Technische Universiteit
Eindhoven
University of Technology

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Biomedical Engineering

Eindhoven University of Technology
Room: Ceres 0.04
Den Dolech 2, 5612 AZ Eindhoven
The Netherlands
Tel. no. +31 50 247 55 59
2015.igem.org/Team:TU_Eindhoven

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

Estimated bench time: 15 minutes
Estimated total time: 3 hours
Purpose: This medium is used for small cultures of competent cells containing desired DNA.

1.1.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- dH_2O
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

1.1.2 Setup & Protocol

- For 1L LB medium, the correct amounts are:
  - 10 g NaCl
  - 10 g paptone
  - 5 g yeast extract
- Collect them in in a bottle and add 1L of dH_2O.
- Autoclave the LB medium at 121 °C for 20 minutes (sterilisation).

1.2 2YT

Estimated bench time: 15 minutes
Estimated total time: 3 hours
Purpose: This medium is used for the protein expression of competent cells with more than one plasmid.

1.2.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- dH_2O
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

1.2.2 Setup & Protocol

- For 1L LB medium, the correct amounts are:
  - 10 g yeast extract
  - 16 g peptone
  - 5 g NaCl
- Collect them in in a bottle and add 1L of dH_2O.
- Autoclave the 2YT at 121 °C for 20 minutes (sterilisation).

2 Agar Plates

2.1 Preparation of LB-agar

Estimated bench time: 15 minutes
Estimated total time: 3 hours
Purpose: Making LB-agar for the pouring of LB-agar plates.

2.1.1 Materials

- Autoclave
- Autoclave tape
- Bacto-agar
- Balance
- Bottle (glassware)
- H_2O
- NaCl (Sodium chloride)
- Peptone
- Yeast extract

2.1.2 Setup & Protocol

With 200 ml LB-agar you can make 8 plates (1 l for ~40 plates).

- For 200 ml LB-agar, the correct amounts are:
  - 1 g yeast extract
  - 2 g NaCl
  - 2 g peptone
  - 3 g bacto-agar
- Collect them in in a bottle and add 200 ml of H_2O.
- Autoclave the LB-agar at 121 °C for 20 minutes (sterilisation).

2.2 Pouring the plates

Estimated bench time: 20 minutes
Estimated total time: 80 minutes
Purpose: Making LB-agar plates where bacteria with an additional plasmid can grow.

It is essential to work sterile, thus disinfect your hands and work near a Bunsen Burner.

2.2.1 Materials

- Antibiotic stock
- Autoclaved LB-agar
- Bunsen burner
- Petri-dishes
- Pipettes and tips

2.2.2 Setup & Protocol

- After autoclaving the LB-agar (at 121 °C for 20 minutes), let the agar cool down to ~50 °C (autoclave can be opened at 90 °C). Make sure the agar does not start solidifying.
- Add antibiotic stock (200 µl for 200 ml) 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 Antibiotic stocks

3.1 Ampicillin stock (50 mg/ml)

Estimated bench time: 15 minutes
Estimated total time: 15 minutes
Purpose: Making antibiotic stock, necessary for LB-agar plates and the LB medium for small culturing.

3.1.1 Materials

- 0.22 µm filter
- Ampicillin
- Balance
- Eppendorf tubes
- Falcon tube
- MiliQ
- Syringe
- Vortex

3.1.2 Setup & Protocol

- Dissolve 0.25 g ampicillin in 5 ml MiliQ.
- Mix/vortex so that all the ampicillin goes into solution.
- Filter into a falcon tube using a syringe and a 0.22 µm filter for sterilization.
- Aliquot into smaller Eppendorf tubes.
- Store at -20 ^{0}C under dark conditions.

3.2 Chloramphenicol stock (35 mg/ml)

Estimated bench time: 15 minutes
Estimated total time: 15 minutes
Purpose: Making antibiotic stock, necessary for LB-agar plates and LB medium for small culturing.

3.2.1 Materials

- 0.22 µm filter
- Balance
- Chloramphenicol
- Eppendorf tubes
- Ethanol (100%)
- Falcon tube
- Syringe
- Vortex

3.2.2 Setup & Protocol

- Dissolve 0.136 g of chloramphenicol into 4 ml 100% ethanol.
- Mix/vortex so that all the chloramphenicol goes into solution.
- Filter into a falcon tube using a syringe and a 0.22 µm filter for sterilization.
- Aliquot into smaller Eppendorf tubes.
- Store at -20 ^{0}C.

4 General necessities

4.1 50% Glycerol

Estimated bench time: 10 minutes
Estimated total time: 3 hours
Purpose: Preparing glycerol for a glycerol stock, which can be made to store bacteria

4.1.1 Materials

- Autoclave
- Autoclave tape
- Balance
- Bottle (glassware)
- Glycerol (100%)
- MiliQ

4.1.2 Setup & Protocol

- Add 10 ml of glycerol (100%) to a bottle.
- Add 10 ml of MiliQ to the glycerol.
- Autoclave the glycerol stock at 121 °C for 20 minutes (sterilisation).

4.2 Sterile H_2O

Estimated bench time: 5-10 minutes
Estimated total time: 3 hours
Purpose: Making sterile H_2O (nuclease free water), necessary for making dilutions.

Aliquot needs to be done sterile, thus disinfect your hands and work near a Bunsen Burner.

4.2.1 Materials

- Autoclave
- Autoclave tape
- Bottle (glassware)
- Bunsen Burner
- Eppendorf tubes (sterile)
- MiliQ
- Pipette and tips

4.2.2 Setup & Protocol

- Fill a bottle with 100 ml MilliQ.
- Autoclave the MiliQ at 121 °C for 20 minutes (sterilisation).
- Optional: aliquot a part into sterile Eppendorf tubes for easy usage.

4.3 Sterile Eppendorf, PCR and sequencing tubes

Estimated bench time: 10 minutes
Estimated total time: 3 hours
Purpose: Making the Eppendorf tubes, PCR tubes and sequencing tubes sterile so that they can be used during experiments.

4.3.1 Materials

- Autoclave
- Autoclave tape
- Beaker
- Eppendorf tubes
- PCR tubes
- Sequencing tubes
- Tin foil

4.3.2 Setup & Protocol

- Fill a beaker with the tubes, for each type, use another beaker.
- Cover the upside with tin foil.
- Autoclave the beakers with the tubes at 121 °C for 20 minutes (sterilisation). Use a dry sterilization program or let the tubes dry in an incubator set at 50 °C.