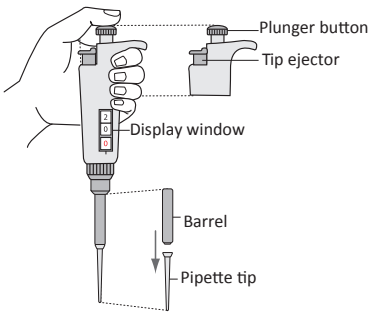
Lab 1 Flow Chart : Learning basic laboratory skills

RD	Red dye solution
S1	Dye 1
S2	Dye 2
S3	Dye 3
H_2O	Water
1X TAE	1X TAE buffer

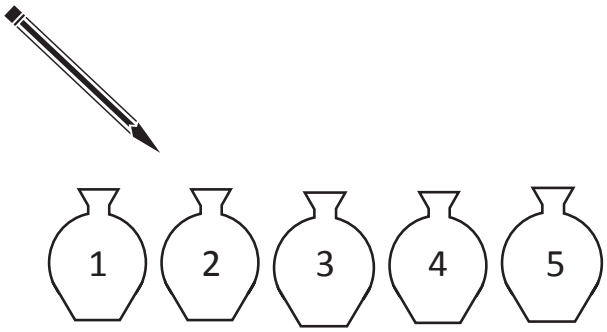
Lab 1.1: Basic pipetting and serial dilution

1



- Pick up and inspect the micropipettes on your bench, identify the parts.

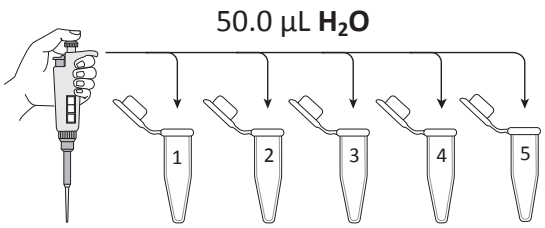
2



- Label 5 microfuge tubes 1 through 5 using a marker.

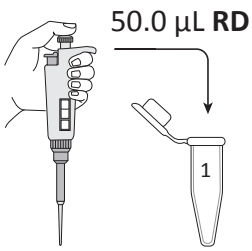
3

Which pipette to use?



- Transfer 50 µL water **(H_2O)** into the 5 tubes.

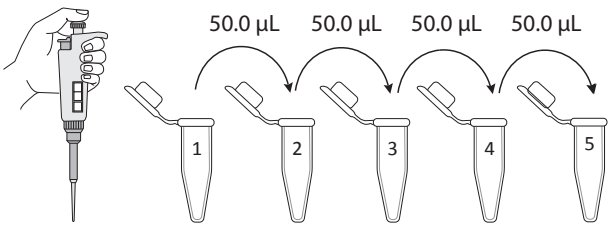
4



- Transfer 50 µL red dye **(RD)** into tube 1.
- Pipette up and down several times to mix.

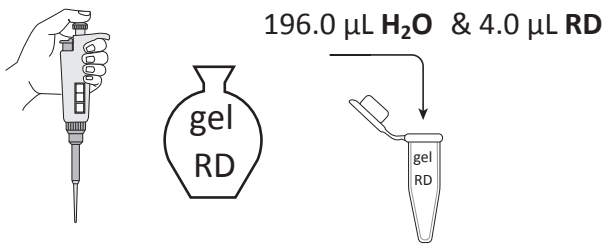
Avoid contamination:
- *Change a new tip.*

5 & 6



- Use a new tip, transfer 50 µL solution from tube 1 into tube 2 and mix well.
- Repeat the process for tube 2 through 5.
- Observe the decreasing intensity of colour of redness from tube 1 through 5.

