Lab 1.1 Flow Chart: Basic Pipetting and Serial Dilution

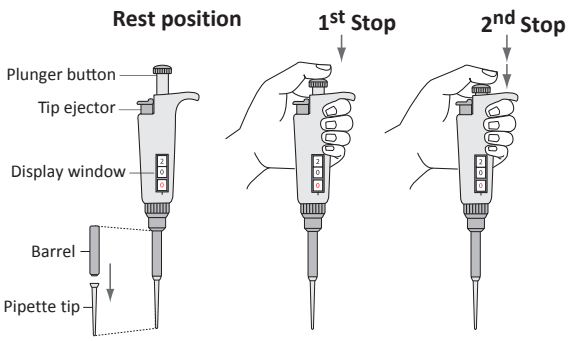
50-fold Dye

Dye

Water

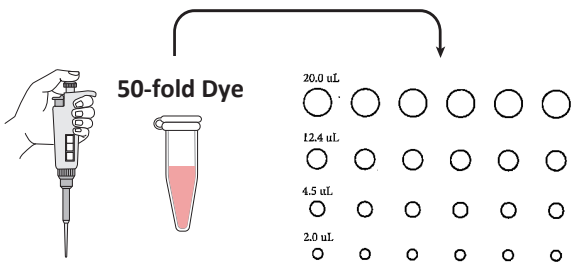
a. Pipetting different volume of the solution

1



- Review micropipettes. Feel the **1st stop** and **2nd stop**.

2

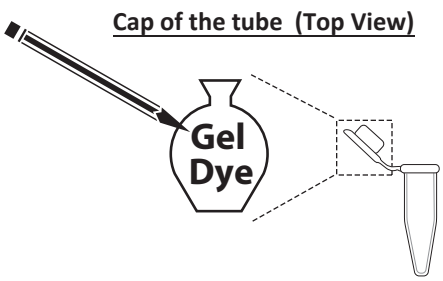


- Pipette different volumes of **50-fold Dye** to the sheet:

  20.0 µL 12.4 µL 4.5 µL 2.0 µL

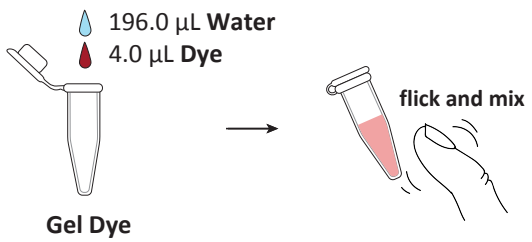
b. Mixing solution: Preparing a 50-fold dye

3



- Label a new microfuge tube, **'Gel Dye'** at the cap.

4



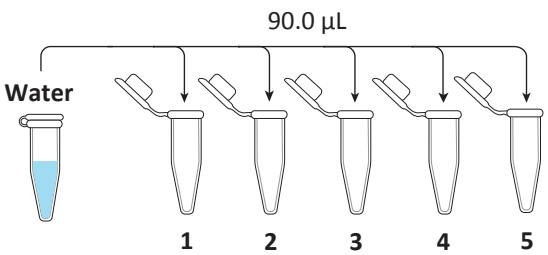
- Add **196.0 µL Water**, and then **4.0 µL Dye** to the tube.
- Flick and mix well.
- Store **Gel Dye** in room temperature for Lab 1.2.

Avoid contamination:

- *Change a new tip.*

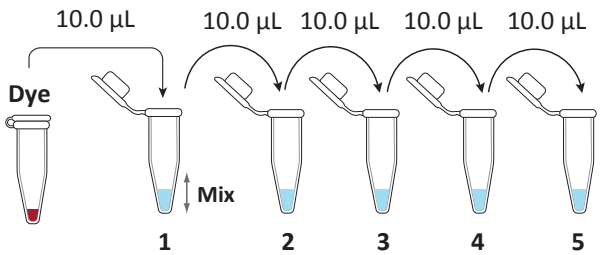
c. Serial dilution: Preparing a 100,000 fold dye

5



- Label 5 microfuge tubes **'1' through '5'** at the cap.
- Add **90.0 µL Water** to all tubes.

6



- Add **10.0 µL Dye** to tube **'1'** and mix well.
- Use a new tip, transfer **10.0 µL solution** from tube **'1'** to tube **'2'** and mix well.
- Repeat the process for tube **'2' through '5'**.
- Observe the change on color intensity.

Lab 1.2 Flow Chart: Gel Electrophoresis — Dye Separation

Gel Dye — from Lab 1.1

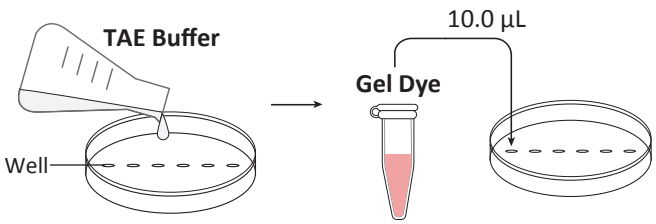
Dye 1

Dye 2

Dye 3

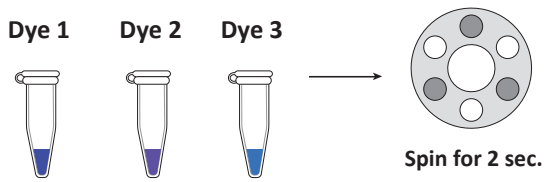
TAE Buffer

1



- Pour **TAE Buffer** into the practice plate (just cover wells).
- Practice gel loading: add **10.0 µL Gel Dye** to each well.

