CHAPTER 4

Result and Discussion

4.1 Plasma power

Plasma generating power was measured by using the V-Q lissajous method. The bio –plasma jet power was measured for 9 conditions by varing intensity and frequency of the plasma generator. The system of the generator used Helium gas. The helium flow rate was controlled by Dwyer at 600 ml/min. The power was measured during the experiment by probes purchased from Tektrinix P 6015A, and was visualized on an oscilloscope. The sinusoidal shaped pulse was generated peak to peak with a repetition rate of 16.67 kHz.

In this experiment, we used 9 conditions consisting of Intensity 1 with Frequency 10, 50, 110 Hertz, Intensity 5 with Frequency 10, 50, 110 Hertz and Intensity 10 with Frequency 10, 50, 110 Hertz. After measurement, the lissajous pattern was shown on the oscilloscope, x-axis representing the applied voltage and y- axis representing the charge as shown in Figure 4.1 to Figure4.9. Then, the lissajous pattern data were printed on paper for calculating inside curve area. The x-axis was converted to ratio of the oscilloscope with actual measured value per one channel by multiplying 1000 in volt (V) and the y-axis was converted to ratio of the oscilloscope with actual measured value per one channel in millivolt (mV) and calculated to find the plasma generating energy and the plasma generating power.

The result showed that the plasma generating energy was in the range between 130 µJ to 180 µJ, as shown in Table 4.1 and Figure 4.10. The plasma generating power was in the range between 2 -3 watts, as shown in Table 4.2 and Figure 4.11. The plasma was powered by a radio frequency supply unit, which consisted of a pulse generator.

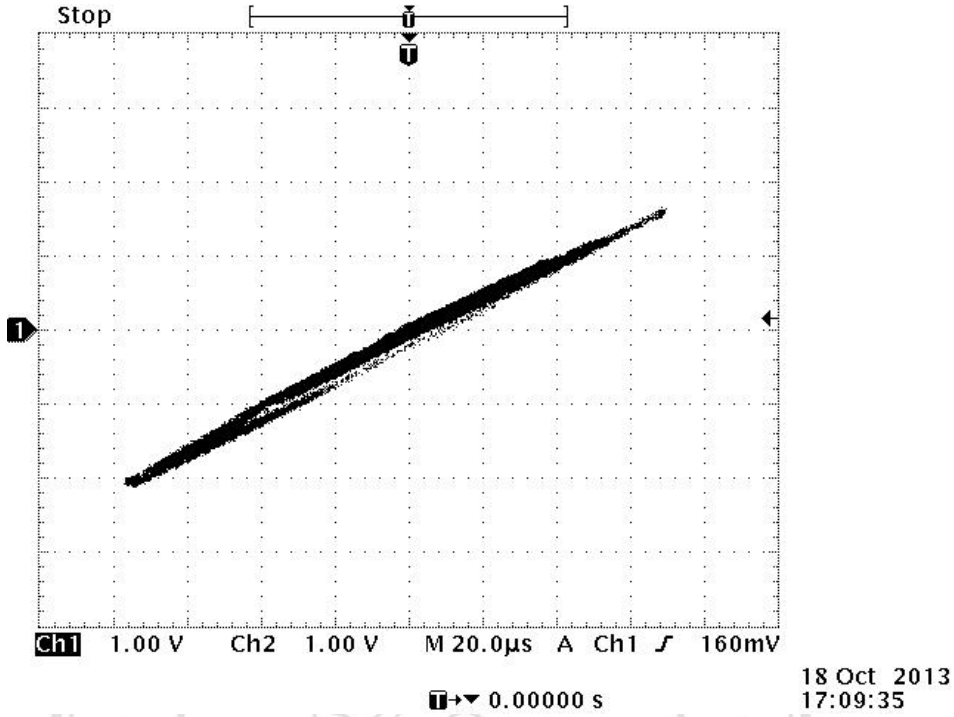

Figure.4.1. Lissajous graph was measured by using the condition of intensity level 1 and frequency 10 Hz

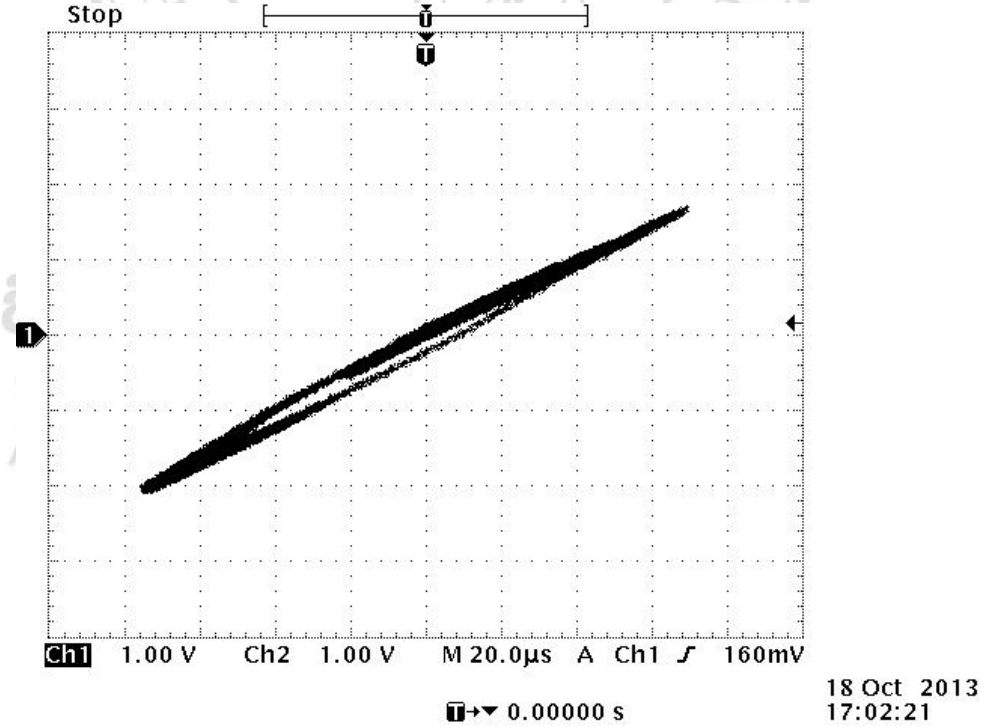

Figure 4.2. Lissajous graph was measured by using the condition of intensity level 1 and frequency 50 Hz