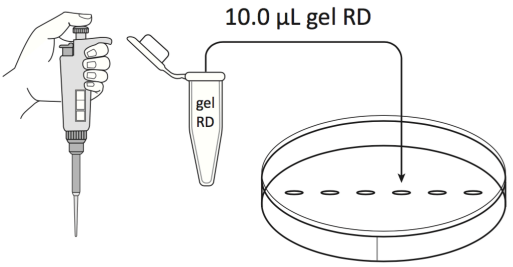
7



- Label a new microfuge tube **gel RD**.
- Prepare 50-fold red dye solution **(gel RD)** by adding 196 µL water **(H_2O)**, then 4 µL red dye **(RD)** to the tube.

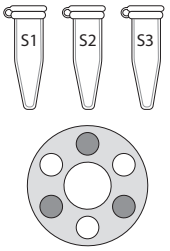
Lab 1.2: Dye separation by gel electrophoresis

8



- Pour 1x TAE buffer **(1x TAE)** into the practice plate.
- Practice your technique by pipetting 10 µL 50-fold red dye solution **(gel RD)** to each well.

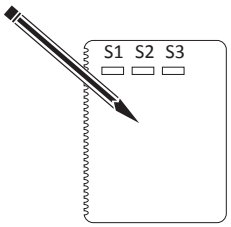
9



- Centrifuge the **S1**, **S2**, and **S3** tubes.

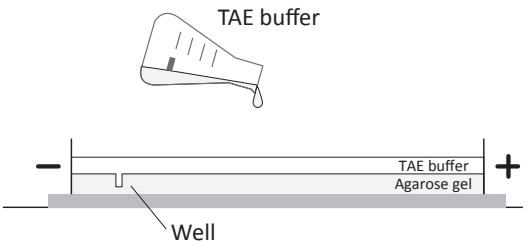
Balance the weight :
- *Arrange the tubes in a triangular pattern to attain uniform weight distribution.*

10



- Draw the location of the wells in your notebook
- Record which solution you will place in each well.

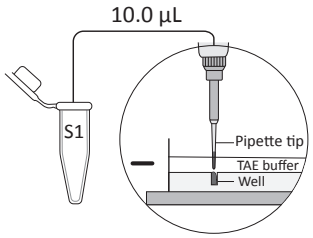
Just cover the surface of the gel



- Pour 1x TAE buffer **(1x TAE)** into the gel tank.
- Put the gel tank into the gel electrophoresis system.

12

Make sure the wells are near the negative electrode.

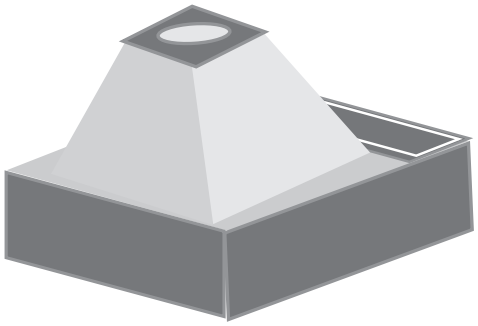


- Add 10 µL **S1** solution into the well.
- Repeat the step for **S2** and **S3**.

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

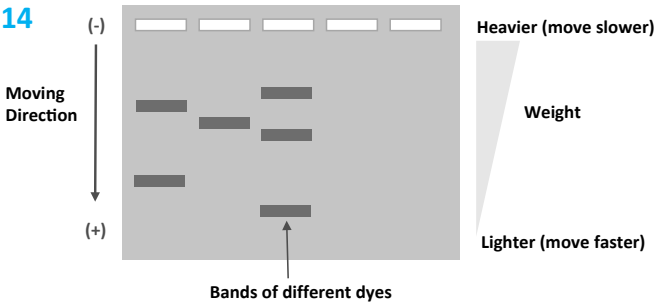
Avoid getting air into the buffer:
- ***Press to 1st stop ONLY and hold the plunger while removing the pipette tip out of the buffer.***

13



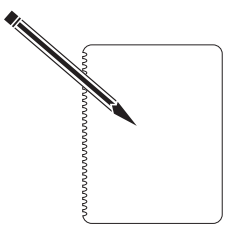
- Place the photo hood on the gel electrophoresis system
- Turn on power supply and press the on/off button.
- Check if bubbles form in the buffer at the (-) electrode.

