APPENDIX A

Plasmid purification

Plasmid purification by Geneaid high-Speed Plasmid Mini Kit

1. Transfer 1.5 ml of bacterial culture to a microcentrifuge tube (not provided).

2. Centrifuge for 1 min at full speed (about 13,000 rpm) and discard the supernatant.

3. If more than 1.5 mol of bacterial culture is used, repeat the Harvesting Step.

4. Add 200 µl of PD1 Buffer (RNase A added) to the tube and resuspend the cell pellet by vortexing or pipetting

5. Add 200 µl of PD2 Buffer and mix gently by inverting the tube 10 times. Do not vortex, avoid shearing genomic DNA.

6. Stand for 2 min at room temperature until lysate is clear.

7. Add 300 µl of PD3 Buffer and mix immediately by inverting the tube 10 times. Do not vortex.

8. Centrifuge for 2 min at full speed

9. Place a PD Column in a 2 ml Collection Tube

10. Apply the clear lysate (supernatant) from Step 4 to the PD Column

11. Centrifuge at full speed for 30 seconds.

12. Discard the flow-through and place the PD Column back in the Collection Tube.

13. Add 400 µl of W1 Buffer in the PD column.

14. Centrifuge at full speed for 30 seconds

15. Discard the flow-through and place the PD Column back in the Collection Tube

16. Add 600 µl of Wash Buffer (ethanol added) in the PD column.

17. Centrifuge at full speed for 30 seconds

18. Discard the flow-through and place the PD Column back in the Collection Tube

19. Centrifuge again for 3 min at full speed to dry the column matrix

20. Discard the Collection Tube with the flow-through, then transfer dried PD Column on a clean 1.5 ml microcentrifuge tube (not provided)

21. Add 50 µl of Elution Buffer or distilled water directly on to the center of the membrane. Avoid residual buffer adhering to the wall of the column

22. Stand for 2 min until Elution Buffer of distilled water is absorbed

23. Centrifuge for 2 min at full speed to elute purified DNA.

APPENDIX B

Plasmid DNA concentration

Naked DNA concentration

Naked DNA was extracted from *Eschericia coli* (*E. coli*) which had 2 types of DNA, chromosomal DNA and plasmids DNA. Plasmid DNA was chosen in this experiment. Bio-drop spectrophotometer was used for analyzing concentration.

Plasmid DNA tube number	Purity A260/A280	Purity A260/A230	Concentration (ng/µl)
1	1.827	2.666	309.3
2	1.818	2.198	236.1
3	1.810	2.243	433.0
4	1.834	2.24	402.8
5	1.841	2.226	185.0
6	1.86	2.215	192

7	1.967	2.212	66.2
8	1.923	2.063	91.9
9	1.886	2.158	97.86
10	1.848	2.129	160.2
11	1.838	2.082	199.7
12	1.923	1.61	65.6
13	1.867	1.784	90.86
14	1.814	1.827	121.5

As shown above is table of naked DNA concentration. Enpendorfs no.1, 2, 3, 4 were incubated for 2 days at 37 °c and the others were incubated for 1 day at 37 °c for comparing concentration.

*** Purity A260/A280 should be between 1.8 to 2.0 ratio and purity A260/A230 should be more than 2.0 ration

APPENDIX C

Electrophoresis

1. Steps of experiment [19]

1. Agar 0.4 g was put on flask and 40 ml 0.5X TBE was added

2. Agarose was put into microwave until the solution was dissolved clear.

3. The solution was allowed to cool to about 55 ºC before pouring. Ethidium bromide was added at this point to a concentration of 0.5 g/ml

4. Gel tray was prepared by sealing ends with tape or other custom made dam.

5. A comb was placed in gel tray about 1 inch from one end of the tray and positioned vertically such that the teeth were about 1-2 mm above the surface of the tray.

6. 50 ºC gel solution was poured into tray to a depth of about 5 mm. Gel was allowed to solidified about 20 minutes at room temperature.

