1. Week April 18-24

SATURDAY, 4/24/2021

Attendace:

	A	B	C
1	**Name**	**Time in**	**Time out**
2	Valeria Martinez De Leon	10:20 am	3:00 pm
3	Adriana Arroyo Fernandez	10:20 am	3:00 pm
4	Luis Garcia Cruz	10:20 am	3:00 pm
5	Solimar Muniz Acevedo	10:20 am	3:00 pm
6	Frances Acevedo Torres	10:20 am	3:00 pm
7	Iris Sanchez Lebron	10:20 am	3:00 pm
8	Rigo Espina Santiago	10:20 am	3:00 pm

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

Experiment: *DNA Extraction Workshop (Part 1)*

Objectives:
- Performed a DNA extraction from a fresh *Escherichia coli DH5a* culture.

Protocols:
Graduate student advisor Victor Lopez conducted a DNA extraction and electrophoresis workshop for the IGEM RUM Biology Team to begin practicing techniques that will be used throughout the project. The laboratory was cleaned and participants prepared according to aseptic techniques. A brief introduction to the topic was given. Each participant and graduate student practiced their own sample (a total of 8 samples).

Protocol for extraction of genomic DNA:
1. 1.5 mL sample of a fresh Escherichia coli culture was introduced into a sterile microtube using a micropipette.
2. The samples were centrifuged for 5 minutes at 13,000 rpm.
3. Supernatant was removed from the microtube and the pellet was resuspended with 200uL of lysis buffer and 66 uL of NaCl.
4. The samples were heated for 10 minutes at 65 C for further cell lysis (Optional)
5. Samples were centrifuged for 10 minutes at 13,000 rpm.
6. The supernatant was transferred to a new microtube, added 1 volume of chloroform and mixed by inversion.
7. Add 2 volumes of absolute ethanol (cold) or 0.6 volumes of isopropanol to precipitate DNA for 15 minutes, followed by centrifugation for 5 minutes at 13,000 rpm. If placed on ice or at -20 C, more DNA could be precipitated.
8. Supernatant was discarded and 500uL of 70% ethanol was added to wash the DNA. Afterwards, centrifuge for 5 minutes at 13,000 rpm.

Materials
- Recent *E. coli* culture (broth or solid medium)
- RNAse
- Electrophoresis chamber
- Centrifuge
- Micropipettes
- Micropipette tips

Notes for this protocol:
- Volume was approximately 266uL
- Modification: Isopropanol was used during the experiment at room temperature. Each participant calculated the volume of isopropanol to be added individually.
- Modification: Due to lack of time, the protocol was stopped at this point and was determined to be continued in the next laboratory visit. The 8 samples were stored inside a small green box at the -20 C fridge in the B-266 laboratory.

Materials storage:
- Micropipettes, micropipette tips, and leftover NaCl, Chloroform and TE 1X in microtubes were stored in the closet at laboratory B-256 for future use.
- All reagents were borrowed from and returned to B-266.

Materials to be returned:
- Pink “Gradilla” with leftover reagents at B-256 closet belongs to B-210.
- Yellow “gradilla” where samples were taken to B-266 belongs to B-210.

To do for the next laboratory visit:
- [ ] Unfreeze samples from DNA extraction. Wash with ethanol 70% again and proceed the protocol (Step 9 and 10)
  - Discard supernatant and dry in Speed Vac, resuspend in 40-70uL of TE 1X.
  - Store at -20 C.
- [ ] Agarose gel preparation and electrophoresis protocol

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.

THURSDAY, 5/6/2021

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.

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

Notes for this protocol:
- Molecular weight ladder used was *Lambda-HindIII*
- 1 uL of loading dye was set to a parafilm strip. 5uL DNA sample was added and mixed with loading dye on the parafilm strip. The micropipette was set to 6uL and samples were added to the wells.

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.