Grown-on-membrane assays

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Abstract

Here we describe a procedure to make bacterial communication assays based on 3OC6HSL and 3OC12HSL quorum sensing signaling. Strains that sense and response to these signals are plated on a permeable membrane printed with a hydrophobic grid, which is placed on top of an agar plate containing growth media and antibiotics. This method is based on Grant et al. 2016 (Grant PK, Dalchau N, Brown JR, Federici F, Rudge TJ, Yordanov B, Patange O, Phillips A, Haseloff J.Orthogonal Intercellular Signaling for Programmed Spatial Behavior. Mol Syst Biol. 2016 Jan 25;12(1):849. doi: 10.15252/msb.20156590)

This assay has been used for low cost and open source fluorescence imaging (please see https://osf.io/dy6p2/ for further information & data, https://github.com/SynBioUC/FluoPi for code and http://docubricks.com/viewer.jsp? for hardware assembly)

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Materials

- P2 micropipette and tips by Contributed by users
- ISO-GRID sterile membrane 0.45 µ by Contributed by users
- Agar plates with proper antibiotics (e.g. Kanamycin and carbenicillin) by Contributed by users
- Incubator with agitation by Contributed by users
- Tweezers by Contributed by users
- Sterile conditions (e.g. laminar flow or a flame) by Contributed by users
- 14 ml round bottom culture tubes by Contributed by users

Protocol

Co-transformation of plasmids into E.coli cells

Step 1.

Co-transform *E.coli* bacteria with plasmids for sender (HSL-C6 or HSL-C12 production together with mBeRFP reporter to be able to visualize the cells) and receiver (HSL-C6 or HSL-C12 quorum sensing response system with two different fluorescent proteins such as sfGFP and CyOFP, respectively):

Receiver Orange High (ROH) = pTet32LasR + pLas8O

Receiver Orange Low (ROL) = pTet32LasR + pLas33O

Receiver Green High (RGH) = 1LU2 + pLux34G

Receiver Green Low (RGL) = 1LU2 + pLux54G

Sender Lux High (SLuxH) = Std34BeRFP + pLac34LuxI

Sender Lux Low (SLuxL) = Std34BeRFP + pLac54LuxI

Sender Las High (SLasH) = Std34BeRFP + pLac34LasI

Sender Las Low (SLasL) = Std34BeRFP + pLac54LasI

ANNOTATIONS

Fernan Federici 08 Sep 2017

It is not necessary to use all sender and receiver versions. All the sequences for these vectors are available at https://osf.io/dy6p2

Growth

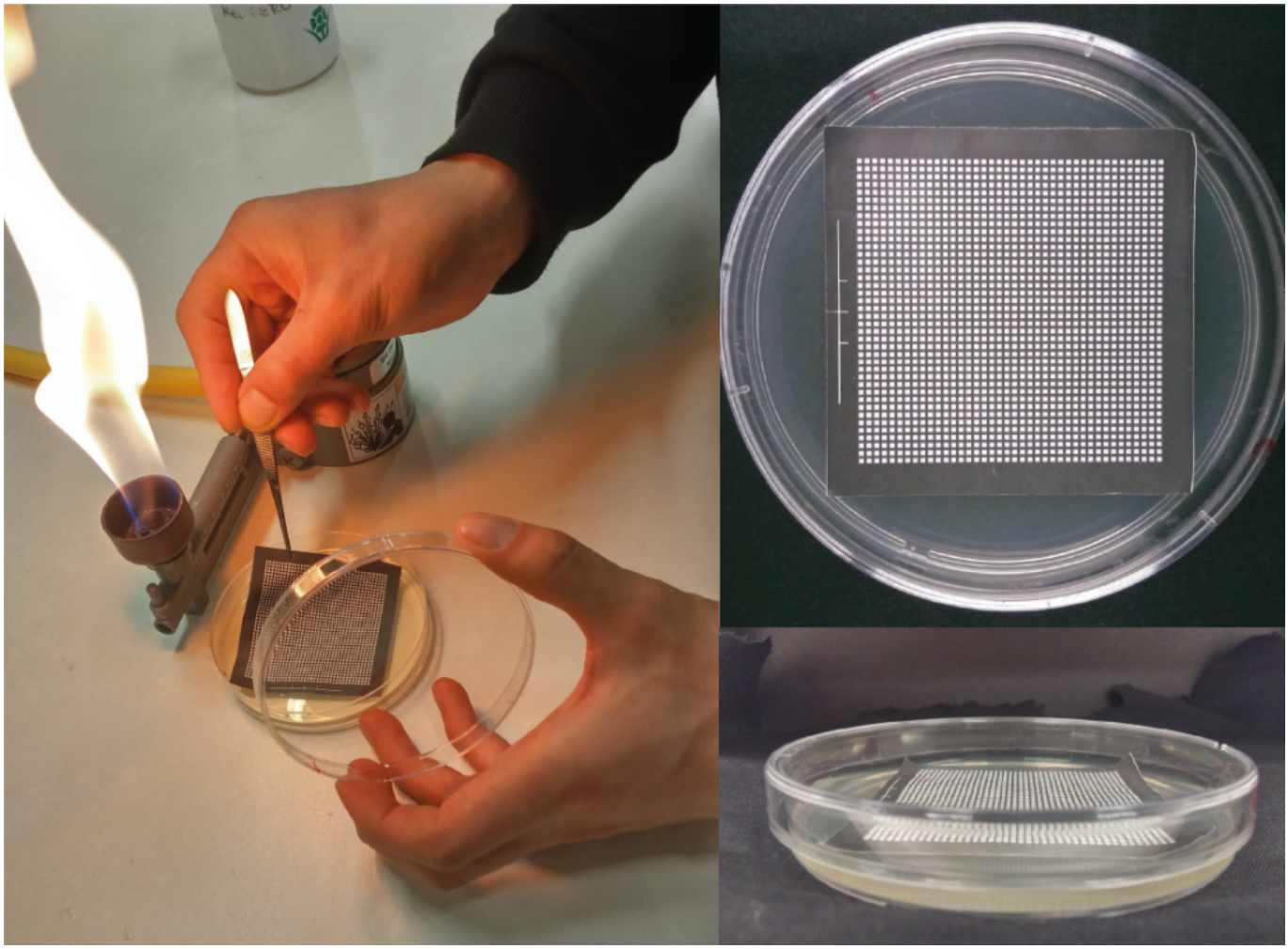
Step 2.

Grow the co-transformed bacteria on 1 ml of liquid LB medium with the **two** appropriate antibiotics (e.g. kanamycin and carbenicillin) at 37°C with agitation until reaching OD600=0.03 (5 hours).

ISO GRID membrane manipulation

Step 3.

In sterile conditions, open the membrane package, take the membrane with the sterilized tweezers and put it over the LB agar of the plate.



Plating

Step 4.

Add 0.5 µl of the corresponding culture from (2) according to the pattern of interest. Special care should be taken to not transfer the culture to the adjacent wells.

NOTES

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To avoid mixing or contaminating of adjacent wells is recommended to recommended to pipet the culture as vertical as possible over each well.

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The arrangement of sender and receiver in the grid is arbitrary, nevertheless it is recommended to leave a space of at least one empty well between different strain cultures (e.g. senders and receivers) to prevent them from mixing.

Incubation

Step 5.

Incubate at 37°C. Results should be visible in the next 24 h.

NOTES

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Avoid using Las/HSL-C12 system at room temperature experiments because his regulatory capacity seems to not work properly on that condition.