Molecular cloning and genetic engineering – LB media

Aim

The culture medium is used to cultivate the strains, which can multiply and expand the strains to meet the application requirements.

Materials

Tryptone

Yeast Extract

NaCl

Agar

ddH_2O

HCl

NaOH

antibiotics

Procedure

1. Solution preparation: Add 800 mL ddH_2O to LB broth powder (10 g/L Tryptone; 5 g/L Yeast Extract; 5 g/L NaCl, 5% Agarose) and then use a magnetic stirrer to make the power totally solved.

2. pH adjustment: measure the pH of the solution with accurate pH test paper and adjust the pH to 7.0 by adding HCl or NaOH to the solution. Add ddH_2O to the solution until the total volume reach 1000 mL.

3. Separate the solution into several narrow neck flasks and plug all flasks tightly.

4. Warp all the flasks in kraft paper and tie it with ropes in a moveable knot, which can be easily untied when using. Use marker to indicate the medium name and date.

5. Sterilization: sterilize the prepared medium with 0.16 MPa (121℃) by high pressure steam sterilizer for 20 minutes.

6. Plate medium making: place the sterilized plates and flasks on the super clean laboratory bench. Quickly pour 10 mL medium into every plate and let it spread the bottom of the plate. Wait until all medium in plates solidified. Turn the plates upside down and place them in a constant-temperature incubator.

7. After 24h, the plates can be used to do bacteria culture if no microorganism occurs on the plates.

Note

Usually we add corresponding antibiotics into media. Here are some most common antibiotics.

Antibiotics	Stock concentration	Work concentration
Ampicillin	100mg/ml	100ug/ml
Chloramphenicol	34mg/ml	34ug/ml
Kanamycin	50mg/ml	50ug/ml