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## Overnight culture in Round-bottomed tubes V.1

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

This protocol describes how to produce an overnight bacterial culture.


## Materials

Round-bottom falcon tubes

Inoculation loops.





LB media (10g/L NaCl)

Shaker at  200 rpm




Incubator at  37 °C







## Prepare overnight culture

- 1 Create a streak plate and incubate at  37 °C  Overnight .
- 2 Label a  14 mL round-bottom falcon tube with name, date, strain and any other contents.  
Transfer  5 mL LB media into the falcon tube.

### Note

- Bacteria prefer growing in the  14 mL round bottom tubes than in the  50 mL conical tubes.
- When creating bacterial spots on an omnitray plate, the bacteria create bumps when grown in the conical tubes compared to round bottom where the spots are smooth.
- Ensure that the tube rack on the shaker in the warm room is slightly tilted when growing cultures.
- No more than  6 mL in the round bottom tubes when growing a culture.
- An overnight culture which was incubated in the late afternoon should be placed in the fridge first thing the following morning.
- Overnight cultures should be replaced daily.

- 3 Using an inoculation loop, pick an *E.coli* isolated colony from the middle of the streak plate and suspend in the  5 mL LB media.
- 4 Incubate the inoculated LB media  Overnight at  37 °C , shaking at  200 rpm .