2



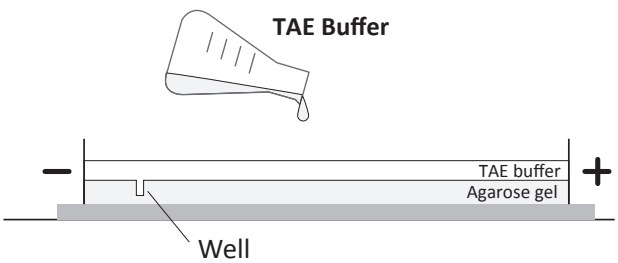
- Check **Dye 1**, **Dye 2** and **Dye 3**.
- If solution stick on the wall, centrifuge the tube for 2 sec..

Balance the weight :

- *Arrange the tubes in the way for uniform weight distribution*

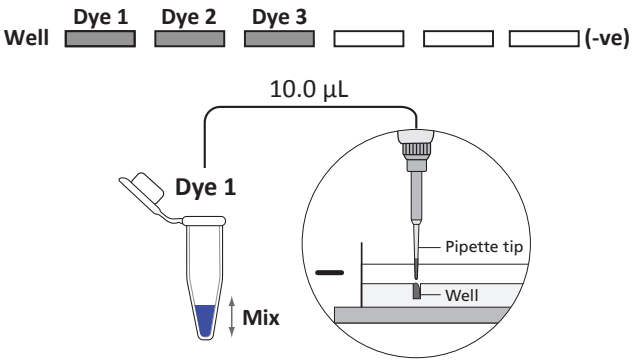
3

Just cover the surface of the gel



- Put the gel into the gel tank.
- Pour **TAE Buffer** into the tank, until it just cover the wells.

4

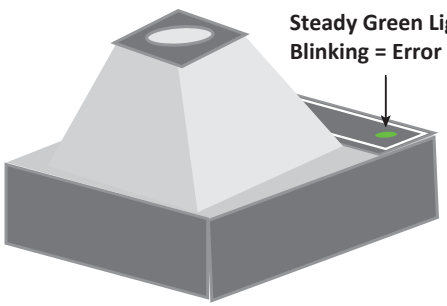


- Add **10.0 µL Dye 1** to the well. Repeat for **Dye 2** and **Dye 3.**
- Record the locations of your samples.

Avoid getting air into the buffer:

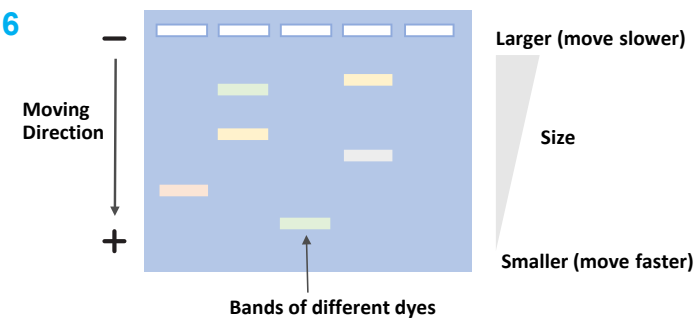
- *Press to 1st stop ONLY and hold the plunger while lifting up the pipette tip out of the buffer*

5



- Put the photo hood on the gel system.
- Press ⏻ to turn on the electric current .
- Observe bubbles come out at the (-) negative electrode.

6



- After **10 minutes**, press ⏻ to turn off the electric current.
- Remove the photo hood and observe the bands.
- Record the relative locations and colors of the bands.

Lab 2.1: Checking Plasmid with PCR (Plasmid A and A-rfp provided)

PCR Master Mix — *Keep on ice*

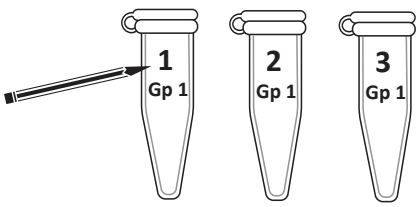
Plasmid A-rfp (Plasmid A with red fluorescent protein gene)

Plasmid A

Water

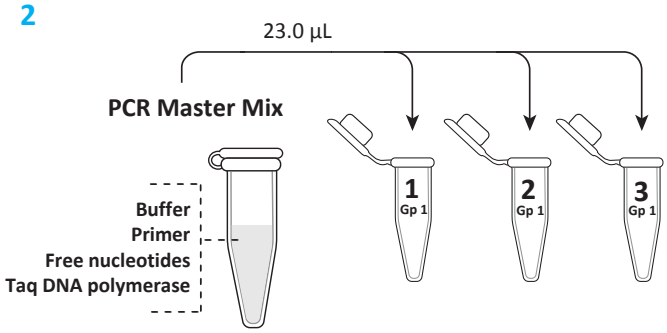
1

Ink may come off the cap in the thermal cycler.



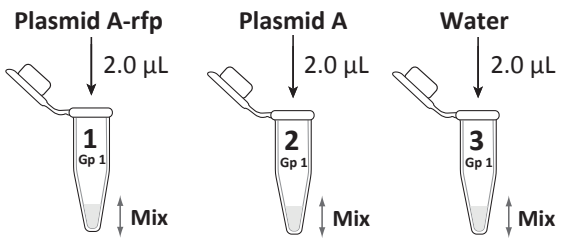
- Label three PCR tubes **'1, 2, 3'** on the **wall** of the tube with your **group number**.

2



- Add **23.0 µL PCR Master Mix** to **PCR tube 1, 2, 3**.

