Experiment 11	**Bacterial genetic exchange : Bacterial Transformation**
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Textbook Chapters	BBOM 9th edition (1999): Chapters 7.2, 9.6, 10.16, and 10.17. BBOM 10th edition (2003): Chapters 8.5, 10.6, and 31.1 BBOM: Madigan M.T., J.M. Martinko and J. Parker: "Brock: Biology of Microorganisms", Prentice Hall.
Objectives	To understand intracellular genetic exchange in bacteria via transformation. To understand the genes which regulate *GFP (green fluorescent protein)* expresssion in *E. coli* cells containing pGlo plasmid.
Background	Genetic transformation occurs when a competent cell takes up (takes inside) and expresses a new piece of genetic material (DNA). In nature, bacteria can transfer plasmids back and forth, allowing them to share genes. Bacterial resistance to antibiotics is transferred by this mechanism. In this experiment *Escherichia coli* K-12:HB101 will be used as recipient strain (transformation host) and plasmid DNA (pGlo) will be transformed into these cells. The gene for the Green Fluorescent Protein (GFP) was originally isolated from the luminescent jellyfish *Aequorea victoria*. The unique three-dimensional conformation of GFP causes it to resonate when exposed to ultraviolet radiation and give off energy in the form of visible green light. The *GFP* gene has been modified by mutagenesis and was inserted into **pGlo** plasmid (Bio-Rad, Catalog Number 166-0003-EDU). Unique pGlo plasmid encodes the gene for the GFP and a beta lactamase, which provides resistance to the antibiotic ampicillin. pGlo also incorporates a special gene regulation system which can be used to control expression of the fluorescent protein in transformed cells. The *gfp* gene can be switched on in transformed cells simply by adding the sugar arabinose to the nutrient medium (see Appendix 1). Selection for cells that have been transformed with pGlo DNA is accomplish by growth on selective media containing the antibiotic ampicillin. Transformed cells will appear white on plates which do not cotain arabinose and fluorescent green under UV light when arabinose is included in the nutrient agar.
Literature	Chalfie, M., Tu Y., Euskirchen G., Ward W.W., Prasher D.C. 1994. Gene Fluorescent Protein as a Marker for Gene Expression. Science (263): 802-805. Hanahan, D. 1983. Studies on transformation of *Escherichia coli* with plasmids. J. Mol. Biol., 166, 557.
www. Links	http://www-bioc.rice.edu/Bioch/Phillips/Papers/gfpbio.html http://faculty.washington.edu/cemills/Aequorea.html
Practical Work	The student will perform a transformation experiment, by introducing pGlo plasmid into competent cells of *Escherichia coli* K-12 strain HB101. General laboratory skills are required to do this experiment, such as working with and culturing bacteria aseptically. It is very important not to introduce any contamination into the experiment. The students will perform 4 different treatments, and they should put the correct components into correct tubes and onto correct plates. After incubating the plates over night at 37°C they will observe and analyse the results.

Materials and Experimental Protocols

Material and equipment needed (per student group):

1. Bacterial strain: over night culture of *E.coli* K12: HB101 in LB broth medium. **LB (Luria Bertani Medium):** bacto tryptone: 10 g/l, yeast extract: 5 g/l, NaCl: 5 g/l, pH: 7.2.
2. Media and solution: poured agar plates: 1 plate LB, 2 plates LB+Amp, 1 plate LB+Amp+Ara, 1 Eppendorf tube containing 2 ml LB broth, 1 ml sterile transformation buffer (50 mM $CaCl_2$ solution).
3. Equipment: 1 set micro pipettes, 1 box sterilized yellow tips, 1 box sterilized blue tips, 1 box sterilized Eppendorf tubes, 1 Eppendorf rack, 1 foam microtube holder (floating microtube rack), 1 forcep, 1 pack sterile inoculation loops, container filled with crushed ice, marking pen (permanent), clock or timer, tissue paper, and waste container.

