

## 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 ml of bacterial culture is used, repeat the Harvesting Step.
4. Add 200  $\mu$ l of PD1 Buffer (RNase A added) to the tube and resuspend the cell pellet by vortexing or pipetting
5. Add 200  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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 or 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 *Escherichia 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. Eppendorfs 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]

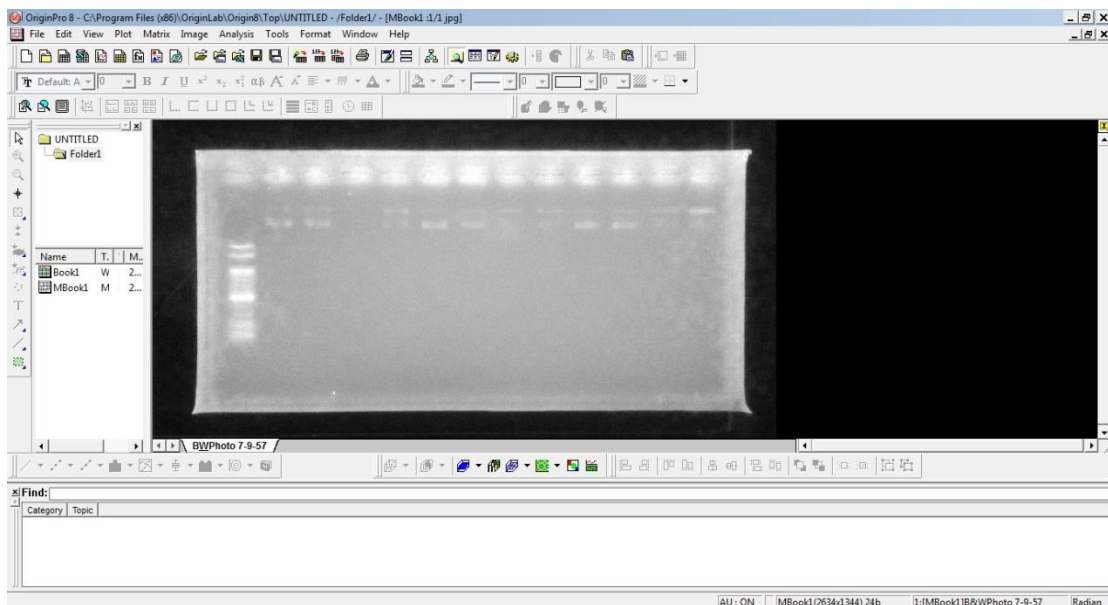
1. Agar 0.4 g was put on flask and 40 ml 0.5X TBE was added
2. Agarose was put into microwave until the solution was dissolved clear.
3. The solution was allowed to cool to about 55 °C before pouring. Ethidium bromide was added at this point to a concentration of 0.5 g/ml
4. Gel tray was prepared by 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 °C 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 covered (just until 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 :