3

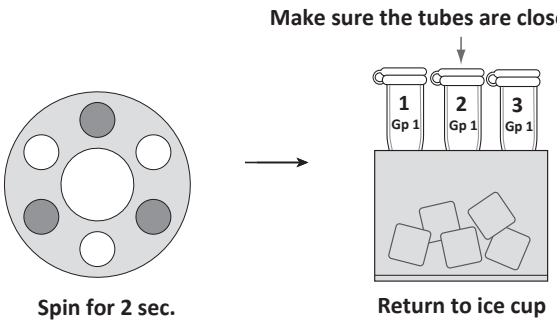


- Add **2.0 µL samples** to the **PCR tube 1, 2, 3.**
- Gently pipette up and down a few times to mix.

Avoid contamination:

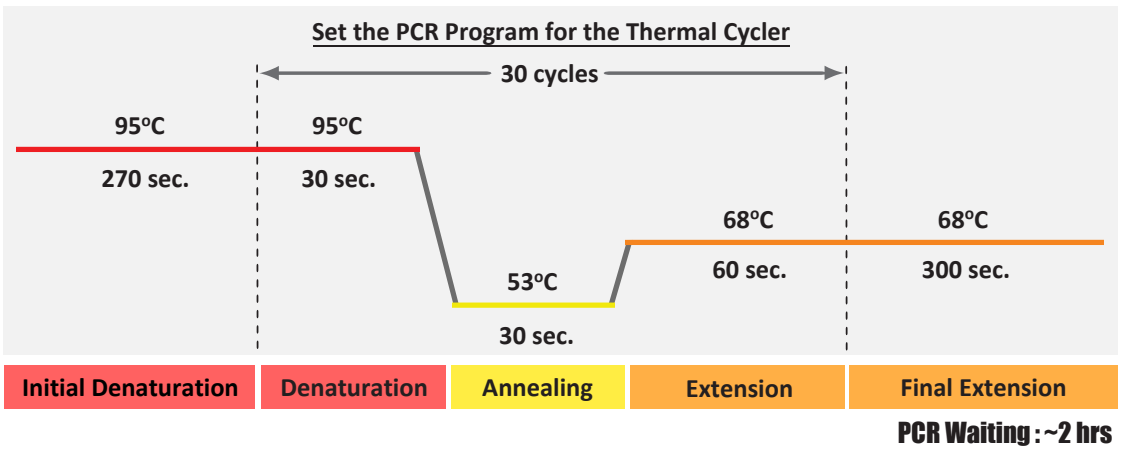
- *Change a new tip every time after adding a solution.*

4



- If there are bubbles, centrifuge the tubes for 2 sec. or gently tap the bottoms of the PCR tubes
- Return all PCR tubes to ice immediately.

5



- Refer to the graph above, **set the PCR program** for the thermal cycler.
- Transfer PCR tubes to the thermal cycler and start the PCR run which takes around 2 hours to complete.
- After **2 hours**, collect the PCR samples and store at -20°C for Lab 2.2.

Lab 2.2: Gel Electrophoresis — Confirmation of PCR Products

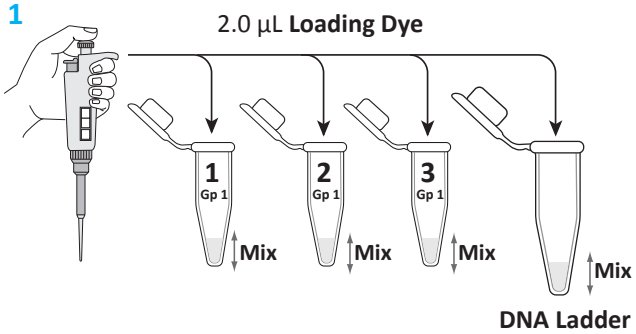
PCR Samples 1, 2, 3 — from Lab 2.1

Loading Dye

DNA Ladder

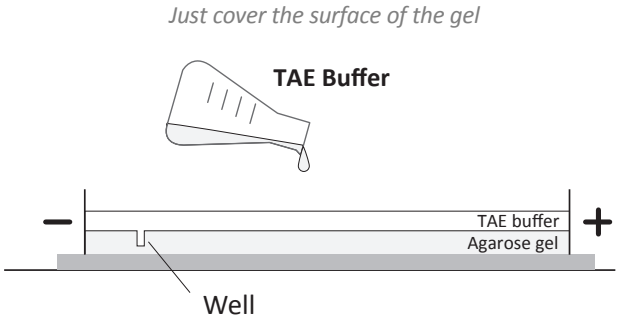
TAE Buffer

1



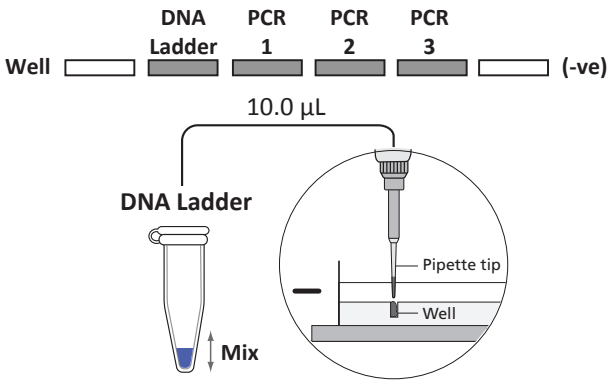
- Add **2.0 µL Loading Dye** to **PCR samples (1, 2, 3)** and **DNA Ladder**.
- Gently pipette up and down a few times to mix.

2



- Put the gel into the gel tank.
- Pour **TAE Buffer** into the tank, until it just cover the wells.

3

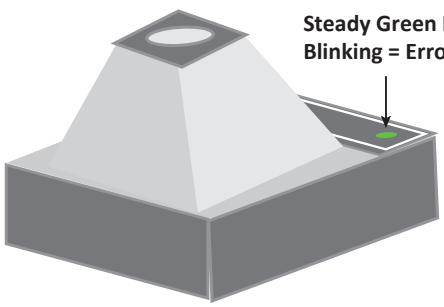


- Add **10.0 µL DNA Ladder** and **PCR samples (1, 2, 3)** into designated wells .
- Record the locations of your samples.