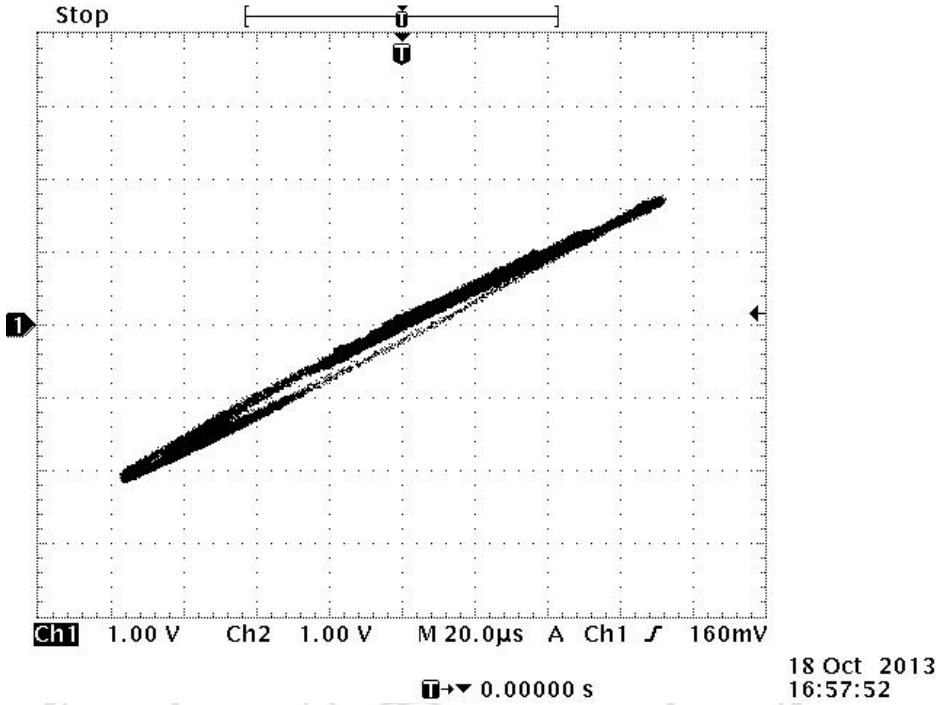

Figure 4.3. Lissajous graph was measured by using the condition of intensity level 1 and frequency 110 Hz

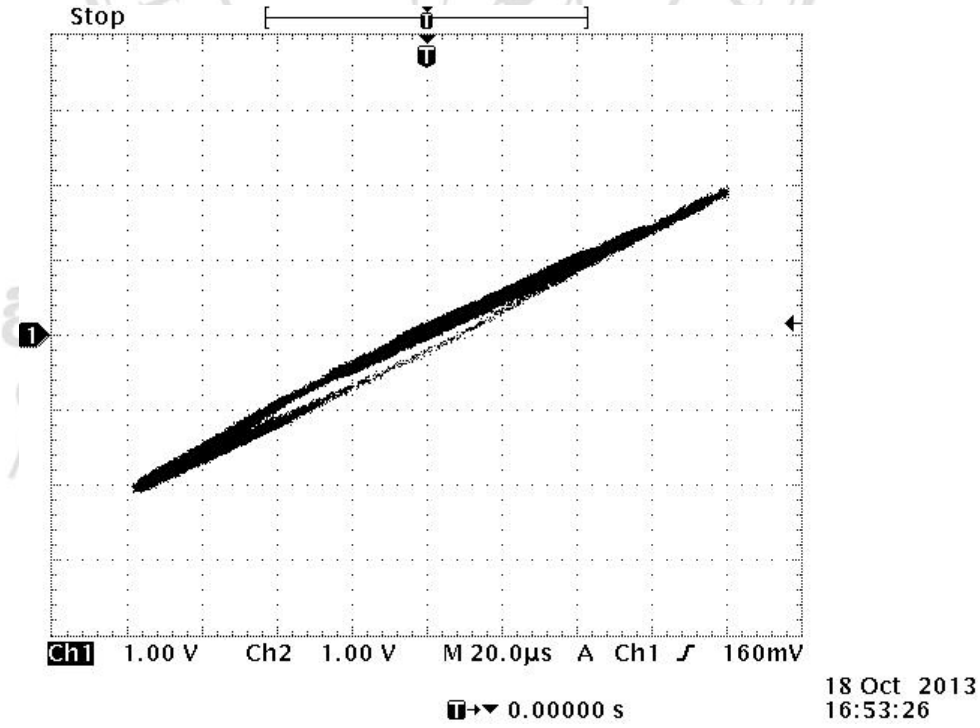

Figure.4.4. Lissajous graph was measured by using the condition of intensity level 5 and frequency 10 Hz

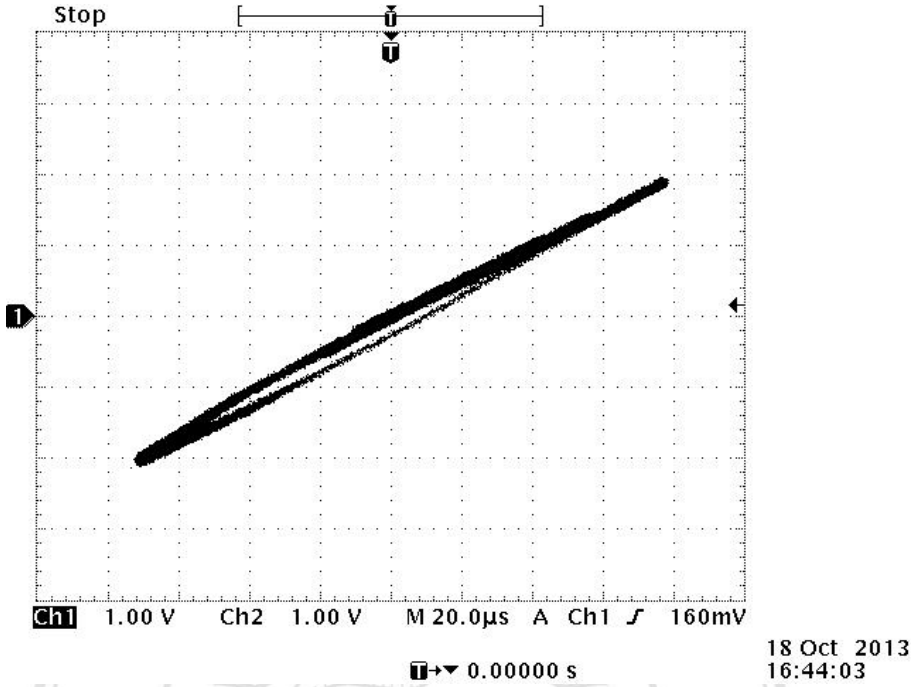

Figure. 4.5. Lissajous graph was measured by using the condition of intensity level 5 and frequency 50 Hz

