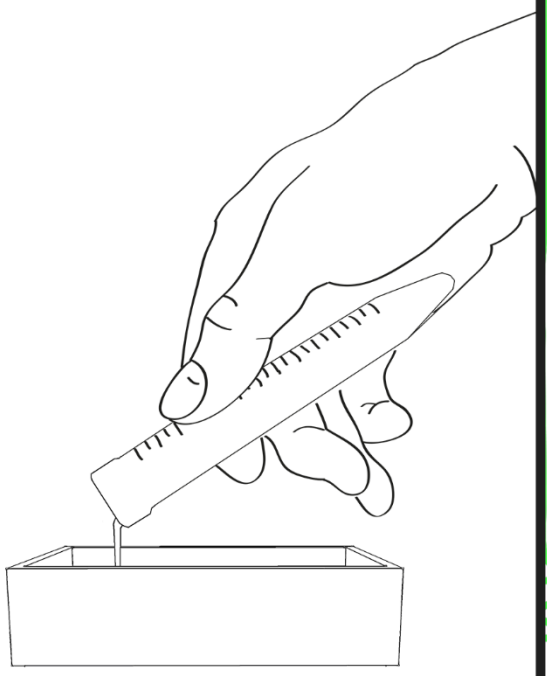
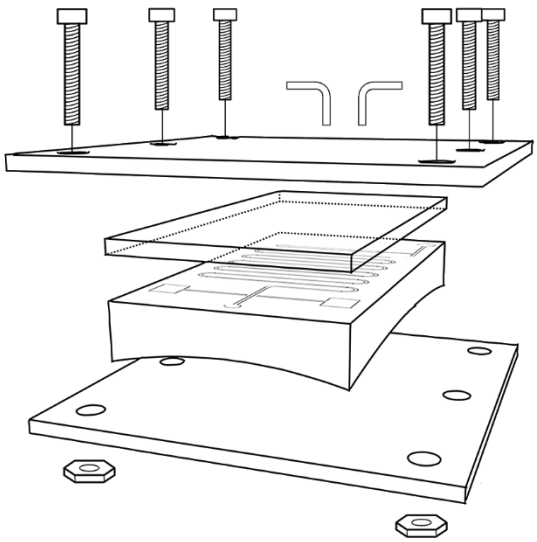
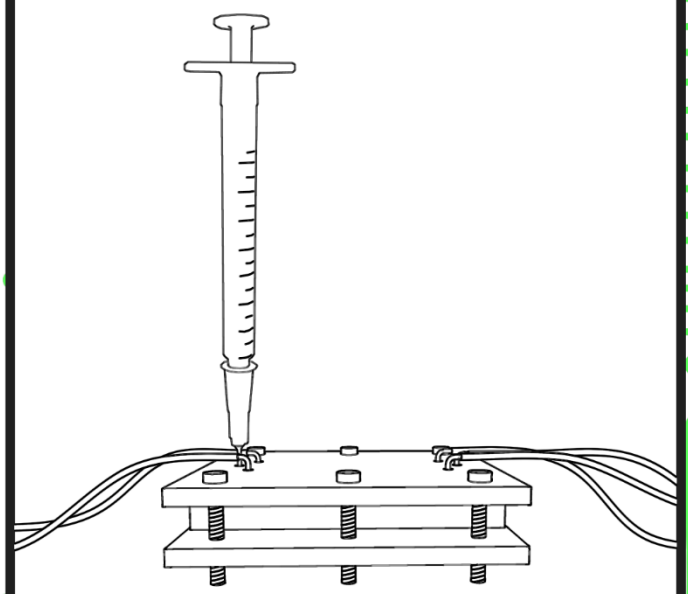




CHIPGINEERING

MANUAL FOR MANUFACTURING A MICROFLUIDIC CHIP FOR HEAT SHOCK TRANSFORMATION





1 TABLE OF CONTENT

2 INTRODUCTION

Microfluidic chips can be used to carry out many laboratory procedures in a micro-scale, effectively downscaling and streamlining laboratory work. The smaller volumes and workload have the potential to lower the cost of bench-top procedures and they are the biggest advantage that microfluidics brings to research. Fabrication methods for microfluidic chips have become faster and easier, making prototyping a rapid task. The method in this manual eliminates the need for a clean room with a 3D-printing alternative and does not require specialized equipment.