14



- After 10 minutes, or when you can distinguish all three dyes, turn off the switch.
- Remove the photo hood from the electrophoresis system and observe the dyes in the gel.

15



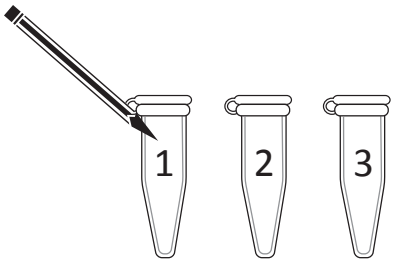
- Draw the relative location of the bands and their colors in each of the lanes containing your samples.

Lab 2 Flow Chart : Identifying a recombinant plasmid pARA-R

MM	PCR master mix
A-rfp	Plasmid A with *rfp* gene (pARA-R)
A	Plasmid A (pARA)
LD	Loading dye
M	DNA ladder
H_2O	Water
1x TAE	1X 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.

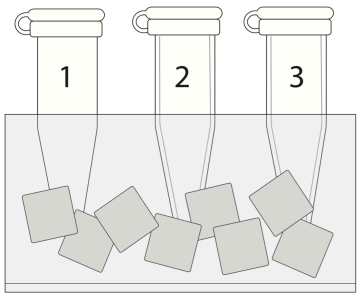
2 Table 2.1: Addition of reagents to the PCR tubes

	1	2	3
(a) PCR master mix **(MM)**	23.0 µL	23.0 µL	23.0 µL
(b) Plasmid A with *rfp* gene **(A-rfp)**	2.0 µL		
(c) Plasmid A **(A)**		2.0 µL	
(d) Water **(H_2O)**			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.*

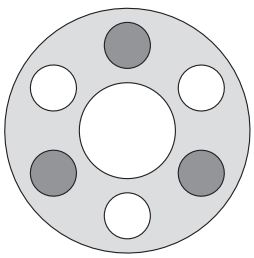
3



- Return all PCR tubes to the ice immediately.

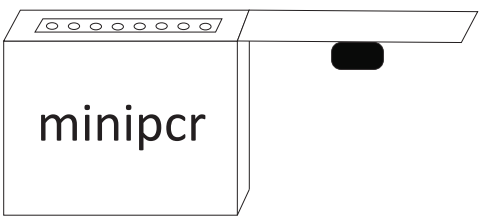
Avoid warming reagents:
- *Tubes must be kept cold in ice.*
- *Hold the tube by the upper rim.*

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

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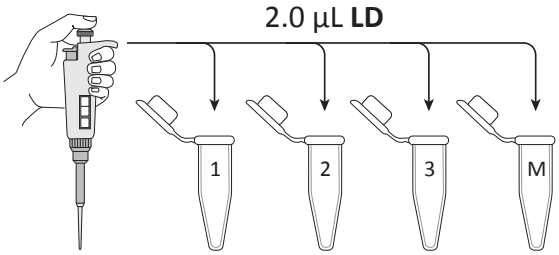
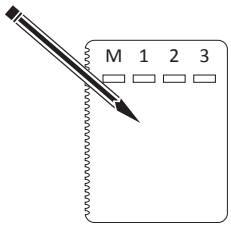


- Add 2 µL of loading dye **(LD)** to each of the three PCR tubes and to the tubes with DNA ladder **(M)**.
- Gently pipette up and down several times to mix.

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

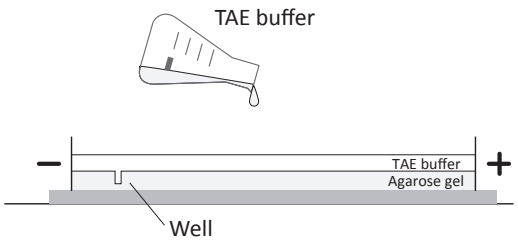
8



- Draw the location of the wells in your notebook.