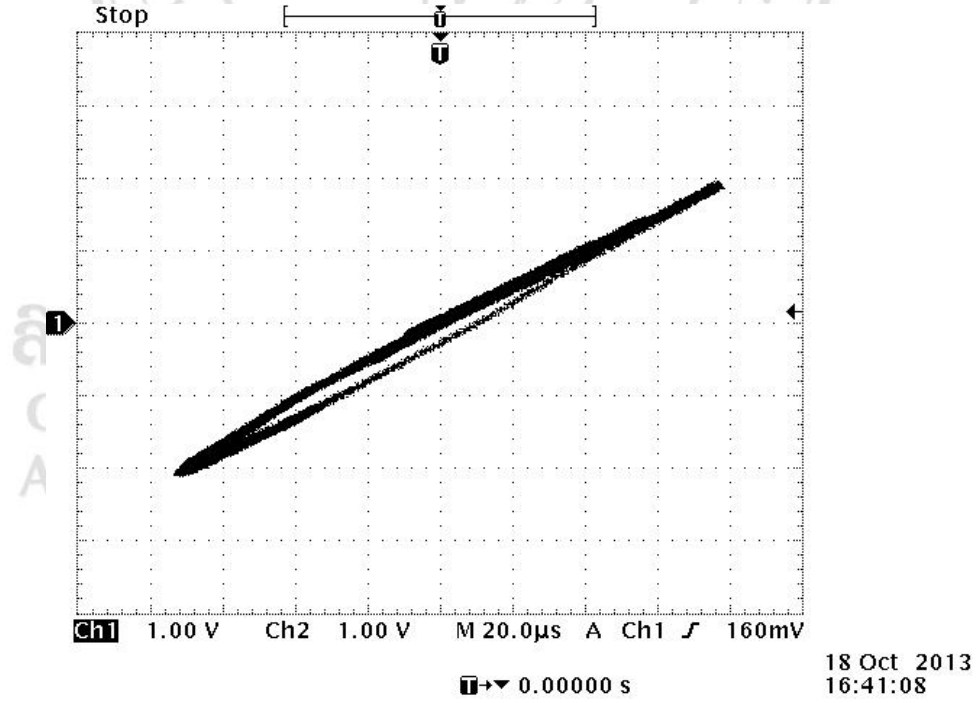

Figure.4.6. Lissajous graph was measured by using the condition of intensity level 5 and frequency 110 Hz

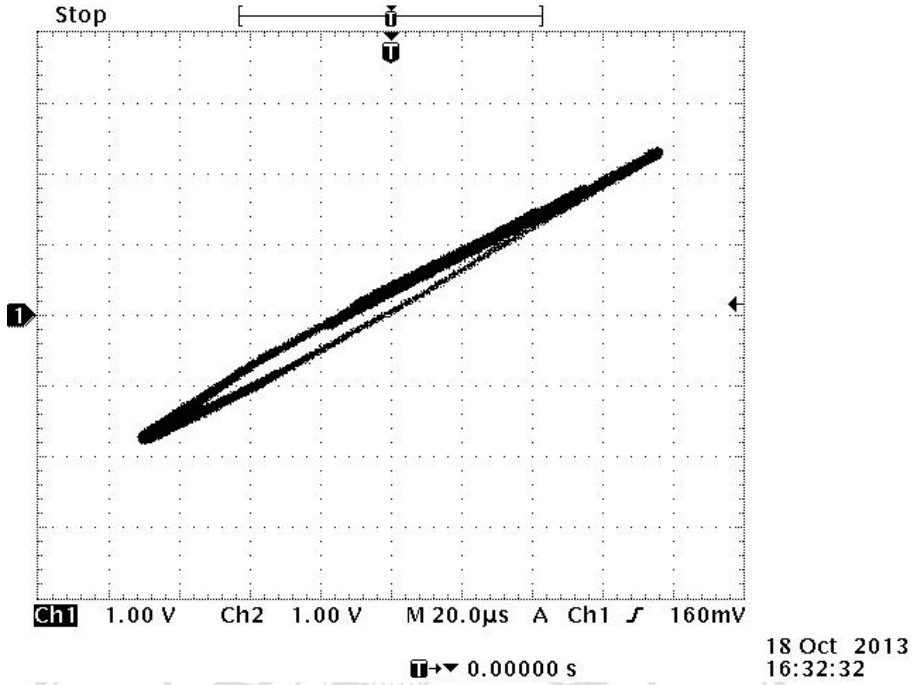

Figure.4.7. Lissajous graph was measured by using the condition of intensity level 10 and frequency 10 Hz

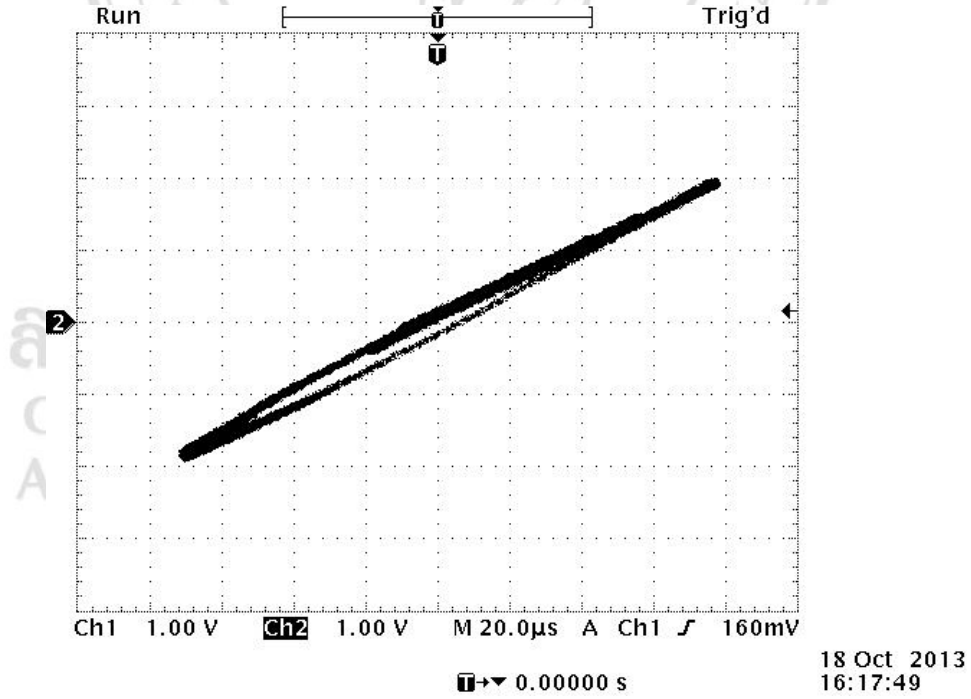

Figure.4.8. Lissajous graph was measured by using the condition of intensity level 10 and frequency 50 Hz

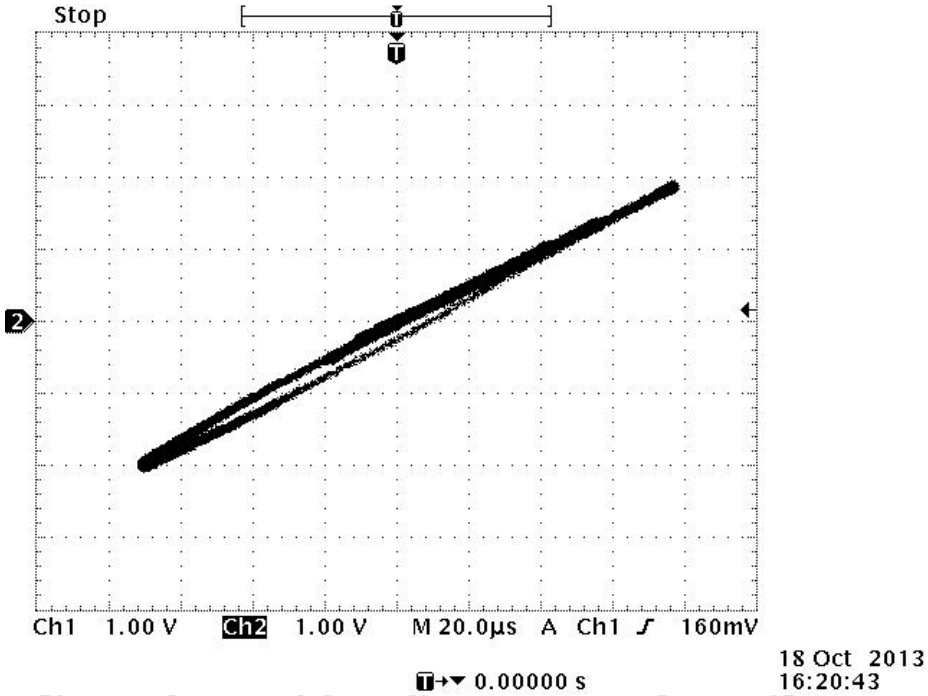

Figure.4.9. Lissajous graph was measured by using the condition of intensity level 10 and frequency 110 Hz

From the figure of the lissajous pattern, the plasma generating energy was calculated from the area inside the lissajous pattern multiplied by the ratio for x-axis and ratio of y-axis. The result of plasma generating energy is shown in table 4.1 and figure 4.10.

