Lab 1.1 Flow Chart: Basic Pipetting And Serial Dilution

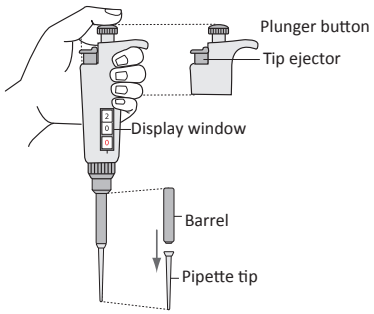
50-fold Dye

Dye

Water

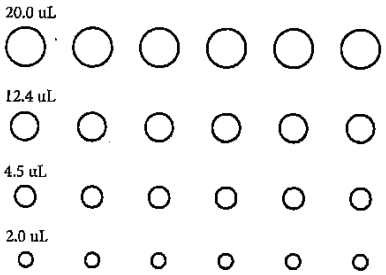
a. Pipetting different volume of the solution

1



- Choose an appropriate micropipette. Feel the first stop and second stop.

2

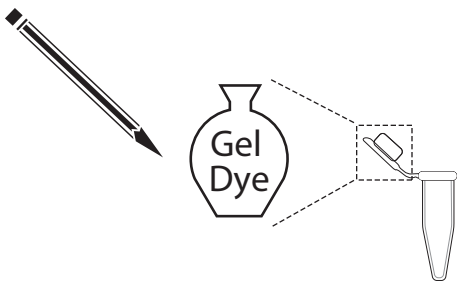


- Pipette the **50-fold Dye** of the volume below to the sheet:

2.0 µL 4.5 µL 12.4 µL 20.0 µL

b. Mixing solution: Preparing a 50-fold Dye

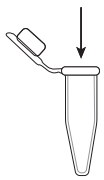
1



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

2

196.0 µL **Water** & 4.0 µL **Dye**



Gel Dye

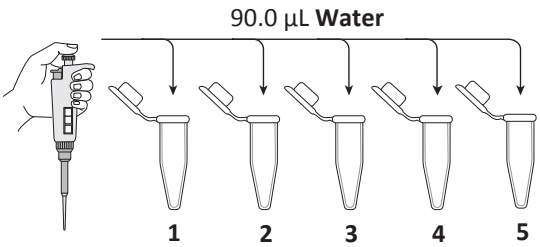
Avoid contamination:

- *Change a new tip.*

- Add 196.0 µL **Water**, and then 4.0 µL **Dye** to the tube.
- Mix well.

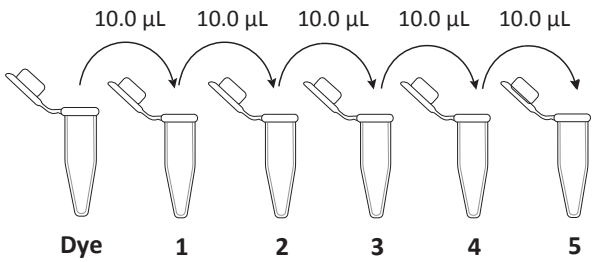
c. Serial dilution: Preparing a 100,000 fold dye

1



- Label 5 microfuge tubes **1** through **5** using a marker at the cap of the tube.
- Transfer 90.0 µL **Water** to the **5** tubes.

2

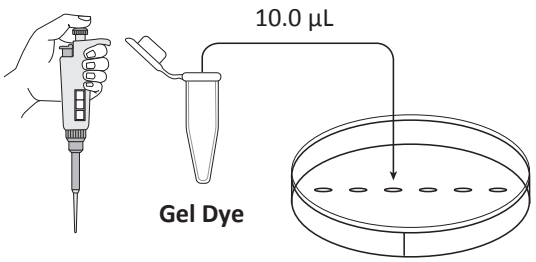


- Transfer 10.0 µL **Dye** to tube **1** and mix well.
- Use a new tip, transfer 10.0 µL solution from tube **1** to tube **2** and mix well.
- Repeat the process for tube **2** through **5**.
- Observe the change on color intensity

Lab 1.2 Flow Chart: Dye Separation By Gel Electrophoresis

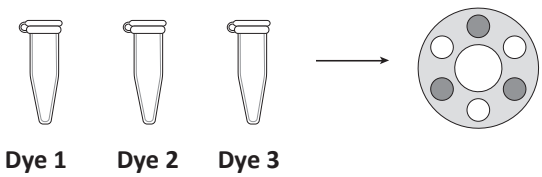
Gel Dye (from Lab 1.1)
Dye 1
Dye 2
Dye 3
TAE Buffer

1



- Pour **TAE Buffer** into the practice plate.
- Practice your technique by pipetting 10.0 µL **Gel Dye** to each well.

