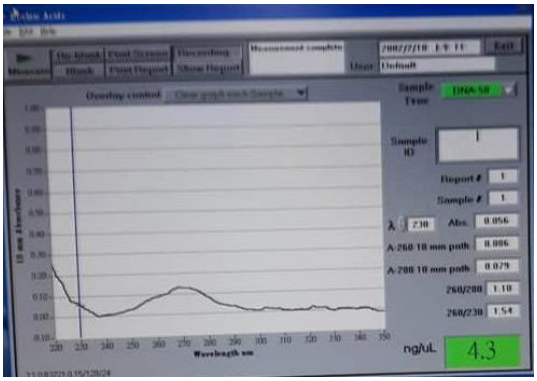
Interlab Debug

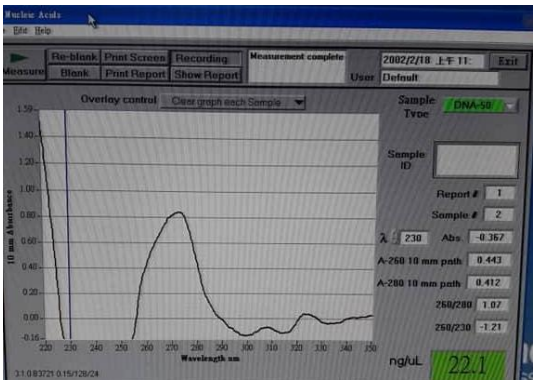
We measured the quality of the DNA distribution kit that we received from iGEM,

Well	2B	2D	2F	2H	2J	2L	2N	2P
$OD_{260/280}$	1.18	1.07	1.09	1.14	1.13	0.91	0.8	1.14
ng/ul	4.3	22.1	6.5	36.5	44.2	2.5	0.9	38.6

