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DMSO stock preparation

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Bacterial cultures in the form of agar plates or liquid cultures are not suitable for long term storage at low temperatures such as 4°C. Long-term storage of bacteria is best at temperatures around -80°C. However, agar plates and liquid cultures cannot be stored at such temperatures, as the water crystallizes in and around the bacterial cells, rupturing and killing them. Glycerol or DMSO (anti-freezing agents) is added to the media to store samples at such temperatures.

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- 1 From the streaked plate of the bacteria, pick one colony and inoculate into a **250 mL** flask containing **100 mL** culture.
- 2 Grow the culture till it reaches OD~ 0.6-0.8

- 3 Take the **100 mL** culture and transfer **40 mL** each into two falcon tubes.
- 4 Leave the remaining **20 mL** culture in the flask. To that, add **80 mL** of micronutrient enriched BG-11 media.
- 5 Take the two falcon tubes and centrifuge them at **5000 rpm, 25°C, 00:07:00** . 7m
- 6 Discard the supernatants of the centrifuged pellets.
- 7 Add **2 mL** of BG-11 into each of the emptied falcon tubes. Use a **1 mL** micropipette to mix such that the pellet is resuspended into a concentrated culture.
- 8 Add **140 µL** of 7% DMSO.
- 9 Take 4 cryovials. Add **1 mL** of the liquid in each cryovial.
- 10 Flash-freeze the vials using liquid nitrogen.
- 11 Store the stocks at **-80 °C**