The Effect of Light on the Epitranscriptome of Plants

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Research Plan/Project Summary Instructions

a. **RATIONALE:**

Plants are dependent on proper light reception for two main reasons. The first is that light perception conveys different cues about geographical location, seasonal timing, and other important aspects of their surroundings. Being able to properly detect changes in light is vital towards proper development as plants are not motile and must be able to adapt quickly to surroundings. The second main reason is that plants utilize light for photosynthesis to provide the main source of energy for green organisms (Artz, et al., 2019).

It is specifically important to study how plants respond to light for agricultural improvements. For example, shade avoidance is a natural botanical adaptation, where plants differ in anatomical development to avoid the shade cast by surrounding plants (Casal, 2012). This mechanism allows for plants to grow more densely, but results in lodging, where the petioles bend. Bent petioles not only make harvesting crops more difficult but also produce less hardy crops as petioles elongate and are more vulnerable to environmental disturbances such as excessive wind. Phytochromes have been discerned to be the photoreceptors mainly responsible for the shade avoidance response (Moglich, Yang, Ayers, and Moffat, 2010).

Most land plants possess two main growth patterns: photomorphogenesis and skotomorphogenesis. Photomorphogenic plants grow in response to light, and develop shorter hypocotyls (etiolation). In contrast, skotomorphogenic plants develop in the absence of light, with elongated petioles, a pale yellowish color, and a bent apical hook. The genetic mutations and photoreceptors responsible for skotomorphogenesis have not all been determined yet, and it is important to determine those involved as it would implicate that they are necessary for proper light perception overall.

Different photoreceptors absorb the most light in specific wavelength ranges. For example, phytochromes, which produce the shade avoidance response, are most active

under high blue light as well as red light (Galvao and Frankhauser, 2015). Cryptochromes, which are mainly responsible for seedling de-etiolation and flowering (Artz, et al., 2019) and have been known to be involved in light perception (Ahmad, Jarillo, Smirnova, and Cashmore, 1998), are most active in blue light (Galvao and Frankhauser, 2015).

A protein of interest is ECT2, or evolutionarily conserved C-terminal region 2. The lack of this protein in the genotype *ect2 v*has been proposed to produce an effect similar to the absence of CRY2, a cryptochrome photoreceptor, where plants do not produce a skotomorphogenic response. ECT2 Vis specifically of interest because of its relationship with m^6A, or N^6-methyladenosine. The RNA-protein complex that results from m^6A has been demonstrated to contribute to many critical RNA modifications and functions such as the nuclear export of RNA, splicing of introns, translation, RNA stability, and localization. Prior to the discovery of this RNA modification, most were known to be static, in contrast to m^6A. However, it has also been discovered that m^6A can be demethylated (Jia, et al., 2011). Currently, the only function in plants it has been determined to have is in RNA stability (Anderson, et al., 2018). So, whether m^6A also so greatly impacts RNA translation in plants as it does in animals is of great interest.

b. **RESEARCH QUESTION(S), HYPOTHESIS(ES), EXPECTED OUTCOMES:**

- Research Questions
    - Does ECT2 phenotypically resemble CRY2? So, does this mutation fail to produce any skotomorphogenic growth pattern?
    - If ECT2 phenotypically resembles CRY2, do the genetic pathways interact to produce photomorphogenic growth?
- Expected Outcomes
    - ECT2 will be significantly different from wildtype hypocotyl length (by t-test, where $p<0.05$), indicating that it is responsible for a significant photomorphogenic response.

- Interactions between ECT2 and CRY2 will be visible with fluorescence, as each will be attached to one half of a yellow fluorescent protein (YFP). If these genes naturally interact in the plant cells, the YFP fragments should join, producing fluorescence in plant nuclei.

c. **Procedures:**

- Preparing Seed Aliquots for Hypocotyl Measurements
    1. Fill one sterile Falcon 50 mL conical centrifuge tube with 100% ethanol.
    2. Fill a second sterile Falcon 50 mL conical centrifuge tube with 35 mL of 100% ethanol and 15 mL of distilled water (for a 70% ethanol mixture).
    3. Add 50 µL of Triton X-100 detergent to the mixture from step 2.
    4. Aliquot approximately fifty seeds of each of the genotypes (wildtype, *cry1*, *cry2*, *cry1cry2*, and *ect2*) from the Columbia (Col) accession into five individual autoclaved 1.7 mL Eppendorf tubes.
    5. Transfer 800 µL of the 70% ethanol with the P2000 pipette to each aliquoet.
    6. Rotate the seed aliquots in a VWR tube rotator for ten minutes.
    7. Immediately remove the 70% ethanol with a P2000 pipette under a flow hood.
    8. Add 800 µL of the 100% ethanol mixture from step 1 to each seed aliquot with the P2000 pipette.
    9. Rotate the Eppendorf tubes for five minutes.
    10. Use the P2000 pipette to remove the ethanol under the flow hood from each Eppendorf tube.
    11. Add 500 µL of distilled water to each seed aliquot.
    12. Use the P2000 pipette to pump water in and out of the pipette.
    13. Hold the Eppendorf tube sideways, so that the seeds settle along one side of the tube.