7. The comb was gently removed. Then, the tray was placed in electrophoresis chamber, and coverd (just untill wells were submerged) with electrophoresis buffer (the same buffer used to prepare the agarose)

8. Samples were prepared for electrophoresis and 2 µl of 6x gel loading dye was added for every 10 µl of DNA solution.

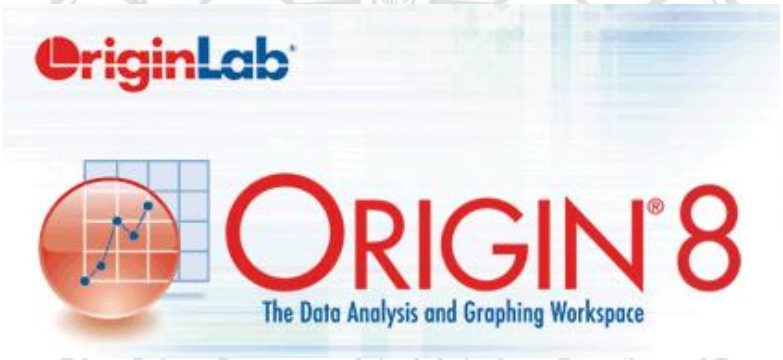
9. Electrophoresed at 100 volts until dye markers were migrated an appropriate distance, depending on the size of DNA to be visualized

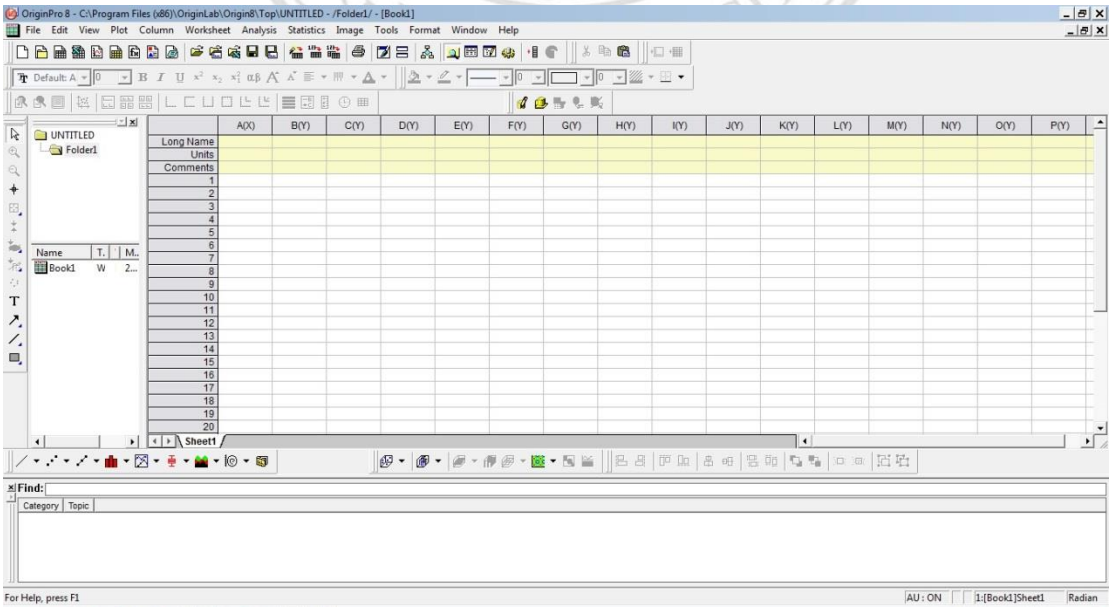
10. The gel was removed to the UV trans-illuminator and placed directly on the gel light box (using gloves and the dedicated “spatula”)

