Segregation of plasmids in bacterial colonies

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Abstract

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Protocol

Step 1.

Co-transform *E. coli* bacteria with two segregating plasmids (e.g. SEG10 and SEG11) and plate them on LB agar plates supplemented with arabinose and the antibiotics specific for each plasmid (e.g. carbenicillin + tetracycline).

NOTES
Tamara Matute 09 Aug 2017

Concentrations used:

Arabinose 10 mM

Chloramphenicol 10 µg/ml

Carbenicillin 100 µg/ml

Tetracycline 10 µg/ml

ANNOTATIONS
Fernan Federici 13 Sep 2017

We use this protocol for competent cells:

(http://openwetware.org/wiki/TOP10_chemically_

competent_cells

Step 2.

Incubate at 37°C overnight.

NOTES
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It is recommended to avoid long periods of growth because it tends to reduce the number of bacteria carrying the two plasmids within the colony (specially when carbenicillin is used).

Step 3.

Check colonies by fluorescence imaging. The homogenous expression of both fluorescent proteins within the colony is a good indicator of co-transformed colonies.

Step 4.

Pick a co-transformed colony and incubate these cells for 5 hours at 37°C with shaking (250 rpm) in liquid LB media supplemented with arabinose and the same specific antibiotics of each plasmid used in step 1 (e.g. carbenicillin + tetracycline).

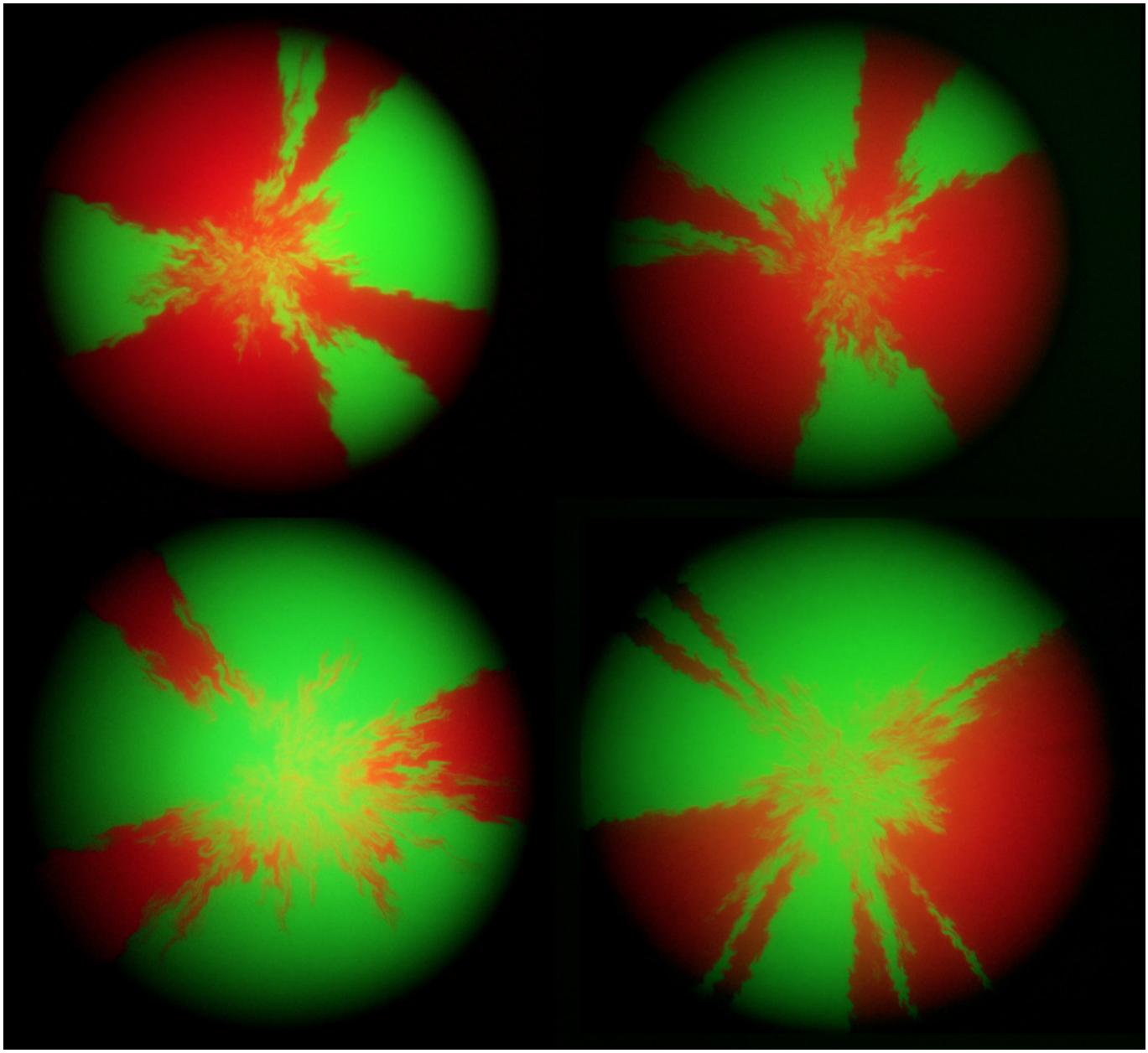
Step 5.