2



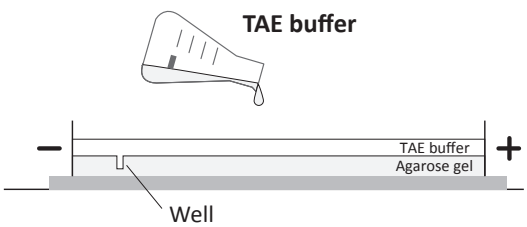
- Centrifuge the **Dye 1**, **Dye 2** and **Dye 3** tubes.

Balance the weight :

- *Arrange the tubes in a triangular pattern for uniform weight distrbution*

3

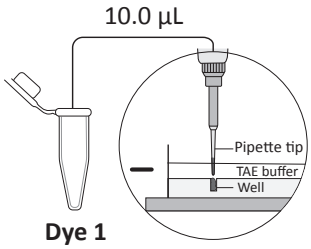
Just cover the surface of the gel



- Put the gel in the gel tank.
- Pour **TAE Buffer** into the gel tank.
- **Test the system:** Put the photo hood on the gel system and turn on the electric current.
- Check if the green light is shining and bubbles come out at the (-) negative electrode.

4

Make sure the wells are near the negative electrode.



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

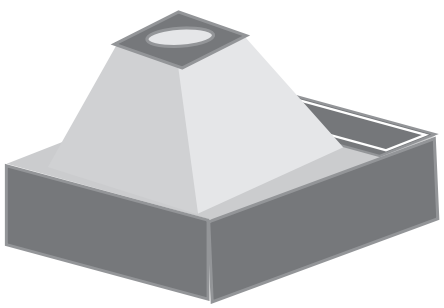
Avoid contamination:

- *Change a new tip every time.*

Avoid getting air into the buffer:

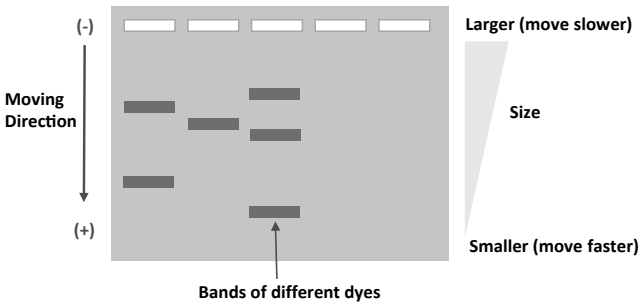
- *Press to 1st stop ONLY and hold the plunger while lifting up the pipette tip of of the buffer*

5



- Put the photo hood on the gel system and turn on the electric current.
- Check if the green light is shining and bubbles come out at the (-) negative electrode.

6



- After 10 minutes, turn off the electric current.
- Remove the photo hood and observe the bands.

Lab 2 Flow Chart: Identifying A Recombinant Plasmid

PCR Master Mix

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

Plasmid A

Loading Dye

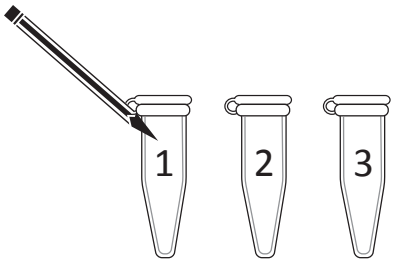
DNA Ladder

Water

TAE Buffer

Lab 2.1: Checking plasmid with PCR

1 *Ink can come off the top of the tube in the thermocycler (PCR machine).*



- Label three PCR tubes **1, 2** and **3** with your group number.

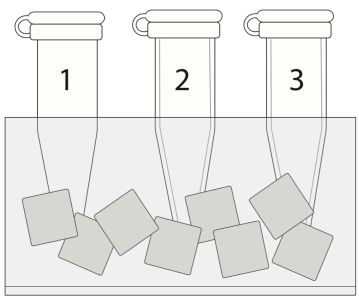
2 Table 2.1: Addition of reagents to the PCR tubes

	1	2	3
(a) **PCR master mix**	23.0 µL	23.0 µL	23.0 µL
(b) **Plasmid A-rfp**	2.0 µL		
(c) **Plasmid A**		2.0 µL	
(d) **Water**			2.0 µL
Total volume	25.0 µL	25.0 µL	25.0 µL

- Add reagents according to the order in Table 2.1.
- Gently pipette up and down several times to mix.

