**Names of each student:** Kaia and Jasmine

**Date:** 9/25/2023

**Protocols Performed:**
Located the E.coli, JM109. Made LB agar and broth
LB Broth:

1. Weigh 5g peptone, 2g yeast, and 5g NaCl into a weigh boat.
2. Pour the powders into a glass bottle containing 500mL of diH2O.
3. Mix the mixture and label it “Knockout | iGEM | 7-12-2023 LB broth.”

Agar:

1. Weigh 5g peptone, 2g yeast, 5g NaCl, and 5g agar onto weigh boat.
2. Add the powders into a glass jar containing 500mL of diH2O.
3. Mix the mixture. Do NOT add antibiotic (ampicillin) yet.
4. Label it “ knockout | iGEM | 7-12-2023 Agar without Ampicillin.”

Place the two glass bottles into the autoclave for 30 min.

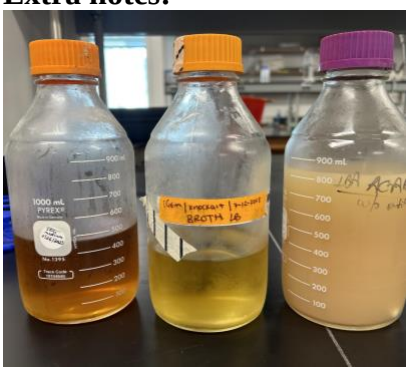
**Purpose:**
The LB agar will be used to culture the E.coli and will also be used to plate a few.

**Supplies used/Materials:**
Peptone, yeast, NaCl, weigh boat, agar, diH2O, 500mL glass bottle, autoclave.

**Results:**
N/A

**Conclusions/Next Lab goals:**
Pour the LB agar into the plates.

**Extra notes:**



**Names of each student:** Tracy

**Date:** 9/26/2023

**Protocols Performed:**
Pouring LB into plate.

1. Completely melt the LB agar on a hot plate.
2. Place the plates upside down before pouring.
3. Keep an open flame and place the plates and agar close to it. Do not open the plates until pouring time.
4. Keeping close to the flame, use one hand to first run the agar bottle through the flame and the other hand to carefully open the culture plate's lid. Pour until the agar is covering all the areas of the plate with no air bubbles.
5. Repeat step 4 sixteen more times. Stay close and below the flame.
6. Label the stack "iGEM Fall 2023 No Amp. KO 9/26/2023.
7. Store in refrigerator for the next use.

**Purpose:**
To prep the culture experiment by pouring the LB agar that was previously made into the culture plates with no ampicillin.

**Supplies used/Materials:**

LB Agar, hot plate, open flame, lighter, 17 culture plates.

**Results:**

N/A

**Conclusions/Next Lab goals:**

Get started with SOC prep.
**Extra notes:**

**Names of each student:** Kaia and Jasmine

**Date:** 9/27/2023

**Protocols Performed:**

SOC media
First prepare the solutions:

Per liter: To 950 mL of deionized H20, add:
*Tryptone* 20g
*Yeast extract* 5g
*NaCI* 0.5g

250 mM solution of KCI.
*KCI*: 1.86g
*Deionized H2O*: 100 mL

2 M MgCl2.
*MgCl2*: 19g
*Deionized H2O*: 90ML

1 M solution of glucose.
*Glucose*: 18 g
*Deionized H2O*: 90ML

Adjust the PH of all solutions to 7.0 and then sterilize all solutions by autoclaving for 20 min at 15 psi (1.05 kg/cm2) on liquid cycle. Just before use, add 10 ml of a sterile solution of 250 mM KCl, 5 mL of a sterile solution of 2 M MgCl2 and 20 mL of a sterile 1 M solution of glucose.

**Purpose:**
SOC will be used run the plasmid and bacteria transformation.

**Supplies used/Materials:**

Tryptone, yeast, NaCl, KCl, MgCl2, glucose, diH2O, pH meter, autoclave

**Results:**

**Conclusions/Next Lab goals:**

Begin heat shock.
**Extra notes:**

**Names of each student:** Nancy

**Date:** 9/28/2023

**Protocols Performed:**
Electroporation trial run

**Purpose:**

To test that the electroporation would be successful, a trial test was ran with a different E.coli strain.

**Supplies used/Materials:**

**Results:**

**Conclusions/Next Lab goals:**

Heatshock.
**Extra notes:**

**Names of each student:** Kaia and Jasmine

**Subteam name:** Knockout

**Date:** 10/4/2023

**Protocols Performed:**

Heatshock

1. Thaw chemically competent bacterial cell such as JM109 on ice.
2. Combine 40 uL of JM109 with 1uL (1ng/ uL) of plasmid to 1.5mL eppendorf tube.
3. Incubate cells on ice for 35 minutes.
4. After ice incubation, place the samples into 42℃ water bath for 4 seconds.
5. Put the samples back to ice for 2 minutes to recover.
6. Quickly take the eppendorf tube out and immediately add 250uL of SOC medium.
7. Place the eppendorf tube into 37℃ shaking water incubator for 1hr and 2omin at 250rpm.
8. After shaking, streak 150uL of solution onto an LB agar plate with the respective antibiotic (ampicillin).

**Purpose:**

To separate the plasmid from E.coli

**Supplies used/Materials:**

Ice bath, eppendorf tube, ampicillin, SOC, shaking incubator, JM109, LB agar plate.

**Results:**

**Conclusions/Next Lab goals:**

**Extra notes:**

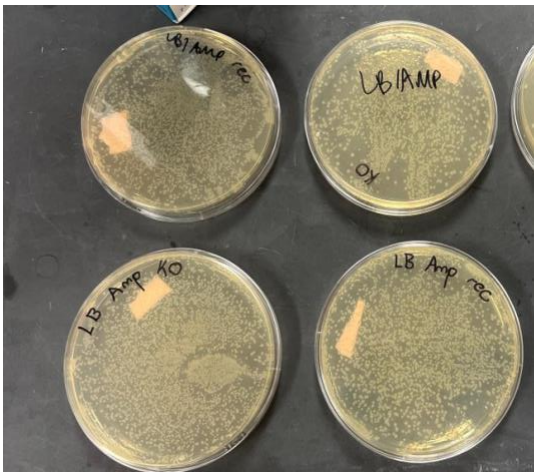
**Names of each student:** Elizabeth Plumart

**Date:** 10.07.23

**Protocols Performed:** Transformation (heat shock)

**Purpose:** Transform recET and ackA knockout into E. coli

**Results:** Success




**Conclusions/Next Lab goals:** Overnight culture for mini-prep

**Extra notes:**