Colony mix assay Version 2

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Abstract

Protocol to co-culture individual colonies of different strains (e.g. transformed with different vectors) on a LB agar plate in a distributed and random manner.

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)

Citation: Isaac Núñez,Tamara Matute Colony mix assay. **protocols.io**
dx.doi.org/10.17504/protocols.io.jsucnew
Published: 08 Sep 2017

Materials

- 14 ml round bottom culture tubes by Contributed by users
- LB agar plates with the proper antibiotic(s) (eg. Kanamycin) by Contributed by users
- Glass beads (~ 5mm diameter) or L shaped spreader by Contributed by users
- Sterile conditions (e.g. laminar flow or a flame) by Contributed by users

Protocol

Growth

Step 1.

Grow TOP10 E.coli cells containing the different vectors (e.g. three bacterial cultures: the first containing CyOFP plasmid, the second containing mBeRFP and the third containing sfGFP). Cells should be grown overnight at 37 °C with agitation in a 14 ml round bottom culture tube with 1-5 ml of LB medium including the proper antibiotics.

NOTES
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If you want to work with a single culture go to step 3.

Mixing strains

Step 2.

Mix all cultures proportionally (eg, 100 µl of each one).

Dilution

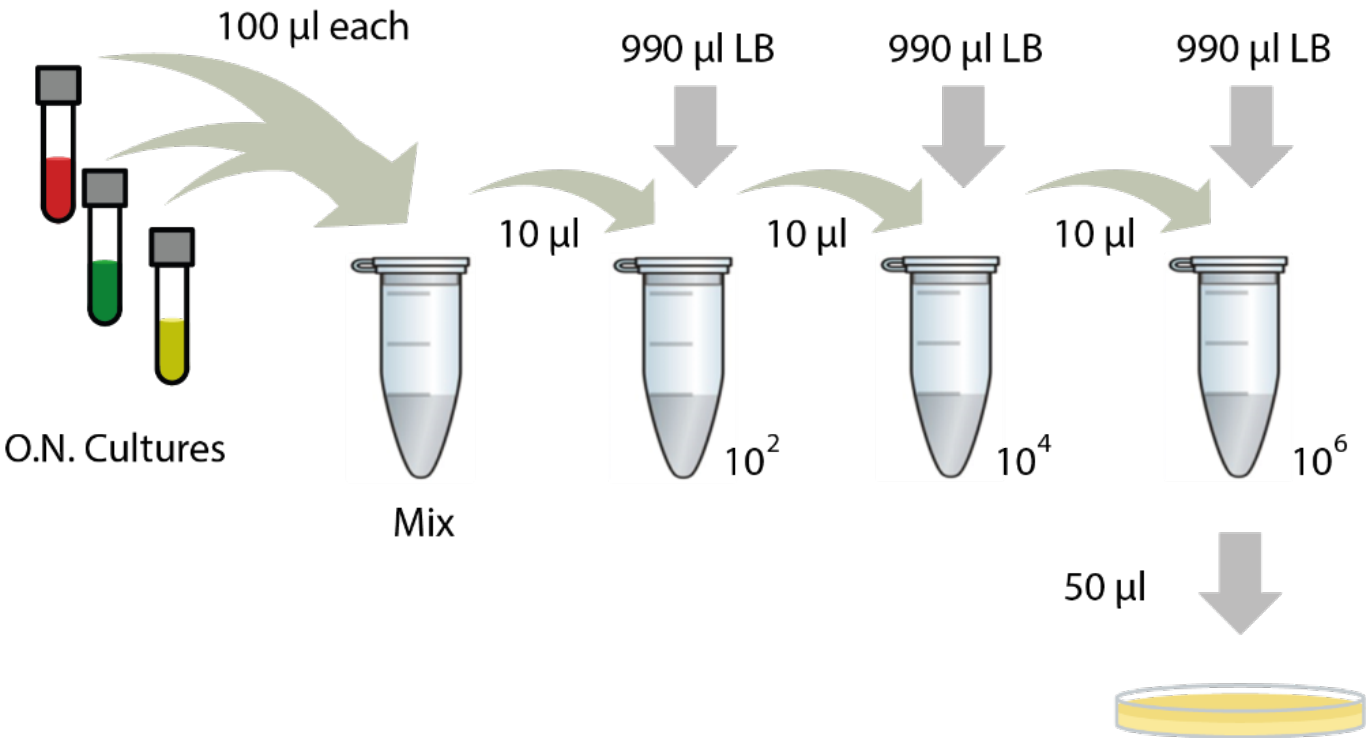
Step 3.

Three consecutive dilutions of the culture (pure or mixture) have to be made:

(1) 10^2 dilution → 1:100 (culture:LB)

(2) 10^4 dilution → 1:100 ((1):LB)

(3) 10^6 dilution → 1:100 ((2):LB)



Step 4.

Spread 50 ul of the dilution performed in the previous step on a LB agar plate with the proper antibiotic(s) (e.g. Kanamycin for these plasmids). To obtain a homogeneous distribution of colonies on the plate it is recommended to use glass beads to spread the dilution. It is possible to use other methods too (e.g an L shaped spreader).

NOTES

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It is important to seal the plates with parafilm to avoid dehydration across the experiment or if is desired to preserve the plate for a long time (e.g. weeks). 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 lid condensation that

ruins the images.

Incubation

Step 5.

Incubate the plate at 37°C for the first 8 hours to accelerate the appearance of colonies (this is an optional step).

Step 6.

Incubate at the desired temperature (e.g. room temperature or 37°C) for a period of 1 to 4 days.

NOTES

Tamara Matute 08 Sep 2017

To accelerate colony growth, it is recommended to incubate the plates at 37 ° C (1-2 days for maximum size). Plates can be left at room temperature if necessary, but growth will take approximately twice as long.