14. Label three square Petri dishes so that one row on each Petri dish corresponds to one genotype, so that there are five rows for WT, *cry1*, *cry2*, *cry1cry2*, and *ect2*.
15. Set the P2000 pipette to 400 µL for the 500 µL in each Eppendorf tub, and draw up seeds along the side of the Eppendorf tube.
16. Hold the pipette vertically so that the dense seeds gather in the bottom of the pipette tip.
17. Dispense approximately fifteen seeds per row to the corresponding genotype on each of the three Petri dishes by touching the surface of the Murashige and Skoog (MS) medium on the Petri dishes.
18. Seal each of the Petri dishes closed with sterile surgical tape and then wrap the plates in two layers of aluminum foil.
19. Place the plates in continual darkness at 4ºC for three days.

- Growing Arabidopsis Plants in Blue Light
    1. Remove the Petri dishes from the cold room.
    2. Insert the probe of light meter in the growth chamber, and adjust the blue light with the control panel until the light meter reads approximately 10 µmol/square meters/s (or whatever desired fluence rate).
    3. Remove the aluminum foil from the Petri dishes in the growth chamber.
    4. Keep the seedlings in the growth chamber (keep it closed for the entirety of this period) for five days.
- Measuring the Arabidopsis Plant Hypocotyls
    1. Remove the Petri dishes from the growth chamber.
    2. Remove the surgical tape from each Petri dish and take the lids off.
    3. Use a pair of tweezers to gently push the plants flat against the agar.
    4. Place the Petri dishes face down (so that the lidless side is flat on the scanner) on the Epson scanner.
    5. Make sure that the ruler is on the scanner, to use for reference distance later.

6. Cover the ruler and Petri dishes with a black cloth to block out light.
7. Preview the images and make sure that the seedlings are visible from the scanner images. Wipe any condensation with a Kim Tech wipe as necessary.
8. Take pictures with the scanner and save images as TIF files.
9. Download images and upload into JImage.
10. Use the straight line setting to measure one centimeter on the ruler in the image. Go to "Analyze", then "Set Scale", and set known distance to 1.0 centimeter and set this pixel to centimeter size as "Global".
11. Use the segmented line setting to measure the hypocotyl distances.
12. Copy the data and paste it into Excel to later analyze.

- Bacterial Inoculation
    1. Remove the antibiotics rifampicin, gentamicin, and kanamycin from the fridge and place them to thaw on a heat block at 37ºC (human body temperature).
    2. Microwave the LB (Luria-Bertani) agar for approximately two minutes. Watch to ensure that it does not explode.
    3. Pour the LB agar into three 50 mL Falcon tubes under a flow hood.
    4. Dispense 50 µL of each antibiotic into separate Falcon tubes (antibiotics are concentrated 1000x).
    5. Mix the Falcon tubes by inverting them approximately ten times each.
    6. Pour the mixture from each Falcon tube onto separate round Petri dishes.
    7. Leave the dishes were to solidify for twenty minutes.
    8. Dispense the agrobacteria on each Petri dish lid in a flowhood with a pipette.
    9. In the flowhood, bend the pipette tip perpendicular to miniprethe pipette on Petri dish lids.
    10. Spread the bacteria around the agar on the Petri dish with the pipette.

11. Leave the bacteria to inoculate overnight, but avoid inoculating only in water.

- Preparing the MES Buffer for Tobacco Infiltration
    1. Place a stir bar in a clean 500 mL beaker.
    2. Fill the beaker with 400 mL of deionized and filtered water.
    3. Place the beaker on the magnetic stirrer.
    4. Place a sterile plastic boat on the balance and set the mass to 0.0 gram.
    5. Use a spatula to scoop 0.48 grams of MES (2-ethanesulfonic acid) hydrate into the plastic boat.
    6. Use 0.48 grams to produce a 10 mM MES solution of volume 500 mL.
    7. Dispense the MES hydrate in the 500 mL beaker on the magnetic stirre.
    8. Dispose of the spatula and throw out the plastic boat.
    9. Place a second sterile plastic boat on the balance and set the mass to 0.0 grams.
    10. Use a clean spatula to scoop 0.98 grams of magnesium chloride into the plastic boat.
    11. Dispense of the magnesium chloride in the solution in the 500 mL beaker on the magnetic stirrer.
    12. Rinse the pH meter probe with distilled water, and gradually add potassium hydroxide until the pH is adjusted to 5.6.