9

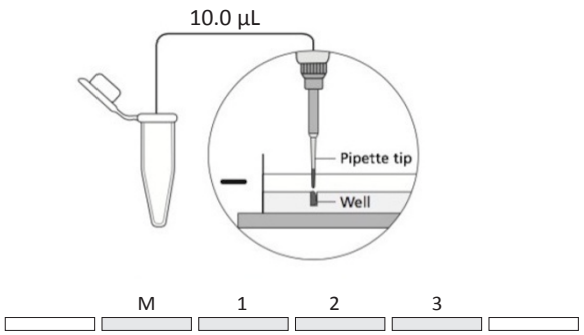
Just cover the surface of the gel



- Pour 1x TAE buffer **(1x TAE)** into gel tank.
- Put the gel tank into the gel electrophoresis system.

10

Make sure the wells are near the negative electrode.

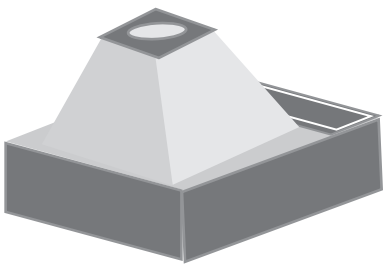


- Add 10 µL of the DNA ladder **(M)** and each samples **(1,2,3)** into designated wells.

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

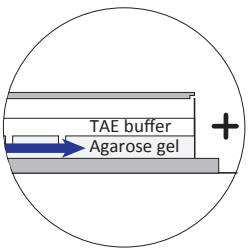
Avoid getting air into the buffer:
- ***Press to 1st stop ONLY and hold the plunger while removing the pipette tip out of the buffer.***

11



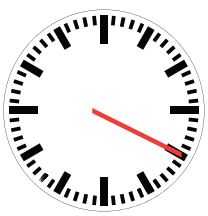
- Place the photo hood on the gel electrophoresis system
- Turn on power supply and press the on/off button.
- Check if bubbles form in the buffer at the (-) electrode.

12



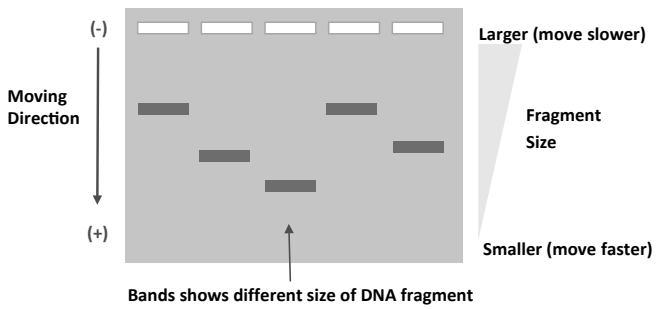
- After two or three minutes, see if the bands are moving towards the positive electrode.

13



~20 min

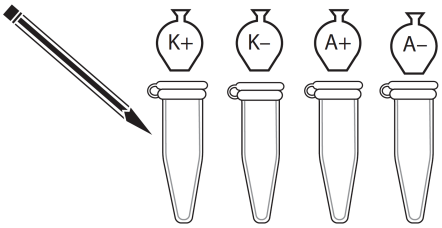
- After 20 minutes, observe the bands.
- Record the location of bands in your notebook



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

2.5x RB	2.5x Restriction Buffer
K	Plasmid K (pKAN-R)
A	Plasmid A (pARA)
RE	Restriction Enzymes (BamHI & HindIII)
H_2O	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 .**

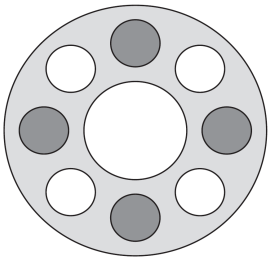
Table 3.1: Addition of reagents to the K+, K–, A+, and A– tubes