This manual covers the full manufacturing process for a microfluidic chip based on a 3D-printed mould. It includes all required materials, step-by-step protocols for the making of the chip and also a protocol for cell transformation on the chip. To make an example this manual is made specifically for heat shock transformation, but the design of the chip can be altered to fit any other purpose.

3 MATERIALS

2-propanol
Disposable container, for example a 50mL Falcon tube
Solid rod (for mixing)
SYLGARD 184 elastomer KIT
50ml Falcon tube (for centrifuge balance)
Vacuum pump (for building advice see attachment) or a fridge
Plexiglas®
M3 screws x6
Saw
Drill
0, 583 mm tubes
Adapters x5
Paper towels
Peristaltic pump x2

4 3D PRINTING

The mould for the chip can be made in design softwares like AutoCAD. The STL files containing the designs of the chip can be downloaded. The moulds to the chip were 3D-printed using a form 2 printer. Please note that that the moulds are quite thin so care should be taken when removing the mould from the printer. Alternatively the moulds can be printed with the integrated support system of the form 2 printer. The support can be cut off with a knife afterwards and preferably filed down with sandpaper to give the mould stability. After printing, the moulds were washed in 2-propanol for 20 minutes to take away liquid resin that comes from the printer and thence put in a UV chamber for approximately 1 hour so that the mould hardens.

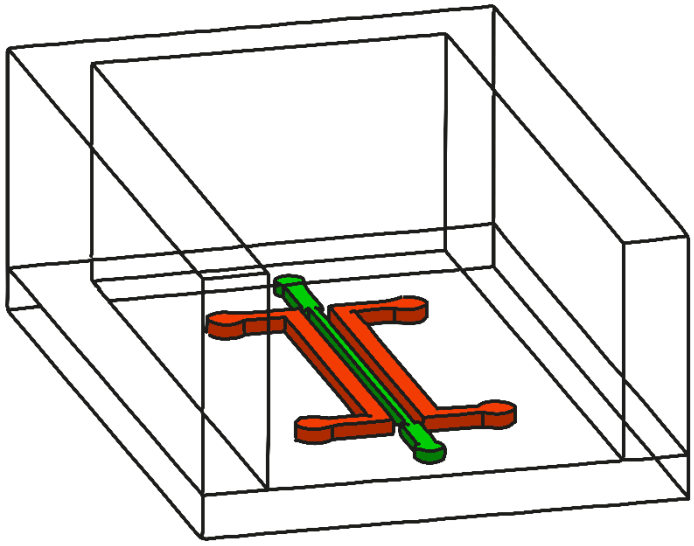


Figure 1: The figure shows the design of the heat shock microfluidic chip. A detachable wall to the open side of the mould.

5 MIXING OF PDMS

First and foremost, cover all workspaces used for mixing in aluminum foil to protect them from untreated PDMS. The preparation of PDMS should preferably be done using unpowdered gloves due to the high risk of contamination. The PDMS for the chip is the SYLGARD® 184 Elastomer KIT. The base and the curing agent comes separately and mixing is required. Mix the base and the curing agent in a falcon tube in the ratio of 1:10. The volume of PDMS for one chip is approximately 2.4 ml, thus about 2,2ml of the base and 0.22 ml of curing agent. The ratio 1:10 is standard but if a stiffer chip is preferred the amount of curing agent can be increased, for example a 2:10 ratio.