- Tobacco Infiltration
    1. After incubating the bacteria, remove them from the cold room and spin them down in a centrifuge at 3800 rpm for fifteen minutes.
    2. Discard the supernatant from each sample.
    3. Set aside a 50 mL aliquot of the 500 mL of MES buffer.
    4. Resuspend the bacteria in MES buffer and incubate them for one hour in semi-dark conditions.

5. Water the three tobacco plants well with a spray bottle one hour before the experiment.
6. After incubating the bacteria, spin them down in the centrifuge at 3800 rpm for fifteen minutes and discard the supernatant from each sample.
7. Resuspend the bacteria in MES buffer.
8. Mix three different combinations.
    a. Each mixture will receive 2 mL of cV-ect2, and 1 mL of p19, the anti-silencing strain.
    b. Each mixture will receive 1 mL of either nvCRY1, nvCRY2, or nv-mock.
9. Use a 1.0 mL HSW syringe to infiltrate the abaxial side of the tobacco leaves, and select leaves that were not too old or too young, and that are large in surface area.
10. Infiltrate one plant with the mixture with nvCRY1.
11. Infiltrate one plant with the nvCRY2 mixture.
12. Infiltrate the the third plant with the nv-mock mixture.

- Genomic DNA Preparation from Plants for PCR Analysis
    1. Remove the *ect2* seedlings from the growth chamber.
    2. Use a pair of tweezers to pick six leaves of approximately 0.5 x 0.5 cm size.
    3. Put the leaves in 1.7 mL Eppendorf tubes and then store them in frozen nitrogen.
    4. Grind the samples to a fine powder using an autoclaved pestle to destroy the cell wall.
    5. Add 400 µL of EB buffer to the samples in the Eppendorf tubes.
    6. Vortex the samples in step five for five seconds each.
    7. Let the samples to sit at room temperature in between vortexing.
    8. Centrifuge the Eppendorf tubes at 13,000 rpm for three minutes.
    9. Transfer 300 µL of the supernatant into a new tube.

10. Add 300 µL isopropanol to the seedling samples.
11. Mix each Eppendorf tube by inverting.
12. Incubate the Eppendorf tubes at room temperature for two minutes.
13. Centrifuge the tubes at 13,000 rpm for ten minutes.
14. Discard the remaining supernatant, and leave the tubes inverted on a Kim Wipe for five minutes to dry.

- Genotyping - PCR setup
    1. Thaw primers at room temperature (not at 37 ºC which would denature primers).
    2. Put primers and GoTaq on ice.
    3. Create two master mixes for each of the primers (according to the chart below), with five times the necessary amount per sample in order to have enough master mix for six reactions.

       MM stands for master mix, Green MM speeds up the PCR and alleviates the necessity to later add a dye for the gel electrophoresis.

Final Concentration	**Component**	**Stock concentration**	**MM x 1**	**MM X 5**
	MQ		3.0	15.00
1 x	Green MM	2X	5.0	25.0
500 nM	Fw Primer 1	10 µ,	0.50	2.50
500 nM	RV Primer 1	10 µM	0.50	2.50
	Template		1.0	n.a.
	GoTaq (1 min/kb)		n.a	n.a
∑			10.0	45.0
MM/Cup				9.0

    4. Vortex then centrifuge each sample for 3-4 seconds.

5. Label tubes for PCR on the side of the Eppendorf tubes.
6. Spin down the Eppendorf tubes in a small centrifuge for several seconds.
7. Conduct PCR with the thermal cycler, repeating the cycles 34 times (35 cycles in total). Enter the volume (10 µL) into the thermal cycler, and adjust the lid to the proper temperature (105ºC). Close all lids and press "Run".

   The PCR cycles:

Temperature	95ºC	95ºC	48ºC	72ºC	72ºC	15ºC
Time	5 minutes	30 s	30 s x 35	1 minute 30 s	5 minutes	unlimited

8. Throw away the master mixes in the biological hazardous waste disposal and return the primers and master mixes to the freezer.

- Preparing for gel electrophoresis
  1. Add 100 mL of 50x TAE buffer (prepared by technician at Cold Spring Harbor Laboratory) to the desired amount of 1% agarose gel.
  2. Microwave the mixture for 1-2 minutes. Swirl a bit to examine if the agarose requires more microwaving.
  3. Add 5 µL of ethidium bromide to the agarose mixture, then swirl it.
  4. Fill ⅔ of the tray with the agarose gel, and let it solidify for twenty minutes.
  5. Discard the extra mold, and place the gel in the tank so covered by the buffer.
  6. Load 10 µL of the DNA into each well and run the PCR.
- Plasmid Miniprep
  1. Add 24 mL of 95% ethanol to the 6 mL of Monarch Plasmid Wash Buffer 2.

