COLONY PCR

PART A: PERFORMING PCR

Label 4 empty PCR tubes 1-4 and initial them. Label both sides and top. Keep on ice.
Tube 1 for pink/red colony; (labeled R).
Tube 2 for white or another pink colony (labeled W).
Tube 3 for pARA-R (labeled +).
Tube 4 for pARA (labeled -).

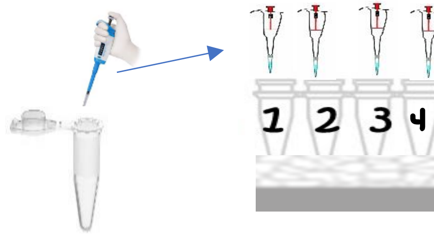
With the P-20 set to 11.5µL, **pipette** 23 µL of the master mix (2 X 11. 5µl) into to each of the tubes. Master Mix is labeled PCR.

If available, you can also pipette 23 µL with a P-200.

Final Volumes after pipetting all reagent and samples.

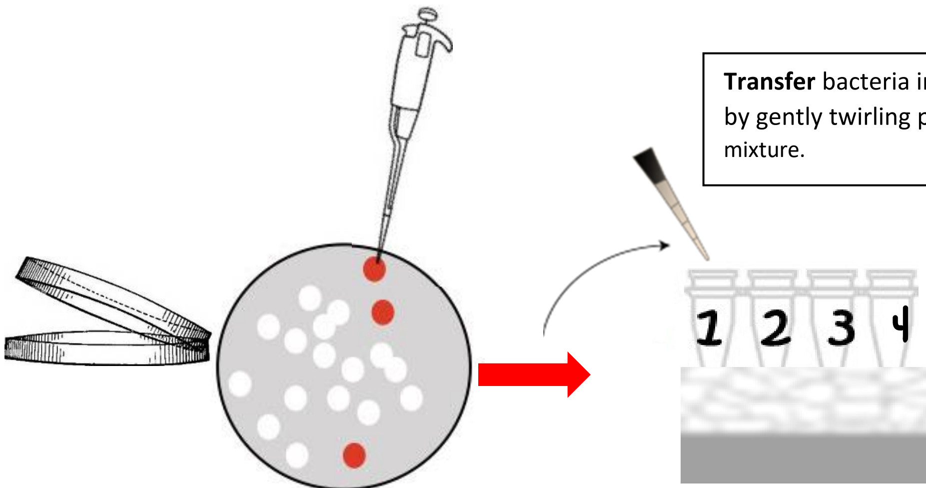
	Step 4: PCR master mix (PCR)	23 µL	23 µL	23 µL	23 µL
1	Step 5: Red colony	2µL			
2	Step 6: White colony	2µL			
3	Step 7: pARA-R (+)	2µL			
4	Step 8: pARA (-)	2µL			
	Total volume	25 µl	25 µl	25 µl	25 µl

R W + -



R W + -

Locate a red colony that is isolated from the other colonies. Open petri dish like clam shell and use the pipette tip to **lightly touch t**he colony.



Transformation Expertment

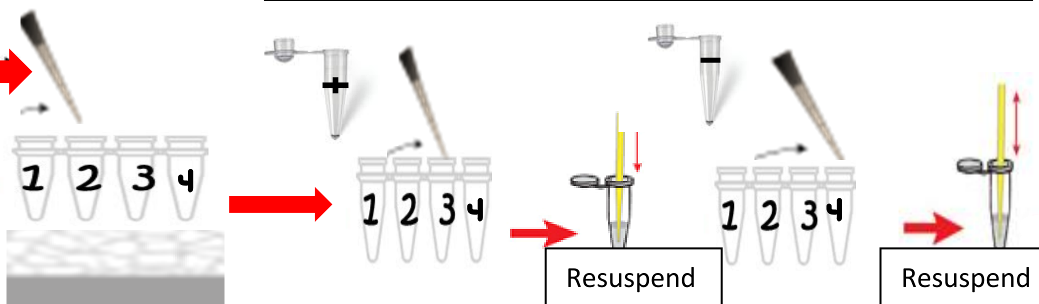
Transfer bacteria into PCR tube # 1 by gently twirling pipette tip in PCR mixture.

Transfer cells from white colony into PCR tube # 2 by gently twirling pipette tip in PCR mixture.

Transformation Expertment

Pipette 2µL of pARA-R into tube 3 (+, positive control) and resuspend.

With a new pipette, **pipette** 2µL of pARA into Tube 4 (-, negative control) into tube 4 and resuspend.



Cap samples and take ice tray/cup with PCR tubes to your teacher to **place** in the thermal cycler. The PREPROGRAMMED thermocycler will run for ~ 70-120 minutes. Thermocycler will **hold** **s**amples at **4^0C** until the samples are transferred to the **freeze**, where they are stored until agarose gel electrophoresis is performed.

-20^0C

PART B: SEPARATE PCR PRODUCTS USING GEL ELECTROPHORESIS

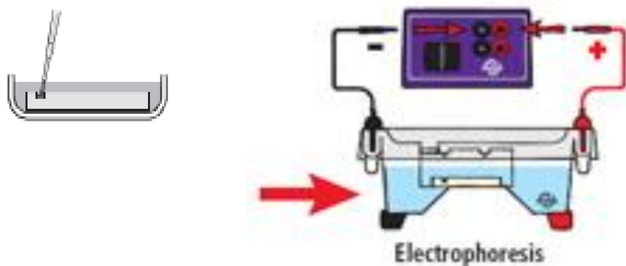
POUR gel (.8% agarose with 1:10,000 dilution of dilution of Sybersafe/gel green dye in 1X Sodium Borate buffer)

Let gel set

Make a drawing in your notebook or paper that shows the location of the wells in the electrophoresis box. Order of samples in each well should be as follows:

Well 1: DNA ladder (M100or M)
Well 2: Red colony (Tube 1)
Well 3: White colony (Tube 2)
Well 4: pARA-R (or pBAD-R)(Tube 3), 1092 bp, positive control (Tube 3)
Well 5: pARA (Tube 4), 662 bp, negative control

Using a fresh pipette tip for each sample, **dispense** 10µL of each prepared sample and the DNA ladder (M) into their designated wells.



Run 120-130 V for 30 min