	K+	K–	A+	A–
(a) Restriction buffer **(2.5x RB)**	4.0 µL	4.0 µL	4.0 µL	4.0 µL
(b) Plasmid K **(K)**	4.0 µL	4.0 µL		
(c) Plasmid A **(A)**			4.0 µL	4.0 µL
(d) Restriction Enzymes **(RE)** and mix	2.0 µL		2.0 µL	
(e) Water **(H_2O)** and mix		2.0 µL		2.0 µL
Total Volume	10 µL	10 µL	10 µL	10 µL

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

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

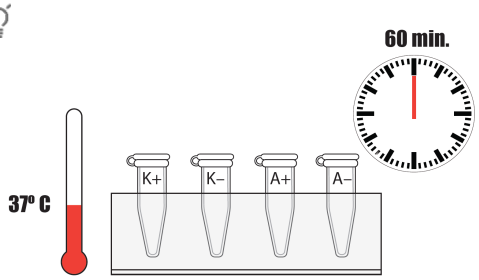
3 *To pool the reagents at the bottom of each tube*



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

Balance the weight:
- *Distribute the tubes evenly*

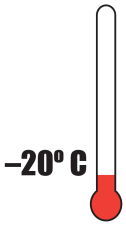
4



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

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

5

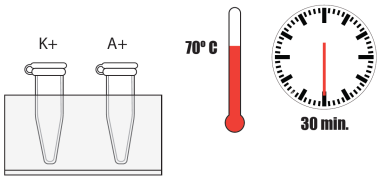


- After the incubation, store 4 tubes in the –20°C freezer for use in *Lab 3.2.*

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

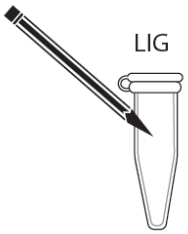
K+	Digested plasmid K (pKAN-R)
A+	Digested plasmid A (pARA)
5x LigB	5x ligation buffer
LIG	DNA ligase
H_2O	Water

1



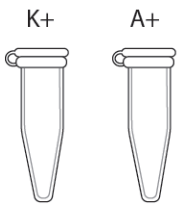
- Place the **K+** and **A+** in 70°C water bath for 30 mins.

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

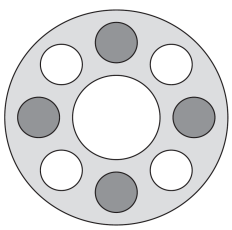
	LIG (with 2.0 µL of DNA Ligase)
(a) Digested plasmid A **(A+)**	4.0 µL
(b) Digested plasmid K **(K+)**	4.0 µL
(c) 5x ligation buffer **(5x LigB)**	3.0 µL
(d) Water **(H_2O)** and mix	2.0 µL
Total Volume	15 µL

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

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

5

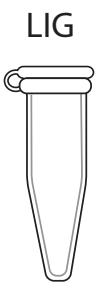
Pool the reagents at the bottom of each tube



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

Balance the weight:
- *Distribute the tubes evenly*

6



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

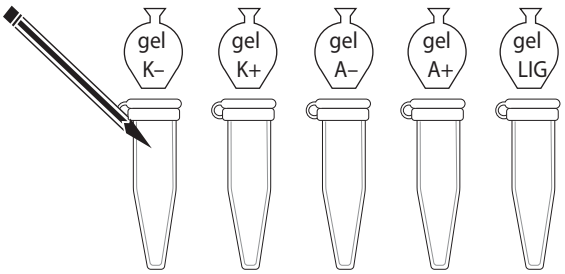
7

- 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-	Nondigested plasmid K (pKAN-R)
K+	Digested plasmid K (pKAN-R)
A-	Nondigested plasmid A (pARA)
A+	Digested plasmid A (pARA)
LIG	Ligated plasmid
LD	Loading dye
H_2O	Water
M	DNA ladder
1x TAE	1x 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**	**M (contain 8 µL DNA ladder)**
(a) Water **(H_2O)**	4.0 µL	4.0 µL	4.0 µL	4.0 µL	3.0 µL	
(b) Loading Dye **(LD)**	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 µL	10 µL	10 µL	10 µL	10 µL	10 µL