2. Pellet 1-5 mL bacterial culture (not to exceed 15 OD units) by centrifugation for 30 seconds. Discard the supernatant.
3. Resuspend pellet in 200 µL Plasmid Resuspension Buffer (B1). Vortex or pipet to ensure cells are completely resuspended. There should be no visible clumps.
4. Lyse cells by adding 200 µl Plasmid Lysis Buffer (B2). Invert tube immediately and gently 5–6 times until color changes to dark pink and the solution is clear and viscous. Do not vortex! Incubate for one minute.
5. Neutralize the lysate by adding 400 µl of Plasmid Neutralization Buffer (B3). Gently invert tube until color is uniformly yellow and a precipitate forms. Do not vortex! Incubate for 2 minutes.
6. Clarify the lysate by spinning for 2–5 minutes at 16,000 x g.
7. Carefully transfer supernatant to the spin column and centrifuge for 1 minute. Discard flow-through.
8. Re-insert column in the collection tube and add 200 µl of Plasmid Wash Buffer 1. Plasmid Wash Buffer 1 removes RNA, protein and endotoxin. (Add a 5 minute incubation step before centrifugation if the DNA will be used in transfection). Centrifuge for 1 minute. Discarding the flow-through is optional.
9. Add 400 µl of Plasmid Wash Buffer 2 and centrifuge for 1 minute.
10. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If there is any doubt, re-spin the column for 1 minute before inserting it into the clean microfuge tube.
11. Add ≥ 30 µl DNA Elution Buffer to the center of the matrix. Wait for 1 minute, then spin for 1 minute to elute DNA.

- LR Clonase II Reaction and Bacterial Transformation
    1. Label empty 1.5 mL Eppendorf tubes with MTA and FIP37 (or the desired combination). Leave the tubes on ice.

2. Spin down the components (entry clone and destination vector) in centrifuge for 3-5 seconds, then put on ice.
3. Use the P20 pipette to pipette 2 µL of the entry clone and 2 µL of the destination vector into each the MTA and FIP37 tubes.
4. Take the LR Clonase II out from the fridge, flick lightly, and put in the centrifuge for 3 seconds.
5. Add 1 µL of LR Clonase II to each Eppendorf tube.
6. Spin down the samples for 1 second, and incubate for 1 hour at room temperature.
7. Add 0.5 µL of ProteinaseK to each sample.
8. Take 2 aliquots of the competent cells from -80ºC and put the two bacteria samples on ice to thaw for ten minutes.
9. Transfer the 5 µL of the MTA and FIP37 mixtures to the corresponding bacteria samples.
10. Tap the samples gently and let them incubate on ice for ten minutes.
11. Put 800 µL of LB on ice.
12. Heat shock the two samples in the incubator for 45 seconds at 42ºC in the Eppendorf Thermomixer C, with no stirring.
13. Let the two samples sit on ice for two minutes.
14. Let the bacteria sit on ice for two minutes.
15. Add 800 µL of LB to each sample.
16. Put the two 1.5 mL Eppendorf tubes in Falcon tubes and incubate the bacteria at 37ºC heat and shake at 1000-1500 RPM for 40-60 minutes.

- Measuring Absorbance to Prepare for Sequencing
    1. Turn on the spectrophotometer (NanoDrop One), and select nucleic acids, then dsDNA.
    2. Load 1.5 µL of the blank (elution buffer). Put the arm down.
    3. Clean both pedestals with a Kim Wipe (gently dab the pedestals), then load samples (1.5 µL each as well).

- Plasmid DNA Purification Using the QIAprep Spin Miniprep Kit and a Microcentrifuge
    1. Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube. Ensure that RNase A has been added to Buffer P1. No cell clumps should be visible after resuspension of the pellet. If LyseBlue reagent has been added to Buffer P1, vigorously shake the buffer bottle to ensure LyseBlue particles are completely dissolved. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
    2. Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4–6 times. Mix gently by inverting the tube. Do not vortex, because this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. Do not allow the lysis reaction to proceed for more than 5 min. If LyseBlue has been added to Buffer P1, the cell suspension will turn blue after addition of Buffer P2. Mixing should result in a homogeneously colored suspension. If the suspension contains localized colorless regions, or if brownish cell clumps are still visible, continue mixing the solution until a homogeneously colored suspension is achieved.
    3. Add 350 µL Buffer N3. Mix immediately and thoroughly by inverting the tube 4-6 times. To avoid localized precipitation, mix the solution thoroughly, immediately after addition of Buffer N3. Large culture volumes (e.g., ≥5 mL) may require inverting up to 10 times. The solution should become cloudy. If LyseBlue reagent has been used, the suspension should be mixed until all trace of blue has gone and the suspension is colorless. A homogeneous colorless suspension indicates that the SDS has been effectively precipitated.
    4. Centrifuge for 10 min at 13,000 rpm (~17,900 x *g*) in a table-top microcentrifuge. A compact white pellet will form.

