OSMOLARITY PROTOCOL

MATERIALS:

Recipe for minimal media with 0% sucrose (100 mL)

- 20 mL 5x MMA
- 0.1 mL (100 µL) $MgSO_4$, $7H_2O$
- 0.1 mL (100 µL) Chloramphenicol
- 1 mL 20% Glucose
- 78.8 mL ddH_2O
- 0.3 g L-leucine (add at the very end)

Recipe for minimal media with 5% sucrose (100 mL)

- 20 mL 5x MMA
- 0.1 mL (100 µL) 1M $MgSO_4$, $7H_2O$
- 0.1 mL (100 µL) Chloramphenicol
- 1 mL 20% Glucose
- 50 mL 10% Sucrose
- 28.8 mL ddH_2O
- 0.3 g L-leucine (add at the very end)

Recipe for minimal media with 10% sucrose (100 mL)

- 20 mL 5x MMA
- 0.1 mL (100 µL) $MgSO_4$, $7H_2O$
- 0.1 mL (100 µL) Chloramphenicol
- 1 mL 20% Glucose
- 50 mL 20% Sucrose
- 28.8 mL ddH_2O
- 0.3 g L-leucine (add at the very end)

Recipe for minimal media with 15% sucrose (100 mL)

- 20 mL 5x MMA
- 0.1 mL (100 µL) $MgSO_4$, $7H_2O$
- 0.1 mL (100 µL) Chloramphenicol
- 1 mL 20% Glucose
- 50 mL 30% Sucrose
- 28.8 mL ddH_2O
- 0.3 g L-leucine (add at the very end)

Recipe for 8 samples 5x MMA (200 mL)

Why 8 samples?
Answer: Because we have 4 media for samples (0%, 5%, 10% and 15%), 2 media for positive controls (0% and 15%) and 2 media for negative controls (0% and 15%). 20 mL for each and some extra.

- 1 g $(NH_4)_2SO4$
- 4.5 g KH_2PO_4
- 10.5 g K_2HPO4
- 0.5 g Sodium citrate dehydrate (Na-citrate-$2H_2O$)
- Add ddH_2O to 200 mL

Recipe for 8 samples 20% glucose (10 mL)

- 2 g D-Glucose
- Add ddH_2O to 10 mL

Recipe for 8 samples 1M $MgSO_4$, $7H_2O$ (2 mL)

- 0.25 g $MgSO_4$ anhydrous
- Make in 2 mL in Falcon tube and vortex.

Recipe for 8 samples 10% sucrose (50 mL)

- 5 g D-Sucrose
- Add 50 mL ddH_2O to it.

Recipe for 8 samples 20% sucrose (50 mL)

- 10 g D-Sucrose
- Add 50 mL ddH_2O to it.

Recipe for 8 samples 30% sucrose (50 mL)

- 15 g D-Sucrose
- Add 50 mL ddH_2O to it.

Kanamycin 340 µg/mL

- Use 6.8 mL of stock Kanamycin 50 µg/mL.

PROCEDURE:

- Prepare fresh minimal media with 0%, 5%, 10% and 15% sucrose.
- Replace Chloramphenicol with other antibiotics if applicable and with ddH_2O for negative control.
- Choose sample with similar fluorescent protein as a positive control and sample without fluorescent protein as a negative control.
- Use only 0% and 15% minimal media for both the positive and the negative controls.
- Prepare overnight culture of the bacteria in 1.2 mL minimal media in Eppendorf tubes. Inoculate one fresh colony in each tube and incubate overnight at 37 ^{o}C with open lids (cover the lids with aluminum foil). Avoid using Rota mixer or Falcon tube for overnight culture as this method is proven to show maximum growth in our lab.
- Do not forget to include negative and positive controls. Check the OD next morning and note it down.
- Dilute the overnight culture using 1:100 dilutions (200 µL of overnight cultured bacteria into 20 mL of respective media in Erlenmeyer flask) and let them grow in the incubator at 37 ^{o}C until they reach OD ~ 0.3. The dilution ratio could be altered to 1:500 or 1:1000.
- Add Kanamycin 340 µg to 1 mL culture to inhibit protein synthesis and immediately perform fluorescence analysis using plate reader. Replace Kanamycin with other antibiotics if your cells contain Kanamycin resistance gene.
- Use excitation and emission length accordingly (depending on the fluorescent protein). An example that we used is the excitation length 580 nm ± 10 and emission length 627 nm ± 30 to measure RFP. The same will go with the controls.
- Do not forget to include negative and positive control during measurement. Use only media as a blank.
- Troubleshoot - Use the same gain for all the samples; do not adjust gain in the settings.
- Repeat the experiment at least three times to have statistical relevance.

DATA ANALYSIS:

Measure the fluorescence and compare with the controls and blank. Plot it as a graph to observe relative change with different osmole concentrations.