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Growth of mixed *E. coli* colonies

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Works for me

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ABSTRACT

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

PROTOCOL CITATION

Wolfram Moebius 2021. Growth of mixed *E. coli* colonies . [protocols.io](https://protocols.io/view/growth-of-mixed-e-coli-colonies-bqqjmtun)
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Make plates

- 1 Autoclave Lennox LB (10 g/l tryptone, 5 g/l NaCl, 5 g/l yeast extract) with 1.5 % agar (w/v) for 15 to 20 minutes.
- 2 **Optional:** Add antibiotics at desired concentration after medium has cooled down sufficiently.
- 3 Pour **10 mL** of LB and agar in Petri dishes with diameter of **6 cm**.
- 4 Leave 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, preheat plates at 37°C for about 00:00:00 min .

Prepare bacterial cultures

- 7 Grow overnight culture (colony picked, or directly from glycerol frozen stocks) in Lennox LB (10 g/l XXX, 5 g/l NaCl, 5 g/l yeast extract).

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.