The experiments were conducted mainly by our WET team (Kento Tsuruzoe, Kazuma Tsurumaki, Rintaro Moriyama, and Misa Iizawa).

During the following period, the number of people allowed to enter the laboratory was limited to two.
5/12~6/20 State of emergency announced by the government
6/21~7/11 preventive measures announced by the government
8/2~8/16 preventive measures announced by the government
8/17~9/30 State of emergency announced by the government

6/15 15:00~17:00
Member: Tsuruzoe, Tsurumaki, Moriyama, Iizawa
“Genetic Recombination Experiment Education and Training”

Experiment Day 1~7
We tried to assemble the DNA by Gibson Assembly and ligation for transformation.

8/23 12:00~15:00
Experiment Day 1
Member: Tsuruzoe, Tsurumaki
・Preparation of TE Buffer
・Preparation of LB medium

8/24 9:00~13:00
Experiment Day 2
Member: Tsuruzoe, Moriyama
・Preparation of Ampicillin
・Preparation of LB Agar Plates

8/25~9/13
The plasmid we were originally going to use took longer than expected to be delivered, so we changed the plasmid and waited for delivery.

9/14 13:00~18:00
Experiment Day 3
Member: Tsuruzoe, Iizawa
・Resuspending gBlocks® Gene Fragments
・Gibson Assembly
・Digestion

9/15 14:00~18:00
Experiment Day 4
Member: Tsuruzoe, Tsurumaki
・Agarose Gel Electrophoresis
・Ligation
NOTE:
・We checked the Gibson Assembly in Electrophoresis to see if it was successful. The band could not be confirmed, but we went ahead with the experiment anyway.

9/20 16:00~18:00

Experiment Day 5
Member: Tsuruzoe, Tsurumak
・Transformation

9/21 16:00~18:00
Experiment Day 6
Member: Tsuruzoe
・Inoculation

9/22 9:00~15:00
Experiment Day 7
Member: Tsuruzoe, Tsurumaki
・Miniprep
・NanoDrop
・Digestion
・Agarose Gel Electrophoresis
NOTE:
・The results of the NanoDrop measurements were as follows.
Nucleic Acid Conc.=42.1ng/$\mu\ell$
$O.D._{260}/O.D._{280}$=0.95
・We used Electrophoresis to check if the transformation was successful. As a result, we found that the plasmid without insert was introduced.

Fig. 1 Day 7 electrophoresis results

Experiment Day 8~12
After considering the cause of the previous failure, we decided to perform DNA purification after Gibson Assembly.

9/29 9:00~11:00
Experiment Day 8
Member: Tsurumaki, Iizawa
・DNA purification
・Gibson Assembly
NOTE:
・To increase the success rate of the experiment, we purified Part 1 (CooA) and Part 2 (luciferase) DNA.

10/4 13:00~19:30
Experiment Day 9
Member: Tsuruzoe, Tsurumaki
・Agarose Gel Electrophoresis
・DNA purification
・NanoDrop

NOTE:
・On the fourth day, insert, for which no band could be seen, was electrophoresed again. As a result, no band could be seen. In addition, when the concentration was measured, it was abnormally high, so it was purified and the concentration was measured again. The following results were obtained.
Nucleic Acid Conc.=79.8ng/$\mu\ell$

$O.D._{260}/O.D._{280}$=2.02

10/5 17:00~20:00
Experiment Day 10
Member: Tsuruzoe
・Digestion
・Ligation
・Transformation

10/6 18:00~19:30
Member: Tsuruzoe, Tsurumaki, Iizawa
Experiment Day 11
・Inoculation

10/7 10:30~16:30
Member: Tsuruzoe, Tsurumaki
Experiment Day 12
・Miniprep
・NanoDrop
・Digestion
・Agarose Gel Electrophoresis
・Preparation of Ampicillin

NOTE:
・The results of the NanoDrop measurements were as follows.
Nucleic Acid Conc.=176ng/$\mu\ell$
$O.D._{260}/O.D._{280}$=1.90
・We used Electrophoresis to check if the transformation was successful. As a result, we found that the plasmid without insert was introduced as before.