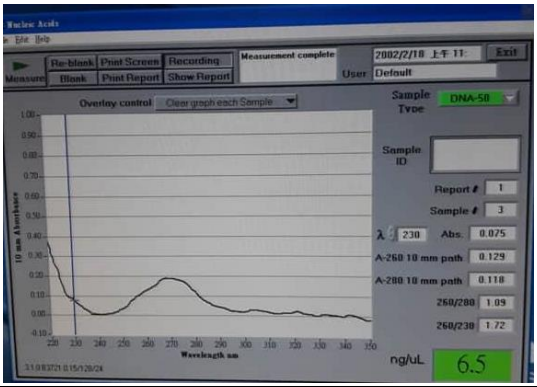
well2B



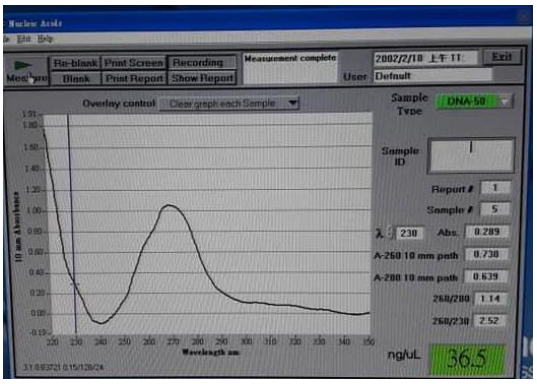
well2D



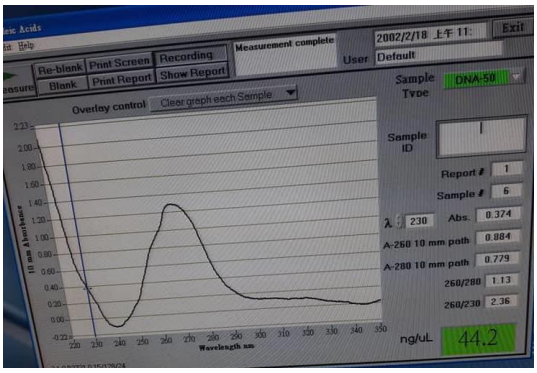
well2F



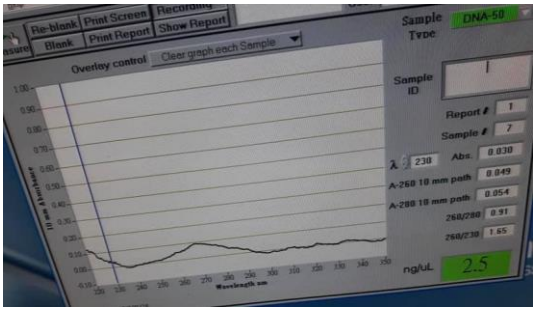
well2H



well2J

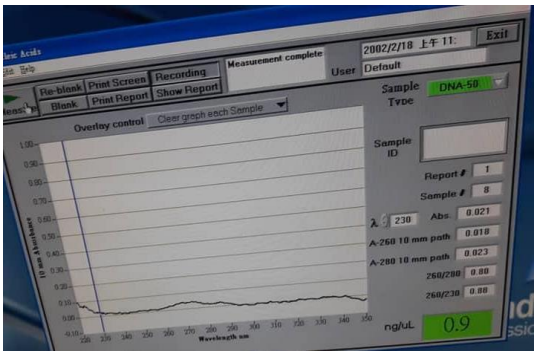


well2L



well2N

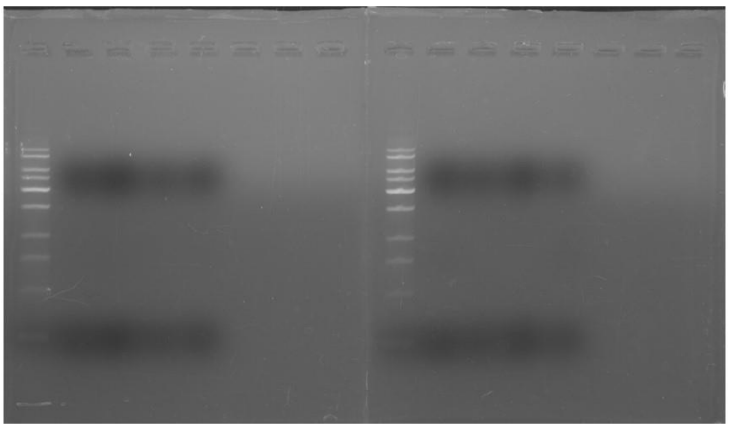
well2P



Also, we have run gel electrophoresis for debugging. However, there was no band on the agarose gel.

Marker 2B 2D 2F 2H

Marker 2J 2L 2N 2P



Materials:

Competent cells (Escherichia coli strain DH5α)
LB (Luria Bertani) media
Chloramphenicol (stock concentration 25 mg/mL dissolved in EtOH)
50 ml Falcon tube (or equivalent, preferably amber or covered in foil to block light)
Incubator at 37°C
1.5 ml eppendorf tubes for sample storage
Ice bucket with ice
Micropipettes and tips
96 well plate, black with clear flat bottom preferred (provided by team)
Devices (from Distribution Kit, all in pSB1C3 backbone):
Device Part Number Plate Location
Negative control BBa_R0040 Kit Plate 7 Well 2D
Positive control BBa_I20270 Kit Plate 7 Well 2B
Test Device 1 BBa_J364000 Kit Plate 7 Well 2F
Test Device 2 BBa_J364001 Kit Plate 7 Well 2H
Test Device 3 BBa_J364002 Kit Plate 7 Well 2J
Test Device 4 BBa_J364007 Kit Plate 7 Well 2L
Test Device 5 BBa_J364008 Kit Plate 7 Well 2N
Test Device 6 BBa_J364009 Kit Plate 7 Well 2P