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

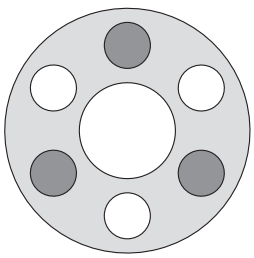
3



- Return all PCR tubes to the ice immediately.

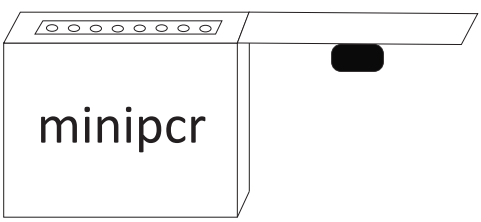
Avoid warming reagents:
- *Tubes must be kept cold in ice.*
- *Hold the tube by the upper rim.*

4



- Gently tap the bottoms of the PCR tubes or centrifuge the tubes if there are bubbles.

5



- Take your ice cup with PCR tubes to your teacher.
- Transfer your PCR tubes from the ice into the thermocycler.

6

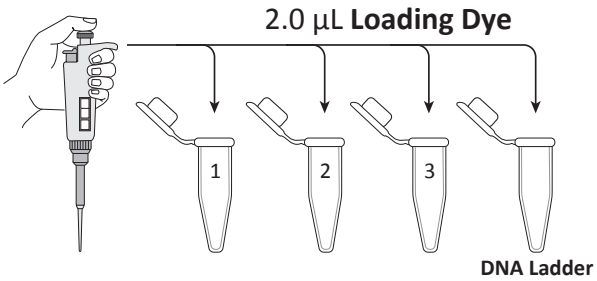
Table 2.2: PCR thermocycler program for ABE

		Temperature (°C)	Time (sec)
Initial hold		4	Indefinite
Initial denaturation		95	270
30 cycles	Denaturation	95	30
	Annealing	53	30
	Extension	68	60
Final extension		68	300

- The thermocycler has been pre-programmed for the reaction.

Lab 2.2: Confirmation by gel electrophoresis

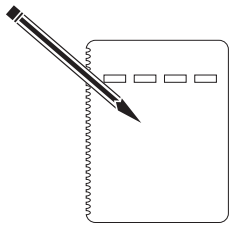
7



- Add 2.0 µL of **Loading Dye** to each of the three PCR tubes and to the tubes with **DNA ladder**.
- Gently pipette up and down several times to mix.

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

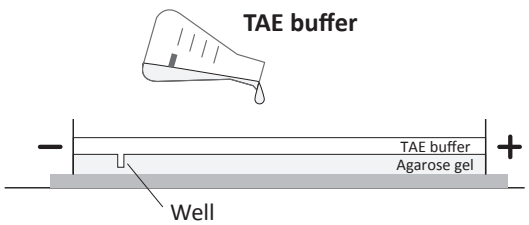
8



- Draw the location of the samples in your notebook.

9

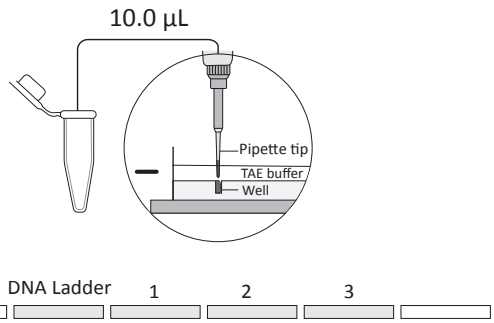
Just cover the surface of the gel



- Put the gel in the gel tank.
- Pour **TAE Buffer** into the gel tank.
- **Test the system:** Put the photo hood on the gel system and turn on the electric current.
- Check if the green light is shining and bubbles come out at the (-) negative electrode.

10

Make sure the wells are near the negative electrode.

