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Over-Agar Antibiotic Plating

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External link: https://www.addgene.org/protocols/over-agar-antibiotic-plating/

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

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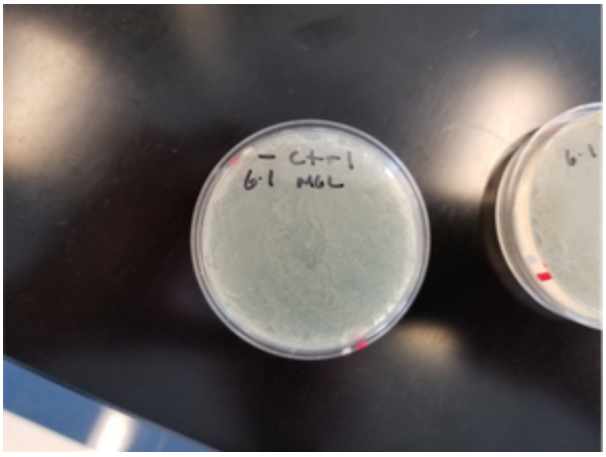
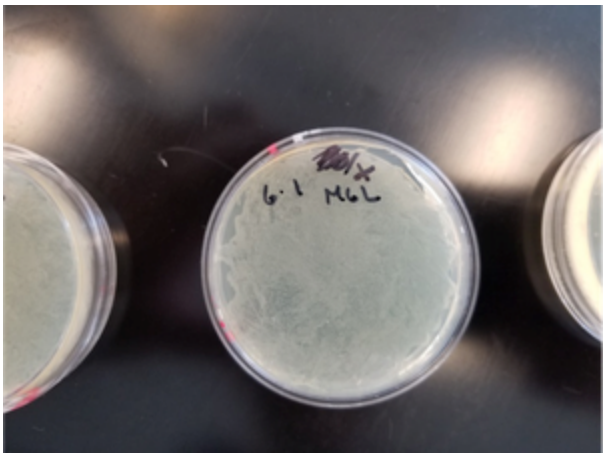
Abstract

This protocol is for over-agar antibiotic plating. To see the full abstract and additional resources, visit https://www.addgene.org/protocols/over-agar-antibiotic-plating/

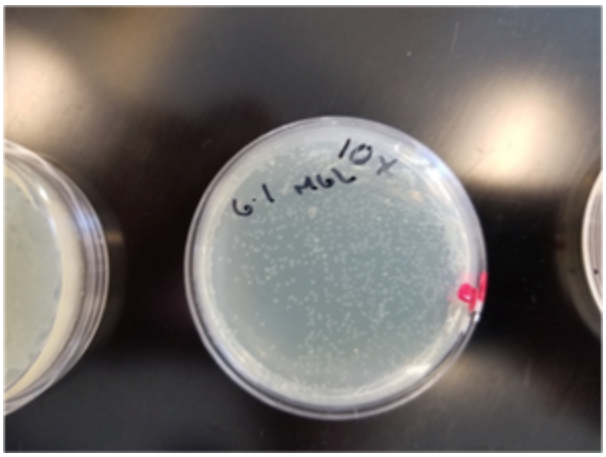
Sample Data: Selection of E.coli on LB-agar using different concentrations of carbenicillin plated over-agar.



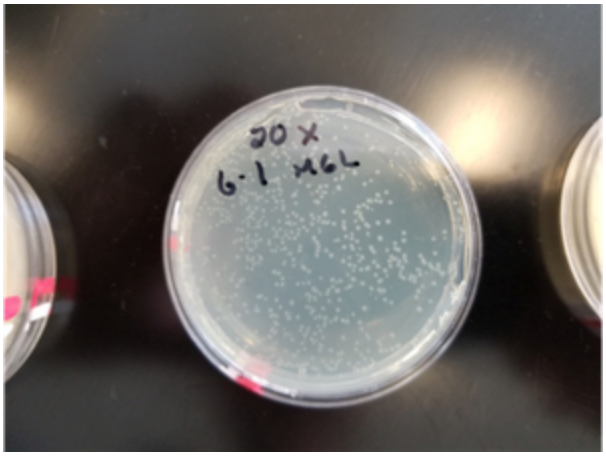
Control Plate with No Carbenicillin
Plate shows a lawn of *E. coli* and no selection.



