

Date: 20260702

Title: Chlorophyll Extraction

Purpose: To isolate the green photosynthetic pigment from *Arabidopsis thaliana*

Procedure:

1. Weight the fresh weight of *Arabidopsis thaliana* (9 at a time)
2. Take the plant from the agar plate, careful not to hurt it by squeezing the tweezers.
3. Put the 9 plants on a tissue paper, lightly press to remove the agar striking to the plates
4. Pick up the plants and put them on a weighting plate to weight
5. Weight the plants
6. Pick up the plants and put them in a microtube, take care that the roots need to go in too
7. Repeat these steps for as many times as the sets of *Arabidopsis thaliana* you are testing
8. Place 3 glass bits in each of the microtubes, place in -80 °C for 10 min
9. Dilute acetone to 80%, add 1 mL to each tube (10 mL in total)
10. Wait for the chlorophyll to extract, and can use a vortex to crush the plants
11. Add the liquid to the circus well, 200 µL in each well, 3 wells for each sample
12. ELISA reader insert the 96 well, run the result through the equation below:

*Total chlorophyll content equation:

$$(20.2 \cdot OD_{645} + 0.02 \cdot OD_{663}) / (1000 \cdot fw)$$

Done by Phoebe, Abby T.

Recorded by Sophie H.

Date: 07/04

Title: Making salinity agar plates

Purpose: prepare salinity agar plates for plant model Experimental Set #5 0,1,5,10 mM trehalose vs. 150, 250 mM NaCl

Procedure:

The following protocol is for 600ml of agar

- 12 agar plates
 - control(no trehalose) x control(no NaCl)
 - control(no trehalose) x 150 mM NaCl
 - control(no trehalose) x 250 mM NaCl
 - 1 mM trehalose x control(no NaCl)
 - 1 mM trehalose x 150 mM NaCl
 - 1 mM trehalose x 250 mM NaCl
 - 5 mM trehalose x control(no NaCl)
 - 5 mM trehalose x 150 mM NaCl
 - 5 mM trehalose x 250 mM NaCl
 - 10 mM trehalose x control(no NaCl)
 - 10 mM trehalose x 150 mM NaCl
 - 10 mM trehalose x 250 mM NaCl

1. Prepare the following:

½ MS medium powder(w/o sucrose)	1.38 g
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MES buffer	0.3 g
Distilled water	600 mL
Sucrose(1%)	6 g
Agar powder(1%)	6 g

2. Add the contents into a 1000 mL flask
3. Use a hot plate to stir (with a magnetic stirring rod) until no sediments are left in the solution
4. Use the pH probe and measure the pH of the solution, make sure the pH is 5.7-5.8
 - a. If the pH is too low, add 0.1 M KOH slowly to increase it
5. Pour 200 mL of mixture out into another flask to separate into three flasks, label with [0 mM], [150 mM] and [250 mM]
6. Add 2.192 g of NaCl into the flask labelled [150 mM NaCl]
7. Add 3.652 g of NaCl into the flask labelled [250 mM NaCl]
8. Prepare 4 flasks for different trehalose concentrations(0, 1, 5, 10 mM)
9. Prepare 12 flasks and pour the respective trehalose and NaCl concentration in each of the flasks
 - a. making sure there is 50ml in each of the flasks(12)
 - b. label each flask clearly according to their trehalose concentrations
10. To their respective trehalose concentrations, add

Trehalose concentration	Amount of trehalose (for 50ml of medium)
0 mM	0 μ L
1 mM	500 μ L
5 mM	2500 μ L
10 mM	5000 μ L

11. Wrap the necks of the flasks with aluminum foil and stick a piece of indicator tape on top
12. Autoclave for 20 min at 127 °C
 - a. Wait for the pressure to reach 0 before retrieving the flask
13. During the autoclave, label 8 of the petri dishes with a marker in the biosafety cabinet
 - a. right top: date and arrow pointing up to show direction for pictures
 - b. bottom lid: different trehalose concentrations and NaCl concentration or control
14. Transfer half of the flasks to BSC
 - a. Store the other half of the flasks in the hot bath while pouring so that it does not solidify
15. Make sure its surface is dry by wiping the flasks with a paper towel before spraying 70% EtOH on them
16. Pour each of the medium mixtures into their square petri dishes (around 50 mL)
17. Transfer the other half of the flasks into the BSC

18. Pour each of the medium mixtures into their square petri dishes
19. Let the petri dishes solidify at room temperature under the BSC

Done by Abby K, Abby T, Audrey H

Date: 20260707

Title: *Arabidopsis thaliana* transformation

Purpose: To verify and transform the healthy *Arabidopsis thaliana* from the rest

Procedure:

1. Place the cap holder in 2000 mL of 10% bleach for 10 min (200 mL bleach and 1800 mL of water)
2. After bleached for 10 min, take the cap holder out and cleanse it under ddH₂O tab(slightly tilt the holder to let the water flow through every corner of it) until no bleach smell left(at least 3 min)
3. Placed the cleansed holder in biosafety cabinet along with the plants you want to transform and tip box (blue)
4. Assemble the cap holder with tip box, than pour MS medium inside the tip holders to almost full, but never pour over the front side of the *Arabidopsis thaliana* holder
5. Select and place the desired plants on the cap holder

	1	2	3	4	5	6	7	8	9	10	11	12
A	DT	SPA	PA	PA	SPA	SPA	PA	PA	PA	PA	PA	PA
B	DT	PA	SPA	DT	PA	PA	DT	PA	SPA	SPA	DT	SPA
C	DT	DT	SPA	DT	PA	DT	PA	PA	DT	DT	DT	SPA
D	PA	PA	DT	DT	DT	DT	DT	SPA	DT	DT	DT	DT