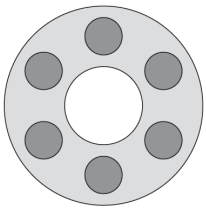
Avoid contamination:

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

3

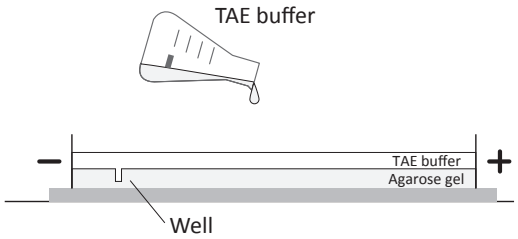
- Return the **LIG** tube to your teacher to store in the –20°C freezer for use in *Lab 4.*

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



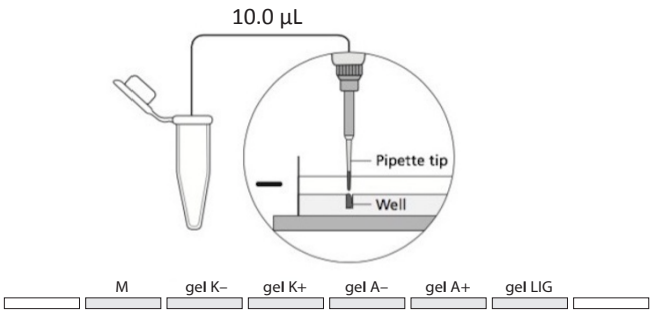
- Spin the six tubes (**gel A–**, **gel A+**, **gel K–**, **gel K+**, **gel LIG** and **M**) for a few seconds.

5 *Just cover the surface of the gel*



- Pour **1x TAE** buffer **(1x TAE)** into gel tank.
- Put the gel tank into the gel electrophoresis system.

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

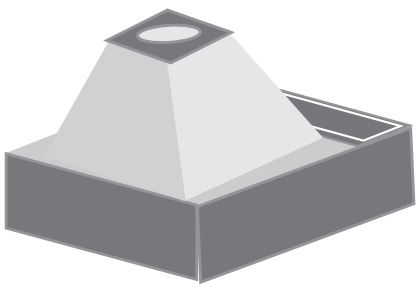


- Add 10 µL of the **M** and five samples (**gel A–**, **gel A+**, **gel K–**, **gel K+** and **gel LIG**) into the wells

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

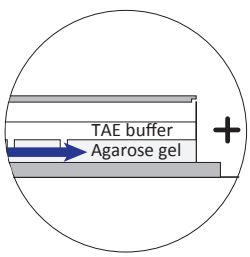
Avoid getting air into the buffer:
- ***Press to 1st stop ONLY and hold the plunger while removing the pipette tip out of the buffer.***

7



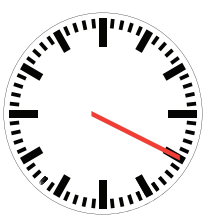
- Place the photo hood on the gel electrophoresis system
- Turn on power supply and press the on/off button.
- Check if bubbles form in the buffer at the (-) electrode.

8



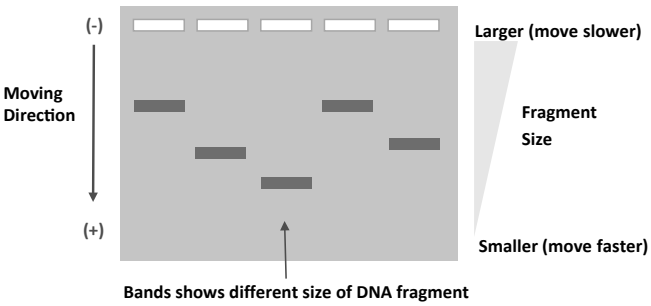
- After two or three minutes, see if the bands are moving towards the positive electrode.