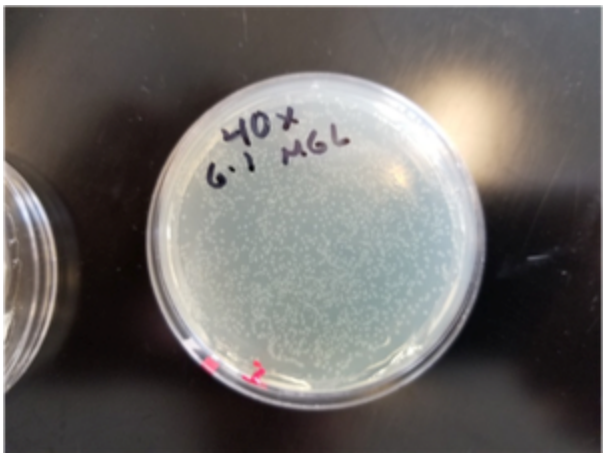
150 µL of 0.1 mg/mL Carbenicillin plated over-agar
Plate shows a lawn of *E. coli* and no apparent selection.



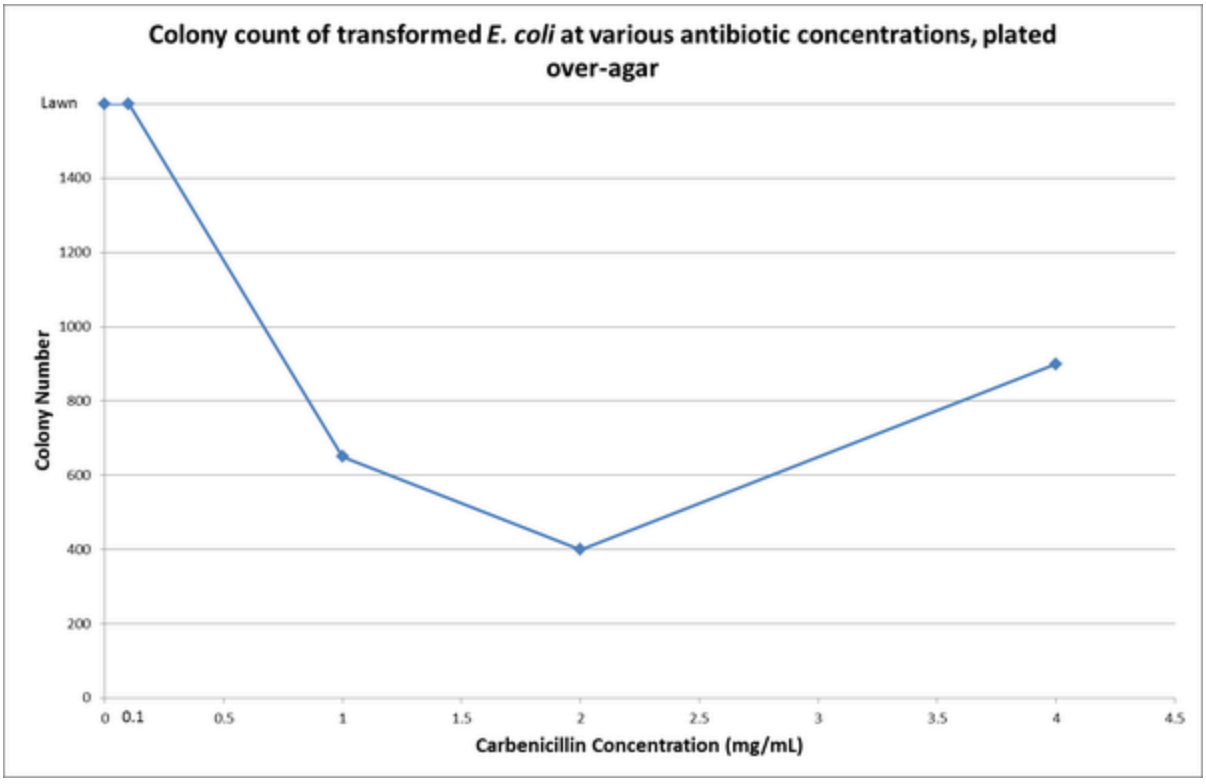
150 µL of 1 mg/mL Carbenicillin plated over-agar
Plate shows several individual colonies and effective selection.



150 µL of 2 mg/mL Carbenicillin plated over-agar
Plate shows less individual colonies than the 1 mg/mL plate and effective selection.



150 µL of 4 mg/mL Carbenicillin plated over-agar
Plate shows several individual colonies with smaller size than the 1 mg/mL and 2 mg/mL plates and effective selection.



Selection Curve of Transformed E. coli after Over-Agar Plating of Carbenicillin. The above graph displays the stock concentration of Carbenicillin stock used (150 µL per plate). Please note we have found that there is generally a broad range of antibiotic concentrations that will work for this assay, and the above result represents a single experiment. For publishable data, the experiment would need to be repeated to account for variability.