*DT: Didn't Transform *PA(17caps): Prioritize *Arabidopsis thaliana* *SPA(10caps): Second Prioritize *Arabidopsis thaliana*

Done by Sarah, Sophie H, Sophie C, Alex, Naomi

Supervised by Alex, Gabriel
Recorded by Sophie H

Date: 20260707

Title: Making ½ MS medium

Purpose: Make a total of 1 liter of hydroponic medium for hydroponic box

1. Measure and add to a flask:

½ MS medium (without sucrose)	2.3 g
MES buffer	0.5 g
ddH ₂ O	1 L

2. Use the hot plate to stir(with a stirring rod) until no sediments are left in the solution
3. Determine pH of the solution and adjust the pH using a pH probe to around 5.6-5.7 using 1M KOH
 - a. Record original and final pH
 - i. original: 4.0
 - ii. final: 5.6
4. Wrap the neck with aluminum foil and place the indicator tape on top
5. Autoclave at 127 °C for 20 min
6. Wait for the pressure to reach 0 before opening the autoclave
7. Pour 300 mL of medium into each of the hydroponic boxes

Done by Abby K/T

Date: 20260707

Title: Transfer *Arabidopsis Thaliana* FA to the salinity plates

Purpose: transfer the germinated seedlings from neutral plates to salinity plates to conduct salinity stress tests (experimental set 5).

Procedure:

1. Draw a standardized baseline on each of the salinity plates to signal where the roots will be lined up after transferring
2. Place all equipments into the BSC after sanitizing
3. Get the neutral plate inside the BSC one at a time
4. Lay a template sheet of paper underneath the salinity plate so that it is clear where to place the seedlings
5. Use the tweezer to slowly lift the *Arabidopsis thaliana* plant from the top of the roots
6. Place the seedling softly onto the salinity plate, with the bottom of the root precisely onto the baseline ; 9 seedlings per row, per plate.

- a. make sure to pick seedlings that have around the same root length
 7. Repeat step 3-6 until all seedlings transferred into all of the salinity plates
 8. Place into the growth chamber
- Done by Abby K/T

Date: 20260707

Title: Remake the incorrect concentrations salinity plates

Purpose: prepare salinity plates for experimental set 5

1. Prepare the following in a 500ml flask:

½ MS medium powder(w/o sucrose)	0.69 g
MES buffer	0.15 g
Distilled water	300 mL
Sucrose(1%)	3 g

2. Use a hot plate to stir (with a magnetic stirring rod) until no sediments are left in the solution
3. Use the pH probe and measure the pH of the solution, make sure the pH is 5.7-5.8
 - a. If the pH is too low, add 0.1M KOH slowly to increase it
4. Pour 50ml of mixture into the 6 flasks
5. Prepare 6 flasks and pour the respective trehalose and NaCl concentration in each of the flasks
 - a. making sure there is 50ml in each of the flasks (6)
 - b. label each flask clearly according to their trehalose concentrations
6. Add:

150mM NaCl, 5mM trehalose

trehalose (µL)	NaCl (g)	Agar powder (g)
2500	0.548	0.5

150mM NaCl, 10 trehalose

trehalose (µL)	NaCl (g)	Agar powder (g)
5000	0.548	0.5

250mM NaCl, 5mM trehalose

trehalose (μL)	NaCl (g)	Agar powder (g)
2500	0.913	0.5

250mM NaCl, 0mM trehalose

trehalose (μL)	NaCl (g)	Agar powder (g)
0	0.913	0.5

0mM NaCl, 0mM trehalose

trehalose (μL)	NaCl (g)	Agar powder (g)
0	0	0.5

0mM NaCl, 1mM trehalose

trehalose (μL)	NaCl (g)	Agar powder (g)
500	0	0.5

7. Wrap the necks of the flasks with aluminum foil and stick a piece of indicator tape on top

8. Autoclave for 20 min at 127 °C

a. Wait for the pressure to reach 0 before retrieving the flask

During the autoclave, label 6 of the petri dishes with a marker in the biosafety cabinet

b. right top: date and arrow pointing up to show direction for pictures

c. bottom lid: different trehalose concentrations and NaCl concentration or control

2. Transfer half of the flasks to BSC

a. Store the other half of the flasks in the hot bath while pouring so that it does not solidify

3. Make sure its surface is dry by wiping the flasks with a paper towel before spraying 70% EtOH on them

4. Pour each of the medium mixtures into their square petri dishes (around 50ml)

5. Transfer the other half of the flasks into the BSC

6. Pour each of the medium mixtures into their square petri dishes

7. Let the petri dishes solidify at room temperature under the BSC

Done by Abby K/T

Date: 20260712

Title: Fresh weight of *Arabidopsis thaliana*

Purpose: To measure stress's impact to the plant's fresh weight

Procedure:

Control-1(contains 3 plants) 0.0154 g

Control-2 // 0.0111 g

Control-3 // 0.014 g

1mM trehalose // -1 0.0095 g

1mM trehalose // -2 0.0014 g

1mM trehalose // -3 0.0087 g

5mM trehalose // -1 0.0101 g

5mM trehalose // -2 0.1050 g

5mM trehalose // -3 0.0112 g

10mM trehalose // -1 0.0114 g

10mM trehalose // -2 0.0134 g

10mM trehalose // -3 0.0024 g

150mM NaCl // -1 0.0090 g

150mM NaCl // -2 0.0013 g

150mM NaCl // -3 0.0024 g

150mM NaCl+1mM trehalose // -1 0.0020 g

150mM NaCl+1mM trehalose // -2 0.0015 g

150mM NaCl+1mM trehalose // -3 0.0025 g

150mM NaCl+5mM trehalose // -1 0.0029 g

150mM NaCl+5mM trehalose // -2 0.0023 g

150mM NaCl+5mM trehalose // -3 0.0035 g

150mM NaCl+10mM trehalose // -1 0.0040 g

150mM NaCl+10mM trehalose // -2 0.0036 g

150mM NaCl+10mM trehalose // -3 0.0022 g

250mM NaCl (contains 7 plants) 0.0013 g

250mM NaCl+1mM trehalose // -1 0.0008 g

250mM NaCl+1mM trehalose // -2 0.0009 g

250mM NaCl+1mM trehalose // -3 0.0014 g

250mM NaCl+5mM trehalose // -1 0.0008 g

250mM NaCl+5mM trehalose // -2 0.0009 g

250mM NaCl+5mM trehalose // -3 0.0011 g

250mM NaCl+10mM trehalose // -1 0.0008 g
 250mM NaCl+10mM trehalose // -2 0.0004 g
 250mM NaCl+10mM trehalose // -3 0.0008 g

Done by Sophie H, Sophie Y, Naomi
Supervised by Gina

Date: 20260713

Title: Chlorophyll Extraction

Purpose: To isolate the green photosynthetic pigment from *Arabidopsis thaliana*

Procedure:

1. Take out the microtubes with *Arabidopsis thaliana* from -80 °C (34 microtubes)
2. Dilute acetone to 80%, add 200 µL to each tube
3. Use a crusher the plants in the acetone, crush until no visible green plants/leaves
4. Add 800 µL of acetone to the tubes
5. Vortex and store in a dark place
6. Centrifuge at 7000 g for 30 sec
7. Add the liquid to the circus 96 well, 200 µL in each well. 3 wells for each sample

	1	2	3	4	5	6	7	8	9	10	11	12
A	Con trol 1	1T 1	5T 1	10T 1	150 NaC 1	150 NaC 1T 1	150 NaC 5T 1	150 NaC 10T 1	250 NaC 1	250 NaC 1T 1	250 NaC 5T 1	250 NaC 10T 1
B	Con trol 2	1T 2	5T 2	10T 2	150 NaC 2	150 NaC 1T 2	150 NaC 5T 2	150 NaC 10T 2		250 NaC 1T 2	250 NaC 5T 2	250 NaC 10T 2
C	Con trol 3	1T 3	5T 3	10T 3	150 NaC 3	150 NaC 1T 3	150 NaC 5T 3	150 NaC 10T 3		250 NaC 1T 3	250 NaC 5T 3	250 NaC 10T 3

8. ELISA reader, insert 96 well, run the results through the equation below, make sure the select the correct wavelength (A663 and A645)

*Total chlorophyll content equation (mg/g): $(20.2 \cdot OD_{645} + 8.02 \cdot OD_{663}) / (1000 \cdot fw)$,

V= 1 ml

*250 NaCl 1T 3 mly use 200 µL acetone

Done by Abby T, Sophie C, Audrey H, Abby K.

Supervised by Gabriel

Date: 20260719

Title: Plant seeds for Exp set 6 and exp set 7 vernalization

Purpose: plant 4 neutral plates (27 seeds/plate)

Procedure:

1. Prepare 4 12x12cm, ½ MS medium, pH 5.8, 1% square agar plates (neutral)
2. Sanitize your hands and all equipments:
 - a. ½ MS medium square agar plates
 - b. Autoclaved toothpicks
 - c. Sterile water
 - d. Planting grid line paper then place in BSC
3. Open the aluminum foil of the autoclaved toothpicks and take one out
4. Dip the tip of the toothpick in the sterile water
5. Attach seeds one at a time and tip it on neutral plates base on the template at the bottom
6. Seal with tape and label the date of vernalization

Done by: Abby T, Audrey H

Recorded by: Audrey H

Date: 20260719

Title: Plant seeds for set A and vernalization in hydroponic system

Purpose: stabilizing plant growth

Procedure:

1. Prepare 2000 mL ½ MS medium using the following protocol
 - a.

Material	volume
½ MS medium powder (w/o sucrose)	4.6 g
MES buffer	1.0 g
Distilled water	2000 mL

- b. pH: 5.72
2. Microwave the centrifuge tubes with 1X ½ MS agar from 7/12 and heat it until it melts (agar is 1% agar)
3. Wait until the agar cools down a little bit
4. Prepare 48 eppendorf lids
5. Add 250 µl agar into each eppendorf lids
6. Wait until the agar solidifies
7. Add 2.2mL of medium into 48 tube rack
8. Put the lids with agar onto the tube rack with medium (hole facing up)
9. Use an autoclaved toothpick to pick up one seed and add the seed into the hole of the lid
10. Repeat step 9 until all lids have seeds
11. Wrap the tube rack with aluminium foil

12. Place the tube rack with aluminium foil into the refrigerator in 4 °C
13. Wait 4 days for seeds to stratify

Done by: Alex

Recorded by: Audrey

Date: 20260720

Title: Transfer plants from tube rack to Box 1,2,3

Purpose: to assemble the following parts to STK level 0 vector

Procedure:

1. Prepare 2000 mL of 10% bleach and add into 2000 mL beaker
2. Place the 3D boards into the beaker and use plastic wrap to cover it to prevent the 3D board from floating too high
3. Wait for 10 min
4. Use ddH₂O to rinse the 3D boards until there are no smell of the bleach
5. Put one tweezer, 3 tip boxes that are autoclaved, 3 3D printer boards, and the tube rack with 41 plants into BSC
6. Put 1000ml of ½ MS medium from 7/19 into the BSC and add about 300ml medium into each tip box
7. Put the 3D printer boards onto the medium in the tip boxes
8. Use the tweezer to pick up one lid and transfer it to the 3D printer boards in the tip boxes. Remember to choose the lids with plants that are growing well.
9. Take everything out of BSC
10. Label the 3 tip boxes as Box 1, Box 2, Box 3
11. Put Box 1, 2, and 3 into the growth chamber at 22.2%

Done by Alex, Ryan

Recorded by: Audrey H

Date: 20260722

Title: Issue of Box B1

Purpose: Summarize and analyze the problem of hydroponic box and find these problem before next round of testing

Problems:

Problem 1: The 3D printer board in Box B1 started to sink into the medium, This is because the medium leads into the 3D printer board. This caused the lids to float on medium, and not on the board.

Problem 2: Two of the lids seemed to be contaminated by red bacteria or fungi

Problem 3: The medium is contaminated and it looks turbid

Problem 4: 8 out of 13 plants are not growing, including the 2 that had been contaminated by red bacteria

Done by plant model team

Date: 20260725

Title: Planting *Arabidopsis thaliana* in soil

Purpose: To plant the *Arabidopsis thaliana* seed in the soil

Procedure:

1. Soak pots and water trays in 1:100 diluted bleach for 10 mins, then rinse it thoroughly with ddH₂O (make sure every corner was rinsed thoroughly until no bleach smell left)
2. Mix peat soil into a 2 L flask and autoclave it. Fill 3-inch pots until 80% full, lightly press down and thoroughly water from the surface to fully moisturize the soil before transplanting the *Arabidopsis thaliana* seeds.
3. Make a small hole (around one knuckle deep) in the soil using finger (the holes should be spaced evenly to prevent plants interrupting each other from growing)
4. Use tweezers to gently lift the healthy seeds (put for 7 days)
5. Place the root against the sidewall of the hole
6. Use a wash bottle to gently rinse soil over roots so they are fully covered by the soil (must avoid direct contact of the root with fingers or tweezers, support seedlings only with tweezers only from beneath the cotyledons)

Done by Alex

Record by Sophie H.

Date: 20260725

Title: ½ MS medium preparation (2000mL)

Purpose: Prepare ½ MS medium for further *Arabidopsis thaliana* planting(hydroponics)

Procedure:

1. Measure and add to the flask
 - a. ½ MS medium(without sucrose): 4.6 g(2.3*2)
 - b. MES buffer: 1 g(0.5*2)
 - c. ddH₂O : 2 L(1*2)
2. Use the hot plate to stir(with a stirring bar) until no sediments are left in the solution
3. Determine pH of the solution and adjust the pH of the ½ MS medium using a pH probe to around 5.7-5.8 pH
 - a. Add 1M KOH to increase the pH
 - i. Original pH: 4.1
 - ii. Final pH: 5.7
4. Split the 2000mL medium into 500 mL flask*2 and the original beaker
5. Wrap the neck with aluminum foil and place the indicator tape on top
6. Autoclave at 127 °C for 20 min
7. Wait for the pressure to reach 0 before opening the autoclave
8. Pour 300 mL of medium into each of the hydroponic boxes

Done by Sara and Sophie H

Supervised by Alex

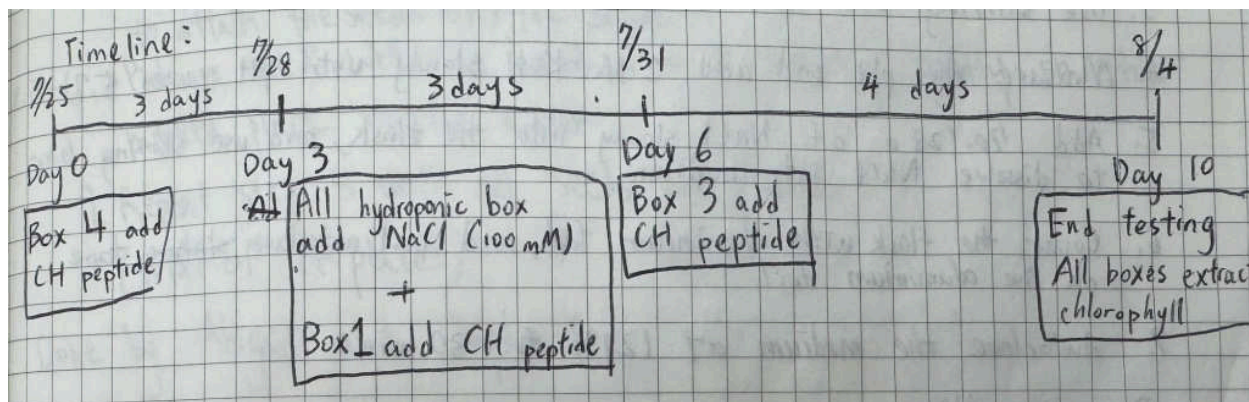
Date: 20260725

Title: CH biotech protectant testing on box 1, 2, 3, 4

Purpose: test on when we should add protectants

Procedure:

1. Autoclave one tip box and cleanse the 3D printed board with 10% bleach
2. Add 300 mL of $\frac{1}{2}$ MS medium into the tip box, and label this box, and label this box as box 4
3. Add 600 μ L of CH biotech protectant into the medium of box 4
4. Place the 3d printed board onto the medium in box 4
5. Transfer 3 plants each from box 1 and box 3 to box 4
6. Transfer 4 plants from box 2 to box 4