Avoid getting air into the buffer:

- *Press to 1st stop ONLY and hold the plunger while lifting up the pipette tip out of the buffer*

4

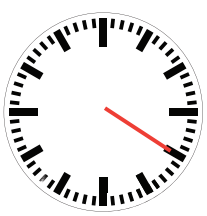


- Put the photo hood on the gel system.
- Press ⏻ to turn on the electric current .
- Observe bubbles come out at the (-) negative electrode.

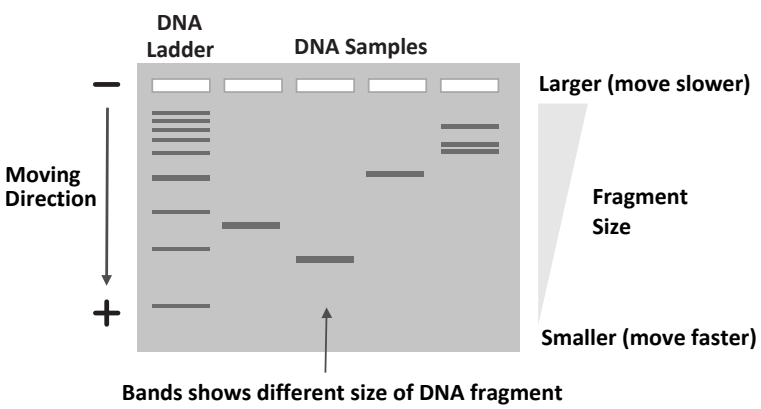
Use weak blue light when running the gel:

- *Extensive exposure to blue light may weaken the fluorescent signal*

5



Waiting : ~20 min.



- After **20 minutes**, press ⏻ to turn off the electric current. Use 💡 **strong blue light** to observe the bands.
- **Take a gel photo** and record the relative locations of the DNA bands.

Lab 3.1 Flow Chart : Cutting the two plasmid (Restriction Digestion)

Restriction Enzymes (BamHI & HindIII) — *Keep on ice*

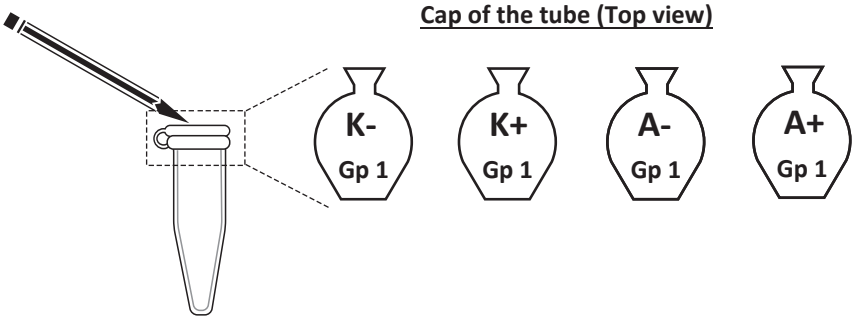
Restriction Buffer

Plasmid K

Plasmid A

Water

1



- Label 4 new microfuge tubes as **K-, K+, A-, A+** with **class** and **group number.**
- **'+'** represents **with** restriction enzymes; **'–'** represents **without** restriction enzymes.

2 *Think of the order... How would you add the reagents?*

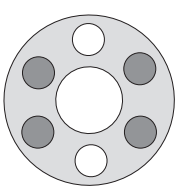
Table 3.1: Addition of reagents to the K-, K+, A- and A+ tubes

	K-	K+	A-	A+
Restriction Buffer	4.0 µL	4.0 µL	4.0 µL	4.0 µL
DNA Sample	4.0 µL **Plasmid K**	4.0 µL **Plasmid K**	4.0 µL **Plasmid A**	4.0 µL **Plasmid A**
Enzymes (with/ without)	2.0 µL **Water**	2.0 µL **Restriction Enzyme**	2.0 µL **Water**	2.0 µL **Restriction Enzyme**
Total volume:	10.0 µL	10.0 µL	10.0 µL	10.0 µL

- Add reagents according to the above table, pipette up and down a few times to **mix well**.
- **Close the tubes tightly** to prevent evaporation of the samples.

3

To pool the reagents at the bottom of each tube



Spin for 2 sec.

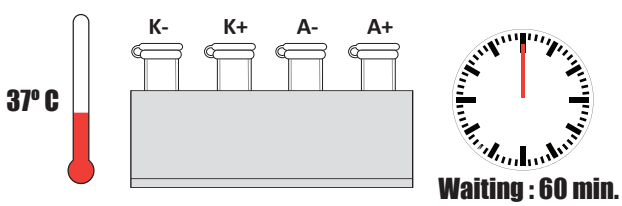
- Centrifuge the tube **K-, K+, A-, and A+** for 2 sec..

Balance the weight :

- *Arrange the tubes in the way for uniform weight distribution*

4

Make sure the tubes are closed tightly



- Incubate the 4 tubes in **37°C** water bath for **60 mins.**
- Store the tubes in the –20°C freezer for Lab 3.2.

