Protocol 1: Production of Luria Broth/ Kanamycin media for agar plates

Purpose: Production of growth media for bacteria for agar plates and liquid cultures

Materials:

- PPE in accordance with BSL 1 standards
- Tryptone
- Yeast Extract
- Sodium Chloride
- Scale
- DI Water
- Autoclave
- Autoclave bin
- Kanamycin
- Thermometer
- 2L beaker
- Tinfoil
- 70% Ethanol Solution
- Hot gloves

Conditional:
Sodium hydroxide
Agar
100mm x 15mm Petri Dishes

Methods:

Amount Made = 1L

1. Weigh out and then add 10g tryptone, 5g of yeast extract, and 10g of sodium chloride to a 2L beaker (use a larger beaker than the volume you are making to prevent boiling over in the autoclave).
2. Add 1000mL of DI water.
3. **Optional:** Add 950mL of DI water then test the pH with a pH meter before adjusting with sodium hydroxide until the pH is 7.0. Then add DI water until the total volume is 1000mL. This will tell you the exact pH of the solution, but we have found that the pH is normally very close to 7.0 anyway and small variations in pH have not affected bacterial growth on the plates.
4. Cover the top of the beaker with tin foil to prevent boiling over in the autoclave.
5. Put the beaker in an autoclave bin and autoclave for 20 minutes on the liquid cycle.
6. After removing from the autoclave with hot gloves add a thermometer to the solution and allow to cool to 55℃. (If you do not have a thermometer, a good indicator if the solution is cool enough is if you can touch and hold it for 10 seconds).
7. Add 50 mg of kanamycin to the solution and mix.

Conditional Methods for Agar Plates:

1. Before autoclaving add 15g of agar.
2. Later pour approximately 15mL of media into a 100mm by 15mm petri dish.
3. When pouring multiple plates stack subsequent plates on top of each other to prevent condensation from occurring.
4. Let set approximately 30 minutes at room temperature to set before storing in a refrigerator (4℃) for up to one month.
5. Test the antibiotic in the plates by plating an antibiotic resistant strain on one plate and a non-antibiotic resistance strain on another and then incubate overnight before checking for growth.

Analysis:
If your agar plates set then you added agar properly to it, if not you can just use the media for liquid cultures or another use. Note that you cannot autoclave it again as that will destroy the kanamycin. For your control test, if you see growth on the antibiotic resistant plate and no growth on the normal strain plate then your antibiotic is functioning correctly.

References:

Green, Michael R. & Sambrook, Joseph. & Cold Spring Harbor Laboratory. (2012). *Molecular cloning : a laboratory manual*. Cold Spring Harbor, N.Y : Cold Spring Harbor Laboratory Press