Fig. 2 Day 12 electrophoresis results

Experiment Day 10~12
In order to reduce the experimental process and increase the success rate, we tried to assemble the DNA using only Gibson Assembly without ligation.

10/5 10:00~12:00
Experiment Day 10
Member: Tsurumaki
・Resuspending gBlocks® Gene Fragments
NOTE:
・The newly ordered DNA arrived and we resuspended it.

10/6 18:00~19:30
Member: Tsurumaki
Experiment Day 11
・Digestion (Vector)
NOTE:
・Digestion of the vectors to assemble the DNA by Gibson Assembly.

10/7 10:30~16:30

Member: Tsuruzoe, Tsurumaki
Experiment Day 12
・Gibson Assembly
・Transformation

10/8 17:00~21:00
Member: Tsuruzoe
Experiment Day 13
・Miniprep
・Preparation of LB Agar Plates

NOTE:
・There were no colonies in the LB Ager Plates coated with E. coli the day before.

Experiment Day 14~17
We thought that the reason why the colony did not form in the previous experiment was because there was a problem in the Gibson Assembly stage, so we decided to split the Gibson Assembly into two stages.

10/11 13:30~19:30
Member: Tsuruzoe, Tsurumaki
Experiment Day 14
・The first Gibson Assembly
・DNA purification
・NanoDrop

NOTE:
・The results of the NanoDrop measurements were as follows.
Nucleic Acid Conc.=25.9ng/$\mu\ell$
$O.D._{260}/O.D._{280}=1.87$

10/12 10:30~12:00, 16:30~18:30
Member: Tsuruzoe
Experiment Day 15
・Agarose Gel Electrophoresis
・The second Gibson Assembly
・Transformation
NOTE:
・We checked the Gibson Assembly in Electrophoresis to see if it was successful. However, the band could not be confirmed. (Was it because the DNA was too thin?)

Fig. 3 Day 15 electrophoresis results

10/13 16:00~17:00
Member: Tsurumaki
Experiment Day 16
・Inoculation

10/14 11:00~15:00
Member: Tsuruzoe, Tsurumaki
Experiment Day 17

・Absorbance measurement
・Miniprep
・NanoDrop
・Digestion
・Agarose Gel Electrophoresis

NOTE:
・The results of the NanoDrop measurements were as follows.
Nucleic Acid Conc.=182ng/$\mu\ell$
$O.D._{260}/O.D._{280}$=1.91
・We used Electrophoresis to check if the transformation was successful. As a result, we found that the plasmid without insert was introduced as before.

Fig. 4 Day 17 electrophoresis results

10/15 11:00~14:00, 17:00~18:00
Member: Tsuruzoe, Tsurumaki
Experiment Day 18
・Miniprep
・NanoDrop
NOTE:
・The results of the NanoDrop measurements were as follows.
Sample 1
Nucleic Acid Conc.=185.3 ng/$\mu\ell$
$O.D._{260}/O.D._{280}$=1.91
Sample 2
Nucleic Acid Conc.=230.1 ng/$\mu\ell$
$O.D._{260}/O.D._{280}$=1.91

10/16 16:00~19:00
Member: Tsuruzoe
Experiment Day 19
・Agarose Gel Electrophoresis
・Inoculation

10/19 10:30~12:30, 17:00~21:00
Member: Tsuruzoe
Experiment Day 20
・The first Gibson Assembly
・DNA purification
・NanoDrop
・Agarose Gel Electrophoresis
・Digestion (Vector)
・The second Gibson Assembly
・Transformation
NOTE:
・We checked the Gibson Assembly in Electrophoresis to see if it was successful. However, the band could not be confirmed.

Fig. 5 Day 20 electrophoresis results