Table 4.1. Summary of plasma energy measurement.

Intensity / Frequency(Hz)	1	5	10
10	1.29×10^{-4} J	1.33×10^{-4}J	1.35×10^{-4}J
50	1.38×10^{-4}J	1.45×10^{-4}J	1.49×10^{-4}J
110	1.52×10^{-4}J	1.66×10^{-4}J	1.81×10^{-4}J

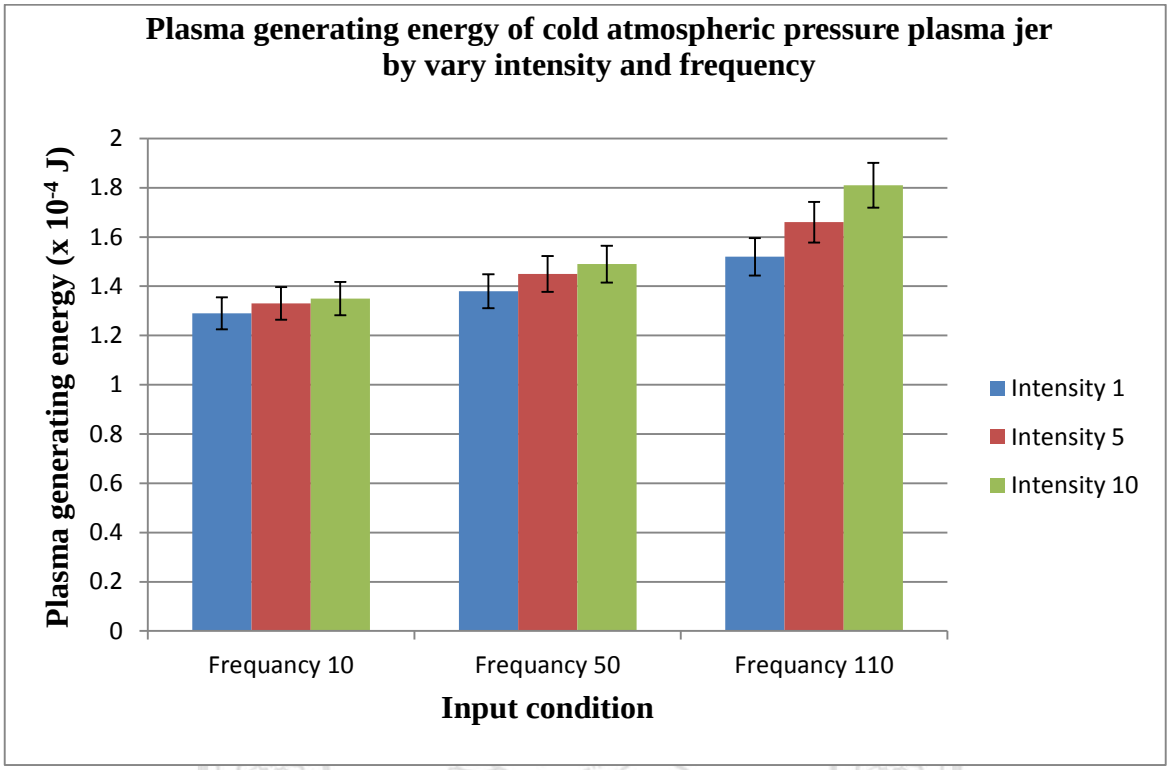

Figure.4.10. Graph of measured plasma generating energy, as a function of control parameter combinations of intensity in I number and frequency in Hz, i.e. I/Hz.

From the result of plasma generating energy, the plasma generating power can be calculated from the plasma generating energy multiplied with the frequency of radio. The result is shown in table 4.2 and figure 4.11

Table 4.2. Summary of plasma power measurement.

Intensity / Frequency(Hz)	1	5	10
10	2.15 W	2.22 W	2.26 W
50	2.31 W	2.42 W	2.49 W
110	2.54 W	2.77 W	3.02 W

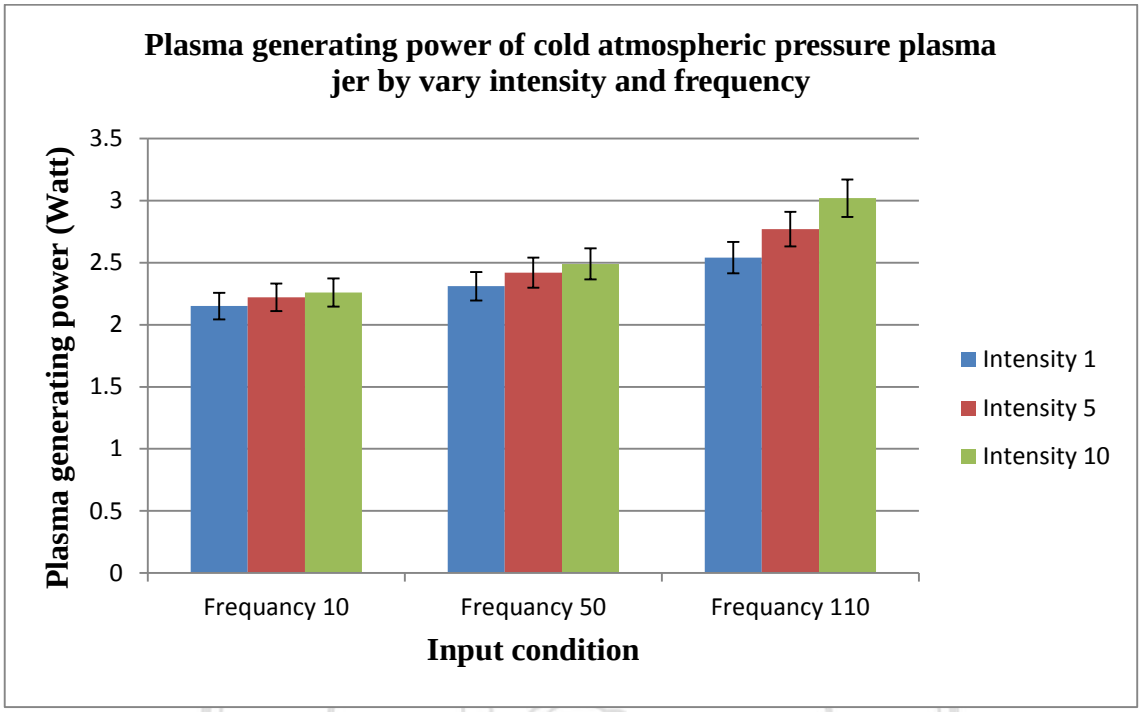

Figure.4.11. Graph of measured plasma generating power, as a function of control parameter combinations of intensity in I number and frequency in Hz, i.e. I/Hz.

