2. Week May 2-8

FRIDAY, 5/7/2021

Attendace

	A	B	C
1	**Name**	**Time in**	**Time out**
2	Valeria Martinez De Leon	10:30 am	2:15pm
3	Elimar Uzcategui Calderón	10:30 am	1:00 pm
4	Luis Garcia Cruz	10:30 am	2:15 pm
5	Solimar Muniz Acevedo	10:30 am	1:30 pm
6	Frances Acevedo Torres	10:30 am	2:15 pm
7	Iris Sanchez Lebron	10:30 am	2:15 pm
8	Rigo Espina Santiago	10:30 am	2:15 pm

Place of work: University of Puerto Rico - Mayaguez - Biology Building - Microbiology Laboratory B-210

Experiment: *DNA Extraction Workshop (Part II)*

Objectives:

- Complete DNA extraction from a fresh *Escherichia coli DH5a* culture and practice gel electrophoresis procedure.

Protocols:

Graduate student advisor Victor Lopez conducted the second part of DNA extraction and electrophoresis workshop for the IGEM RUM Biology Team, to complete the previous workshop of techniques that will be used throughout the project. The laboratory was cleaned and participants prepared according to aseptic techniques. Biology Team Members consulted the protocol that was used for April 24,2021 and determined the starting point. Samples were recovered from the -20 C fridge at laboratory B-266.

Protocol continuation for extraction of genomic DNA:

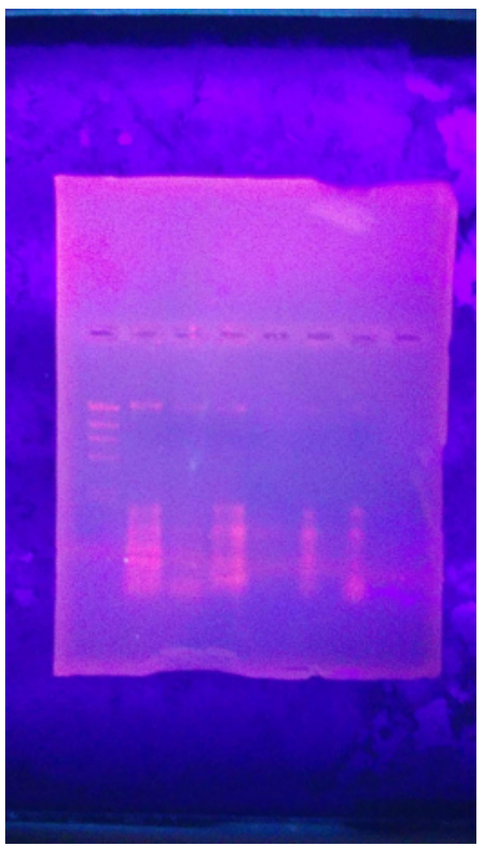
1. Unfreeze samples from DNA extraction. Wash with ethanol 70%.
2. Discard supernatant and dry in Speed Vac, resuspend in 50uL of TE 1X.
3. Agarose gel preparation and electrophoresis protocol

Protocol for gel electrophoresis

1. Measure 1.0g of agarose. Mix agarose powder with 100mL TAE 1X in a microwavable flask.
2. Microwave for 1-3 minutes until agarose is completely dissolved (but not over boil the solution as some buffer would evaporate and thus alter the final percentage of the gel).
3. Let agarose solution cool down to about 50 C (about 5 minutes). Add ethidium bromide (EtBr) to a final concentration of 0.2-0.5 ug/mL. (If added, must be added to running buffer as well)
4. Pour the agarose into a gel tray with the well comb in place. Pour slowly to avoid bubbles that could disrupt the gel. Any bubbles could be pushed away from the well comb or towards the side/edges of the gel with a pipette tip.
5. Place newly poured gel at 4 C for 10-15 minutes or let sit at room temperature for 20-30 minutes until it has completely solidified.
6. Add the loading buffer to each of the DNA samples.
7. Once solidified, place the agarose gel into the gel box (electrophoresis unit)
8. Fill gel box with TAE 1X until gel is covered
9. Carefully load the molecular weight ladder into the first lane of the gel.
10. Carefully load the samples into the additional wells of the gel.
11. Run the gel at 80-150V until the dye is approximately 75-80% of the way down. Typical run time is 1-1.5 hours, depending on gel concentration and voltage.
12. Turn off power, disconnect the electrodes from the power source and then carefully remove the gel from the box.
13. Using any device that has a UV light, visualize your DNA fragments.

Results:

Resulting bands from Gel Electrophoresis



Materials

- *E. coli* DH5a DNA samples
- Electrophoresis chamber
- Centrifuge
- Micropipettes
- Micropipette tips
- Parafilm
- Gloves

Reagents

- Lysis Buffer
- Tris-Acetate-EDTA (TAE 1X)
- *Lambda-HindIII*
- Loading dye
- Cold absolute ethanol
- TE 1X

Materials storage:

- TAE 1X, Agarose, micropipettes,electrophoresis chamber, centrifuge, gloves, isopropanol and micropipette tips were returned to B-256.
- Ethanol was returned to B-266.
- DNA extraction leftovers and Lambda were stored in a green box inside -20 C fridge at B-266.
- Pink and yellow “gradillas” were returned to B-210.

Reference: Rios Velazquez, C., López, G., Rivera, M., Flores, K., Cardona, V., Torres, I., . . . Rullán, J. (2013). Manual de laboratorio de Genética de Bacterias (3rd ed.). Mayaguez, PR: UPRM.

3. Week May 23-30

WEDNESDAY, 5/26/2021

Attendance:

Table1

	A	B	C
1	Name	Time in	Time out
2	Marieli Ruiz Cortés	10:30 am	5:10pm
3	Elan Reynoso	10:30 am	5:10pm
4	Luis Garcia	12:10 pm	5:10pm

Place of work: University of Puerto Rico - Mayaguez - Biology Building - B-266

Experiments:

1. LB Broth

Objectives:

1. Do inventory
2. Autoclave Micropipettes tips, Assay Tubes
3. Prepare 500ml of LB Broth

Protocols:

a. LB Prepatarion Protocol

1. Calculate the grams required to make 25 Petri dishes containing 20 ml of medium each.
2. Add 500 mL of dH2O to a 1000 mL flask and place a magnet.
3. Add 12.5 g of agar to the water while gently shaking and heating in a hot plate/stirrer.
4. Shake continuously until homogenization.
5. Cover the flask and place a piece of sterilization indicator (autoclave tape).
6. Sterilize the flask in an autoclave at a cycle of 15 min at 121 ° C and 15 p.s.i. This step will be carried out with the laboratory technique. Once the cycle is finished, wait for the temperature and pressure in the autoclave to drop before you can open it.
7. Store at room temperature.

Materials:

- 500mL of dDNA
- 12.5mL Liria Bertani Broth, Miller
- Spatula
- Scale
- 1000mL Flask

Results and their descriptions

- Obtained 500mL of LB broth.

Reference: Rios Velazquez, C., López, G., Rivera, M., Flores, K., Cardona, V., Torres, I., . . . Rullán, J. (2013). Manual de laboratorio de Genética de Bacterias (3rd ed.). Mayaguez, PR: UPRM.