Avoid over cutting:

- *Do not incubate more than 2 hours.*

Lab 3.2 Flow Chart : Putting the rfp gene into the plasmid (Ligation)

LIG (with DNA Ligase) — ***Keep on ice***

Ligation Buffer — ***Keep on ice***

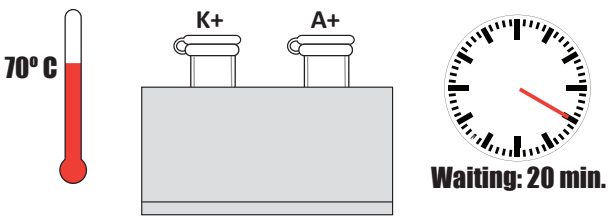
K+ (Digested plasmid K) — **from Lab 3.1**

A+ (Digested plasmid A) — **from Lab 3.1**

Water

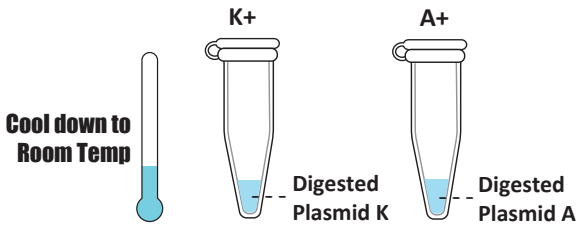
1

To inactivate the restriction enzymes



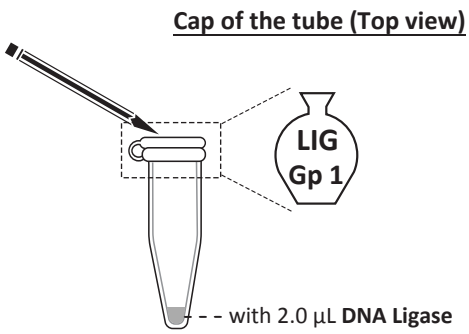
- Place tube **K+** and **A+** in **70°C** water bath for **20 mins**.

2



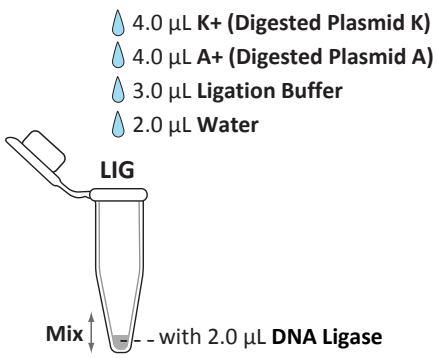
- After 20 minutes, remove **K+** and **A+** from water bath.
- Allow it to **cool down** to room temperature.

3



- Write your **class** and **group number** on the **LIG tube**.

4



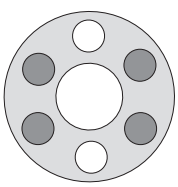
- Add reagents to the **LIG** tube as shown above.
- Pipette up and down a few time to **mix well**.

Avoid contamination:

- *Change a new tip every time after adding a solution.*

5

To pool the reagents at the bottom of each tube



Spin for 2 sec.

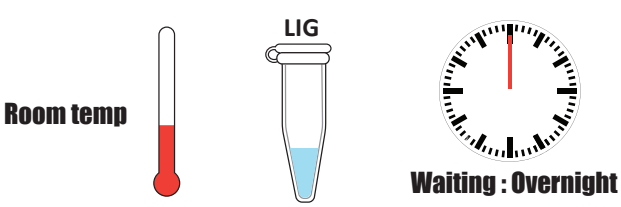
- Centrifuge the **LIG** tube for 2 sec..

Balance the weight :

- *Arrange the tubes in the way for uniform weight distribution*

6

Make sure the tubes are closed tightly



- Incubate the **LIG** tube at **room temperature overnight**.
- Store the tubes in the –20°C freezer for Lab 3.3.

Lab 3.3 Flowchart:
Gel Electrophoresis — Confirmation of Digestion and Ligation Products

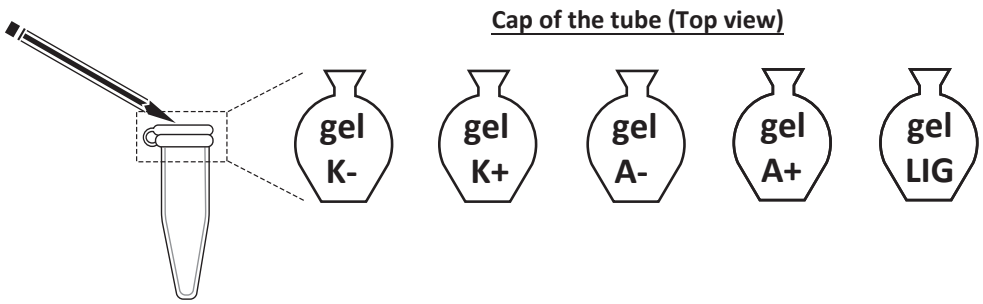
K- (Non-digested plasmid K) & **K+** (Digested plasmid K) — **from Lab 3.1**

A- (Non-digested plasmid A) & **A+** (Digested plasmid A) — **from Lab 3.1**

LIG (Ligated plasmid) — **from Lab 3.2**

Water	**Loading Dye**
TAE Buffer	**DNA Ladder**

1



- Label 5 new tubes as **gel K-, gel K+, gel A-, gel A+** and **gel LIG**.
- **'gel'** represents gel electrophoresis samples.

