VERSION 1

NOV 19, 2019

Seeding V.1

Francisco Javier Quero¹, Alfredo L´Homme Iriarte¹, Laura Armero¹

¹OpenFIESTA

Bio-X-Space

Laura Armero

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.9g7h3zn

Protocol Citation: Francisco Javier Quero, Alfredo L´Homme Iriarte, Laura Armero 2019. Seeding . **protocols.io** <https://dx.doi.org/10.17504/protocols.io.9g7h3zn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: In development
We are still developing and optimizing this protocol

Created: Nov 19, 2019

Last Modified: Nov 19, 2019

PROTOCOL integer ID:
29951

MATERIALS

- 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 K₂HPO₄+ 0.0464M KH₂PO₄).
- 100 mM MOPS buffer ;pH 7.0, adjusted using NaOH (10X: 0.2M MOPS free acid+ 0.05M Sodium Acetate+ 0.01M Na₂EDTA).
- 2 mM MgCl₂
- 700 µM CaCl₂
- 50 µM MnCl₂
- 100 µM FeCl₃
- 1 µM ZnCl₂
- 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 START INSTRUCTIONS

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.