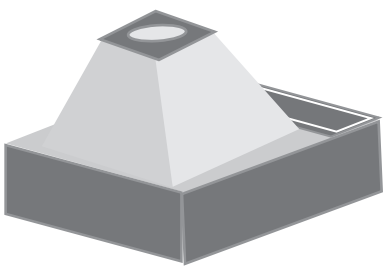


- Add 10.0 µL of the **DNA ladder** and each samples **(1, 2 ,3)** into designated wells.

Avoid contamination:
- *Change a new tip every time.*

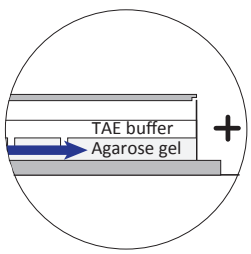
Avoid getting air into the buffer:
- *Press to 1st stop ONLY and hold the plunger while lifting up the pipette tip of of the buffer*

11



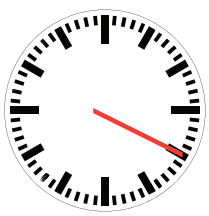
- Put the photo hood on the gel system and turn on the electric current.
- Check if the green light is shining and bubbles come out at the (-) negative electrode.

12



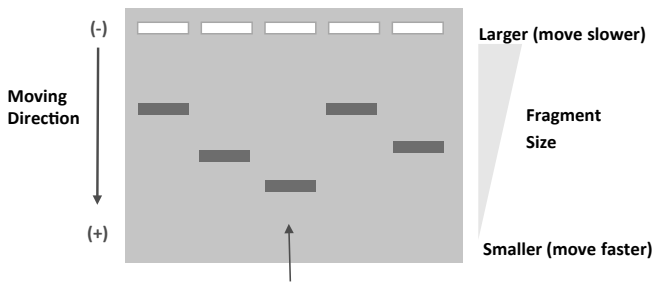
- After two or three minutes, see if the bands are moving towards the (+) positive electrode.

13



~20 min

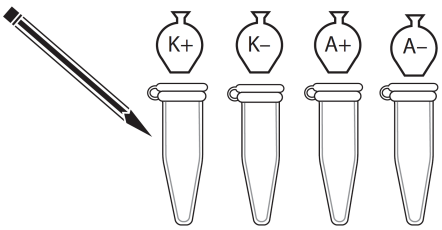
- After 20 minutes, observe the bands.
- Record the location of bands in your notebook



Lab 3.1 Flow Chart: Cutting The Two Plasmids (Restriction Digestion)

Restriction Buffer
Plasmid K
Plasmid A
Restriction Enzymes (BamHI & HindIII)
Water

1 *'+' means present ; '–' means absent*



- Label 4 new tubes as **K+**, **K–**, **A+**, and **A–** with class and group no. .

2

- Add reagents according to the order in **Table 3.1 .**

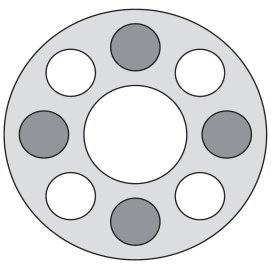
Table 3.1: Addition of reagents to the K+, K–, A+, and A– tubes

	K+	K–	A+	A–
(a) **Restriction buffer**	4.0 µL	4.0 µL	4.0 µL	4.0 µL
(b) **Plasmid K**	4.0 µL	4.0 µL		
(c) **Plasmid A**			4.0 µL	4.0 µL
(d) **Restriction Enzymes** and mix	2.0 µL		2.0 µL	
(e) **Water** and mix		2.0 µL		2.0 µL
Total Volume	10.0 µL	10.0 µL	10.0 µL	10.0 µL

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

Mix well:
- *Gently pipette up and down three times.*

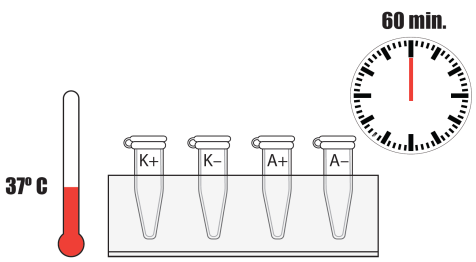
3 *To pool the reagents at the bottom of each tube*



- Spin the four microfuge tubes **(K+, A+, K–, and A–)** for few seconds.

Balance the weight:
- *Distribute the tubes evenly*

4



- Incubate 4 tubes in 37°C water bath for 60 mins.