7/3

LB Broth and LB Agar preparing

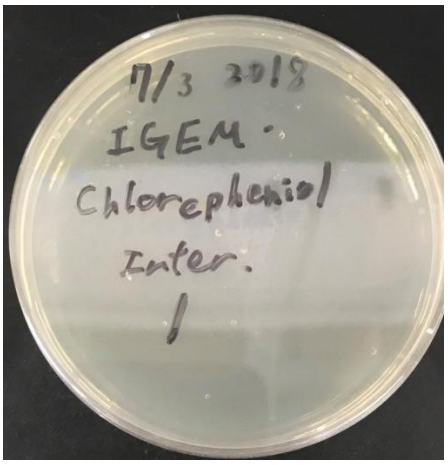
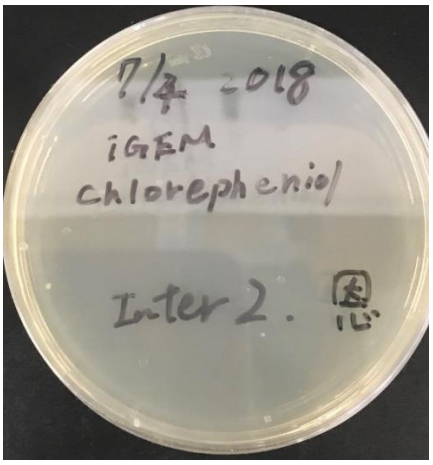
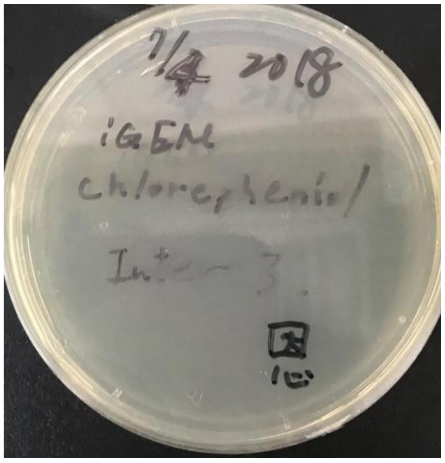
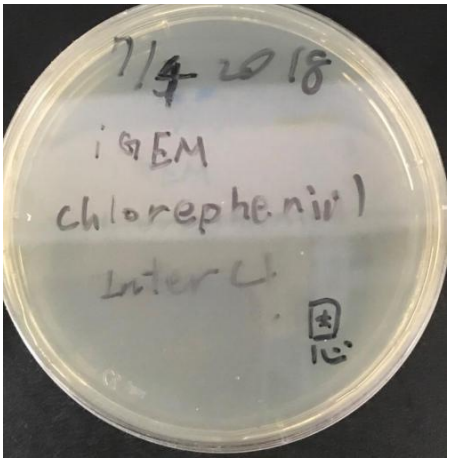
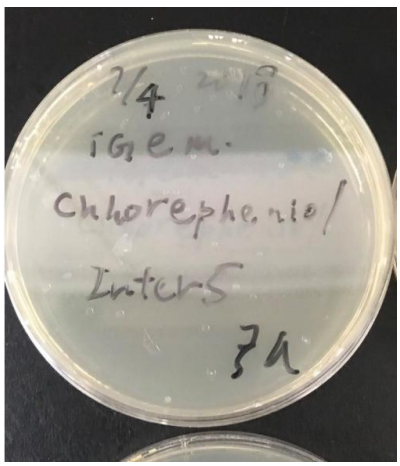
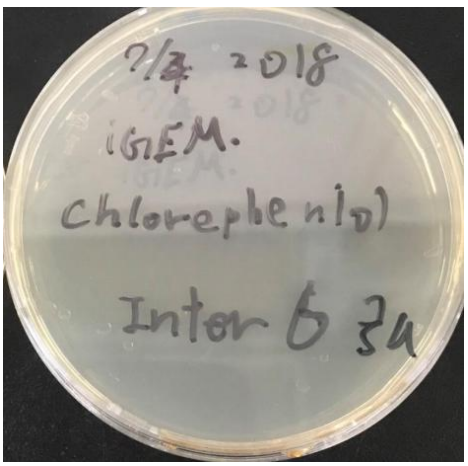
1. Prepare two 1L beaker and add Tryptone 5 g, NaCl 5 g, Yeast extract 2.5 g in each beaker, then add MiliQ water to 500ml, stir by magnetic stirrer.
2. Prepare 1L Serum bottle and add Tryptone 5 g, NaCl 5 g, Yeast extract 2.5 g, then add MiliQ water to 500ml, stir by magnetic stirrer.
3. Autoclave for 30min.
4. Prepare 5ml 1000 X Stock Chloramphenicol
5. Add 500µl 1000 X Stock Chloramphenicol when temperature down to 60°C(without pipetting)
6. Pour LB Agar into dish
7. Store at 4°C