2

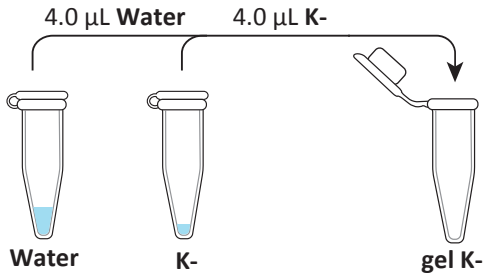
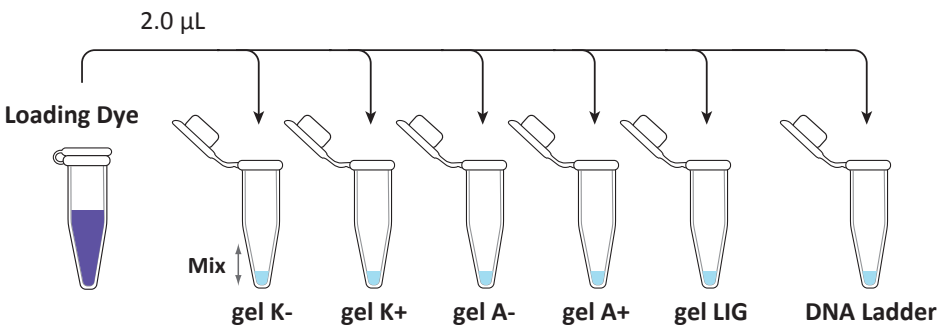


Table 3.3: Dilution of digestion (K-, K+, A-, A+) and ligation (LIG) products

	gel K-	gel K+	gel A-	gel A+	gel LIG
Water	4.0 µL	4.0 µL	4.0 µL	4.0 µL	4.0 µL
DNA Sample	4.0 µL **K-** (Non-digested)	4.0 µL **K+** (Digested)	4.0 µL **A-** (Non-digested)	4.0 µL **A+** (Digested)	4.0 µL **LIG** (Ligated)
Total volume:	8.0 µL	8.0 µL	8.0 µL	8.0 µL	8.0 µL

- Dilute the DNA samples according to the above table.
- **Return the LIG tube to your teacher** and store the tubes in the –20°C freezer for Lab 4.

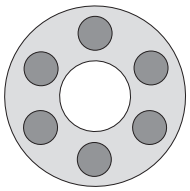
3



- Add **2.0 µL Loading Dye** to tube **gel K-, gel K+, gel A-, gel A+, gel LIG** and **DNA Ladder**.
- Gently pipette up and down few times to **mix well**.

4

To pool the reagents at the bottom of each tube



Spin for 2 sec.

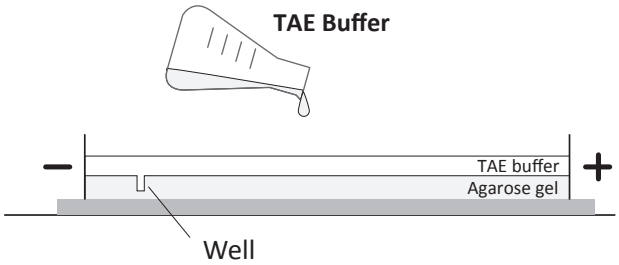
- Centrifuge **gel K-, gel K+, gel A-, gel A+, gel LIG** and **DNA Ladder** tubes for 2 sec..

Balance the weight :

- *Arrange the tubes in the way for uniform weight distribution*

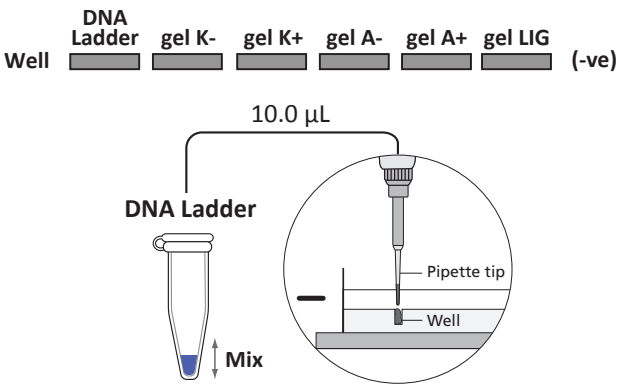
5

Just cover the surface of the gel



- Put the gel into the gel tank.
- Pour **TAE Buffer** into the tank, until it just cover the wells.

6

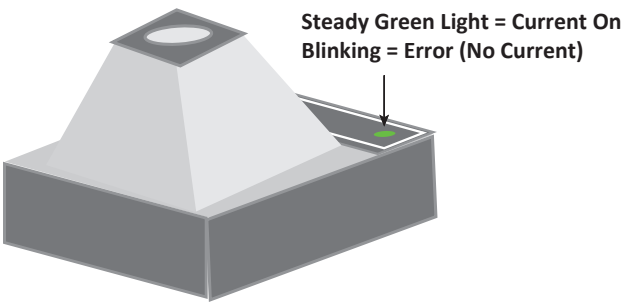


- Add **10.0 µL DNA Ladder**, **gel K-, gel K+, gel A-, gel A+ and gel LIG** into designated wells .
- Record the locations of your samples.

Avoid getting air into the buffer:

- *Press to 1st stop ONLY and hold the plunger while lifting up the pipette tip out of the buffer*

7



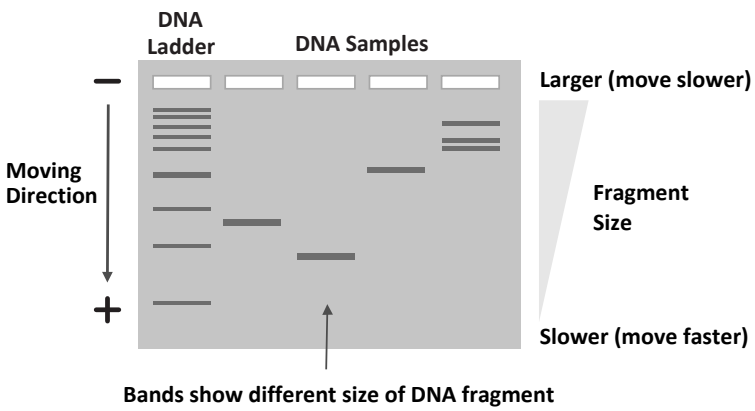
- Put the photo hood on the gel system.
- Press ⏻ to turn on the electric current .
- Observe bubbles come out at the (-) negative electrode.

