

iGEM TU/e 2015
Biomedical Engineering

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Preparation of culture media, agar plates, antibiotics and general necessities

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1 Preparation of Culture Media

1.1 Luria-Bertani (LB) medium

This medium is used for small cultures of competent cells containing desired DNA.

For 1 L:

- 10 g peptone
- 10 g NaCl
- 5 g yeast extract
- Add 1 L H₂O

The LB medium needs to be autoclaved (sterilization).

1.2 2YT

This medium is used for the protein expression of competent cells with more than one plasmid.

For 1 L:

- 16 g peptone
- 5 g NaCl
- 10 g yeast extract
- Add 1 L H₂O

The 2YT needs to be autoclaved (sterilization).

2 Agar Plates

2.1 LB-agar

For 1 L:

- 10 g peptone
- 10 g NaCl
- 5 g yeast extract
- 15 g bacto-agar
- Add 1L H₂O

The LB-agar needs to be autoclaved (sterilization).

2.2 Pouring agar plates

- After autoclaving the LB-agar at 121 °C for 20 minutes, let the agar cool down to ~50°C. Make sure the agar does not start solidifying.
- Add the appropriate antibiotics to the liquid LB-agar and slowly mix.
- Pour the LB-agar in the petri dishes until the bottom is well covered. Work near the Bunsen burner flame.
- Close the lid after filling the plate. Let the agar solidify for ~1 hour on the bench.
- Transfer the plates to a bag, in which they should be placed upside down.
- Store the plates in the fridge (4°C).

3 Antibiotics

3.1 Ampicillin (5 mL)

- Weigh 0.25 g ampicillin in a small weigh boat (1000x stock concentration, 50 mg/mL).
- Add 5 mL of milliQ to the glassware.
- Add the ampicillin to the MilliQ.
- Mix/vortex so all the ampicillin goes into solution.
- Filter sterilize into a falcon tube using a syringe and a 200 nm filter.
- Aliquot into smaller eppendorf tubes.
- Store at -20 °C under dark conditions.

3.2 Chloramphenicol

25 mg/mL stock solution in ethanol. Final concentration in cultures and LB-agar: 25 µg/mL
Filter stock solution using 0.2 µm filter.

4 General necessities

4.1 Glycerol Stock 50% (20 mL)

- Add 10 mL of glycerol (100%) to glassware.
- Add 10 mL of deionized water to the glycerol.
- Autoclave the glycerol stock.

4.2 Sterile H₂O

Autoclave 100 mL of milliQ using a liquid sterilization program.

4.3 **Sterile Eppendorf tubes and PCR tubes**

Autoclave eppendorf tubes and PCR tubes using a dry sterilization program.