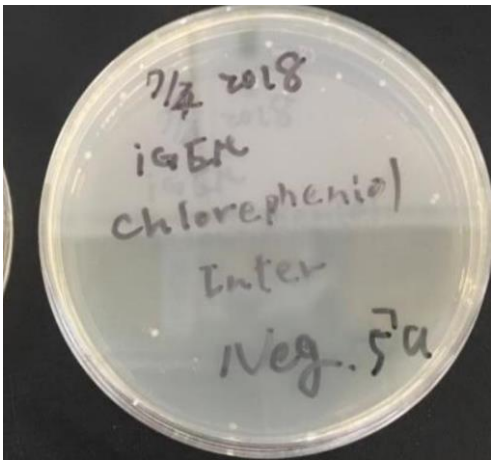
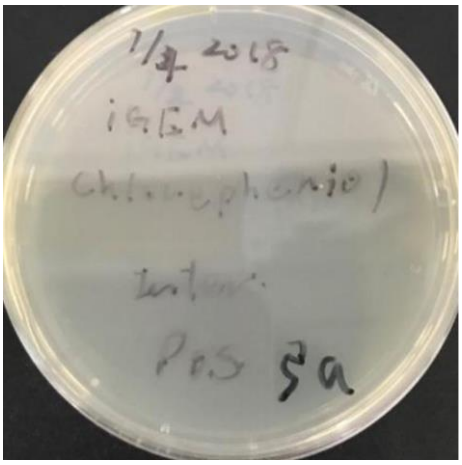
7/4 (2:30~6:30)

Transformation(0)

1. Resuspend DNA in selected wells in the Distribution Kit with 10µl dH20. Pipet up and down several times, let sit for a few minutes. Resuspension will be red from cresol red dye.
2. Label 1.5ml tubes with part name or well location.
3. Take competent cells out of -80°C and thaw on ice.(150µl commercial competent cell)
4. Remove agar plates (containing the appropriate antibiotic) from storage at 4°C (without warm at 37°C incubator)
5. Pipette 25µl of commercial competent cell into 1.5ml tube.
6. Pipette 1µl of resuspended DNA into 1.5ml tube. (without 輕彈 three time)
7. Close 1.5ml tubes, incubate on ice for 40min. (should be 30min, but we take 10min to take 50µl competent cell for D5, D6, and add it.)
8. Heat shock tubes at 42°C for 45 sec.
9. Incubate on ice for 5min
10. Pipette 950µl LB media to each transformation(we don't use SOC)
11. Incubate at 37°C for 1 hours, shaking at 200-300rpm.
12. WITHOUT Spin down cells at 6800g for 3mins and discard 800µL of the supernatant. Resuspend the cells in the remaining 100µL, and pipette each transformation onto petri plates.
13. Incubate transformations overnight (15hr) at 37°C.

Results:

 7/3 2018 IGEM. Chlorephenicol Inter. 1	 7/4 2018 iGEM Chlorephenicol Inter 2.
 7/4 2018 iGEM Chlorephenicol Inter 3.	 7/4 2018 iGEM Chlorephenicol Inter 4.
 7/4 2018 iGem. Chlorephenicol Inter5	 7/4 2018 iGEM. Chlorephenicol Inter 6

 7/4 2018 iGEM Chlorephenicol Inter Neg. 5a	 7/4 2018 iGEM Chlorephenicol Inter Pos 5a

7/5 (2:00~7:30)

Transformation(1)

1. Label 1.5ml tubes with part name or well location.
2. Take competent cells out of -80°C and thaw on ice.(175µl commercial competent cell)
3. Remove agar plates (containing the appropriate antibiotic) from storage at 4°C (without warm at 37°C incubator)
4. Pipette 25µl of commercial competent cell into 1.5ml tube.
5. Pipette 1µl of DNA into 1.5ml tube. (without resuspended)
6. Close 1.5ml tubes, incubate on ice for 35min. (should be 30min, but we take 5min to take 25µl competent cell for D3, and add it.)
7. Heat shock tubes at 42°C for 45 sec.
8. Incubate on ice for 5min
9. Pipette l LB media to 1000µL each transformation(we don't use SOC)
10. Incubate at 37°C for 1 hours, shaking at 200-300rpm.
11. Spin down cells at 6800g for 3mins and discard 800µL of the supernatant. Resuspend the cells in the remaining 200µL, and pipette each transformation onto petri plates.

