Making 10x TBE (500mL):

1. Obtain a clean 500mL Erlenmeyer Flask
2. Add 400mL of ELGA H_2O to the Flask
3. Dissolve 54g of Tris Base [tris(hydroxymethyl)aminomethane], 27.5g of Boric Acid, and 3.75g of EDTA into the flask.
4. Swirl the solution (5min) until no more clumps can be visible.
5. Fill the flask up to 500mL with ELGA H_2O. Swirl again to dissolve all the clumps.
6. Store at room temperature. Do not place in refrigerator.

Making 1x TBE (1L) from 10x TBE:

1. Obtain a clean 1L Erlenmeyer Flask
2. Mix 100mL of 10x TBE with 900mL of ELGA H_2O in the 1L flask. (Only do this if there is no other 1x TBE available. The same TBE can be reused for many gels if it is saved.)
3. Make sure there is enough 1x TBE to be used for both the gel and the gel box. You do not want to mix different batches of buffer when pouring the gel and covering it.

Making a 1% Agarose Gel for Electrophoresis of Plasmids:

1. Pre-cool the gel casting tray by placing at 4°C.
2. Obtain a clean 125 - 250mL Erlenmeyer Flask
3. Mix 0.75g of Agarose with 75mL of TBE in the flask. (Loosely plug top of flask with a kimwipe. Make sure the plug leaves holes for air to escape)
    a. Microwave for 20 seconds. Use hot gloves to swirl the flask.
    b. Put back into the microwave for another 20 seconds. Use hot gloves to swirl the flask.

   c. Continue to microwave (20 sec) and watch the flask very closely. Once bubbles start forming, stop the microwave immediately. Let cool in microwave (5 min).
   d. Cooling can be accelerated by placing the flask in a tray for water for 1 minute

4. When the temperature has reached about 55°C, add 7.5µL of SYBR Safe DNA Gel Stain and swirl to mix. (1µL to 10mL of gel mixture).
5. Pour all 75mL of agarose gel into the pre-cooled gel tray. Make sure the well comb is already in place. Pour slowly to prevent bubbles. Use a pipette tip to move bubbles to the side of the gel. Do this right after pouring, before the gel has a chance to set.
6. Let sit at room temperature for 20 minutes. The gel should know be solid.
7. Remove the comb and position the gel so the wells are closer to the black (negative side). The DNA should run towards the opposite side of the gel (towards the red-positive side).
8. Fill gel box with 1x TBE until gel is covered.

Loading Samples and Running Agarose Gel:

1. Fill up ice box and place 1kb NEB ladder and Purple gel loading dye (6x) inside. (The loading dye used a 1:5 ratio of dye to DNA.)
2. Cut a strip of parafilm. Separate the parifilm into different sections, depending on how many samples will be loaded into the gel. Use a sharpie to create the separations and label each section. (a 3cm x 3cm square for each sample will suffice).
3. Add 1µL of purple loading dye onto a square. Add 5µL of the NEB ladder on top of the dot of purple loading dye and use the pipette to mix the two (5-10 up/down). Load the ladder first and into the first well.

4. Add 1μL of purple loading dye to 4 more sections of parafilm. (Do not wait long to add the DNA sample as the dye can eventually adhere to the parafilm).
5. Add the corresponding 5μL of the DNA on top of the purple loading dye and use the pipette to mix the two (5-10 up/down). Load the 6μL onto gel immediately. Repeat for rest of samples. (Remember to change pipette tips to avoid contamination. Or cleanse the same tip in TBE buffer well before using on next sample) Mark down which sample is going into each well.
6. Run the gel at 120 V until the dye line is approximately 75-80% of the way down the gel. A typical run time is about 1-1.5 hours. You should see bubbles and the dyes moving across the gel after 10 minutes if everything is working properly.
7. Turn of electrodes and carefully remove gel box. Place in tray and transport to Gel Digital Imaging System.
8. Use the imager to photograph the gel under the white light setting and use the software to annotate the gel bands/wells.
9. Save the image onto the lab computer and backup onto the google drive. After this is done, the gel can be thrown away in the Biohazard waste bin.