Use weak blue light when running the gel:

- *Extensive exposure to blue light may weaken the fluorescent signal*

8

Waiting: ~20 min.



- After **20 minutes**, press ⏻ to turn off the electric current. Use 💡 **strong blue light** to observe the bands.
- **Take a gel photo** and record the relative locations of the DNA bands.

Lab 4 Flowchart: Transforming Bacteria with Recombinant Plasmid (A-rfp)

E. coli — *Keep on ice*

Plasmid A-rfp — Plasmid A with red fluorescent protein gene

Luria-Bertani Broth (LB)

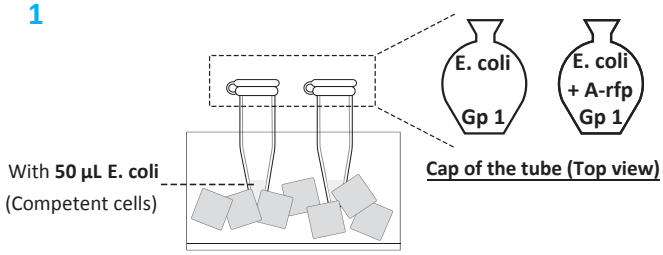
LB Plate — Contains LB Broth (LB)

LB/amp Plate — Contains LB Broth (LB) and ampicillin (amp)

LB/amp/ara Plate — Contains LB Broth (LB), ampicillin (amp) and sugar arabinose (ara)

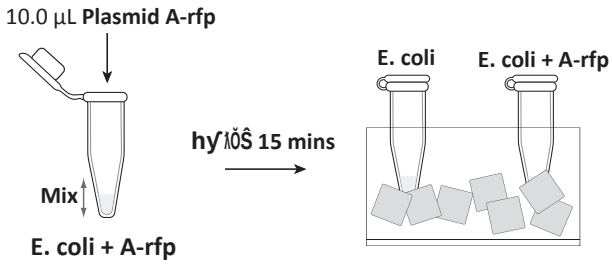
Part 1: Sample Preparation

1



- Write **your group number** on the **E.coli tubes**.
- Label **one** of E.coli tubes as **"E. coli + A-rfp".**

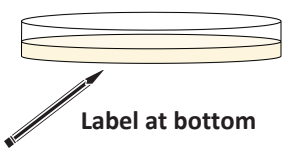
2

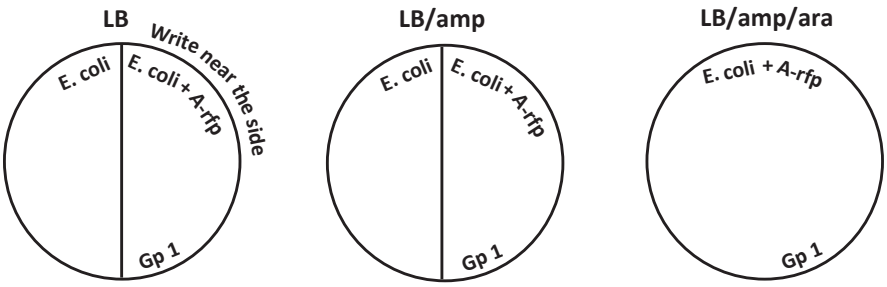


- Add **10.0 µL Plasmid A-rfp** to **"E. coli + A-rfp"** and **mix well.**
- Incubate all E.coli tubes **on ice** for **15 mins**.

3

Do not open the lid while labeling.

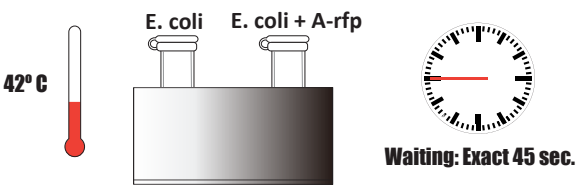




- Write your **class and group no.** on the pertri plates.
- **LB and LB/amp plate:** Draw a line in the middle. Label half as **"E. coli"** and the other half as **"E. coli + A-rfp"**.
- **LB/amp/ara plate:** Label as **"E. coli + A-rfp"**.

Part 2: Transformation (Heat Shock and Recovery)

4

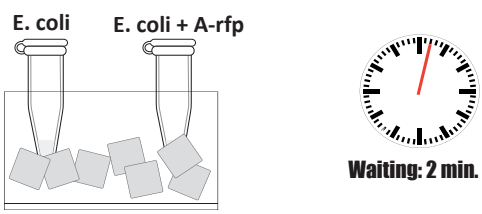


- Incubate **E.coli tubes** in **42°C** water bath for **45 sec**.

Avoid warming cells:

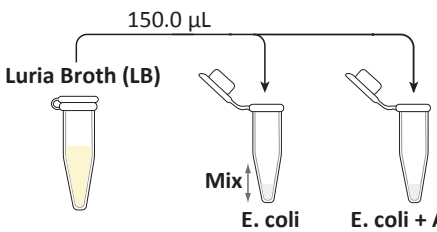
- *Carry tubes in the ice cup to water bath.*

5



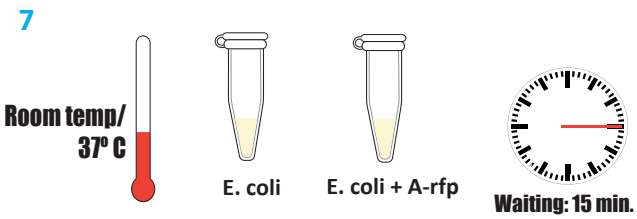
- Immediately place the tubes back **on ice** for **2 mins**.

