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Growth of mixed *E. coli* colonies V.2

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Wolfram Moebius[1]

[1]University of Exeter

Wolfram Moebius
University of Exeter

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Protocol status: Working
We use this protocol and it's working.

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Abstract

Growth of mixed *E. coli* colonies on agar plates and between an agar plate and an agar pad

Make plates

1 Autoclave Lennox LB (10 g/l tryptone / casein digest peptone, 5 g/l NaCl, 5 g/l yeast extract) with 1.5 % agar (w/v).

2 **Optional:** Add antibiotics at desired concentration after medium has cooled down sufficiently.

3 Pipette 10 mL of LB and agar in Petri dishes with diameter of 6 cm .

4 Leave plates plates to dry Overnight at Room temperature . Optionally enclose to avoid nonuniform drying.

Store and prepare plates

5 If not used the day after pouring plates, store at 4 °C . Enclose in plastic container or bag to avoid further drying of plates.

6 Before usage of plates that have been refrigerated, warm up plates at 37 °C .

Prepare bacterial cultures

7 Grow overnight culture (colony picked or directly from glycerol frozen stocks) in Lennox LB.

Inoculate colonies and incubate

8 Mix 900 µL Lennox LB and 50 µL of each of the two overnight cultures in an Eppendorf tube.
Vortex.

9 Inoculate 1 µL in centre of plate and let dry.

10 **Optional:** Cover colonies with agar pad. Cut about 18 mm x 18 mm agar pad inside plate. Use spatula to lift pad. Place vertically next to colony and let fall upside down onto colony. Use spatula to remove bubbles by pressing on top.

11 Place plates into container, together with sufficiently wet paper towels. Incubate for 7-8 days in a dark environment.