5. Apply 800 µL of the supernatant from step 4 to the QIAprep 2.0 spin column by pipetting.
6. Centrifuge for 30-60 s. Discard the flow-through.
7. Recommended: Wash the QIAprep 2.0 spin column by adding 0.5 mL Buffer PB and centrifuging for 30-60 s. Discard the flow-through. This step is necessary to remove trace nuclease activity when using *end*A+ strains such as the JM series, HB101 and its derivatives, or any wild-type strain, which have high levels of nuclease activity or high carbohydrate content. Host strains such as XL-1 Blue and DH5®α do not require this additional wash step.
8. Wash QIAprep 2.0 spin column by adding 0.75 mL Buffer PE and centrifuging for 30-60 s.
9. Discard the flow-through, and centrifuge at full speed for an additional 1 minute to remove residual wash buffer.
10. Place the QIAprep 2.0 column in a clean 1.5 mL microcentrifuge tube. To elute DNA, add 50 µL Buffer EB (10 mM Tris·Cl, pH 8.5) or water to the center of each QIAprep 2.0 spin column, let stand for 1 minute and centrifuge for 1 minute.

- Bacterial Transformation Electro Competent Cells (Electroporation)
    1. Thaw plasmid on ice.
    2. Take cells from -80 ºC and put them on ice (3-5 minutes until “thawed”).
    3. Dilute plasmid 1:100.
    4. Add 1 µL of diluted plasmid to cells.
    5. Add entirety of cell and plasmid mix (~55 µL) to cuvette with a pipette. Ensure that the inserted material is touching both plates. Try to avoid bubbles.
    6. Turn the electroporator (BioRad MicroPulser) on and set to the appropriate microorganism setting.
    7. Dry the cuvette with a paper towel. Insert it into the electroporator.

8. Set the electroporator to maximum time (2.5), push the 2 red buttons at the same time, release upon hearing beep (~2-3 seconds). To ensure it worked, press "Time Const." number should be 6-6.5.
9. Add 0.5 mL LB to cuvette (quickly in order to destress the cells).
10. Pipette the entire contents of the cuvette into a 1.5 mL Eppendorf tube. Set cuvette aside to later clean.
11. Repeat steps 7-10 for each sample.
12. Plating
    a. Pipette desired amount onto the LB+antibiotic plate, or centrifuge tube with cells and LB at 5000 RPM for five minutes.
    b. Pour off the supernatant.
    c. Resuspend pellet in 50 µL LB.
    d. Pipette cell solution onto LB plate with the necessary antibiotic.
    e. Remove the spreader from ethanol, burn off ethanol with Bunsen burner, cool for a moment, touch spreader to agar before spreading cells.
    f. Incubate the cells at 37 ºC overnight.

- **Data Analysis:**
    - A t-test will be used on the data copied into Excel for hypocotyl measurements. This will be used to compare the CRY2 data to the wildtype measurements for all plates in each trial, and to compare the ECT2 measurements to the wildtype measurements for all plates in each trial.
    - The measurements will be used to create column charts or violin charts of the hypocotyl measurements with standard error bars to visualize hypersensitivity or hyposensitivity to light in the plants.

d. **BIBLIOGRAPHY:**

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1. **Human participants research: N/A**
2. **Vertebrate animal research: N/A**
3. **Potentially hazardous biological agents research:**

- All cells are established cell lines and biosafety level 1.
- All bacteria will be handled under a sterile flow hood and exposed skin will be protected by wearing gloves under supervision by the mentor.
- The flow hood surfaces will be cleaned with ethanol before and after experimentation.
- All pipette tips, centrifuge tubes, and Eppendorf tubes that come in contact with these bacteria will be discarded in biological hazardous waste after they have been used.
    - *Agrobacterium tumefacians* strain GV3101 was purchased from VWR.
    - *E. coli* strain DH5-ALPHA Cell was purchased from Thermo Fisher Scientific.
- The Gateway pDONR 221 vector was purchased from Invitrogen through Thermo Fisher Scientific.
- The p19 vector was purchased from Addgene.
- The plasmid containing the Ac6 gene and the plasmid containing the Dn6 gene (vectors) were purchased from Addgene.
- The mCherry vector was purchased from Addgene.
- The vectors for FIP37, MTA, and MTTB were created by Oliver Artz.

4. Hazardous chemicals, activities, and devices

- For all chemicals toxic to inhale, avoid breathing dust/fume/gas/mist/vapours/spray, and use only outdoors in a well-ventilated area. If inhaled, remove person to fresh air and keep comfortable for breathing. Call a Poison Center or a doctor.
- Wash hands thoroughly after handling all chemicals.
- Wear protective gloves/protective clothing/eye protection/face protection while handling all chemicals in order to avoid contact with skin and eyes.

- Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed.
- If hazardous chemicals come in contact with the skin, wash with plenty of soap and water.
- If hazardous chemicals come in contact with eyes, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
- If skin irritation occurs, get medical advice/attention.
- If eye irritation persists after washing eyes out, get medical advice/attention.
- If hazardous chemicals are swallowed, call a poison center/doctor if you feel unwell. Rinse mouth.
- Take off clothing contaminated by hazardous chemicals and wash before reuse.
- Properly collect all spillage and dispose of contents/container to an approved waste disposal plant, and in a biological hazardous container as appropriate.
- Triton X-100 for molecular biology
    - From: Sigma Aldrich
    - GHS classification
        - Acute toxicity, oral (Category 4).
        - Skin irritation (Category 2).
        - Serious eye damage (Category 1).
        - Short-term (acute) aquatic hazard (Category 1).
        - Long-term (chronic) aquatic hazard (Category 1).
    - Hazard statements:
        - Causes skin irritation.
        - Causes serious eye damage.
        - Very toxic to aquatic life with long lasting effects.
    - Handling:
        - When cleaning spillage, soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closeed containers for disposal.
        - Avoid contact with skin and eyes. Avoid inhalation of vapor or mist.

- Ethanol: 200 proof anhydrous ethyl alcohol USP
    - From: Warner-Graham Corporation.
    - GHS classification
        - Flammable liquids (Category 2).
        - Serious Eye Damage/Eye Irritation (Category 2).
        - Specific target organ toxicity (single exposure) (Category 1).
        - Target Organs - Central nervous system (CNS), optic nerve, Respiratory system.
        - Specific target organ toxicity - (repeated exposure) (Category 1).
        - Target Organs - Kidney, Liver, spleen, blood.
    - Hazard Statements
        - Highly flammable liquid and vapor.
        - Causes serious eye irritation.
        - Causes damage to organs.
        - Causes damage to organs through prolonged or repeated exposure.
    - Handling
        - Store locked up.
        - Store in a well-ventilated place.
        - Keep cool.
        - Dispose of contents/container to an approved waste disposal plant.
- Luria-Bertani (LB) broth/Lysogeny broth
    - From: VWR.
    - Not a hazardous substance or mixture according to the GHS.
    - Keep containers tightly closed in a cool, well-ventilated place.
    - Keep away from strong oxidizing agents and strong acids.
- Acetosyringone (3',5'-Dimethoxy-4'-hydroxyacetophenone)
    - From: Sigma Aldrich.
    - GHS Classification:
        - Skin irritation (Category 2).

        - Eye irritation (Category 2A).
        - Specific target organ toxicity - single exposure (Category 3), Respiratory System.
    - Hazard statements
        - Causes skin irritation.
        - Causes serious eye irritation.
        - May cause respiratory irritation.
    - Precautions for safe handling
        - Normal measures for preventive fire protection.
- Rifampicin antibiotic
    - From: VWR.
    - GHS Classification:
        - Acute toxicity - oral (Category 4).
        - Skin corrosion/irritation (Category 2).
        - Eye damage/irritationi (Category 2A).
    - Hazard statements
        - Causes serious eye irritation.
        - Causes skin irritation.
        - Harmful if swallowed.
    - Handling
        - Store away from oxidizing agents.
        - Avoid prolonged storage periods.
        - Store under inert gas. Moisture sensitive. Store in refrigerator.
- Gentamicin antibiotic
    - From: VWR (Gentamycin Sulfate).
    - GHS Classification
        - Respiratory sensitization (category 1).
        - Skin sensitiziation (category 1).
    - Hazard statements

        - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
        - May cause an allergic skin reaction.
    - Handling
        - Recommended to store at an ambient temperature.
        - Keep container tightly closed and in a well-ventilated place.
- Kanamycin antibiotic
    - From: VWR (Kanamycin Sulfate).
    - GHS Classification
        - Reproductive toxicity (Category 1B).
    - Hazard statements
        - May damage fertility or the unborn child.
    - Handling
        - Recommended to store at an ambient temperature.
        - Keep container tightly closed and in a well-ventilated place. Keep/Store only in original container.
- Carbenicillin antibiotic
    - From: VWR.
    - GHS Classification: Hazardous substance or mixture
    - Hazard statements:
        - May cause an allergic skin reaction.
        - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
    - Handling
        - Provide exhaust ventilation at places at places where dust is formed. Normal measures for preventive fire protection. Avoid contact with skin and eyes. Avoid formation aerosols.
        - Keep labeled container tightly closed and upright. Keep frozen.
        - Keep away from strong oxidizers.
- Spectinomycin antibiotic
    - From: VWR.