4.2 Plasma species result (OES)

The Bio-plasma jet used Helium gas to be the gas source. It used a flow rate of gas about 600 ml per min. This experiment proposed to utilize cold atmospheric pressure helium plasma by varying of frequency and RF power.

Optical emission spectroscopy was used to measure the optical emission of the cold atmospheric pressure plasma jet. An optical fiber was inserted through a small hole (with a diameter of 2 mm). The distance between the head and detector the cold atmospheric pressure plasma jet was almost 1 centimeter in order to avoid any effect on the discharge. This experiment had three conditions to measure the plasma species. The first condition measured the normal plasma jet. The second and third condition used the electric field. We used a pair of parallel electrodes and bias DC voltage at the electrodes as shown in Figure 4.12.

The optical emission spectroscopy result shown that the normal plasma jet was found to have OH, O_2 species as shown in Figure 4.13. The electric field conditions was found to have OH, O_2 species too, but the intensity of the species from the area directly under the plasma jet exit after the plasma traversed the electrical field was lower than

the intensity of the species from the bent plasma jet after the plasma traversed the electrical field. The results of both conditions are shown in Figure 4.14 and Figure 4.15, respectively.

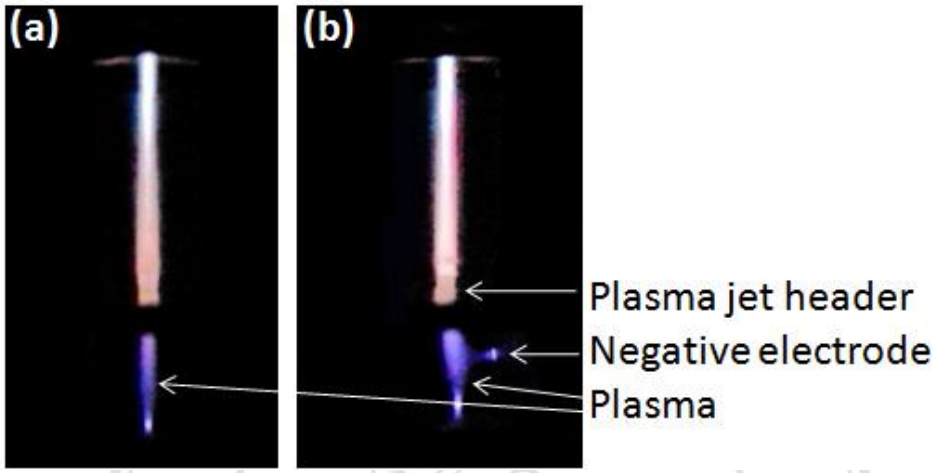

Figure.4.12**.** Photograph of the plasma jet when it traverses a pair of parallel electrodes when the electrical field is (a) off and (b) on

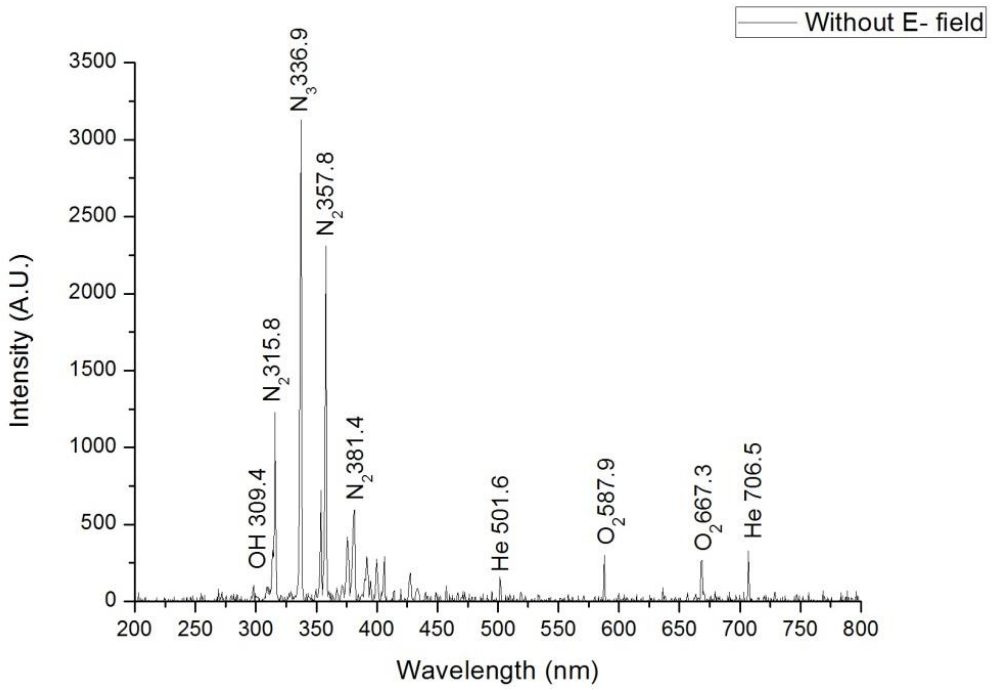

Figure.4.13. Optical emission spectra (OES) of the CAPPJ, OES detect from the original plasma jet**.**

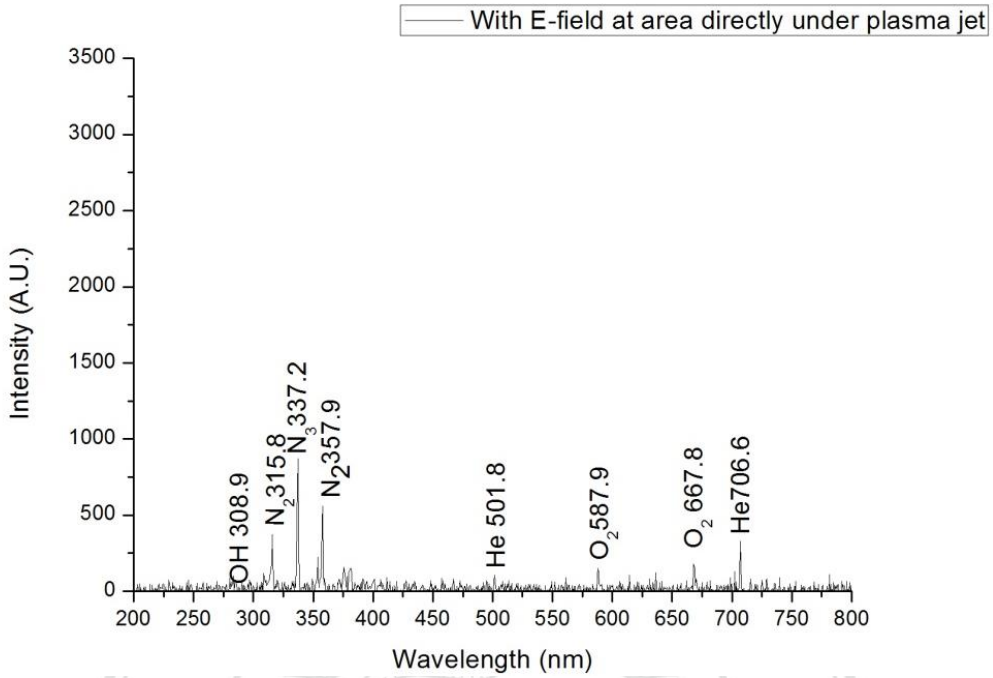

Figure.4.14 Optical emission spectra (OES) of the CAPPJ, OES detect from the area directly under the plasma jet exit, where the main part of the original plasma jet was bent away after it traversed the electrical field.