Harvest cells by centrifugation at 3000 rpm for 10 minutes and discard the supernatant.

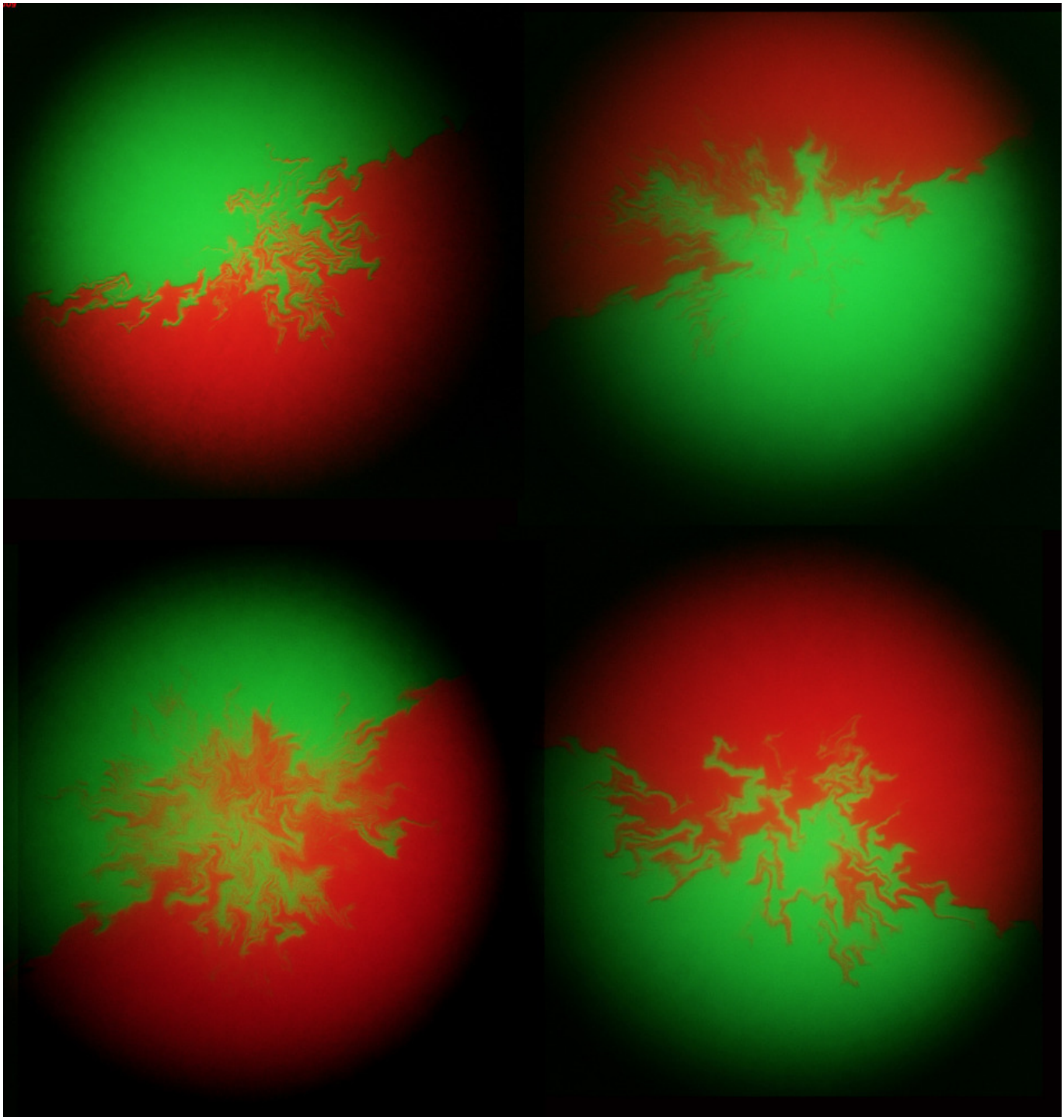
Step 6.

Re-suspend the cells in 5 ml of fresh LB media supplemented with the antibiotic in common to both SEG plasmids (e.g. chloramphenicol for SEG10 and SEG11).