Common work station: 1 vial pGlo plasmid (Bio-Rad, Catalog Number 166-0003-EDU), 42°C water bath, thermometer, 37° C incubator oven, UV lamp (long wavelength), refrigerator, centrifuge for Eppendorf tubes, pH meter, autoclave, Petri dishes, ice making machine, vortex mixer, 500 ml graduate cylinder, 250 ml flask, distilled water.

Procedures: See also Appendix 2.

-Period 1 (transformation experiment): Use sterile technique.

a. Take 2 Eppendorf tubes, label them with **+DNA** and **-DNA**, respectively. Fill them with 1.5 ml of the over night *E. coli* culture. Centrifuge at 7000 rpm for 2 minutes to collect the cells, then pour off the supernatant.

b. Using a 1000 µl micro pipette, transfer 1000 µl of 50 mM $CaCl_2$ into each tube. Resuspend the pelleted cells. Place tubes on ice for 15 minutes.

c. Centrifuge at 7000 rpm for 2 minutes to collect the cells, then pour off the supernatant. Add 200 µl of transformation solution into each tube. Resuspend the pellet cells. Close and place tubes on ice for 10 minutes.

d. Take 10 µl of plasmid-containing solution and mix (resuspend) well into the competent cell suspension of the +DNA tube. Close the tube and return it to the rack on ice. **Do not add plasmid DNA to the -DNA tube**. Incubate the tubes on ice for 10 minutes. Make sure the bottom of the tubes make contact with the ice.

e. Meanwhile, label 4 agar plates on the bottom as follows: **LB+Amp (+ DNA); LB+Amp +Ara (+ DNA); LB+Amp (-DNA) and LB (- DNA)**

f. Using the floating racks as the holder, transfer both tubes into the water bath set at 42°C and incubate for 50 seconds. Make sure that the bottom of the tubes make contact with the warm water. After the incubation, place both tubes back onto ice. For best results, the change from the ice to 42° C and then back to the ice **must be rapid**. Incubate tubes on ice for 2 minutes.

g. Add 250 µl LB broth to the tube and close it. Repeat with a new steril tip for the other tube. Incubate the tubes for 10 minutes at room temperature.

h. Tap the closed tubes with the finger to mix. Using a new steril pipette tip for each tube, pipette 100 µl of the transformation suspension (+DNA) and control suspension (-DNA) onto the appropriate plates.

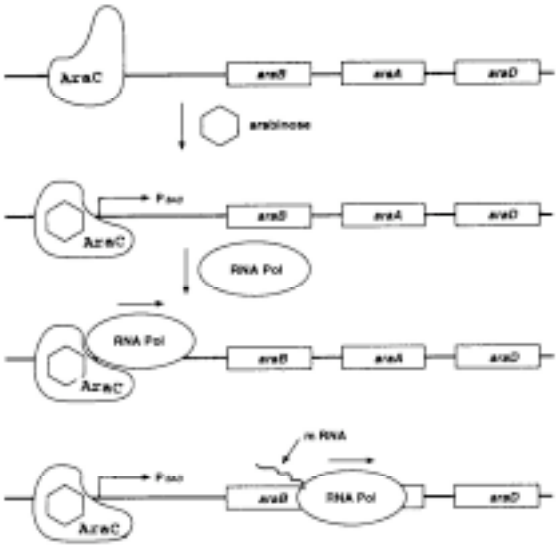
i. Use a new sterile loop for each plate. Spread the suspensions on the surface of the agar by quickly skating the flat surface of a new sterile loop back and forth across the plate surface.

j. Stack up your plates and tape them together. Put your group name and the date on the bottom of the stack and place it upside down, **over night** in the 37°C incubator.

