Growing Medium of *E.coli* :

Overview :

We used LB broth (Luria broth, Luria-Bertani medium) for plasmid DNA production (cloning, transformation and miniprep). Otherwise, we used only M63 media for curdlan production because it is a minimal medium, so we could control all the media parameters for production.

Media :

- **Luria-Bertani (LB) broth: 1L**

Component	Volume & Mass	Procedure
Bactotryptone	10 g	1) Adjust pH to 7.5 with NaOH 2) Adjust volume to 1 L 3) Sterilize by autoclave
Yeast Extract	5 g	
NaCl	10 g	

- For LB plates : add 12 g/L of agar

- **Preparation of 5X M63 Medium**

Component	Volume & Mass	Procedure
$(NH_4)_2SO_4$	10 g	1) Add the following reagents to a 2-liter flask 2) Adjust volume to 1 L 3) Once the ingredients are added, heat with stirring until the components are completely dissolved. 4) Adjust pH to 7.0 with Acid 5) Sterilize by autoclave
KH_2PO_4	68 g	
$FeSO_4.7H_2O$	2,5 **mg**	

- **Preparation of 1X M63 Medium Working Solution**

Aseptically dilute 200mL of 5X stock solution with 789 mL of sterile distilled water.
Aseptically add the following sterile solutions:

- 1 mL of 1 M $MgSO_4.7H_2O$ (*directly in the 1X medium, not in the 5X*)
- 10 mL of 20% carbon source *(final concentration: 0,2 %)*
- 0.1 mL of 0.5% vitamin B1 (thiamine)
  « *Vitamins should be added to a final concentration of 1µg/mL* or 1mg/L»
- Antibiotic

Optional :
Add 5 mL of 20% Casamino Acids or L amino acids to 40 µg/mL or DL amino acids to 80 µg/mL

- **Preparation of Stock Carbohydrate Solution (Glucose) :**

Add 20 g of carbohydrate to distilled water and bring volume to 100mL.
Mix Thoroughly.
Filter sterilize.

- **Preparation of Stock $MgS0_4$-$7H_20$ Solution :**

Add 24,65 g of $MgS0_4$-$7H_20$ to distilled water and bring volume to 100mL.
Mix Thoroughly.
Filter sterilize.

Antibiotic :

Antibiotic	Stock concentration	Working concentration	Dissolve in
Ampicillin	50 $mg.mL^{-1}$	50 $\mu g.mL^{-1}$	H_20
Kanamycin	50 $mg.mL^{-1}$	50 $\mu g.mL^{-1}$	H_20
Chloramphenicol	34 $mg.mL^{-1}$	10 $\mu g.mL^{-1}$	95% Ethanol
Tetracycline	12,5 $mg.mL^{-1}$	12,5 $\mu g.mL^{-1}$	50% Ethanol

- Cool down the medium to 50°C before adding antibiotics.

Curdlan Production with *E.coli* :

1. Pre-warm the medium at 37°C to decrease the time of lag-phase
2. Take colonies and inoculate 50 mL of complete M63 (1X) + Antibiotic at 37°C overnight
3. Grow cells until A600 : 0.7-0.9
4. Inoculate 30 mL inoculum for 120 mL of the M63 complete medium + antibiotic in a 500 mL Erlenmeyer flask (in order to have A600: 0.2)
5. Put the flask at 37°C, 180 rpm
6. Take out 10 mL of culture, centrifuge 5min at 14,000 rpm and 4°C, discard supernatant and store pellet at -20°C (this is the uninduced time point).
7. During stationary phase, re-incubate remaining cultures at 25°C shaking with 180rpm during 21h.