9



~20 min

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



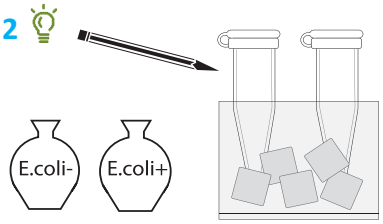
Lab 4 Flowchart: Transforming Bacteria with Recombinant Plasmid

LIG/A-rfp	Ligated plasmid/ Plasmid A with *rfp* gene
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)

1 Use **ASEPTIC** techniques throughout the whole lab.

Part 1: Sample Preparation

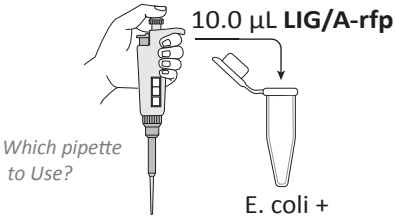
2



Label two tubes of **E. coli** cells with "**–**" and "**+**" and your group no. .

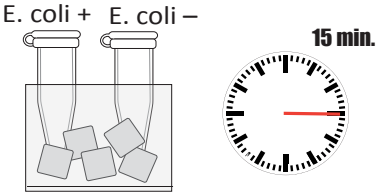
Avoid warming cells:
- *Keep two tubes on ice.*
- *Do not hold the bottom of the tubes.*

3



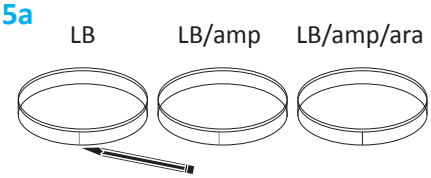
- Add 10 µL of **LIG** (or **A-rfp)** to **"E. coli+"**.
- Gently flick the tube three times to mix.
- Return the tube immediately to ice.

4



Keep **E. coli–** and **E. coli+** on ice for 15 mins.

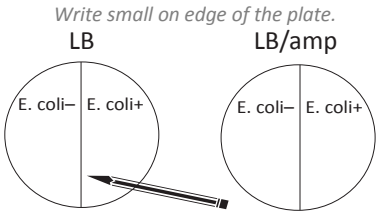
5a



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

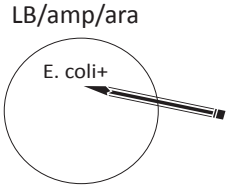
Avoid contamination:
- *keep the plates closed while labelling*

5b



- 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+".

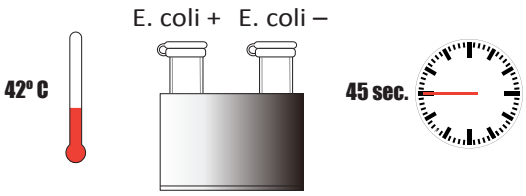
5c



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

Part 2: Transformation (Heat Shock and Recovery)

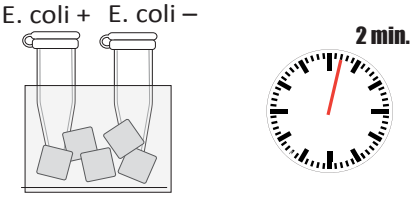
6



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

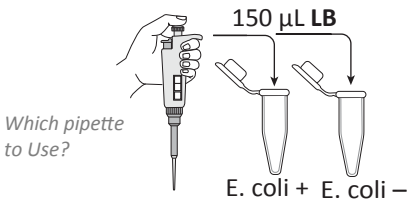
Avoid warming cells:
- *Carry tubes in the cup of ice to water bath.*

7



Immediately place the tubes back on ice for 2 mins.

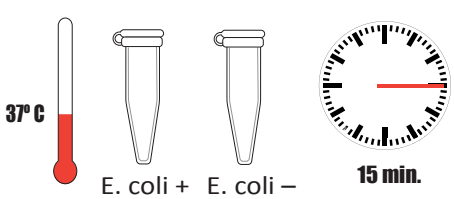
8



- Add 150 µL of **LB** to the **E. coli–** and **E. coli+** tubes
- Gently flick it three times to mix.