Materials

Equipment

- Pipette tips for both pipetting and spreading
- Bunsen burner (or other small flame source)
- Incubator

Reagents

- 6 cm LB/agar plates without antibiotic
- High concentration (100 mg/mL, 1000x) carbenicillin stock solution in sterile water (or other antibiotic)
- *E. coli* transformed with a plasmid containing the carbenicillin (amp) resistance gene (or other antibiotic resistance)

Safety warnings

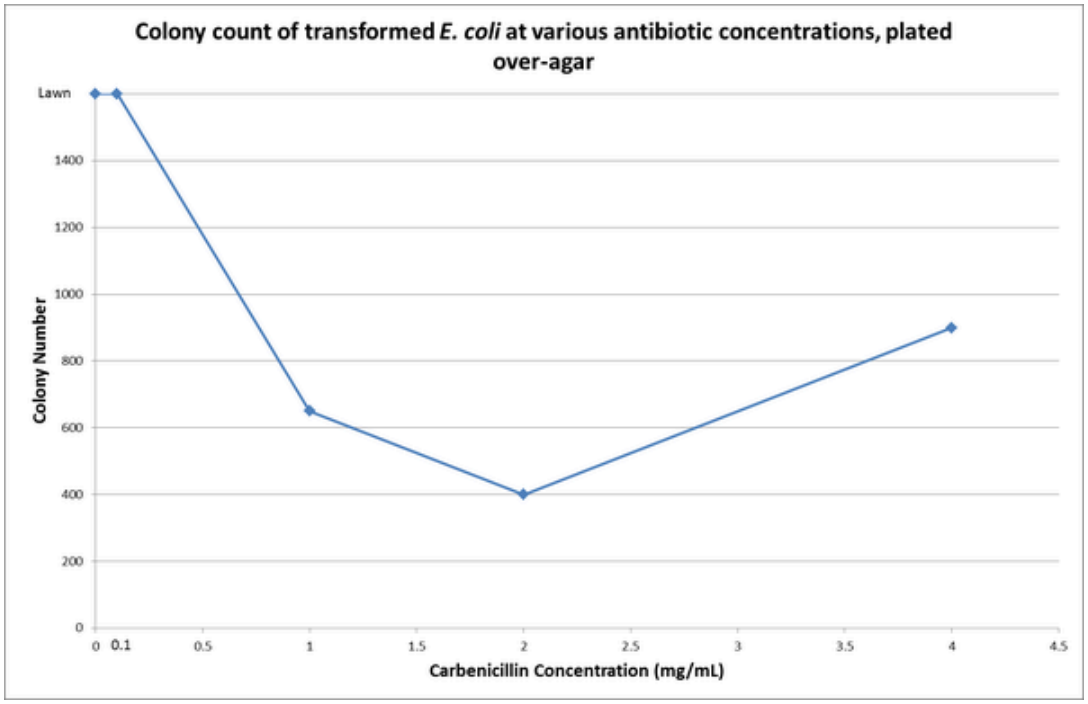
See SDS (Safety Data Sheet) for safety warnings and hazards.

Day 1

1 Prepare carbenicillin to a concentration of 1 mg/mL – 4 mg/mL in LB medium.

Note

The concentration of antibiotic required for effective over-agar selection has been empirically determined. See selection curve below.



Note

Carbenicillin is used here in place of ampicillin because carbenicillin is more stable, so it is potentially more effective at selecting only bacteria containing the plasmids of interest (for example, fewer satellite colonies will grow). It is, however, more expensive.

2 With a 6 cm diameter petri plate containing solidified LB-agar, pipette 150 µL of carbenicillin on top of the agar and gently spread over the surface until the liquid is

mostly absorbed (there is a very small visible volume of pooled liquid remaining on the surface).

Note

We use the micropipette tip itself to do the spreading; the tip is gently bent to create an “L” shape, and then used like a cell spreader. Several other devices may be used for this purpose, provided that they fit your petri plate.

3 Incubate the plate at Room temperature for at least 00:30:00 with the lid on to give the antibiotic time to more fully absorb.

4 During the incubation, transform DH5α *E. coli* cells by heatshock with the plasmid of interest. See our **transformation page** for a detailed heatshock transformation protocol.

5 Plate 50 µL of transformed *E. coli*/rescue media suspension onto the agar and gently spread over the surface until the liquid is mostly absorbed.

Note

The spreading of cells can be done in the same way as the antibiotic, using either a bent micropipette tip or other cell spreading device that fits the plate.

6 Incubate plates at 37 °C for 18:00:00 .

Day 2

7 Observe plates for colony formation. Shown below are the results from an experiment optimizing the concentration of carbenicillin, plated over-agar for selection of transformed *E. coli*.