Avoid non-specific cutting:
- *Incubate not longer than 2 hours*

5

- After the incubation, store 4 tubes in the –20°C freezer for use in *Lab 3.2.*

Lab 3.2 Flow Chart: Putting The rfp Gene Into The Plasmid (Ligation)

K+ (Digested Plasmid K)

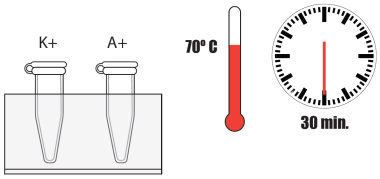
A+ (Digested Plasmid A)

Ligation buffer

LIG (with DNA Ligase)

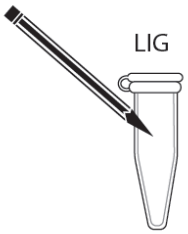
Water

1



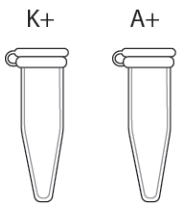
- Place the **K+** and **A+** in 70°C water bath for 30 mins.

2



- Label **LIG** with class and group no. .

3



- After 30 minutes, remove **K+** and **A+** from the water bath.

4

- Add reagents directly into **LIG** according to the order in **Table 3.2**.

Table 3.2: Addition of reagents LIG tubes

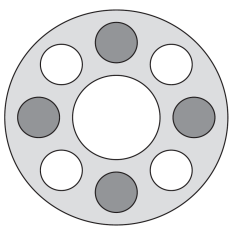
	LIG (with 2.0 µL of DNA Ligase)
(a) Digested plasmid A **(A+)**	4.0 µL
(b) Digested plasmid K **(K+)**	4.0 µL
(c) **Ligation buffer**	3.0 µL
(d) **Water** and mix	2.0 µL
Total volume	15.0 µL

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

Mix well:
- *Gently pipette up and down three times.*

5

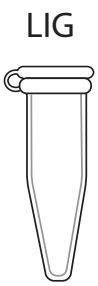
Pool the reagents at the bottom of each tube



- Spin the **LIG** tube for a few seconds.

Balance the weight:
- *Distribute the tubes evenly*

6



- Incubate **LIG** tube at room temperature overnight.

7

- Store **K+** and **A+** tubes in the –20°C freezer for use in *Lab 3.3.*

Lab 3.3 Flow Chart: Confirmation By Gel Electrophoresis

K- (Non-digested Plasmid K)

K+ (Digested Plasmid K)

A- (Non-digested Plasmid A)

A+ (Digested Plasmid A)

LIG (Ligated Plasmids)

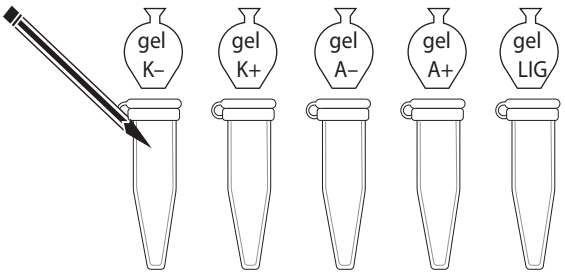
Loading Dye

Water

DNA Ladder

TAE buffer

1 *'gel' indicates gel electrophoresis samples*



- Label five new microfuge tubes **gel A–**, **gel A+**, **gel K–**, **gel K+** and **gel LIG** with class and group no. .

2

- Add reagents according to the order in **Table 3.3**.
- Pipette up and down several times to mix.

Table 3.3: Addition of reagents to the gel K–, gel K+, gel A–, gel A+, gel LIG and M tubes

	gel K–	gel K+	gel A–	gel A+	gel LIG	DNA Ladder
(a) **Water**	4.0 µL	4.0 µL	4.0 µL	4.0 µL	3.0 µL	
(b) **Loading Dye**	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL	2.0 µL
(c) Nondigested plasmid K **(K–)** and mix	4.0 µL					
(d) Digested plasmid K **(K+)** and mix		4.0 µL				
(e) Nondigested plasmid A **(A–)** and mix			4.0 µL			
(f) Digested plasmid A **(A+)** and mix				4.0 µL		
(g) Ligated plasmid **(LIG)** and mix					5.0 µL	
Total volume	10.0 µL	10.0 µL	10.0 µL	10.0 µL	10.0 µL	10.0 µL

Avoid contamination:

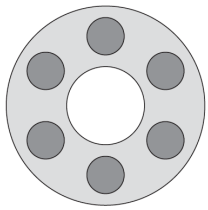
- *Change a new tip every time after adding a solution.*

3

LIG

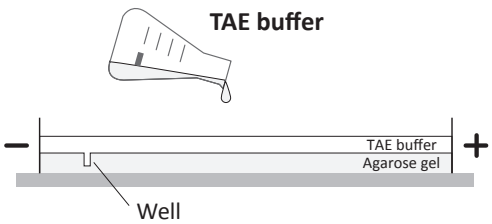
- Return the **LIG** tube to your teacher to store in the –20°C freezer for use in *Lab 4.*

4 *Pool the reagents at the bottom of each tube.*



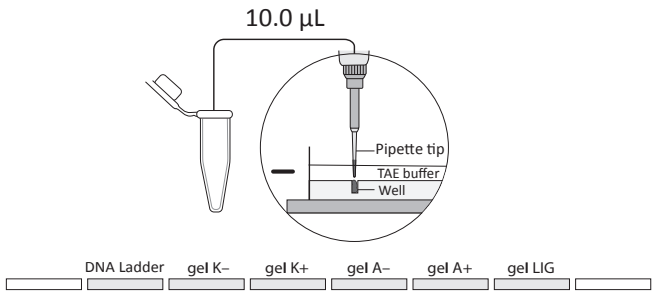
- Spin the six tubes (**gel A–**, **gel A+**, **gel K–**, **gel K+, gel LIG** and **M**) for a few seconds.

5 *Just cover the surface of the gel*



- Put the gel in the gel tank.
- Pour **TAE Buffer** into the gel tank.
- **Test the system:** Put the photo hood on the gel system and turn on the electric current.
- Check if the green light is shining and bubbles come out at the (-) negative electrode.

6 *Make sure the wells are near the negative electrode.*

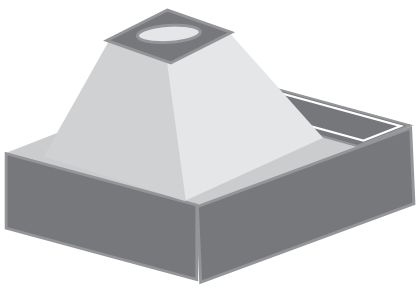


- Add 10.0 µL of the **DNA Ladder** and five samples (**gel A–**, **gel A+**, **gelK–**, **gelK+** and **gel LIG**) into the wells.

Avoid contamination:
- *Change a new tip every time.*

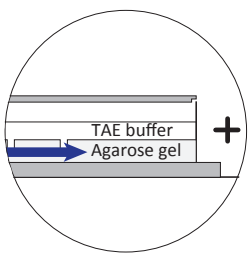
Avoid getting air into the buffer:
- ***Press to 1st stop ONLY and hold the plunger while removing the pipette tip out of the buffer.***

7



- Put the photo hood on the gel system and turn on the electric current.
- Check if the green light is shining and bubbles come out at the (-) negative electrode.

8

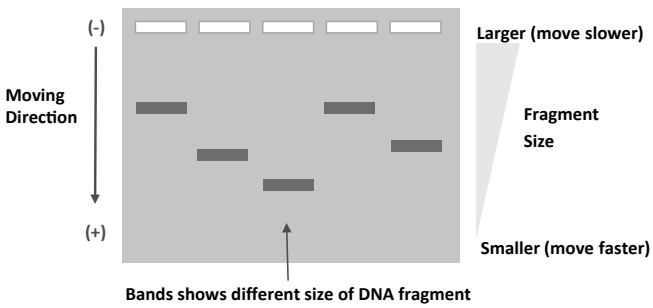


- After two or three minutes, see if the bands are moving towards the (+) positive electrode.

