Colony-sectoring assay

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Abstract

This protocol describes the procedure to obtain single bacterial colonies composed of multiple sectors of different strains. Each sector is originated from different founder cells (situated in close proximity to each other at the moment of plating). This protocol is based on Hallatschek et al. 2007 (Hallatschek O, Hersen P, Ramanathan S, Nelson DR. Genetic drift at expanding frontiers promotes gene segregation. Proc Natl Acad Sci U S A. 2007 Dec 11;104(50):19926-30).

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
- P20 micropipette and tips by Contributed by users
- P200 micropipette and tips by Contributed by users
- LB agar plates with the proper antibiotic (e.g. Kanamycin) by Contributed by users
- 1.5 ml tubes to perform the dilutions by Contributed by users
- Shaker incubator 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

Growth

Step 1.

Grow independent liquid cultures of the bacterial strains (e.g. three bacterial cultures: E.coli Top10 with CyOFP expression plasmid, mBeRFP expression plasmid and sfGFP expression plásmid) overnight in 5 ml LB media with the proper antibiotic (e.g. Kanamycin for the plasmids mentioned above) at 37°C with agitation.

Mixing

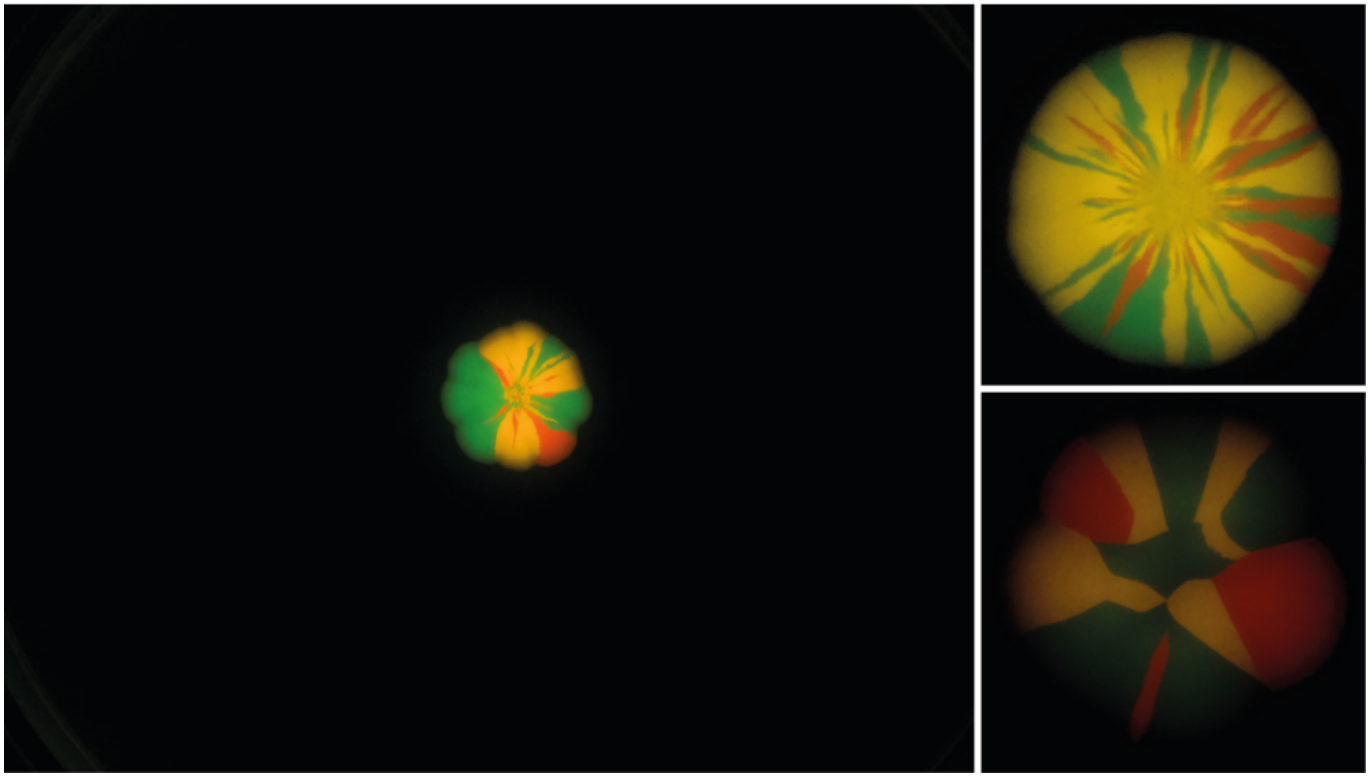
Step 2.

Mix all cultures proportionally (eg, 100 µl of bacterial culture containing the CyOFP plasmid, 100 µl of bacterial culture containing the mBeRFP plasmid and 100 µl of bacterial culture containing the sfGFP plasmid).

Dilution

Step 3.

Dilute the mixture from step 2 in order to start the colony with fewer cells and number of sectors. It is recommended to dilute 1: 100 (top right image) or 1: 10,000 (bottom right image) with LB media and place a drop of 0.5 ul in the center of a LB agar plate with corresponding antibiotics (e.g. Kanamycin for the plasmids used here).



Plating

Step 4.

Take 0.3 µl of the previous step and place it in the center of a LB agar plate with the proper antibiotic(s) in common of the strains (e.g. Kanamycin).

ANNOTATIONS

Fernan Federici 08 Sep 2017

It is important to seal the plates with parafilm to avoid dehydration during the experimentation time and preserve the colony for several days. The above does not apply if you want to do

timelapse of the plate, in which case it is advisable not to seal it to reduce condensation on the lid that affects imaging.

Incubation

Step 5.

Incubate the plate at room temperature for a period of 2 to 10 days (or 37°C for a shorter period of time). Take into account that the incubation temperature is a key determinant of colony growth rate.

ANNOTATIONS

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In case the experimental conditions are not the optimal (e.g. room temperature not high enough) is recommended to incubate the first 12 h in optimal conditions (e.g. 37ºC) before transferring the plates to the experimental conditions.