When we Spread plate for D3, we discard 800µL, Resuspend ,and pipette 200µL onto petri plates.

when we Spread plate for D4, we discard 850µL, Resuspend ,and make mistake to discard ~120µL.We add 70µL LB, Incubate at 37°C for 1 hours, shaking at 200-300rpm, and pipette 100µL onto petri plates.

For other plates, we discard 850µL, Resuspend, and pipette 150µL onto petri plates.)

Results:

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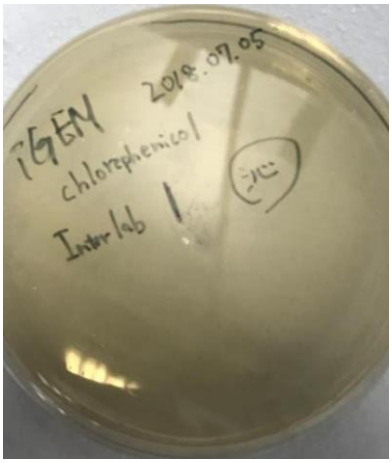
<td> 
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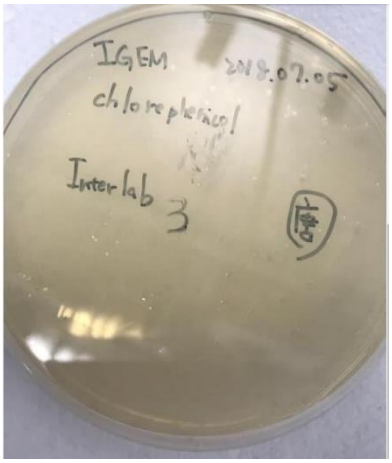
<td> 
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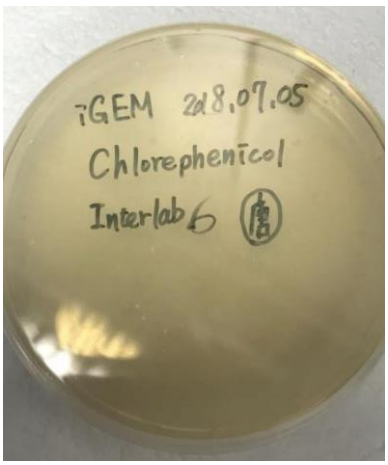
<td> 
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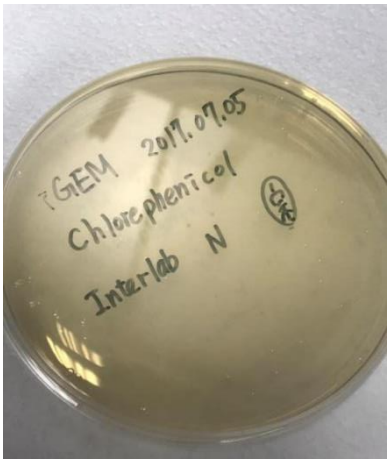	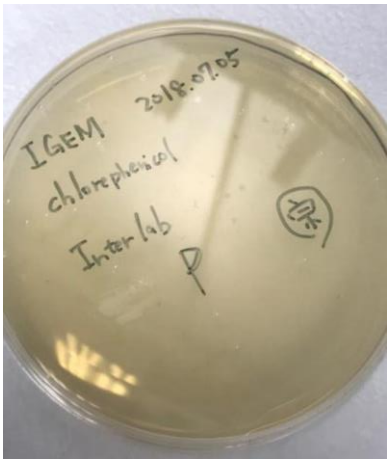

Point: Add 500µl 1000 X Stock Chloramphenicol when temperature down to 60℃(without pipetting)

Chloramphenicol is not well-mixed that leads to some of E.coli can grow on the plate while others cannot (not well-distributed growing on the plate).