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

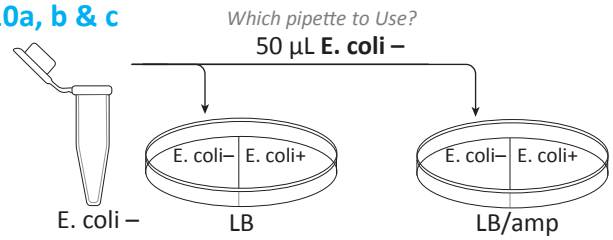
9



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

Part 3: Spread the Cells on Plates for Incubation

10a, b & c

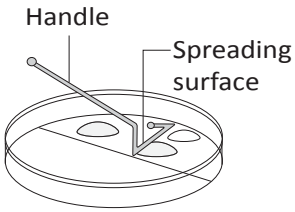


- Suspend **E. coli–** cells by gently pump the pipette two or three times.
- Add 50 µL of **E. coli–** cells to "E. coli–" on **LB** plate and **LB/amp** plate.

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*

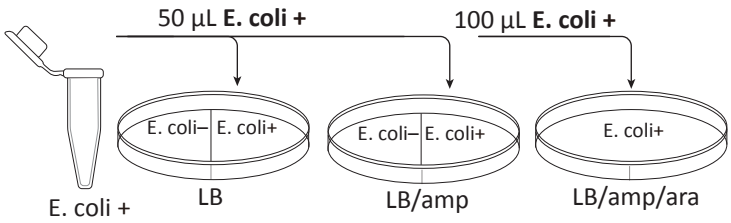
11a, b & c



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

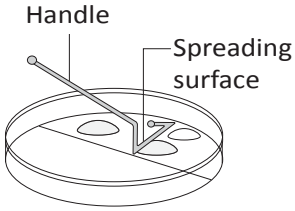
Avoid contamination:
- *Hold the spreader by the handle.*
- *Do not allow the bent end to touch any surface.*
- *Open lid just big enough to add the cells (like a clamshell)*

12



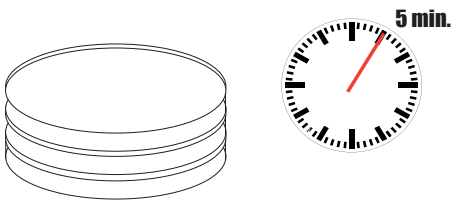
- Repeat step 10 for **E.coli+.**
- Add 50 µL **E. coli+** cells "E. coli+" on **LB** and **LB/amp** plates.
- Add 100 µL **E. coli+** cells to the **LB/amp/ara** plate.

13



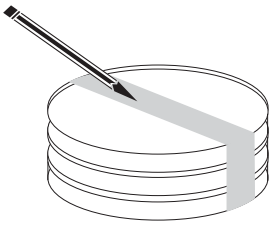
- Repeat step 11 for **E.coli+.**
- Spread the **E. coli+** cells evenly across the entire "E. coli+" section on **LB**, **LB/amp**, and **LB/amp/ara plates**.

14



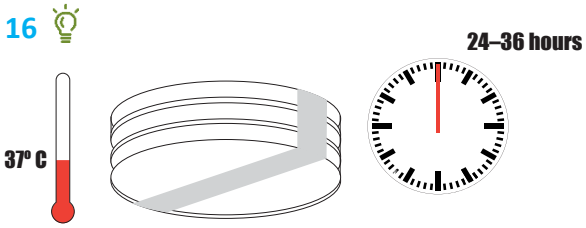
Leave all plates right side up for 5 mins.

15



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

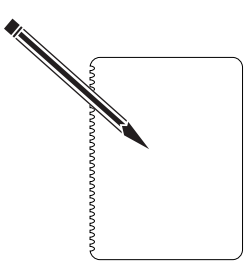
16



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

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

17



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