	-Period 2 (Observations): Observe the phenotype of the colonies under the long-wave UV light. Observe what you see on each of the four plates. Record your data and compare the +DNA cells with the non-transformed *E. coli*
Laboratory Rules & Precautions	Handling the *E. coli* K-12 requires the use of standard microbiological laboratory techniques. Collect all of the waste materials in a biohazard bag, and autoclave them. All contaminated liquid or solid wastes will have to be decontaminated before disposal, as well. Please wash your hands after you have handled material containing recombinant DNA molecules and before leaving the laboratory. All procedures are performed carefully to minimize the creation of aerosols. Eating, drinking, smoking and applying cosmetics are not permitted in the work area. UV radiation can cause damage to eyes and skin. Short-wave UV is more damaging than long-wave UV light. Use UV protection glasses.
Experiences gained	Familiarity with genetic transfer process from one organism to another with the aid of plasmid DNA. Understanding the *Green Fluorescent Protein* gene expression, that was originally isolated from the bioluminescent jellyfish *Aequorea victoria*, in *Escherichia coli* cells.
Timing	90 minutes.
Reporting	Keep proper notes on experimental steps and observations in your laboratory book and use them for your written report.

Appendix 1.

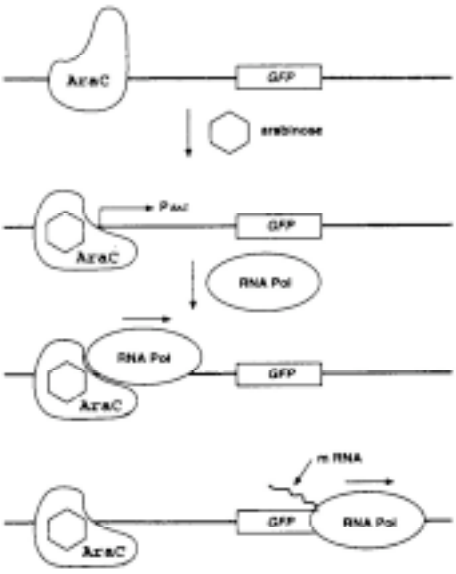
The Arabinose Operon



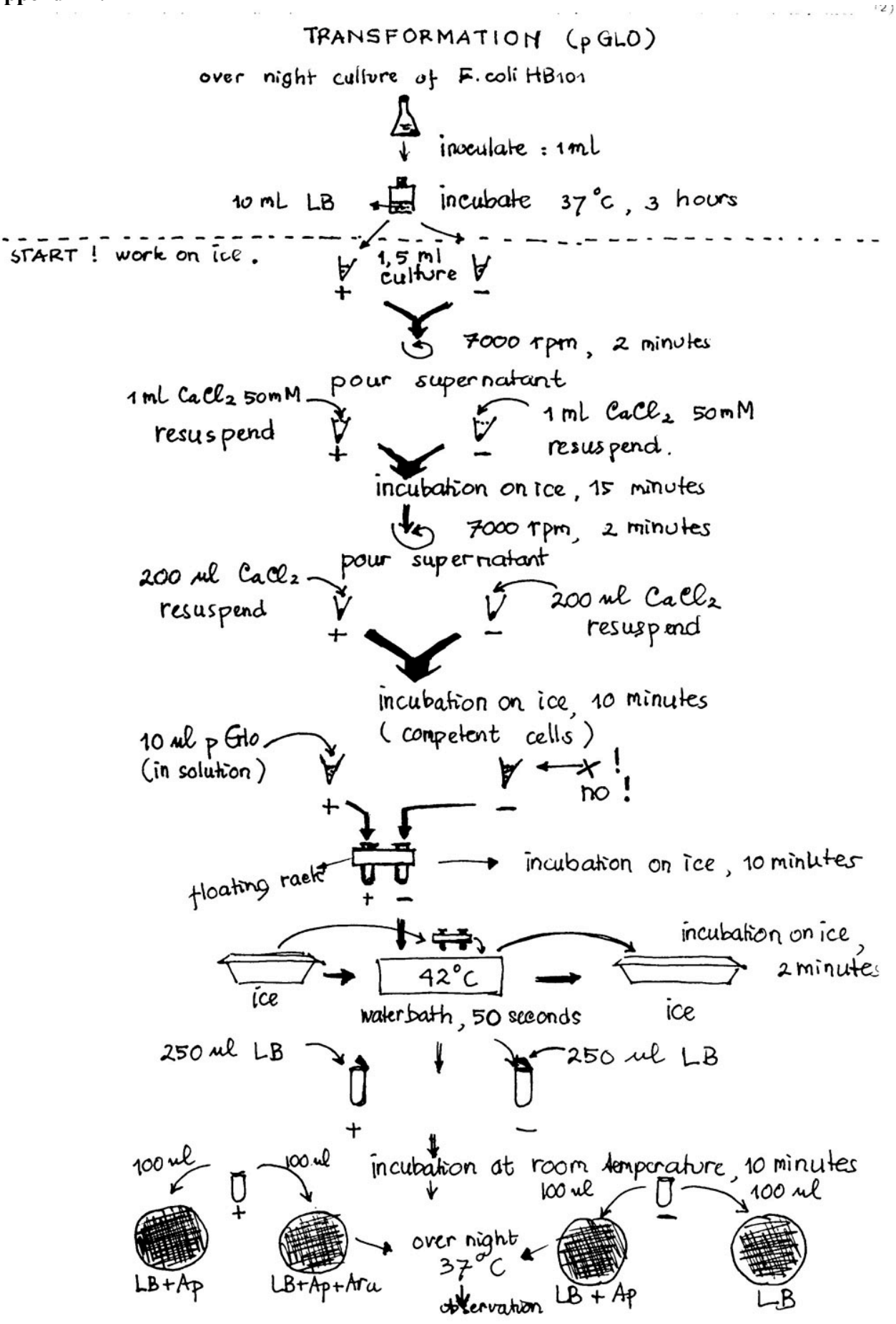
The three genes (*araB, araA* and *araD*) code three digestive enzymes that are involved in the breakdown of arabinose. They are clustered together as arabinose operon. These three proteins are dependent on initiation of transciption from a single promotor (P_{BAD}). Transciption of these three genes requires the presence of the DNA template (promotor and operon), RNA Polymerase, a