iGEM Grand Tokyo × ADvanceLab Experimental class

「What is genetic modification?」

Overview

Soybean DNA extracts are subjected to a PCR cycle to increase the number of specific genes, electrophoresis to observe that gene.

※We had originally planned to start with DNA extraction of soybeans, but due to time constraints and reagents, we will use DNA that we have already extracted.
We will explain our DNA extraction process later!!

Time schedule

12:00 Start
12:00~12:30 Ice break
12:30~13:15 Description of Experiment
　　Overall picture and brief explanation of the experiment
　　Explanation of DNA extraction operations and principles
　　Description of PCR operation
13:15~13:45 PCR operation
13:45~15:00 PCR cycle run
13:45~14:30 Lecture
　　Explanation of the principle of PCR operation
　　Lectures about synthetic biology and iGEM
14:30~14:45 Break time
14;45~15:00 Explanation of ADvance Lab
15:00~15:15 Explanation of electrophoresis
　　Explanation of the principle of electrophoresis
　　Description of operation
15:15~15:45 preparation of electrophoresis
15:45~16:15 electrophoresis
16:15~16:30 Observation of electrophoresis results (UV irradiation)
16:30~17:00 Reflection, questionnaire response, Q&A
17:00 Termination, Dismissal

iGEM Grand Tokyo × ADvanceLab Experimental class

「What is genetic modification?」

Protocol of the experiment

1.Preparation and execution of PCR operation

1.Dilute the soybean DNA extract.

DNA extract	1μl
MilliQ	14μl

1/10 => DNA 0.5μl + MilliQ 4.5μl => 5μl
1/20 => DNA 0.5μl + MilliQ 9.5μl => 10μl

2.Dilute the primer

Primer(F)	0.5μl
Primer(R)	0.5μl
MilliQ	1μl

Primer 0.5μl + MilliQ 0.5μl => 1μl

3.Prepare solution for PCR

DNA(1/1, 1/10, 1/20)	1μL each
Diluted primer(F,R)	0.6μl each
KOD Master Mix	15μl
MilliQ	12.8μl

DNA 1μl + Primer(F) 0.6μl + Primer(R) 0.6μl + KOD Master Mix 15μl + MilliQ 12.8μl => 30μl

4.Combine the solution you just made into three portions.

30 μl => 10 μl of the solution made in 3.

「What is genetic modification?」

PCR cycle(Operated by TA!!)

98℃ 3 minutes

98℃ 10 seconds
50℃ 5 seconds ×30 cycle
68℃ 3 seconds

68℃ 7 minutes

12℃ ∞

2.Electrophoresis

1.Combine the three aliquoted PCR products into one.

10μl × 3 => 30μl

2.Set the gel in the electrophoresis apparatus.(Operated by TA!!)

3.Apply to gel.

Marker 4μl

Each PCR products 5μl
Loading Dye 1μl
計 6μl

Applying each to a well as follows