9

~20 min

- After 20 minutes, observe the bands.
- Record the location of bands in your notebook

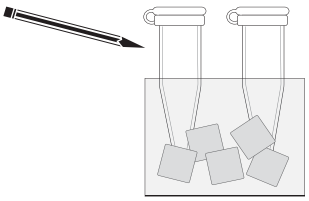


Lab 4 Flowchart: Transforming Bacteria With Recombinant Plasmid

LIG/ Plasmid A-rfp	Ligated plasmid/ Plasmid A with red fluorescent protein gene
Luria Broth (LB)	Luria Broth
E. coli	50 µL of chilled competent *E. coli* cells x 2
LB	Plate contains Luria Broth (LB)
LB/amp (one stripe)	Plate contains Luria Broth (LB) and ampicillin (amp)
LB/amp/ara (two stripes)	Plate contains Luria Broth (LB), ampicillin (amp) and sugar arabinose (ara)

Part 1: Sample Preparation

1

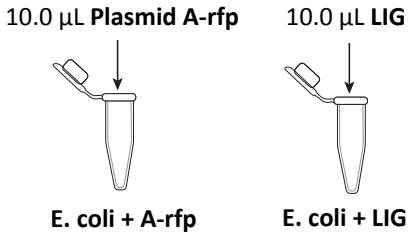


Label the **E. coli** tube:

- Without any plasmid as **"E. coli"**
- with Plasmid A-rfp as **"E. coli + A-rfp"**
- with LIG as **"E. coli + LIG"**

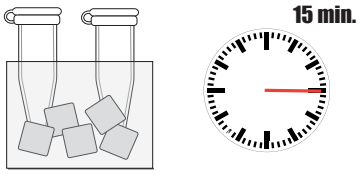
Avoid warming cells:
- *Keep two tubes on ice.*
- *Do not hold the bottom of the tubes.*

2



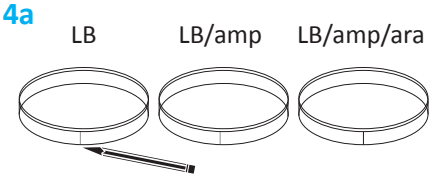
- Add 10.0 µL of **Plasmid A-rfp (or LIG)** to the E. coli tubes.
- Gently flick the tube three times to mix.
- Return the tube immediately to ice.

3



Keep all the E. coli tubes on ice for 15 mins.

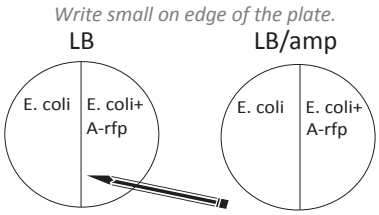
4a



- Prepare three agar Petri plates— **LB**, **LB/amp**, and **LB/amp/ara**.
- Label the bottom of each plate with class & group no. .

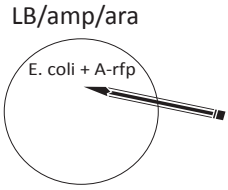
Avoid contamination:
- *keep the plates closed while labelling*

4b



- Draw a line in the middle of **LB** and **LB/amp** plate.
- Label half of each plate **"E. coli"** and the other half **"E. coli + A-rfp"**.

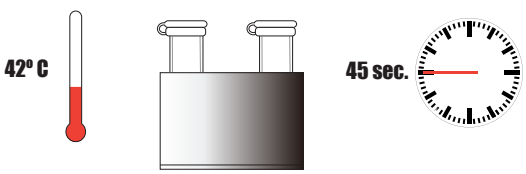
4c



Label **LB/amp/ara** plate as **"E. coli + A-rfp"**.

Part 2: Transformation (Heat Shock and Recovery)

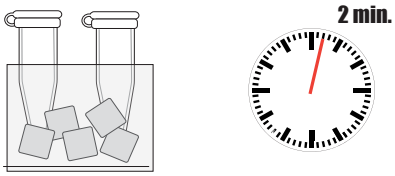
5



After 15-min incubation on ice, incubate the **E. coli** tubes in 42°C water bath for exactly 45 sec.

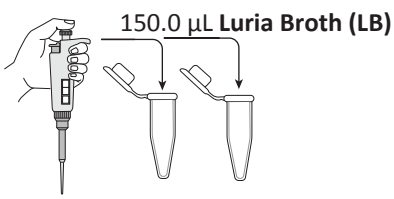
Avoid warming cells:
- *Carry tubes in the cup of ice to water bath.*

6



Immediately place the tubes back on ice for 2 mins.

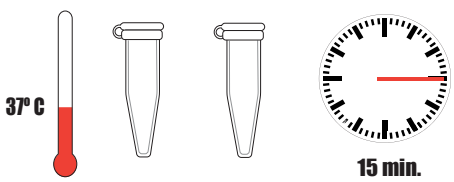
7



- Add 150 µL of **Luria Broth (LB)** to the **E. coli** tubes
- Gently flick it three times to mix.