Done by Alex

Supervised by Gina

Date: 20260725

Title: Prepare 3M NaCl with $\frac{1}{2}$ MS medium

Purpose: hydroponics testing with CH biotech protectant

Procedure:

1. Prepare 400ml $\frac{1}{2}$ MS medium with the following protocol

$\frac{1}{2}$ MS powder (w/o sucrose)	0.92 g
MES buffer	0.2 g
Distilled water	400 mL

2. Add the components into 50 mL flask
3. Use stirring bar to dissolve the powder into water
4. Measure the pH and add 1 M KOH slowly until pH reaches 5.7-5.8
5. Add 70.128 g of NaCl slowly into the flask, and use stirring bar to dissolve NaCl into medium
6. Cover the flask with aluminum foil, and put autoclave tape on the aluminum foil
7. Autoclave the medium at 127 °C for 30 min.

Done by Alex

Supervised by Gina

Date: 20260727

Title: Plant seeds in circular ½ MS medium (neutral) plates

Purpose: plant seeds to stratify for 4 days before testing on CH bio protectants

Procedure:

1. Distribute (columbia-0 kyle seeds into individual tubes
2. Open bsc and put the circular 0.8% ½ ms medium plates from 0726, see template (horizontal 5 seeds x vertical 5 seeds = 25 seeds / plate), seeds in tube sterilized water and bamboo sticks in the BSC
3. Place the plate on the seed template
4. Stick the bamboo stick in sterilized water so it is easier to attach the seeds on the stick
5. Pick a seed with the bamboo stick and tap lightly on the marked place on the agar
6. Repeat step 5 until all seeds are planted (total of 14 plates)

Done by Abigail and Sophia Y.

Date: 20260727

Title: Make experiment set 6 salinity plates

Purpose: to prepare salinity plates for experiment set 6

Procedure:

1. Melt the solidified ½ MS agar (in tubes 30 mL) by putting the 2 tubes into microwave

For 300 mL of agar:

6 agar plates — only made 2 agar plates (control X control and 150 mM NaCl x control with the stored ½ MS solidified agar, 3M NaCl liquid and 100 mM trehalose liquid)

- Control (no trehalose) × 75 mM NaCl
- Control (no trehalose) × 100 mM NaCl
- 5 mM tre × control (no NaCl)
- 5 mM tre × 75 mM NaCl
- 5 mM tre × 100 mM NaCl
- 5 mM tre × 150 mM NaCl

1. Prepare the following
 - a. ½ MS medium powder (w/o sucrose): 0.6 g
 - b. MES buffer: 0.15 g
 - c. Distilled water: 300 mL
 - d. Sucrose (1%): 3 g
 - e. Agar powder (1%): 3 g
2. Add the contents into a 1000 mL flask
3. Use a hot plate to stir (with a magnetic stirring rod) until no sediments are left in the solution
4. Use the pH probe and measure the pH of the solution, make sure the pH is 5.7–5.8
 - a. If pH too low, add 0.1 M KOH slowly to increase it

5. Pour 300 mL of mixture out into another flask to separate into six flasks, label with:
 - a. [0 mM] NaCl, 5 mM trehalose
 - b. [75 mM] NaCl, 5 mM trehalose
 - c. [100 mM] NaCl, 5 mM trehalose
 - d. [150 mM] NaCl, 5 mM trehalose
 - e. [75 mM] NaCl, 0 mM trehalose
 - f. [100 mM] NaCl, 0 mM trehalose
6. Add 219.2 mg of NaCl into the flask labelled [75 mM NaCl]
7. Add 292.2 mg of NaCl into the flask labelled [100 mM NaCl]
8. Add 438.3 mg of NaCl into the flask labelled [150 mM NaCl]
9. To their respective trehalose concentrations, add:

Trehalose concentration	Amount of trehalose
0 mM	0 mg
5 mM	85.575 mg

10. Wrap the necks of the flasks with aluminum foil and stick a piece of indicator tape on top
11. Autoclave for 20 min at 127°C
12. Wait for pressure to reach 0 before retrieving flasks

Done by: Abby, Abigail
Supervised by: Gabriel
ELN by Naomi

Date: 20260728

Title: Transfer plants from growth chamber to salinity plates

Purpose: start salinity stress testing of experiment 6 (0, 75, 150, 150mM NaCl, 0, 5mM trehalose)

Procedure:

1. Take plates (incubated for 4 days) out of growth chamber
2. Open BSC
3. Place 2 vertical plate holders, tweezers, [unclear/crossed out], the salinity plates and plates that incubated for 4 days into the BSC
4. Choose plants that have similar root lengths to transfer after visually comparing the incubated plants
5. Lightly lift the chosen plant using a tweezer below the leaves
6. Place the plant on the salinity plates
7. Use a marker to draw a straight line along a ruler using the seed template
8. Align the root endpoints with the drawn line and use a marker to indicate the endpoint for further image analysis
9. Seal the plates with breathable tape and label the test group and transferred date

10. Place the vertical plates on the racks in the growth chamber at a slanted angle towards the back (front faces forward)

Done by: Sara, Sophie H, Abby T

Date: 20260728

Title: Change medium for hydroponic box 1, 2, 3, 4

Purpose: change medium to test on when we should add protectant

Procedure:

1. Take 4 new tip boxes, 3 M NaCl, CH protectant, and 1000 μ L pipette, P100 pipette gun, and serological pipettes to BSC
2. Add 290 mL of medium to Box 1, 2, 3 and 4 and use the beaker with a label of 290 mL to measure the amount of medium
3. Add 600 μ L of CH protectant into Box 1
4. Add 10 mL of 3M NaCl to Box 1, 2, 3, and 4
5. Take the 3D printed rack from the corresponding boxes and put them into the corresponding boxes
6. Label the new boxes
7. Wrap your phone with plastic wrap and spray alcohol on it and use it to take test pictures of the 4 boxes

Done by: Abby T, Audrey H

Date: 20260730

Title: Plant seeds for set C and stratify in hydroponic system

Purpose: heat stress testing

Procedure:

1. Create lids with 3 mm diameter holes, and autoclave for 20 min at 121°C
2. Prepare processed Eppendorf
3. Add 0.8% agar into 48 lids (270 μ L each)
4. Wait until agar solidifies
5. Add 2.2 mL of $\frac{1}{2}$ MS medium into 48 tube racks
6. Put lids onto the tube rack (hole facing up)
7. Use an autoclaved toothpick to pick one seed, and add the seed into the hole of the lid
8. Repeat step 6 until all lids have seeds
9. Close the lid of the tube rack
10. Wrap the tube rack with aluminium foil
11. Place the tube rack into the refrigerator at 4°C

Done by: Alex

Date: 20260730

Title: Prepare 50 mL 0.8% $\frac{1}{2}$ MS agar

Purpose: add agar into lids for new set of seeds

Procedure:

The following protocol is for 50 mL $\frac{1}{2}$ MS agar (0.8%)

1. $\frac{1}{2}$ MS powder (w/o sucrose): 0.115 g
2. MES buffer: 0.025 g
3. Distilled water: 50 mL
4. Sucrose: 0.5 g
5. Agar: 0.5 g
6. Add $\frac{1}{2}$ MS powder, MES buffer, sucrose, and distilled water into a flask
7. Shake the flask with hands until no sediments are in the solution
8. Use the pH probe to measure the pH of the solution, ensure the pH is between 5.7–5.8
9. If pH is too low, add 0.1 M KOH slowly to increase it
10. Add agar into the flask
11. Wrap the beaker with aluminium foil and stick a piece of indicator tape on top
12. Autoclave for 20 min at 127 °C

Done by: Alex

Date: 20260730

Title: Prepare 2 1500 mL $\frac{1}{2}$ MS medium

Purpose: prepare medium for changing medium and salinity test's medium

Procedure:

The following protocol is for 1500 mL $\frac{1}{2}$ MS medium

1. $\frac{1}{2}$ MS powder (w/o sucrose): 3.45 g
 - a. MES buffer: 0.75 g
 - b. Distilled water: 1500 mL
2. Add the contents into a beaker
3. Use hot plate to stir (using a magnetic stirring bar) until no sediments are left in the solution
4. Use the pH probe and measure the pH of the solution, ensure the pH is between 5.7 and 5.8
5. If pH is too low, add 1 M KOH to slowly increase it
6. Wrap the beaker with aluminium foil and stick a piece of autoclave indicator tape on top
7. Repeat steps 1–6 to prepare the second 1500 mL $\frac{1}{2}$ MS medium
8. Autoclave for 20 min at 121 °C

Done by: Alex

Date: 20260804

Title: Add CH bio protectant to exp7. Circular plates (pretreatment group: 0, CH bio / 100 NaCl, CH bio)

Purpose: to test when to add the protectant is the best

Procedure:

1. Dilute CH bio protectant by 1/500 (add 6986 μ L water + 14 μ L protectant) into the 15 mL tube in the BSC
2. Add 1mL of the diluted protectant into the 2 agar plates (0, CH bio/100 mM NaCl, CH bio)
3. Evenly distribute the liquid protectant by filling the plate sideways until all the plants are covered with a thin layer protectant
4. Seal the plates with tape and label the pretreatment date

Done by Sophia Y.

Supervised by Audrey H, Abby T., Abigail

Date: 20260804

Title: Agar exp set 6 fresh weight

Purpose: compare different groups' growth results

Procedure:

1. Using a tweezer, lift and pick all 9 *Arabidopsis thaliana* simultaneously from the agar plate in a single motion
2. Careful not to hurt the plants by squeezing the tweezer
3. Lift from below the leaf area
4. Put the 9 plants on a tissue paper, lightly press to remove the agar sticking to the plants
5. Careful not to press the leaf areas too hard
6. Place all 9 plants in a weighing plate, weight the fresh weight of *Arabidopsis thaliana* (a plate at a time)
7. Pick them up and put them(plants) in a 2 mL microtube, take care that the entire plant and the roots need to go in too
8. Label the group concentration as well as the respective fresh weight on the microtube
9. Repeat these steps for as many times as the sets of *Arabidopsis thaliana* (8 sets)

Done by Audrey H

Supervised by Abby T

Result:

Group	Weight(g)
150 mM NaCl	0.0175
Control	0.01756

100 mM NaCl 5mM Trehalose	0.0517
75 mM NaCl 5mM Trehalose	0.0655
75 mM NaCl	0.0655
5 mM Trehalose	0.1755
100 mM NaCl	0.0298
150 mM NaCl 5 mM Trehalose	0.0187

Date: 20260804

Title: Stratify seeds(x4 plates) on vertical plates

Purpose: heat stress testing(pretrest)

Procedure:

1. Prepare 4 12x12cm, ½ MS medium, pH 5.8, 1% square agar plates (neutral)
2. Sanitize your hands and all equipments:
 - a. ½ MS medium square agar plates
 - b. Autoclaved toothpicks
 - c. Sterile water
 - d. Planting grid line paper then place in BSC
3. Open the aluminum foil of the autoclaved toothpicks and take one out
4. Dip the tip of the toothpick in the sterile water
5. Attach seeds one at a time and tip it on neutral plates base on the template at the bottom
6. Seal with tape and label the date of vernalization.