Mix the sample thoroughly with a solid rod until the solution is full of bubbles. Avoid mixing with a glass rod since they easily break. For balance, fill a second falcon tube with water until both tubes weigh the same. Centrifuge both tubes for 30 seconds at 3000g force.

Prepare the complete 3D-printed mould by taping the detachable wall to the rest of the mould. Pour roughly 2.4 ml of the mixture into the mould until it covers the whole bottom and halfway up the walls. Any excess mixture can be stored in a -20°*C* freezer for a couple of days and used for other chips.

Figure 2: The figure shows a mould being filled with PDMS.

6 DEGASSING

The PDMS needs to be degassed to get rid of the bubbles. Depending on the amount of bubbles this can be done by using a vacuum chamber, a fridge or both ways simultaneously. Construction advice for the vacuum chamber can be found in the appendix. When using the vacuum chamber put the mould with PDMS in the chamber and connect it to a vacuum pump. The mould or the whole vacuum chamber can also at times be put in the fridge overnight. Remove any excess topmost bubbles with nitrogen gas (often not needed when using a vacuum chamber). During degassing preheat the oven and prepare a baking sheet by taping two strips and placing the mould between them for stability in the oven.

7 BAKING

The baking of the PDMS can be done in several ways. It can be left at room temperature for approximately 48 hours, baked for two hours at 100°*C* or baked for three hours at 80°C. If the chip is baked at 100°*C* tin foil should be used instead of a petri dish made of plastic, since it melts at 100°*C*. Depending of the amount of curing agent that has been used the PDMS will cure at different times and temperatures. If the PDMS is not cured the mould can be left longer in the oven, for instance overnight at a lower temperature.

8 TAKING OUT THE CHIP AND MAKING THE HOLES

Once the chip is baked remove the tape and cut out the chip from the mould with a scalpel. Attach a hole maker in form of a needle with a blunt end to a syringe, and fill thereafter the syringe with air. The needle should be compatible with the tubing connectors for the chip. Create the holes by sticking the sharpened, blunt needle into the PDMS all the way to the other side. Blow out the air from the syringe with force in order to take out the little piece of gel inside of the created hole. Take out the needle from the PDMS and repeat the procedure for all six inlet and outlet holes in the design.

9 ASSEMBLY & CLEANING

Clean the side of the chip that contains the channels by covering them with scotch tape to get rid of dust. Take the tape off straight away and wash the chip in isopropanol. Make two 4x6 centimeter glass slides using a saw. Prepare the glass slides by making holes compatible with the holes on the PDMS with a drill. Make 6 additional holes along the side of the slides. Furthermore, wash the glass slides in isopropanol and dry them with nitrogen together with the PDMS. Put the two slides together with M3 screws in the additional holes and add the PDMS in the middle.

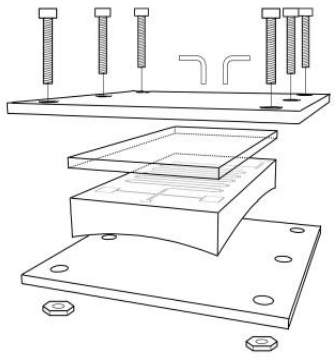


Figure 3: The figure shows the assembly of the chip.

10 TRANSFORMATION PROTOCOL

10.1 MATERIALS

50 µL of $CaCl_2$ competent cells
0.8 µL of DNA
75 % ethanol
SOB medium
Agar plate with appropriate antibiotic

10.2 PREPARING FOR CELL TRANSFORMATION

1. Set up the chip by connecting 0.583 mm tubes insulated with paper towels to the heat channels. Use bent metal connectors. Connect tubes to two peristaltic pumps; one for each heating channel. Place a syringe needle in the inlet of the transformation channel and a tube in its outlet. (See figure 4)
2. Heat the chip by running water at 65°C and 300 mLh^{-1} through the heating channels.
3. Take out 50 µL of $CaCl_2$ competent cells from -80°C freezer and thaw them on ice for 5 minutes.
4. Take 10 µL of competent cells into a separate tube for negative control. Add 0.8 µL of DNA (at a concentration of approximately 70 $ng\mu L^{-1}$) to the remaining 40 µL of cells. Incubate cell and DNA suspension for 20 min on ice.