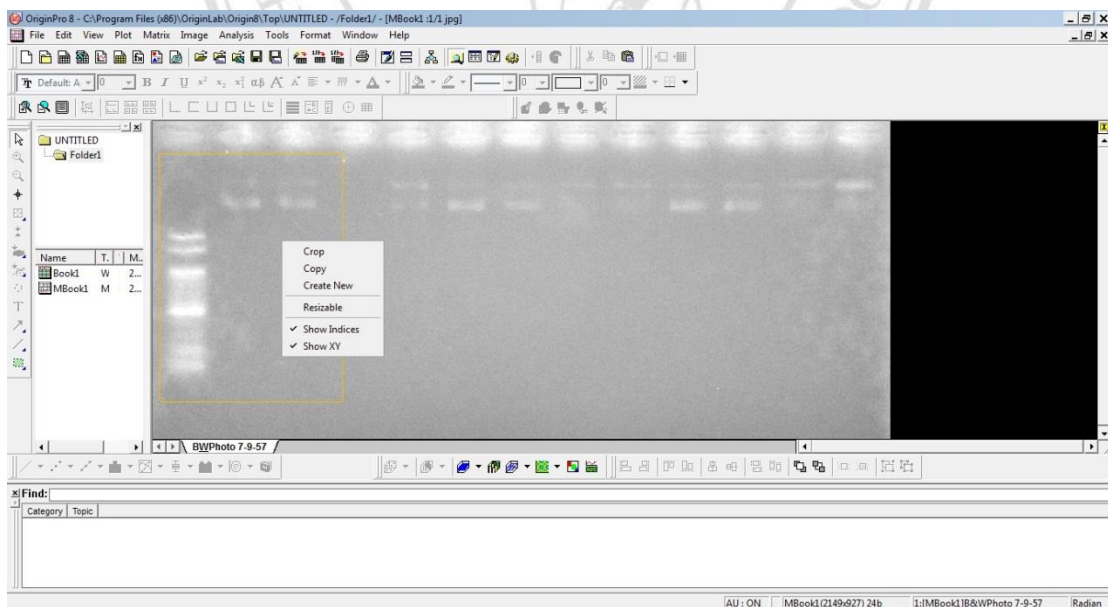


Picture of OriginLab 8.0 interface

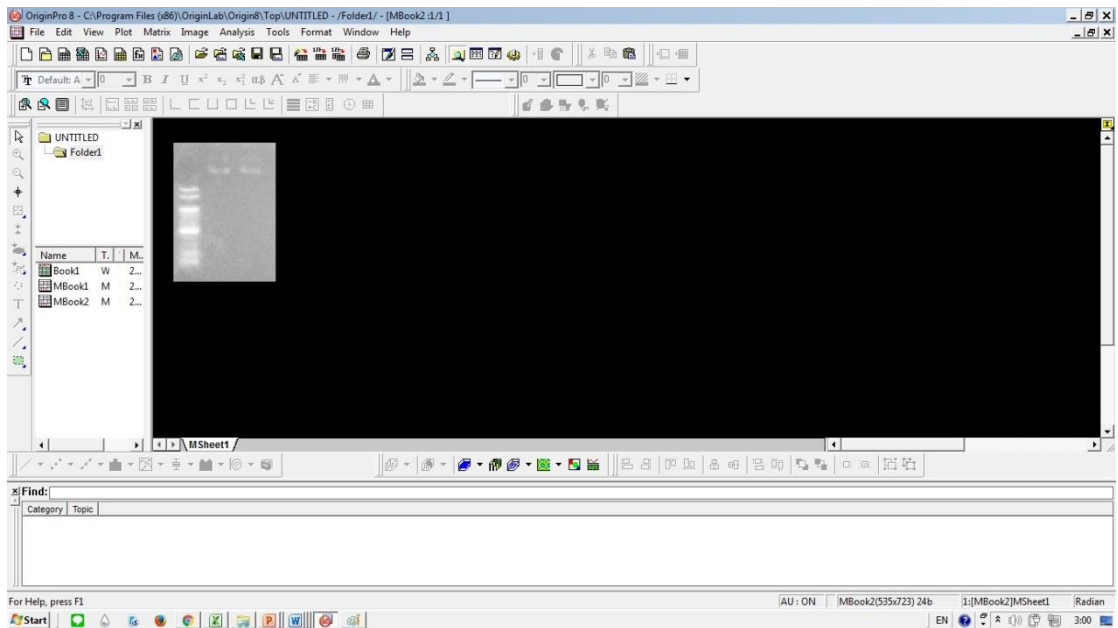


1. Drag the picture of gel electrophoresis to OriginLab 8.0 program

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

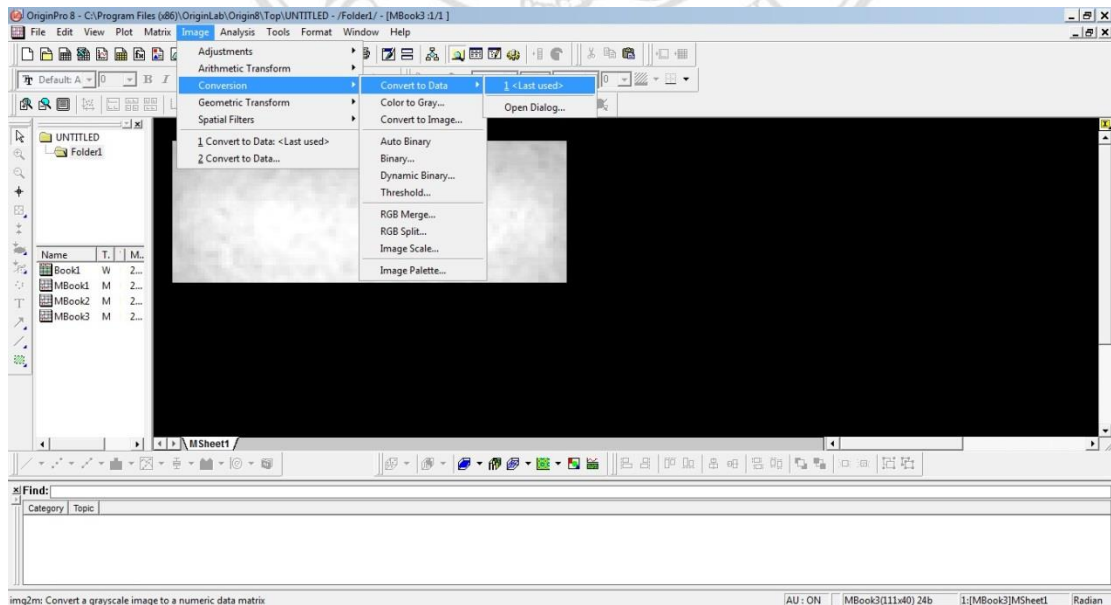


2. Create the selection area in a new window.



3. Select brand which you want to analyzation.

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



4. Convert Image Mode to Data Mode.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	49087	49087	47802	47545	47288	47802	48573	50886	50372	49344	48830	49601	49858	49344	48573	49601	49
2	48316	49344	48573	47802	47031	47031	47031	49344	49344	49858	49087	50115	50115	49858	50115	50115	49
3	46250	48316	49344	48830	47288	47545	47288	48316	47802	47545	49601	50886	51657	50372	50629	52428	52
4	47545	48830	50372	51143	50372	51400	50886	50115	49087	49858	50372	53970	54998	54998	55255	54227	54
5	48316	49087	49601	50629	50886	52685	52685	52171	50886	51143	51914	53970	55255	54227	53199	53456	53
6	49087	49601	49601	50115	50372	52428	53199	53199	52171	51914	52428	53713	53713	52171	51400	51914	52
7	49344	50115	50372	50372	50629	52942	52942	53199	52171	52428	52428	53199	52685	51400	50886	51657	51
8	49087	50115	50372	50629	51143	52685	53970	53970	53199	53456	53713	55512	55512	53713	53713	53713	53
9	49344	50115	50115	50372	50629	52428	53970	54741	53456	53713	54741	56797	56797	55255	54998	54998	56
10	51143	51143	51657	52171	51657	52942	53199	53713	54741	55769	56540	57568	57054	56540	56540	56283	56
11	51400	52171	52942	52942	52685	53713	53713	52428	54998	56797	57311	57311	56540	56283	56540	56026	55
12	50886	52428	52685	53456	53713	53970	53713	53456	54998	56283	56026	56797	56540	56540	55769	55512	55
13	50886	51914	52171	53456	53970	54227	53199	56255	56026	56283	56026	56283	56797	57054	56540	56797	56
14	50886	51657	51400	52942	53970	55255	54484	56540	56797	57568	57054	56797	56540	57311	57825	59367	59
15	51143	51400	51657	54484	55512	56283	56540	57825	57825	58596	57825	57311	57311	58339	58339	59881	60