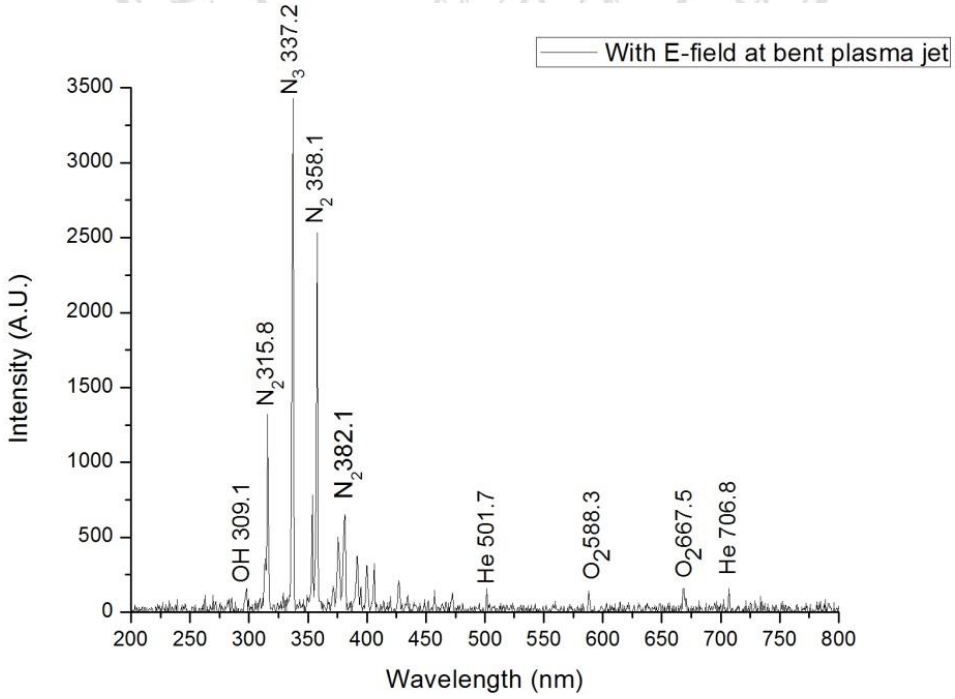

Figure.4.15. Optical emission spectra (OES) of the CAPPJ, OES detect from the bent plasma jet after it traversed the electrical field

4.3. Gel electrophoresis (Changed plasmid form)

In this part after plasma treatment of naked DNA changed plasmid DNA form was analyzed. In this experiment there were 2 main conditions of the sample, dry condition and wet condition, with using the same plasmid DNA. After the cold atmospheric pressure plasma jet treated plasmid DNA, the plasmid DNA was analyzed by using gel electrophoresis. Gel was prepared before treatment because gel took about 40 minutes for coagulating.

Plamid DNA (pGFP: plasmid green fluorescent protein, 3344 base pairs) would be seen green color under ultra violet light. Treated plasmid DNA was separated into 2 parts, the first part was used for checking form and another part used for transferring into competent bacterial cells. This part was used 5 µl per gel well.

All wells had pGFP about 100 ng/µl and the amount of 5 µl per well combine with gel red and loading dry. "A": markers of 100 base pair. "B": intensity 10 and frequency 10 Hz. "C": intensity 10 and frequency 50 Hz. "D": intensity 10 and frequency 110 Hz. "E": intensity 1 and frequency 10 Hz. "F": intensity 1 and frequency 50 Hz. "G": intensity 1 and frequency 110 Hz. "H": intensity 5 and frequency 10 Hz. "I": intensity 5 and frequency 50 Hz. "J": intensity 5 and frequency110 Hz. "K": control. All results are shown in Figure 4.16

After gel electrophoresis, all result was analyzed by Origin 8. Origin 8 converted a light intensity of gel electrophoresis to a numerical percentage for comparing percentage of changed plasmid DNA form. The data from Figure 4.16 were analyzed for percentage of the light intensity as shown in Table 4.3. The control had most relaxed and supercoiled forms. After plasma treatment, the supercoiled form concentration decreased while the relaxed form concentration increased. All numerical result was converted to a graph of the changed plasmid DNA form percentage as shown in Figure 4.17. It shows that in the condition of intensity 1 with frequency 50 Hz found a linear form but not in other conditions. We choose this generating condition for comparing dry and wet conditions.

The dry and wet condition used electric field for comparing differentiation of plasmid DNA when treated by the cold atmospheric pressure plasma jet through the electric field. The gel electrophoresis's result is shown in Figure 4.18 and Table 4.4. In

the wet condition, the percentage of light intensity shows that the supercoiled form concentration decreased while the relaxed form concentration is more than that in the dry condition. Comparison of both conditions shows that a little water has an effect to changing rate of plasmid DNA form, as shown in Figure 4.19.

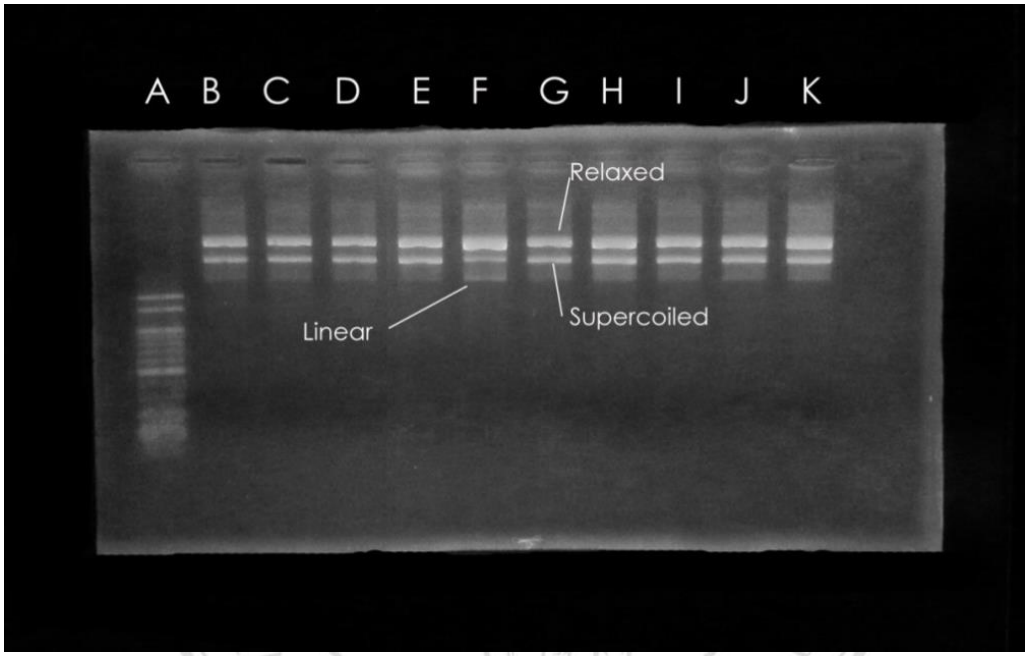

Figure.4.16. Photograph of gel electrophoresis result, it has 11 lanes.

