“Let’s observe the DNA in foods” (Held on 6/2 Sun.) Protocol

Host：iGEM Grand Tokyo
Sponsors：Leave A Nest Co., Ltd.

[Experiment 1]

<Extraction of DNA in Bacillus subtilis natto>

[Materials]

Natto	¼ of a pack
DNA extraction solution - Water - Neutral detergent - NaCl	Total:50ml 45ml 5ml 5g
Ethanol（cooled）	20ml
Wooden chopsticks	1
TE buffer	500μl
100ml Beaker	2
1.5ml Tube	1

[Methods]

1. Add 50mL of DNA extraction solution to a beaker containing natto.

2. Mix gently with wooden chopsticks, and wait for 5 minutes.

3. Avoiding the beans, remove 10mL of the solution into another beaker, using a micropipette.

4. In that solution, gently pour 20mL of ethanol. Confirm that the solution separates into two layers.

5. Using chopsticks, collect the filamentous DNA from the ethanol layer and transfer it into a 1.5mL tube. Centrifuge the tube at room temperature at 10,000rpm for about 20 to 30 seconds.

※Make sure to balance the tube, and position it so that the fixed part of the lid faces outward

6. Using a micropipette, suck up the layer of liquid at the top that was centrifuged. Remove as much as possible.
※ Avoid the precipitated DNA

7. Add 100μl of TE buffer into the tube with the precipitated DNA. Mix with the tip of the micropipette. Then, add an additional 400μl of TE buffer.

<Extraction of DNA from broccoli>

[Materials]

Broccoli	Around 100g
DNA extraction solution - Water - Neutral detergent - NaCl	Total:50ml 45ml 5ml 5g
Ethanol（cooled）	20ml
Wooden chopsticks	1
TE buffer	500μl
100ml Beaker	2
1.5ml Tube	1

[Methods]

1. Add 50mL of DNA extraction solution to a beaker containing broccoli.

2. Mix gently with wooden chopsticks, and wait for 5 minutes.

3. Centrifuge to precipitate the broccoli parts. Transfer the top layer liquid into another beaker.

4. Gently pour 20mL into the beaker with the liquid. Confirm that the solution separates into two layers.

5. Collect the DNA from the ethanol layer by measuring 2mL using a micropipette and transfer it into a 1.5mL tube. Centrifuge the tube at room temperature at 10,000rpm for 1 minute.

6. Using a micropipette, suck up the layer of liquid at the top that was centrifuged. Remove as much as possible.
※ Avoid the precipitated DNA

7. Add 100μl of TE buffer into the tube with the precipitated DNA. Mix with the tip of the micropipette. Then, add an additional 400μl of TE buffer.

[Experiment 2]

<Polymerase Chain Reaction (PCR)>

<100-fold dilution of template DNA>

[Materials]

MilliQ	198µl + 18µl
Extracted DNA	2µl each (natto/broccoli)
1.5mL tube	4

Perform PCR using diluted DNA to observe whether the thickness/darkness of electrophoretic bands changes depending on the DNA concentration.

1. Add 198µl of MilliQ into 2 1.5mL tubes
2. Take 2µl of the extracted DNA and put it into the tubes marked with three lines. (Natto has a blue sticker, Broccoli has a pink sticker on the lid）
3. Mix by tapping the tube, and centrifuge for several seconds to remove the water droplets from the lid.

<1000-fold dilution of template DNA>

Perform PCR using diluted DNA to observe whether the thickness/darkness of electrophoretic bands changes depending on the DNA concentration.

4. Add 18µl of MilliQ into 2 1.5mL tubes.
5. Take 2µl of the 100-fold diluted DNA and put it into the tubes marked with four lines.
   (Natto has a blue sticker, and Broccoli has a pink sticker on the lid）
6. Mix by tapping the tube, and centrifuge for several seconds to remove the water droplets from the lid.