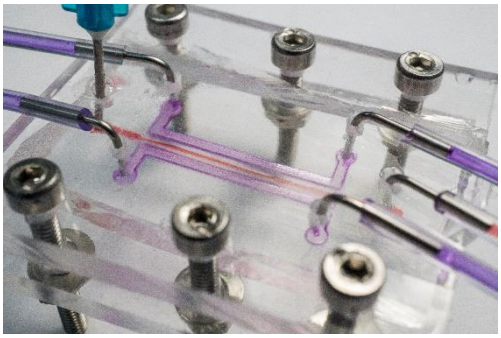


Figure 4: The figure shows the finished chip.

10.3 RUNNING THE TRANSFORMATION CHIPS

1. Flush the cell channel with 2 mL of 75 % ethanol with a syringe. Dry the cell channel with air. This cleaning step should be repeated after each transformation to avoid contamination.
2. For each transformation: pipette 6 µL of cell and DNA suspension into the syringe connector on the chip.
3. Push the suspension into the transformation channel by pushing air in with a syringe.
4. Heat shock the suspension for 45 seconds.
5. Push the cell suspension through and collect in an Eppendorf tube filled with 100 µL SOB.
6. Place the tube on ice immediately.
7. Incubate for 1.5 hours at 37°C and then spread on an agar plate with the appropriate antibiotic.

11 APPENDIX

11.1 CONSTRUCTION ADVICE OF VACUUM CHAMBER

11.1.1Materials

PVC tube
1 piece of SAN plastic
Rubbercloth
Saw
Drill
Vacuum pump
Adapter

11.1.2 Building

The PVC tube should be big enough to cover the chip. Cut out two circles of the rubbercloth that are slightly bigger than the diameter of the PVC tube and cut a hole inside them. The hole should be made so that it leaves two centimeter at the sides of the circle. Cut the SAN plastic into two parts by using a saw. Drill a hole in the PVC tube, the hole should be compatible with the connecting tubing to the vacuum pump. Put an adapter compatible with the tubing to the vacuum pump in the hole and connect it to the vacuum pump. Place the prepared rubbercloths followed by the two pieces of SAN plastic on the two open sides of the PVC. Open one side to place the object that should be degassed in the chamber.

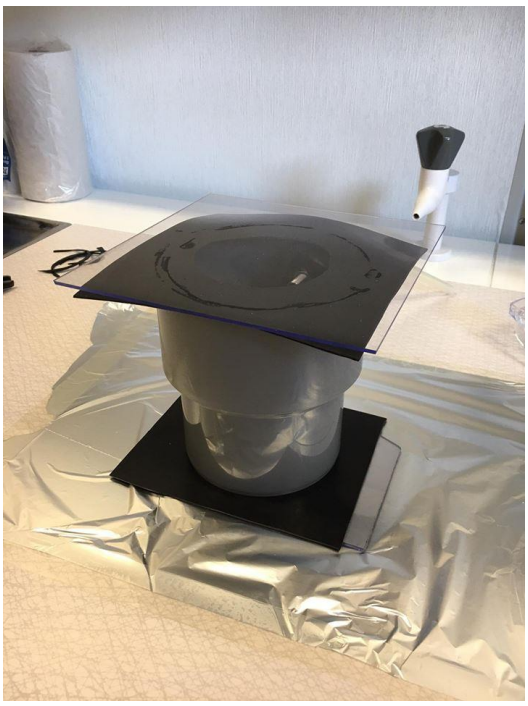


Figure 5: The figure shows a vacuum chamber built with the technique described above.