    - Not a hazardous substance or mixture according to the GHS.
    - Handling
        - Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.
        - Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.
        - Recommended storage temperature: -20 ºC.
        - Store under inert gas.
- GoTaq Green Master Mix
    - From: Promega.
    - Not a hazardous substance or mixture according to the GHS.
- 1% agarose gel
    - Agarose powder from VWR.
    - Hazards identification:
        - May cause allergic skin reaction.
    - Handling:
        - In case of a spill or leak: Eliminate all ignition sources. Absorb and/or contain spill with inert materials. Then place in appropriate container. For large spills, use water spray to disperse vapors, flush spill area. Prevent runoff from entering waterways or sewers.
        - Use only in areas provided with appropriate exhaust ventilation.
        - Avoid oxidizing and spontaneously flammable products.
- Ethidium bromide solution
    - From: Sigma Aldrich.
    - Ethidium bromide is a known carcinogen.
    - All work with ethidium bromide will be limited to a taped off area on the workbench specifically for equipment that comes in contact with ethidium bromide, and work will only be conducted under the supervision of a lab employee. Only equipment continually used, such as trays for the gels, are placed

within this area - other objects are not brought in the vicinity. Gloves will be immediately disposed of in the biological hazardous waste container after usage. All PCR tubes used to dispose samples in the gels will be directly disposed in the biological hazardous waste container after use.

- GHS Classification
  - Acute toxicity, inhalation (Category 3).
  - Germ cell mutagenicity (Category 2).
- Hazard statement
  - Toxic if inhaled.
  - Suspected of causing genetic defects.
- Store in a well-ventilated place. Keep container tightly closed.
- Store locked up.

- BP Clonase II
  - From: Thermo Fisher Scientific in an Invitrogen kit for the Gateway BP Clonase II Enzyme mix.
  - Not a hazardous substance or mixture according to the GHS.
  - Handling
    - Use personal protective equipment as required.
    - Keep in a dry, cool and well-ventilated place. Keep in properly labeled containers.
- LR Clonase II
  - From: Thermo Fisher Scientific in an Invitrogen kit for the Gateway LR Clonase II Enzyme mix.
  - Not a hazardous substance or mixture according to the GHS.
  - May cause eye irritation with susceptible persons.
  - May cause skin irritation in susceptible persons.
  - May be harmful by inhalation.
  - May be harmful if swallowed.
  - Handling

        - Always wear recommended personal protective equipment. No special handling devices are necessary.
        - Keep in a dry, cool and well-ventilated place.
- Proteinase K
    - From: Thermo Fisher Scientific in an Invitrogen kit for the Gateway BP Clonase II Enzyme mix.
    - Product code: 59895.
    - GHS Classification
        - Skin corrosion/irriation (Category 2).
        - Serious eye damage/eye irritation (Category 2A).
        - Respiratory sensiization (Category 1).
    - Hazard Statements
        - Causes skin irritation.
        - Causes serious eye irritation.
    - Handling
        - Always wear recommended personal protective equipment. No special handling devices are necessary.
        - Keep in a dry, cool and well-ventilated place. Keep in properly labeled containers.
- RNase A
    - From: QIAprep Spin Miniprep Kit for Plasmid DNA Purification (From Qiagen).
    - GHS Classification
        - Respiratory sensitization (Category 1).
        - Skin sensitization (Category 1).
    - Hazard statements:
        - May cause an allergic skin reaction.
        - May cause allergy or asthma symptoms or breathing difficulties if inhaled.
    - Precautions
        - Avoid breathing dust/fume/gas/mist/vapors/spray.

        - Wear protective gloves/protective clothing/eye protection/face protection.
        - Wear respiratory protection.
    - Handling.
        - Avoid exposure and contact with skin and eyes.
        - Sufficient air exchange and/or exhaust should be provided in work rooms.
        - Container will be tightly closed in a dry and well-ventilated place.
- Buffer P1
    - From: QIAprep Spin Miniprep Kit for Plasmid DNA Purification (From Qiagen).
    - Not a hazardous substance or mixture according to the GHS.
    - Handling
        - Use personal protective equipment.
        - Keep in suitable, closed containers for disposal.
- Buffer P2
    - From: QIAprep Spin Miniprep Kit for Plasmid DNA Purification (From Qiagen).
    - GHS Classification
        - Corrosive to metals (Category 1).
        - Skin irritation (Category 2).
        - Eye irritation (Category 2A).
    - Hazard statements
        - May be corrosive to metals.
        - Causes skin irritation.
        - Causes serious eye irritation.
    - Handling
        - Use personal protective equipment.
        - Prevent product from entering drains.
        - For containment, soak up with inert absorbent material. Keep in suitable, closed containers for disposal.
        - Normal measures for preventive fire production.
        - Keep container tightly closed in a dry and well-ventilated place.

