


PGlo Transformation Procedure

Read the directions and follow them carefully! You will be graded on your ability to follow directions and get glowing bacteria! Every time someone in your group asks a teacher "What do I do next?" without reading these directions FIRST, your grade on this lab will go down 10 points! Check off each step as you do it.

NOTE: DO NOT TOUCH THE AGAR IN THE PETRI DISHES OR YOU WILL CONTAMINATE THEM WITH OTHER BACTERIA!

Procedure:

1. Go to the pipet station and pipet 250uL of cold TS solution into your empty microfuge tube (the orange tube).
2. Take out a new yellow plastic loop. Do not touch the loop end! Take your loop and microfuge tube to teacher and get some bacteria onto the loop. Swish the loop in the tube 10 seconds. Throw the loop into the trash. loop--> 
3. Bring your microfuge tube to teacher and get 20 uL of PGlo plasmid. Gently stir the orange microfuge tube with a new yellow plastic loop. Put the loop in trash.
4. Close the microfuge tube and float it in ice water for 10 minutes,
5. Take your microfuge tube out of the ice water and IMMEDIATELY put it into the warm water bath. Leave it there for 50 seconds.
6. Take your microfuge tube out of the warm bath and IMMEDIATELY put it back in the ice water for 2 minutes.
7. Put your microfuge tube in the white plastic rack and IMMEDIATELY go to pipet station and add 250 uL of LB to your orange tube. Let sit undisturbed in white rack on lab table for 10 minutes.
8. Get the sterile transfer pipet out of the package. Mix the bacteria by GENTLY squeezing the liquid up and down.
9. Use the transfer pipette to add about 1ml (the top line) of your bacteria solution. Place a third of the liquid into EACH of the three Petri dishes in your basket. Do the petri dishes in this order: 1) LB AMP ARA (purple), 2) LB AMP(blue), 3) LB(green). Use the pipette tip to spread the solution around a little but do not scratch the agar or make holes in it. Do not leave the Petri dishes open to the air or you will get contamination!
9. Tape your three dishes together and give to teacher.
10. Clean up. Throw away your tube and plastic pipet and any garbage. Wash your hands!

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Period _____ Group Number: _____

Group Member Names: _____

Results:

Plate Type	Treatment	Results-Observations	Did it glow?
LB	LB= Luria Broth Food for bacteria		
LB + AMP	Luria Broth and AMP=ampicillin antibiotic		
LB + AMP + ARA	Luria Broth, ampicillin and ARA=arabinose sugar		

Questions:

1. Why did we put ampicillin on some plates?
2. Why did we add arabinose to one plate?
3. Why did more bacteria grow on the LB plate than on the other plates?
4. Predict what would happen to the bacteria on the LB/AMP plate if we added arabinose sugar.
5. Suppose you wanted to make insulin using bacteria. You have E. coli bacteria, the insulin gene, an arabinose operon gene, and an ampicillin resistance gene. You have plates with LB, LB/AMP and LB/AMP/ARA. What would you put in your plasmid to make sure that you only have bacteria that can make insulin when you want them to? Explain what you would do and draw a picture of the plasmid.

SCORE: growth_____/10 glowing_____/10 questions_____/10 technique_____/20 TOTAL:_____/50