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## Seeding V.2

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Bio-X-Space



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### ABSTRACT

This protocol describes the procedures to seed the *Bacillus subtilis* inside the microfluidic chip to create the biofilms.

### MATERIALS TEXT

- Pressure pump.

- Needles

- Microfluidics chip (see design in this link

<https://cad.onshape.com/documents/d01540193290530cad6c1bea/w/108b7d3735006ad86309beb7/e/58c9223ab6c2555e1ce67f02>).

- Shaker.

- 50 ml falcon tubes.

- Centrifuge.

### **MSgg biofilm-forming medium:**

-5 mM potassium phosphate buffer pH 7.0 ( 0.0536 M K<sub>2</sub>HPO<sub>4</sub>+ 0.0464M KH<sub>2</sub>PO<sub>4</sub>).

-100 mM MOPS buffer ;pH 7.0, adjusted using NaOH (10X: 0.2M MOPS free acid+ 0.05M Sodium Acetate+ 0.01M Na<sub>2</sub>EDTA).

-2 mM MgCl<sub>2</sub>

-700 µM CaCl<sub>2</sub>

-50 µM MnCl<sub>2</sub>

-100 µM FeCl<sub>3</sub>

-1 µM ZnCl<sub>2</sub>

-2 µM thiamine HCl

-0.5% (v/v) glycerol

-1X (30 mM) of glutamate.

### **LB medium:**

-1% Bacto tryptone.

-0.5% Bacto yeast extract.

-1% NaCl.

-1 mM NaOH.

Media were solidified through the addition of Bacto agar (Difco) to 1.5%, and the plates were allowed to dry at 25°C for 16 h before use.

### BEFORE STARTING

Make sure to clean all the workspace with alcohol and bleach.

#### Day before experiment

- 1 Cells from stock were streaked onto LB agar plate and incubated at 37 °C overnight.

#### Day of the experiment

- 2 A single colony was picked from the plate and inoculated into 3 ml of LB broth in a 50 ml conical tube, and then incubated at 37 °C in a shaker.
- 3 After 2.5 h of incubation, the cell culture was centrifuged at a relative centrifugal force of 2,100 for 1 min.
- 4 The cell pellet was re-suspended in MSgg and then immediately loaded into microfluidics.
- 5 After the loading, cells in the microfluidic chamber were incubated at 37 °C for 90 min, and then the temperature was kept at 30 °C for the rest of the experiment.



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