Done by Abby K.

Date: 20260804

Title: Hydroponic experiment set 4 fresh weight

Purpose: collect data for *Arabidopsis thaliana* fresh weight analysis and compare between different test groups

Procedure:

1. Use tweezers and lift to pick the *Arabidopsis thaliana*(full plants) from hydroponic box
 - a. Careful not to hurt the plants by using the tweezer too hard)
2. Put the picked up plants in a 1.5 mL tube(remember to calibrate the tube)

Date: 20260805

Title: agar (exp set #6) chlorophyll extraction

Purpose: compare different groups' growth results

Procedure:

1. Take the microtubes from 0804
2. Dilute acetone to 80%, add 4 mL to each tube
3. Add 10 metal bits into each tube
4. Wait for the chlorophyll to extract, and use a vortex to crush the plants to speed up the process (done when the plants are no longer green)
5. Add the liquid to the 96 well, 200 μ L in each well, 3 wells for each sample
6. ELISA Reader, insert the 96 well, run the results through the equation
7. Make sure to select the correct wavelength
 - a. A633 -> used the wrong wavelength this time, it should be 663

Done by Audrey H, Phoebe, Sarah C

Date: 20260805

Title: Seed planting on agar

Purpose: grow the seeds on agar

Procedure:

1. Open the BSC
2. Use the seed template (circular plates) to plant 25 seeds on each agar plate
3. Use breathable tape to seal each of the agar plates
4. Label the vernalization date, in growth chamber date, and seed type
5. Wrap the agar plates with aluminium foil
6. Stratify in the 4°C refrigerator for 4 days and avoid light

Done by Audrey H, Naomi, Sophie H

Date: 20260805

Title: Add CH protectant and NaCl on horizontal agar plates(exp set #7)

Purpose: to test when to add protectant is the best (Co-treatment)

Procedure:

1. Use the following amount to apply for each plate

3 M NaCl	1667 μ L
CH biopeptide	1000 μ L (dilution rate 1/500 -> 6986 μ L sterile water + 14 μ L peptide)

2. Apply NaCl for the following plates

Co-treatment	100 mM NaCl, CH biopeptide (Day 0)
Pre-treatment	100 mM NaCl, CH biopeptide (Day -1)
Post-treatment	100 mM NaCl, CH biopeptide (Day 1)

3. Apply CH biopeptide to the following plates

Co-treatment	100 mM NaCl, CH biopeptide	(Day 0)
	0 mM NaCl, CH biopeptide	

Done by Audrey H

Date: 20260806

Title: Add CH protectant on horizontal agar plates (exp set #7)

Purpose: test CH protectant(posttreatment)

Procedure:

1. Use the following amount to apply for each plates CH biopeptide 1000µL (dilution 1/500-> 6986 µL of sterile water +14 µL of peptide)
2. Apply the protectant for the following plants
 - posttreatment
 - 100 mM NaCL , CH biopeptide (Day 1)
 - 0,CH biopeptide (Day1)

Done by Sophic C and Naomi

Supervised by Abigail

Date: 20260809

Title: Measure *Arabidopsis thaliana* fresh weight

Purpose: collect fresh weight data analysis for agar experiment set 7

Procedure:

1. Prepare seven 1.5 mL tube and label information on the agar plate (stratified, incubated, planted, and the date of measuring fresh weight)
2. Open the electronic scale and tare the scale after putting a labeled 1.5 mL tube inside.
3. Take out the tube
4. Carefully take out all the *Arabidopsis thaliana* from the corresponding agar plate (careful not to break the root) and place them inside the tube
5. Put the tube back onto the scale and measure fresh weight
6. Wait until the weighting value stops changing
7. Took the tube out and label the fresh weight on the tube
8. Repeat step 2~7 until all agar plate sets were finished measuring
9. Store the tubes in -80°C and wait for Chlorophyll extraction

Done by Naomi, Sophia L, Sophie H

Results:

Group	Freshweight (g)	Number of <i>Arabidopsis thaliana</i>
Control(0,0)	0.1537	23
Pretreatment(w/ 100 mM NaCl)	0.0203	24
Pretreatment(no salinity)	0.1013	23
Cotreatment(w/ 100 mM NaCl)	0.0168	25
Cotreatment(no salinity)	0.0406	25
Posttreatment(w/ 100 mM NaCl)	0.0234	25
Posttreatment(no salinity)	0.1830	24

Date: 20260819

Title: Prepare 420 mL 0.8% ½ MS agar

Purpose: add agar into lids for new set of seeds

Procedure:

The following protocol is for 420 mL ½ MS agar (0.8%)

1. ½ MS powder (w/o sucrose): 0.966 g
2. MES buffer: 0.21 g
3. Distilled water: 420 mL
4. Sucrose: 4.2 g
5. Agar: 3.36 g
6. Add ½ MS powder, MES buffer, sucrose, and distilled water into a flask
7. Shake the flask with hands until no sediments are in the solution
8. Use the pH probe to measure the pH of the solution, ensure the pH is between 5.7–5.8
9. If pH is too low, add 0.1 M KOH slowly to increase it
10. Add agar into the flask
11. Wrap the beaker with aluminium foil and stick a piece of indicator tape on top
12. Autoclave for 20 min at 127 °C