Table.4.3. Percentage of light intensity is result of DNA change form

Condition	Relaxed	Supercoiled	Linear
Control	49.904	37.987	12.107
Intensity 1 frequency 10 Hz	52.695	30.225	17.078
Intensity 1 frequency 50 Hz	52.02	29.747	18.234
Intensity 1 frequency 110 Hz	56.996	28.025	14.978
Intensity 5 frequency 10 Hz	56.358	26.626	17.013
Intensity 5 frequency 50 Hz	58.578	24.369	17.052
Intensity 5 frequency 110 Hz	58.693	25.925	15.381
Intensity 10 frequency 10 Hz	64.289	21.698	14.012
Intensity 10 frequency 50 Hz	60.927	23.156	15.92
Intensity 10 frequency 110 Hz	61.245	24.142	14.613

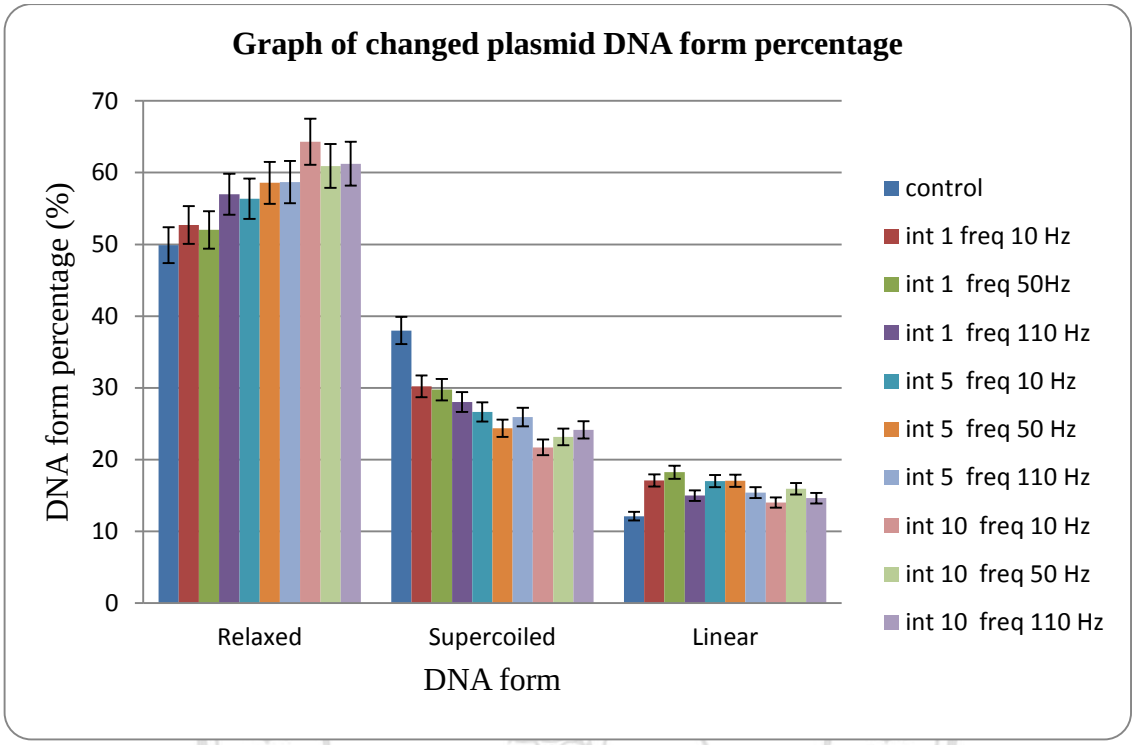

Figure.4.17. Percentages of the DNA forms under varied CCAPPJ treatment conditions, quantified from the gel electrophoresis band light intensity

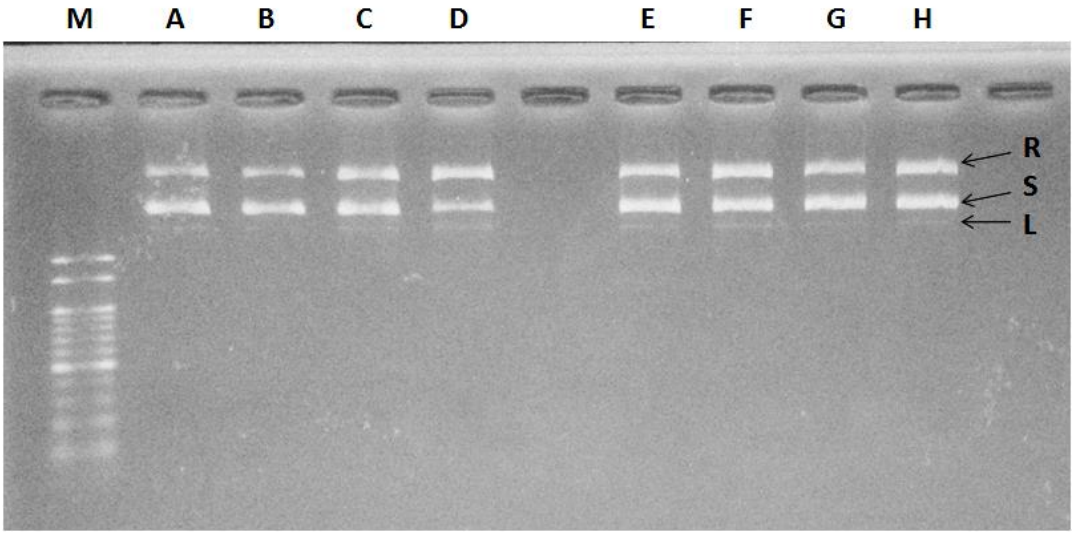

Figure.4.18. Electrophoresis result for comparison. M: Marker. A and E: I1/50Hz, dry. B and F: I1/50Hz, wet. C and G: I5/50, dry. D and H: I5/50Hz, wet. A-D: without electrical field. E-H: with electrical field. R: relaxed form. S: supercoiled form. L: linear form.