Avoid contamination:
- *change a new tip every time after adding a solution.*

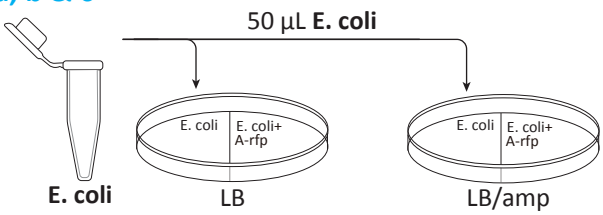
8



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

Part 3: Spread the Cells on Plates for Incubation

9a, b & c

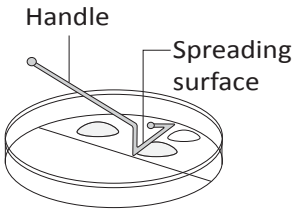


- Suspend **E. coli** by gently pump the pipette two or three times.
- Add 50 µL of **E. coli** cells to **"E. coli"** on **LB** plate and **LB/amp** plate.

Avoid contamination:
- *Change a new tip every time after adding a solution.*
- *Open lid just big enough to add the cells (like a clamshell)*

Avoid the cells slipping to another half of the plates:
- *Add the cells slowly to the section*

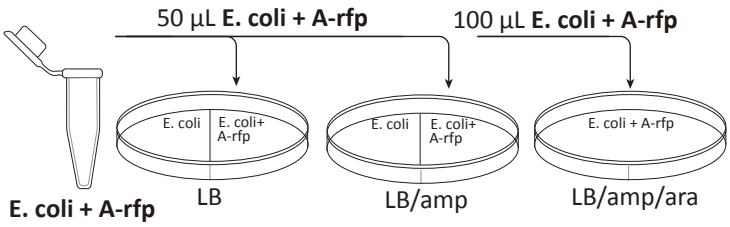
10a, b & c



Use the same spreader to spread the **E. coli** cells evenly across the entire **"E. coli"** section on **LB** and **LB/amp** plate.

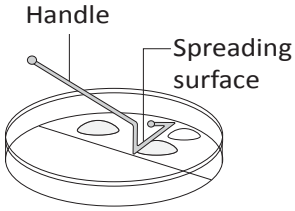
Avoid contamination:
- *Hold the spreader by the handle.*
- *Do not allow the bent end to touch any surface.*
- *Open lid just big enough to add the cells (like a clamshell)*

11



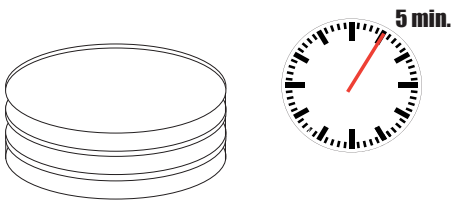
- Repeat step 9 for **E.coli + A-rfp.**
- Add 50 µL **E. coli + A-rfp** cells **"E. coli + A-rfp"** on **LB** and **LB/amp** plates.
- Add 100 µL **E. coli + A-rfp** cells to the **LB/amp/ara** plate.

12



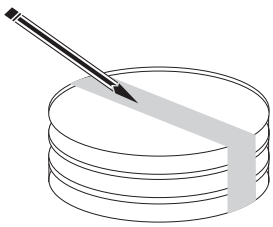
- Repeat step 10 for **E.coli + A-rfp.**
- Spread the **E. coli + A-rfp** cells evenly across the entire **"E. coli + A-rfp"** section on **LB**, **LB/amp**, and **LB/amp/ara plates**.

13



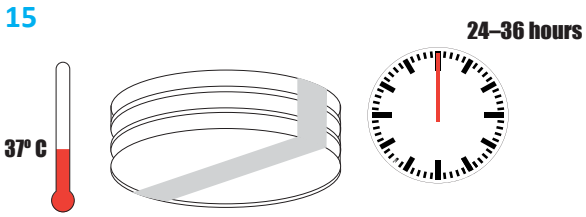
Leave all plates right side up for 5 mins.

14



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

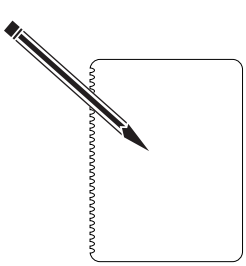
15



Incubate the plates at 37°C upside down for 24–36 hours.

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

16



Examine the plates and record the amount of growth on each half.