Done by: Abigail Abby K

Date:20260903

Title: Enzymatic Hydrolysis of Soybean waste using Papain

Purpose: Hydrolyze soybean waste

Procedure:

1. Use the blender to make the soybean waste into smaller pieces(powder)
2. Pour the soybean powder into a beaker
3. Measured 5 g of soybean powder using a scale
4. Take another beaker and measure 100 mL of distilled water
5. Pour the 5 g soybean powder into the distilled water beaker
6. Use a pH meter to measure the pH, add OH to make its pH 7.0
7. Use a plastic wrap to cover the beaker and secure it with a rubber band
8. Water bath the mixture at 40 °C for 20 min
9. Peel the papaya skin and cut it into smaller pieces
10. After waterbathing for 20 min, take out the beaker and add the papaya skin into the beaker
11. Put the beaker back to water bath at 40 °C for 2 hr
12. Use the water bath to heat the beaker at 85 °C for 10 min
13. Aliquot the supernatant into 24 2 mL microtubes
14. Centrifuge the microtubes at 4 °C for 10 min
15. Open the Bunsen burner to maintain an aseptic field
16. Use a pipette to transfer the supernatant into a syringe fitted with a sterile filter, and filter the solution into a tube
17. Label the tube and store both the tube and the remaining beaker in the refrigerator

Done by Renee and Sophie L

Date:20260911

Title: Hay infusion

Purpose: Grow *Bacillus subtilis*

Procedure:

1. Use the scale to measure 5 g of sugar and mix it with 100 mL of distilled water
2. Disinfect all equipment and move it into the BSC for aseptic operation
3. Use the pipette controller with a serological pipette to draw the solution into a syringe fitted with a sterile filter
4. Filter the sugar solution into a 50 mL centrifuge tube
5. Use the pipette controller to draw the 0821 autoclaved hay infusion in the flask into 2 centrifuge tubes
6. Centrifuge the hay infusion tubes for 10 min
7. Take 7 culture tubes and label them as follows:
5 mL Hay Infusion, 5 mL Sugar Solution, 5 mL Soybean, 5 mL LB,
1 : 1 : 1, 1 : 2 : 1, 1 : 1 : 2(Hay, Soybean, Sugar)
8. Take the Hay infusion, Sugar Solution, and 0903 extracted soybean solution and make the solution as labeled on the tubes
9. Use the pipette to add 200 µl of WB800 *Bacillus subtilis* into each tube

10. Centrifuge all the tubes overnight

Done by Sophia L

Date:20260918

Title: Stratify 12 circular plates in 0.6% agar

Purpose: For ACCD testing

Procedure:

1. Distribute columbia-0 kyle seeds into individual tubes
2. Open bsc and put the circular 0.6% ½ ms medium plates, 3D printed template (horizontal 8 seeds x vertical 5 seeds = 40 seeds / plate), seeds in tube sterilized water and bamboo sticks in the BSC
3. Place the plate on the seed template
4. Stick the bamboo stick in sterilized water so it is easier to attach the seeds on the stick
5. Pick a seed with the bamboo stick and tap lightly on the marked place on the agar
6. Repeat step 5 until all seeds are planted (total of 14 plates)
7. Wrap the circular plates with breathable tape and label the stratification and incubation date on the plates with marker
8. Wrap the plates with aluminum foil to avoid light and place in 4°C for 4 days stratification

Done by Abby T and Abigail

Date:20260925

Title: Hay infusion

Purpose: Grow *Bacillus subtilis*

Procedure:

1. Cut the sugarcane waste to 5-10 cm small pieces
2. Put 10 g of sugarcane and 100 mL of distilled water into a beaker
3. Autoclave for 30-40 min
4. Pour the sugarcane extract into 2 50 mL centrifuge tubes and ensure no sugarcane waste gets into the tubes

Done by Sophia L

Date:20260928

Title: Hay infusion

Purpose: Grow *Bacillus subtilis*

Procedure:

1. Disinfect all equipment and move it into the BSC for aseptic operation
2. Use 12 2 mL microtubes and fill it up with the supernatant
3. Put it into the centrifuge
4. Take out and filter the supernatant using a syringe and filters

Media and Blank Preparation

1. Single Component Tubes
 - a. Culture tubes: Add 5 mL of the specified solution
 - b. 2 mL Blank Tubes: Add a scaled down volume (e.g. 1.5-2.0 mL) of pure solution

2. 1 : 1 : 1 Ratio Tubes

- a. Culture Tube: 1.67 mL Hay + 1.67 mL of Soybean + 1.67 mL of Sugarcane
- b. 2 mL Blank: 0.5mL Hay + 0.5 mL soybean + 0.5 mL Sugarcane

3. 1 : 2 : 1 Ratio Tubes

- a. Culture Tube: 1.25 mL Hay + 2.50 mL of Soybean + 2.50 mL of Sugarcane
- b. 2 mL Blank: 0.375mL Hay + 0.75 mL soybean + 0.375 mL Sugarcane

4. 1 : 1 : 2 Ratio Tubes

- a. Culture Tube: 1.25 mL Hay + 1.25 mL of Soybean + 2.50 mL of Sugarcane
- b. 2 mL Blank: 0.375mL Hay + 0.375 mL soybean + 0.75 mL Sugarcane

5. 1 : 1 : 2 Ratio Tubes

- a. Culture Tube: 1.25 mL Hay + 1.25 mL of Soybean + 2.50 mL of Sugarcane
- b. 2 mL Blank: 0.375mL Hay + 0.375 mL soybean + 0.75 mL Sugarcane

Done by Sophia