16	50629	50115	51914	56283	57568	56797	57568	58596	60138	59881	59110	59881	59624	59881	60395	61680	61
17	50629	50372	52942	56540	57054	56540	57825	58082	59367	59110	59367	59624	59881	60909	61423	62965	62
18	51143	53199	54484	56797	56283	57054	56596	58596	60138	60138	60138	60909	60909	62194	62965	63993	63
19	51657	53456	55255	56540	56797	57054	57825	58853	60395	60652	60652	61166	60909	61680	62194	63479	62
20	52685	53456	56026	56540	58596	59624	59881	60909	60138	61423	62451	62194	60652	59110	58082	61937	62
21	53199	52942	54998	56026	57825	58082	59110	60395	60395	61680	62451	62451	62451	61166	58596	59881	61
22	53199	52942	54998	56283	58339	58596	60909	60909	60395	60395	61166	63222	64507	64507	62194	60652	62
23	53456	53199	56797	57311	59110	58596	60395	60652	58853	58853	60395	62451	63222	63993	63736	62451	62

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  $\mu$ l 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  $\mu$ l 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  $\mu$ F 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 °C.
8. 50  $\mu$ l of the electroporated cells were plated onto LB agar medium which containing 200  $\mu$ g/ml IPTG (isopropyl  $\beta$ -D-thiogalactoside) and 100 $\mu$ g/ml ampicillin.
9. The plates were inverted and incubated at 37 °C After overnight Transformed colonies should be appears.

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



ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved

## CURRICULUM VITAE

Author's Name	Mr.Chaitavat Yaopromsiri
Date/Year of Birth	18 October 1988
Place of Birth	Chiang Mai Province, Thailand
Education	2006 Graduated grade 12 from Montfort collage secondary school, Chiang Mai 2010 Graduated Bachelor degree of Science from Chiang Mai University, Chiang Mai
Publication	C.Yaopromsiri, L.D.Yu, S.sarapirom, P.Thopan, D.Boonyawan,” Effect of cold atmospheric pressure He-plasma Jet on DNA change and mutation”, Nuclear Instruments and Methods in Physics Research Section B Beam Interactions with Materials and Atoms, Vol.365, Part A, 15 December 2015, Page No. 399–403