11. Gel was photographed under UV-light with a digital camera.

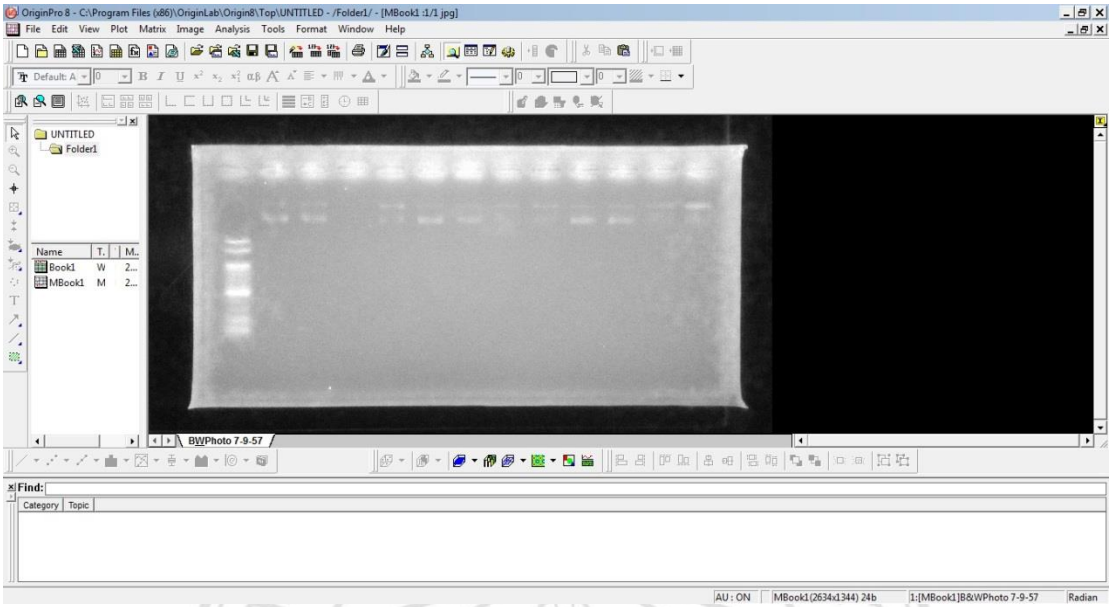
2. Analyzing Electrophoresis Gels

The following is an easy procedure for using OriginLab® software (OriginLab® 8.0) to analyze one dimensional electrophoresis gel. It also demonstrates some of the less obvious features in OriginLab® software, and also a few shortcuts. And this technique can be used to compare bands on different gels because the gels can be calibrated to another figure by deleting background. Any results obtained using this procedure should not be trusted without testing using standards with known concentrations or by comparing with results obtained using a densitometer. The operating steps are :