- Buffer P3
    - From: Qiagen.
    - GHS Classification
        - Skin irritation (Category 2).
        - Eye irritation (Category 2A).
    - Hazard statements
        - Causes skin irritation.
        - Causes serious eye irritation.
    - Handlinig
        - Use personal protective equipment.
        - Avoid contact with skin and eyes.
        - Keep container tightly closed in a dry and well-ventilated place.
- Buffer N3
    - From: QIAprep Spin Miniprep Kit for Plasmid DNA Purification (From Qiagen).
    - GHS Classification
        - Skin irritation (Category 2).
        - Eye irritation (Cattegory 2A).
    - Hazard statements
        - Causes skin irritation.
        - Causes serious eye irritation.
    - Handling
        - Keep container tightly closed in a dry and well-ventilated place.
- Buffer PB
    - From: QIAprep Spin Miniprep Kit for Plasmid DNA Purification (From Qiagen).
    - GHS Classification
        - Flammable liquids (Category 2).
        - Skin irritation (Category 2).
        - Eye irritation (Category 2A).

        - Specific target organ systemic toxicity - single exposure (Category 3) (central nervous system).
    - Hazard statements
        - Highly flammable liquid and vapor.
        - Causes skin irritation.
        - Causes serious eye irritation.
        - May cause drowsiness or dizziness.
    - Precautionary statements
        - Keep away from heat/sparks/open flames/hot surfaces. No smoking.
    - Handling
        - Take necessary action to avoid static electricity discharge (which might cause ignition of organic vapors). Use only explosion-proof equipment. Keep away from open flames, hot surfaces, and sources of ignition
        - Avoid exposure and contact the chemical.
        - Provide sufficientt air exchange and/or exhaust in work rooms. Open drum carefully as content may be under pressure.
        - Keep container tightly closed in a dry and well-ventilated place.
        - Do not store together with oxidizing and self-igniting products.
- Buffer PE
    - From: QIAprep Spin Miniprep Kit for Plasmid DNA Purification (From Qiagen).
    - Not a hazardous substance or mixture according to the GHS.
    - Handling
        - Use personal protective equipment.
        - Avoid breathing dust/fume/gas/mist/vapors/spray.
        - Keep in suitable, closed containers for disposal.
- Monarch Plasmid Resuspension Buffer (B1)
    - From the Monarch Plasmid DNA Miniprep Kit from New England BioLabs.
    - Not a hazardous substance or mixture according to the GHS.
    - Keep containers tightly closed in a dry, cool, and well-ventilated place.

- Monarch Plasmid Lysis Buffer (B2):
    - From the Monarch Plasmid DNA Miniprep Kit from New England BioLabs.
    - OSHA Hazard Communication Standard
        - Skin corrosion/irritation (Category 2).
        - Serious eye damage/eye irritation (Category 2A).
        - Corrosive to metals (Category 1).
    - Hazard statements
        - Harmful if swallowed.
        - Causes severe skin burns and eye damage.
        - May be corrosive to metals.
    - Handling
        - Keep only in original container.
        - Wear protective gloves/eyes protection/face protection.
- Monarch Plasmid Neutralization Buffer (B3)
    - From the Monarch Plasmid DNA Miniprep Kit from New England BioLabs.
    - OSHA Hazard Communication Standard
        - Acute toxicity - oral (Category 4).
        - Skin corrosion/irritation (Category 2).
        - Serious eye damage/eye irritation (Category 2A).
    - Hazard statements:
        - Harmful if swallowed.
        - Causes skin irritation.
        - Causes serious eye irritation.
    - Keep containers tightly closed in a dry, cool and well-ventilated place. Keeep out of the reach of children.
- Monarch Plasmid Wash Buffer 1
    - From the Monarch Plasmid DNA Miniprep Kit from New England BioLabs.
    - OSHA Hazard Communication Standard
        - Acute toxicity - oral (Category 4).

        - Skin corrosion/irritation (Category 2).
        - Serious eye damage/eye irritation (Category 2).
        - Flammable liquids (Category 3).
    - Hazard statements
        - Harmful if swallowed.
        - Causes skin irritation.
        - Causes serious eye irrtation.
        - May cause drowsiness or dizziness.
        - Flammable liquid and vapor.
    - Handling
        - Wear protective gloves/protective clothing/eye protection/face protection.
        - Keep container tightly closed.
        - Use only non-sparking tools.
        - Take precautionary measures against static discharge.
- Monarch Plasmid Wash Buffer 2
    - From the Monarch Plasmid DNA Miniprep Kit from New England BioLabs.
    - Not a hazardous susbstance or mixture according to the GHS.
    - Keep containers tightly closed in a dry, cool and well-ventilated place.
- Monarch DNA Elution Buffer
    - From the Monarch Plasmid DNA Miniprep Kit from New England BioLabs.
    - Not a hazardous substance or mixture according to the GHS.
    - Keep containers tightly closed in a dry, cool and well-ventilated place.