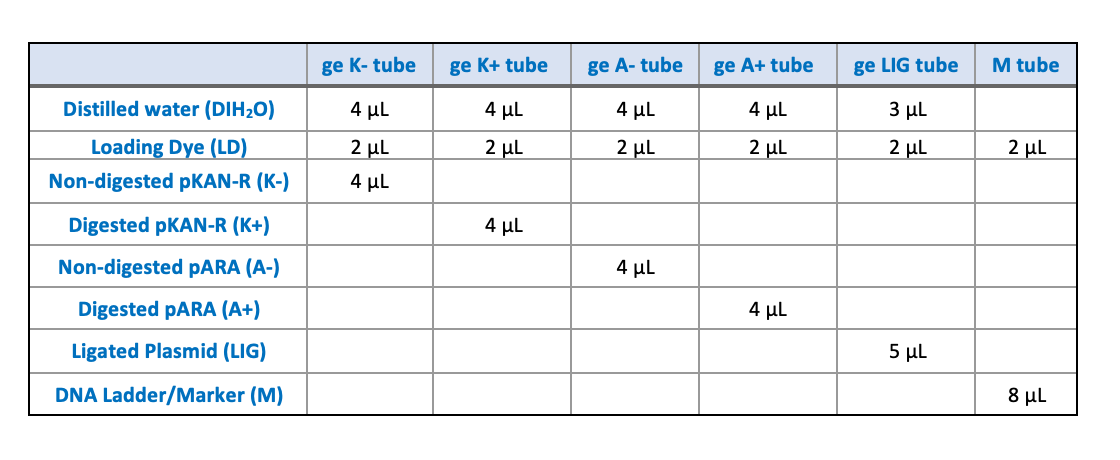
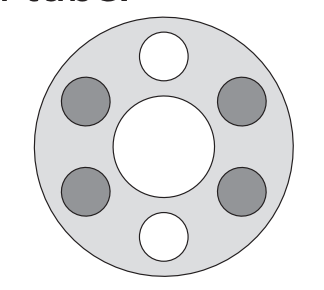
**Lab 4: Verification of Restriction and Ligation Using Gel Electrophoresis**

1. Label five clean microfuge tubes as “geA-”, “geA+”, “geK-”, “geK+”, “geLIG” and your group and period ID. “ge” indicates that the tubes contain samples for gel electrophoresis.

2. Using a fresh tip for each reagent, use the p20 micropipette to add the following reagents to each tube according to the table below. Check them off as you add them. After adding the last reagent to each tube, gently pump the solution up and down to mix.

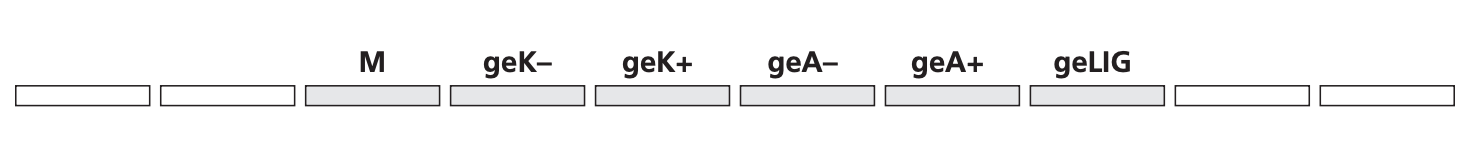


3. Quickly spin all six tubes to pool the reagents.



4. Correctly place a gel in the buffer tank (wells up and near the “-” electrode). Cover the gel with 1X buffer.

5. Using a new tip, load 10µL of each sample into its own well, being careful to lower the pipette tip just inside the buffer but not in the well. Depress the plunger to the first stop and hold it there until pulling it out of the buffer.Load samples as follows:



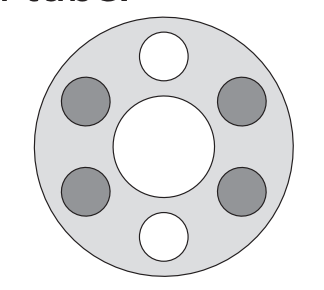
6. If using an OWL or Enduro Gel XL box, set the Voltage to 130V and run for 30-40 minutes. If using a MiniOne or BlueGel, run the gel for 15-30 minutes with the light off and check the bands every five minutes.

7. For OWL and Enduro boxes, transfer the gel to the transilluminator and take a photo. For MiniOne or BlueGel units, turn on the light and take a photo. Return your original LIG tub to your teacher for Lab 5.

**Lab 4A: Verification of the Recombinant Plasmid Using Gel Electrophoresis**

1. Using a fresh tip each time, add 2.0µL of LD to your R+, R- and M tubes. Gently pump up and down to mix the solution.

2. Spin the tubes for five seconds to pool the reagents.



3. Correctly place a gel in the buffer tank (wells up and near the “-” electrode). Cover the gel with 1X buffer.

4. Using a fresh tip, load 10µL of each sample into its own well, being careful to lower the pipette tip just inside the buffer but not in the well. Depress the plunger to the first stop and hold it there until pulling it out of the buffer. Load samples as follows:



5. If using an OWL or Enduro Gel XL box, set the Voltage to 130V and run for 30-40 minutes. If using a MiniOne or BlueGel, run the gel for 15-30 minutes with the light off and check the bands every five minutes.

6. For OWL and Enduro boxes, transfer the gel to the transilluminator and take a photo. For MiniOne or BlueGel units, turn on the light and take a photo.