<Making the PCR solutions>

[Materials]

PCR Mix A - MilliQ - 2× gotaq - Natto FPrimer	22.5µl each 9.5µl 12.5µl 0.25µl

- Natto RPrimer	0.25μl
PCR Mix B - MilliQ - 2× gotaq - Broccoli FPrimer - Broccoli RPrimer	22.5μl each 9.5μl 12.5μl 0.25μl 0.25μl
1/100 DNA	5μl each
1/1000 DNA	5μl each
0.2mL tube	7

[Procedure]

PCR Mix A	Total:112.5μl
Extracted Natto DNA (1/100 fold dilution)	2.5μl
Extracted Natto DNA (1/1000 fold dilution)	2.5μl

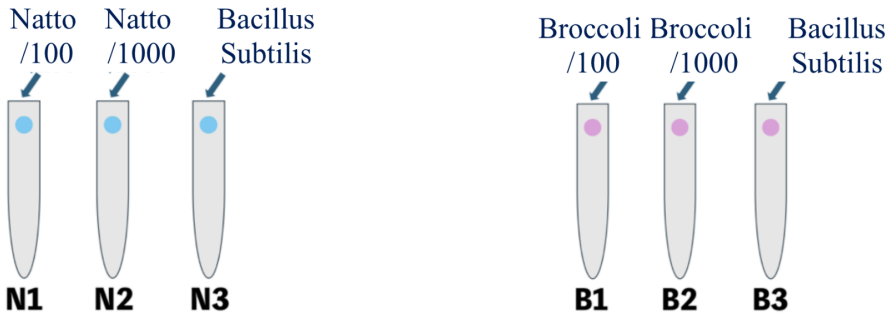
1. Add 22.5μl each of PCR MixA to the 4 tubes(1-3、N).

2. Add the DNA as following instructions.
   1 Extracted Natto DNA (1/100 fold dilution) 2.5μl
   2 Extracted Natto DNA (1/1000 fold dilution) 2.5μl
   3 DNA from Bacillus Subtilis 2.5μl
   N Natto DNA that is prepared 2.5μl

PCR Mix B	Total:112.5μl
Broccoli DNA (1/100 fold dilution)	2.5μl
Broccoli DNA (1/1000 fold dilution)	2.5μl

1. Add 22.5μl PCR MixB each to the 3 tubes(1-3).

2. Add the DNA as following instructions.
   1 Extracted Broccoli DNA (1/100 fold dilution) 2.5μl
   2 Extracted Broccoli DNA (1/1000 fold dilution) 2.5μl
   3 DNA from Bacillus Subtilis 2.5μl



<PCR>

[Materials]

PCR Master Mix	25μl each
Thermal Cycler	1

1.作製したPCR溶液の入った0.2mlチューブをサーマルサイクラーにセットする。

2.以下のサイクル条件を設定し、実行する。

95℃ 1 min 30 sec

95℃ 30 sec

60℃ 30 sec

72℃ 1min ×30 cycles

72℃ 5 min

12℃ keep

[Experiment 3] 電気泳動

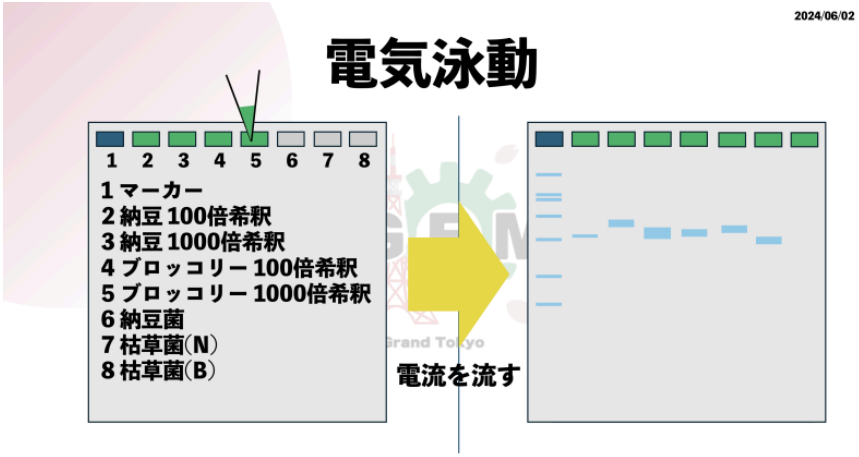
[Materials]

PCR product	5μl
DNA gene ladder	5μ
2% Agarose gel	1piece
TAE buffer	ゲルが浸かるまで(400ml)
Ethidium Bromide	20μl
Electrophoresis tank	1

[Procedure]

※The EtBr in the buffer is carcinogenic, so **DO NOT TOUCH IT WITH BARE HANDS!!!**

1. Place the PCR product in the holes created in the Agarose gel, in the order shown in



the image below.

2. Leave it at 100V for 20 minutes.

3. Observe the gel under a UV lamp, and compare it with the image below to identify the placements of the band. ※Do not look directly at it as much as possible