DNA binding protein called AraC and arabinose. AraC (a regulator protein) binds to the DNA at the binding site for the RNA Polymerase (the beginning of the arabinose operon). When arabinose is present in the environment, bacteria take it up. Once inside, the arabinose interacts directly with AraC which is bound to the DNA. The interaction causes AraC to change ist shape which in turn promotes the binding of RNA Polymerase and the three genes (*araB, araA* and *araD*) are transcribed. In the absence of arabinose, the AraC returns to its original shape and transciption is shut off.

The DNA code of the pGlo plasmid has been engineered to incorporate aspects of the arabinose operon. Both the promoter P_{BAD} and *araC* gene are present. However, the three genes *araB, araA* and *araD* have been replaced by a single gene which codes for the Green Fluorescent Protein (GFP). Therefore, in the presence of arabinose, GFP gene is transcribed, and cells fluoresce a green color under the UV light. In the absence of arabinose, GFP gene is not transcribed, bacteria colonies will appear to have a wild type (natural) phenotype, with no fluoresce.

Expression of Green Fluorescent Protein



Appendix 2:


TRANSFORMATION (pGLO)
over night culture of E. coli HB101
inoculate : 1 ml
10 mL LB
incubate 37 °C, 3 hours
START ! work on ice.
1,5 ml culture
+
−
7000 rpm, 2 minutes
pour supernatant
1 ml CaCl2 50mM
resuspend
1 ml CaCl2 50mM
resuspend.
incubation on ice, 15 minutes
7000 rpm, 2 minutes
pour supernatant
200 ul CaCl2
resuspend
200 ul CaCl2
resuspend
incubation on ice, 10 minutes
(competent cells)
10 ul pGlo
(in solution)
no !
floating raek
incubation on ice, 10 minutes
ice
42°C
waterbath, 50 seconds
ice
incubation on ice,
2 minutes
250 ul LB
250 ul LB
100 ul
100 ul
incubation at room temperature, 10 minutes
100 ul
100 ul
LB+Ap
LB+Ap+Ara
over night
37°C
observation
LB + Ap
LB