6



- Add **150 µL Luria Broth (LB)** to the **E. coli tubes**.
- Gently flick a few times to **mix well**.

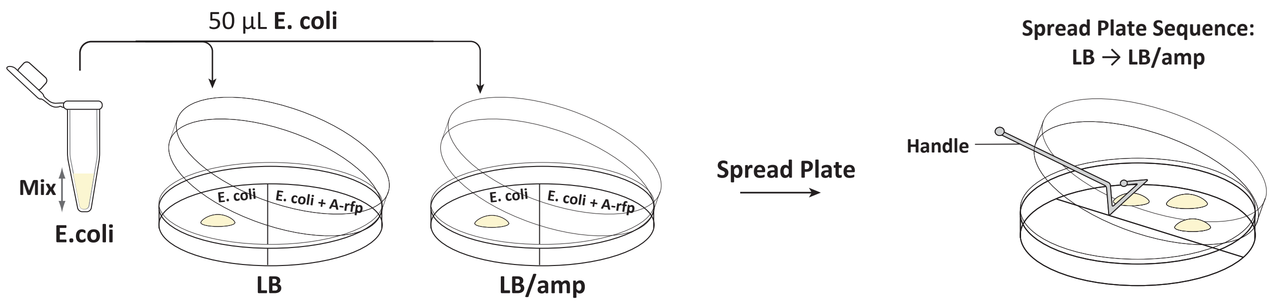
7



- Incubate the **E. coli tubes** at **room temperature (or 37°C)** for **15 mins**.

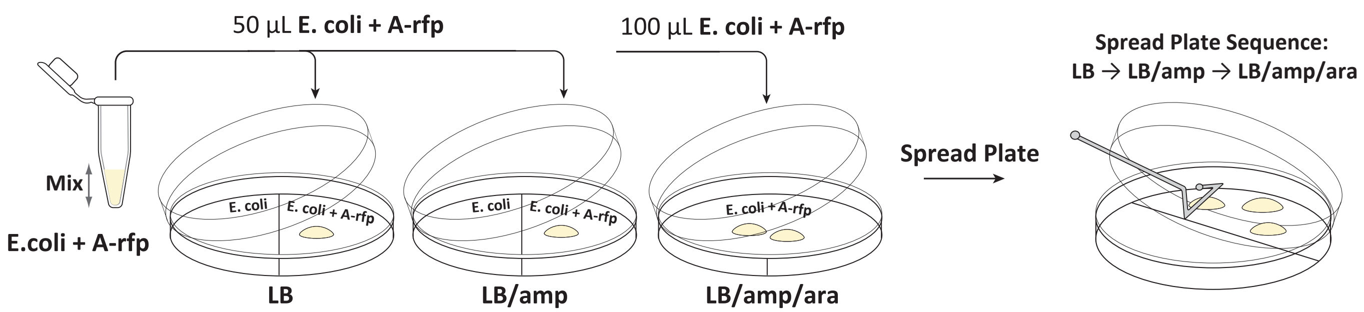
Part 3: Spread the Cells on Plates for Incubation

8 *Open the lid slightly just like a clamshell and add the cells slowly.*



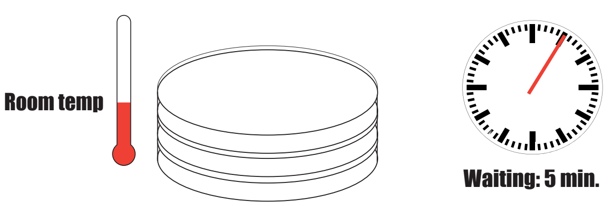
- Pipette up and down the **E. coli** a few times to **mix well**.
- Add **50 µL E. coli** to the **"E. coli" section** on **LB** plate and **LB/amp** plate.
- Use **one spreader** to spread the cells evenly across the **"E. coli" section** on **LB** and **LB/amp** plate.

9



- Pipette up and down the **E. coli + A-rfp** a few times to **mix well**.
- Add **50 µL E. coli + A-rfp** to the **"E. coli + A-rfp" section** on **LB** plate and **LB/amp** plate.
- Add **100 µL E. coli + A-rfp** to the **"E. coli + A-rfp"** section on **LB/amp/ara** plate.
- Use **one new spreader** to spread the cells evenly across the **"E. coli + A-rfp"** section on **LB**, **LB/amp**, **LB/amp/ara** plates.

10



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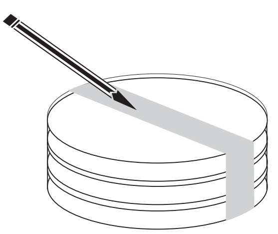
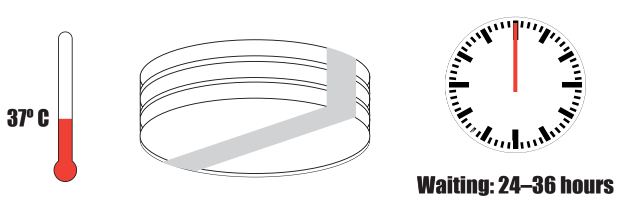
11



- Tape all three plates together
- Label the tape with **class** and **group no**.

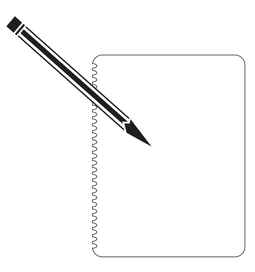
12



- ǇyŎǳōI·űŜ I·ű **37°C upside down** Ŧƻǋ **24–36 hrs.**

Prevent condensation from dripping onto the plate:
- *Incubate the plate upside down*

13



- **Take a photo** for each plate.
- Compare and record the growth on each half.