If you wish to favor the formation of multipartite colonies go to step 8



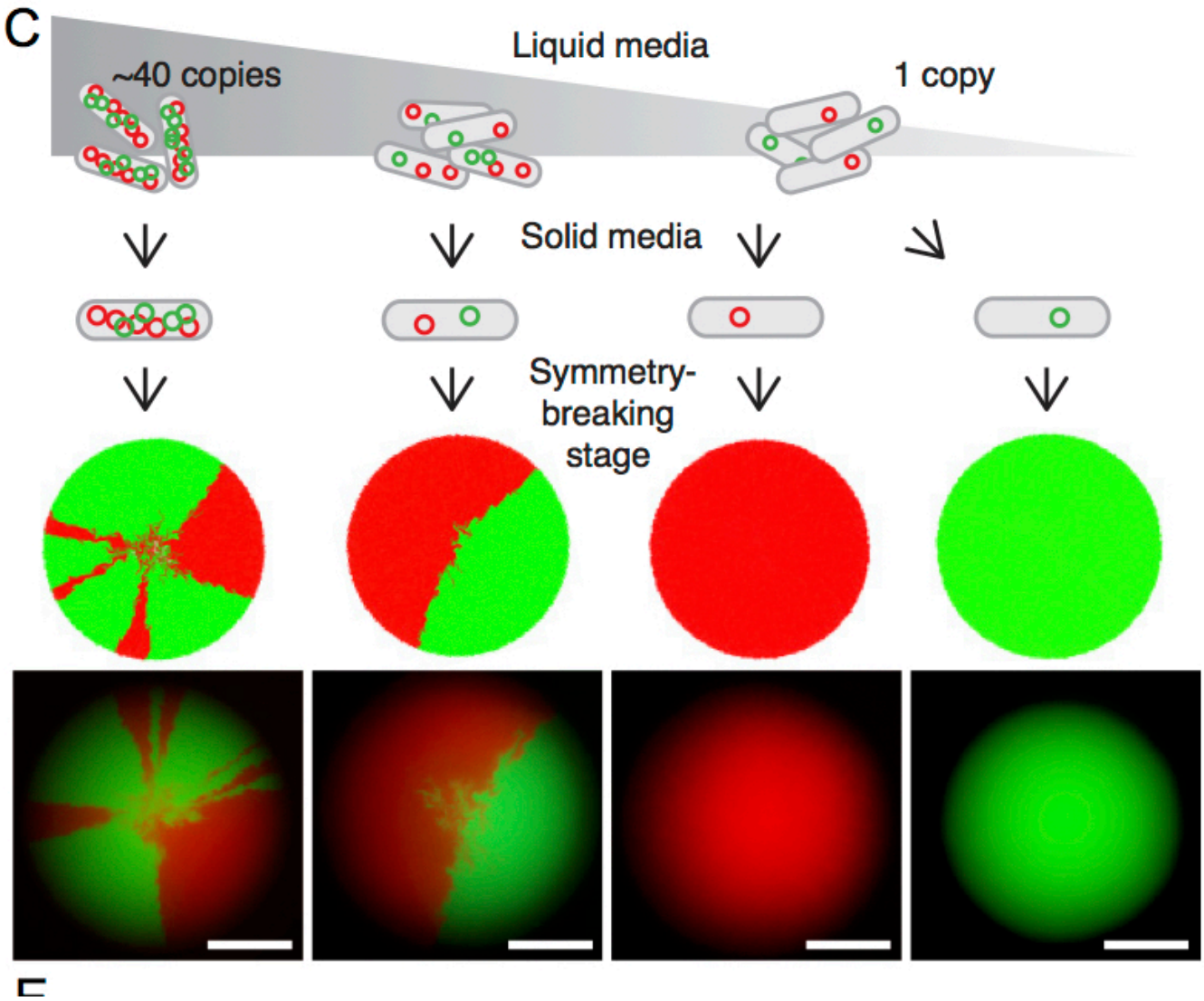
If you wish to favor the formation of bipartite colonies go to optional step 7



(Optional Step) For bipartite colonies

Step 7.

Grow cells on liquid culture for extra 3h before going to step 8.



Step 8.

Dilute this culture 1:5000 with fresh LB media supplemented with the antibiotic in common to both SEG plasmids (e.g. chloramphenicol for SEG10 and SEG11).

Step 9.

Spread 10µl of this dilution on LB agar plates supplemented with the common antibiotic to both SEG plasmids (e.g. chloramphenicol for SEG10 and SEG11).

NOTES

Tamara Matute 10 Aug 2017

Using 10µl produces ~50 colonies per plate. To vary this amount modify the dilution or the spread

volume at will. Fine tuning might be needed when SEG plasmids carry genetic components that impose high metabolic burden on the cell.

Step 10.

Incubate at 37°C until colonies can be seen (8-12 hours).