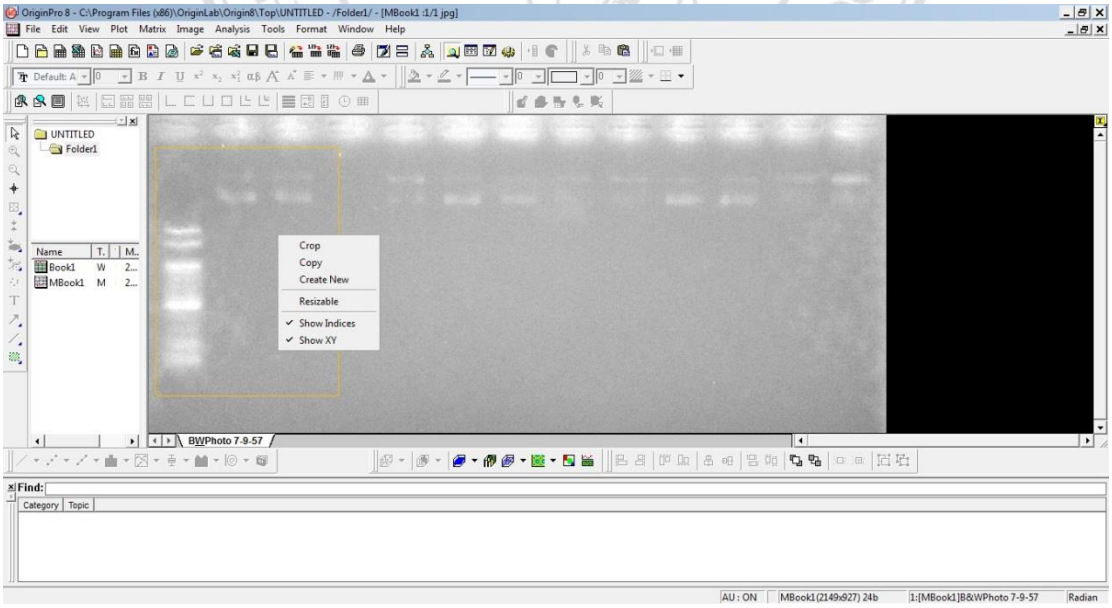


Picture of OriginLab 8.0 interface

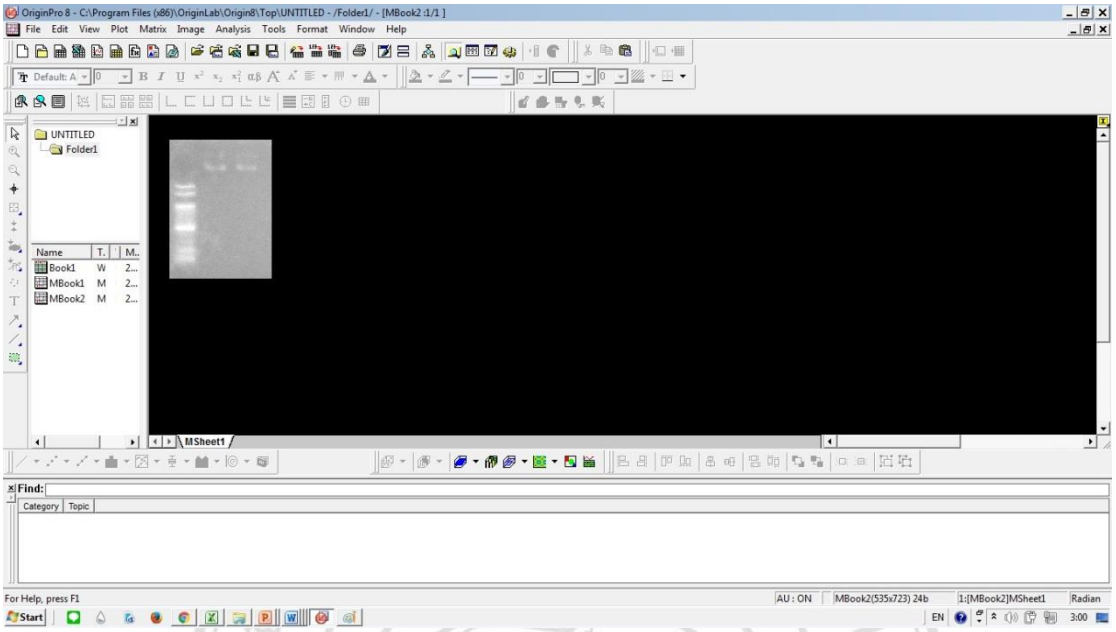


1. Drag the picture of gel electrophoresis to OriginLab 8.0 program

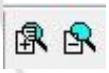
*** Must Convert image to Gray mode first, select area band.

