

05_01_Preparation-of-S.O.C.-medium

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Goal-Setting

- Preparation of S.O.C. medium

Terms / abbreviations

- ddH₂O = MilliQ water
- KCl = Potassium chloride
- MgCl₂ = Magnesium chloride
- MgSO₄ = Magnesium sulfate
- NaCl = Sodium chloride
- S.O.C. = Super optimal broth with catabolite repression

Risk areas

- None

Required materials and / or information

- Chemicals:
 - Glucose, Carl Roth
 - KCl, cell culture grade, AppliChem
 - MgCl₂, Carl Roth
 - MgSO₄, Carl Roth
 - MilliQ water, Sartorius arium pro VF
 - NaCl, Carl Roth
 - Tryptone, AppliChem
 - Yeast Extract, Carl Roth
- Materials:
 - Schottflask(s)
 - Stirring fish

Templates, devices, software

- Analysis balance, Kern ABJ 220-4NM
- pH meter, Knick Digital pH-Meter 646

Preliminary work

- Calculate the needed masses for specific volumina

Operation

- pH meter (ready to use in the chemical room)
 - If it is used for the first time, ask an experienced person around how to use it
 - Regularly calibrate the pH meter according to manufacturer
 - User guide:
 - pH meter should measure about 6.5 when the solution is stored (small Falcon Tube)
 - Take it out and unlock it (small switch at the top)
 - Wash it carefully with some MilliQ water
 - Hold into the solution and measure

- Wash again after use, turn off the switch and put it back into the small Falcon Tube

The following recipe is standardized to 1 L

1. Prepare stock solutions of glucose, MgCl_2 and MgSO_4 and filter sterilize them
 - a. Glucose 2 M: 18 g glucose in 90 mL of ddH₂O, fill up to 100 mL
 - b. MgCl_2 2 M: 19 g MgCl_2 in 90 mL of ddH₂O, fill up to 100 mL
 - c. MgSO_4 2 M: 49.3 g $\text{MgSO}_4 \times 7 \text{ H}_2\text{O}$ in 90 mL of ddH₂O, fill up to 100 mL
 - d. KCl 250 mM: 1.86 g KCl in 90 mL of ddH₂O, fill up to 100 mL
2. Weigh the following components into a schottflask
 - a. Yeast Extract: 5 g
 - b. Tryptone: 20 g
 - c. NaCl: 0.5 g
 - d. KCl: 0.186 g
3. Fill up to 900 mL with ddH₂O
4. Add 10 mL of a 250 mM solution of KCl
5. Adjust the pH of the medium to 7.0 with 5 M NaOH (~0.2 mL)
6. Adjust the volume of the solution to 1 L --> 2% (980 mL, or 970 mL, if KCl solution was used) with ddH₂O
7. Sterilize by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle
8. After the SOC medium has been autoclaved, allow it to cool to 60 °C or less
9. Add following substances under the cleanbench!
 - a. 5 mL of a sterile solution of 2 M MgCl_2
 - b. 5 mL of a sterile solution of 2 M MgSO_4
 - c. 10 mL of a sterile solution of 2 M glucose

Disposal

- Can be discarded in the sink with much water

Troubleshooting

- None

Follow-up work

- [05_03_Seamless-cloning-reaction](#)
- [05_04_pGEM-T-Easy-Vector-System](#)