Yeast Growth

1. Materials:

- 2 Autoclaved shot glases
- 3 Autoclaved Erlenmeyers with pignose
- Magnetic Stirrer
- Ammonium Sulfate (NH₄)₂SO₄
- Glucose (Dextrose) C₆H₁₂O₆
- YNB without amino acids
- Yeast Extract
- Peptone
- milliQ Water

2. Media Preparation:

2.1. Yeast Nitrogen Base (YNB) Minimal Media 500 mL

Ammonium Sulfate

• Molecular Weight: 132.14 g.mol⁻¹

• Location: Above the pH meter (G46)

Mass: 2.5 g

Balance Weight: _________

YNB without Amino Acids

• Location: Above Balance (G46)

• Mass: 0.85 g

Balance Weight: _____

Glucose (Dextrose):

Molecular Weight: 180.156 g.mol⁻¹

• Location: Above the TECAN OD₆₀₀ (G46)

Mass: 10 g

Balance Weight: _____

Add milliQ water to 500 mL and filter

2.2. Yeast Extract, Peptone, Dextrose (YPD) Rich Media 500 mL

Yeast Extract

• Location: Above the Balance (G46)

• Mass: 5 g

Balance Weight:

<u>Peptone</u>

• Location: Below Balance (G46)

Mass: 10 g

Balance Weight:

Glucose (Dextrose):

Molecular Weight: 180.156 g.mol⁻¹

• Location: Above the TECAN OD₆₀₀ (G46)

Mass: 10 g

Balance Weight:

Add milliQ water to 500 mL and filter

3. Yeast Strain

• Name: FY4

• Location: G34 Freezer "The Dragon", 3rd shelf, 3rd Metabolic box, 2rd Column top box.

• Characteristics: Prototrophic strains

4. Culture and Growth:

4.1. Pre-Inoculation YNB:

- 3 Greiner Tube (each 3mL YNB media)
- Incubation:
 - 30°C
 - 250 rpm
 - Around 15h to reach OD of 0.5

4.2. Pre-Inoculation YPD:

- 3 Greiner tubes per previous tube (each 3mL YPD)
- Add 800 uL YNB culture in each tube
- Incubation:
 - 30°C
 - 250 rpm
 - Around 10h to reach OD of 2

4.3. Inoculation YPD:

- 3 Erlenmeyer
- Add 45 mL YPD media in each
- Add 6 mL Culture in each
- Incubation:
 - 30°C
 - 250 rpm
 - Around 10h to reach OD of 3

5. Cell Harvesting

- Fill needed number of Eppendorf tubes (2mL) with culture
- Centrifuge:
 - 4°C
 - Max Speed
 - 1 min
- Discard supernatant
- Freez pellet in Freezer (-80°C)