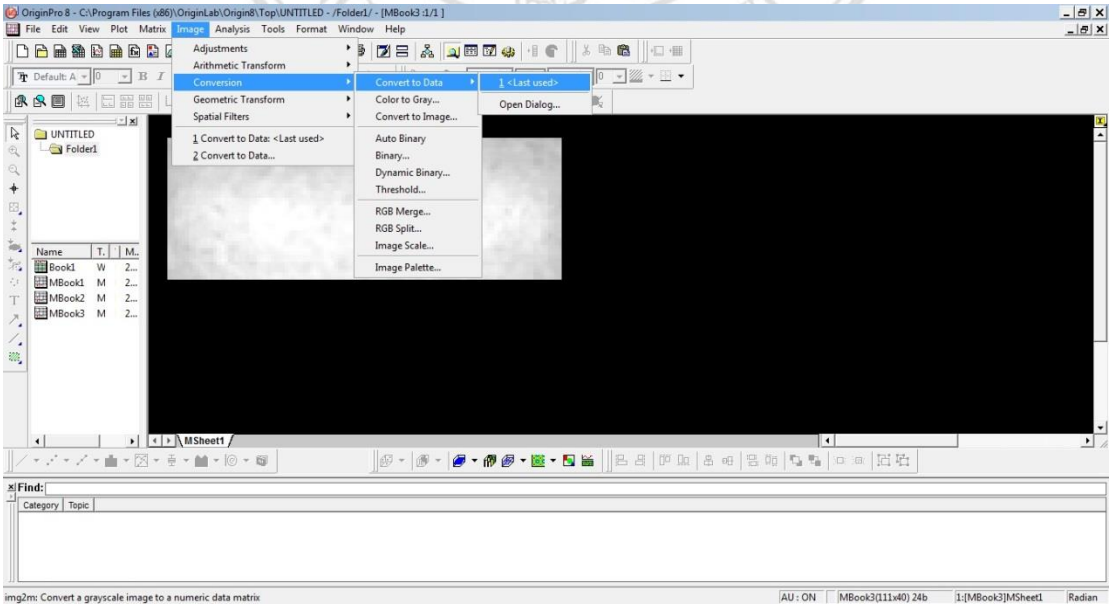


2. Create the selection area in a new window.

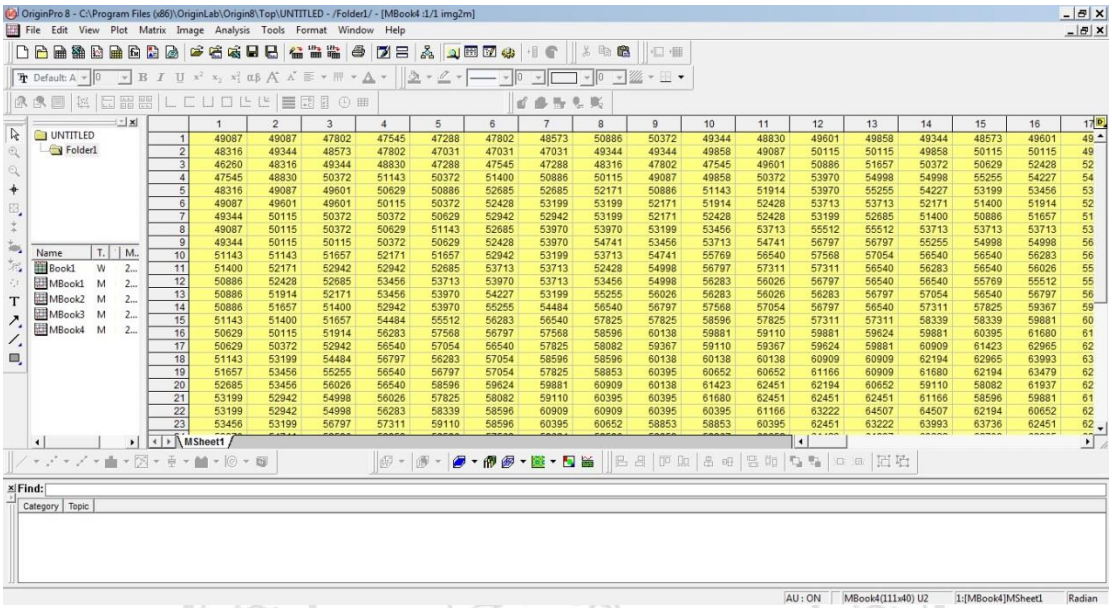


3. Select brand which you want to analyzation.

***** Use**  **bottom for zoom in or zoom out *****



4. Convert Image Mode to Data Mode.



5. Sent data to calculate by Microsoft Excel program.

*** For comparison to another figure, choose background area and calculate average data by the same process and bring it to delete background form figure. ***

APPENDIX D

Electroporation [19]

1. 100 µl of the competent cells were pipetted into ice-cold sterile 0.5 ml micro-centrifuge tubes. The cells were placed on ice, together with 0.1 cm. electrode gap electroporation cuvettes.

2. 50-100 ng of the DNA was added to be electroporated in a volume of 1-2 µl to each micro-centrifuge tube and incubated on ice for 30-60 seconds.

3. The electroporation apparatus was set to deliver an electrical pulse of 25 µF capacitance, 25 kV, and 200 ohm resistance.

4. The DNA and cell mixtures were pipetted into a cold electroporation cuvette. The solution was tapped to ensure that the suspension of bacteria and DNA sit at the bottom of the cuvette. The condensation was dried and moistens from the outside of the cuvette. The cuvette, then, was placed in the electroporation device.

5. A pulse of electricity was delivered to the cells at the setting dicated above. A time constant of 4-5 milliseconds with a field strength of 1.8 kV/cm was registered on the machine.

6. As quick as possible after the pulse, the electroporation cuvette was removed and 1 ml of LB medium was added at room temperature.

7. The cell was transferred to 17x100-mm polypropylene tube and incubated with gentle rotation for 1 hour at 37 ºC.

8. 50 µl of the electroporated cells were plated onto LB agar medium which containing 200 µg/ml IPTG (isopropyl β-D-thiogalactoside) and 100µg/ml ampicillin.

9. The plates were inverted and incubated at 37 ºC After overnight Transformed colonies should be appears.

10. White colonies (mutant) were picked out and plated on plates again to check for their purity.

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