LB Agar Plate preparation Protocol

LB Agar Plate Preparation:

Introduction

Once the plasmid constructs have been developed, it is necessary to transform them into *E.Coli* cells. This way, a library of self-renewable biosensors will provide the necessary Cas12a and gRNA for antibiotic resistance gene detection.

Firstly, we need to prepare all the necessary materials to culture the transformations, such as **LB agar plates with kanamycin** (for cells transformed with gRNA plasmid) and with **carbomycin** (for cells transformed with LbCas12a plasmid). Furthermore, if we want to transform cells with both plasmids, we also need to prepare plates with both kanamycin and carbomycin.

Everything must be performed under sterile conditions.

Materials

- Materials for kanamycin agar plates preparation
  - Kanamycin (50mg/mL stock)
  - LB agar
- Materials for carbomycin agar plates preparation
  - Carbomycin (50mg/mL stock)
  - LB agar
- Materials for carbomycin-kanamycin plates preparation
  - Kanamycin (50mg/mL stock)
  - Carbomycin (50mg/mL stock)
  - LB agar

Procedure

1. LB-kanamycin agar plates

1. **Autoclave** 1L of LB agar.
2. **Cool** to 55ºC.
3. **Add** 1mL of the kanamycin at 1000x
4. **Pour** into Petri dishes.

2. LB-carbomycin agar plates

5. **Autoclave** 1L of LB agar
6. **Cool** to 55ºC
7. **Add** 1mL of the carbomycin at 1000x

8. **Pour** into Petri dishes

3. LB-carbomycin-kanamycin agar plates

9. **Autoclave** 1L of LB agar
10. **Cool** to 55ºC
11. **Add** 1mL of the kanamycin and 1mL of the carbomycin at 1000x
12. **Pour** into Petri dishes

4. 2 Plating

13. **Warm** LB agar plates containing kanamycin at 37°C for 15 min.
14. **Mix** the cells thoroughly by flicking the tube and inverting, then spread 100 μl outgrowth onto each plate. To obtain the maximum number of colonies, spin the 1000 μL cell culture for 1 min at 5000 rpm, remove 800 μL of media and spread cells after re-suspending in the remaining buffer.
15. **Incubate** the plates overnight at 37°C, or 24 hrs at 30°C, or 48 hrs at 25°C [1].

4.3 Notes

16. **Competent cells** are very sensitive to changes in temperature and should be thawed on **ice**. The transformation should be started immediately after the cells are thawed. For best results, each vial of cells should be thawed only once. Although the cells are re-freezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately two-fold.
17. Competent cells must be treated gently. Mix cells by swirling or gently tapping the reaction tube. Do not mix by pipetting or vortexing.
18. Media other than **SOC** can be used, but the transformation efficiency will be reduced. Using LB reduces transformation efficiency a minimum of two- to three-fold.
19. **Transformation** efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid [2].

Bibliography

1. Addgene: Pouring LB Agar Plates. (n.d.). Retrieved October 18, 2021, from Addgene.org website: https://www.addgene.org/protocols/pouring-lb-agar-plates/
2. Transformation Protocol. (n.d.). NZYCompetent cells preparation buffer. Retrieved October 18, 2021, from Nzytech.com website: https://www.nzytech.com/wp-content/uploads/2019/04/mb120_pb_v1901.pdf