Table.4.4. Percentage of light intensity is result of DNA change form, the dry – wet condition and electric field condition

Condition	Relaxed	Supercoiled	Linear
Intensity 1 frequency 50 Hz dry	29.714	66.887	3.199
Intensity 1 frequency 50 Hz wet	29.932	66.825	3.243
Intensity 5 frequency 50 Hz dry	44.682	49.745	5.173
Intensity 5 frequency 50 Hz wet	54.436	35.814	9.750
Intensity 1 frequency 50 Hz dry with E. field	38.873	60.362	0.765
Intensity 1 frequency 50 Hz wet with E. field	50.357	47.362	1.681
Intensity 5 frequency 50 Hz dry with E. field	38.764	59.842	1.394
Intensity 5 frequency 50 Hz wet with E. field	48.103	49.585	2.312

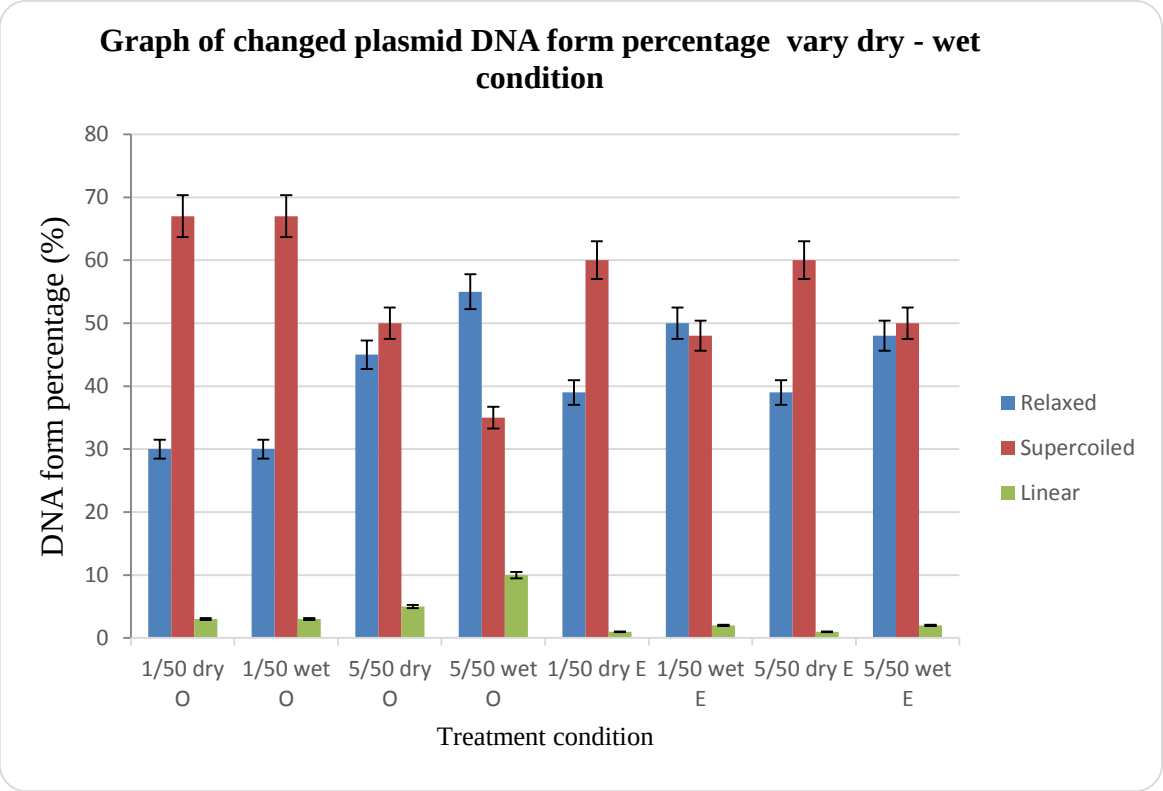

Figure.4.19. DNA form concentrations quantified from the electrophoresis result in Figure 4.18. I/Hz: plasma intensity level/frequency. Dry: dry sample. Wet: wet sample. O: original treatment without the electricla field. E: with the electrical field.

All of the results show that with increasing of the plasma power, the supercoiled form concentration decreased while the relaxed form concentration increased, but on the other hand, linear form concentration remained roughly independent of plasma power. The plasma power only represented the plasma intensity or the number of plasma particles but not the energy of the particle. As SSB could cause relaxed forms, only one hit by the reactive physical particle or agents in the plasma to the atoms of DNA could possibly displace the atom to break the strand. Therefore, the more plasma agents, the more single hits to the DNA atoms. However, to cause a linear form needed a consecutive twice hits onto two atoms at the double strands and this meant that the reactive particle or agent should have higher energy which could make it possible to perform the second hit after first hit.

4.4. Bacterial mutation by using electroporation.

The 5 µl of naked DNA were used for plasmid DNA transfer into competent bacterial cells (*E.coli*). Electroporation was used at 1.8 kV/cm with 5 second per sample to transfer naked DNA. Afterwards, competent bacterial cells were mixed with 1 ml of lb broth. It was shaken in an incubator at 37 °C about 3 hours and added with 2 µl of ampicillin and shaken continuously for 3 hours.

Spread-Plate Technique was used for spreading *E. coli* on agar gel plate. Penicillin enrichment was used for screening mutant cells. *E. coli* in plates was cultured in an incubator at 37°C overnight. On next day, the plate of *E. coli* was observed under UV light by a UV trans-illuminator. In regular *E. coli* bacterial cell (DH-α5) there are colonies of bacteria in punctiform. All spots on agar gel petri-dish were shown green light as shown in Figure 4.20.

We transferred treated plasmid DNA of every condition. Some condition was in error. But also, the intensity 1 with frequency 50 Hertz condition found a small white spot on the petri-dish after observing under UV light by a UV- trans-illuminator, as show in Figure 4.21. This is a preliminary result but showing trends of mutation in E.coli bacterial cells.

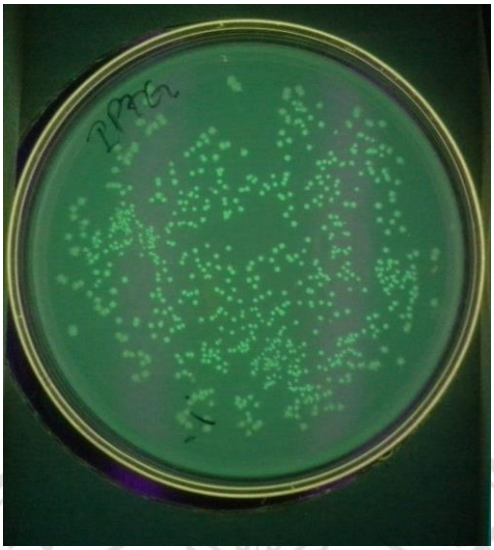

Figure.4.20. Picture of bacteria plate, which is a control. In petri - dish has shown a lot of green spot by using UV trans-illuminator.

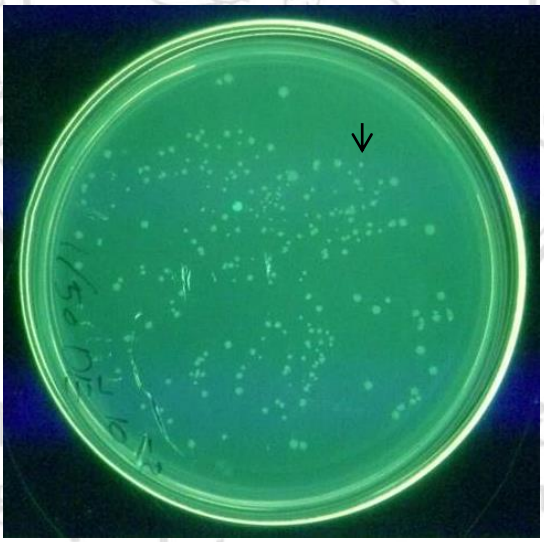

Figure.4.21. Picture of bacterial petri dish, which is intensity 1 with frequency 50 Hz. In petri - dish has shown a small white spot follow the arrow.