Notebook

23/09/2019

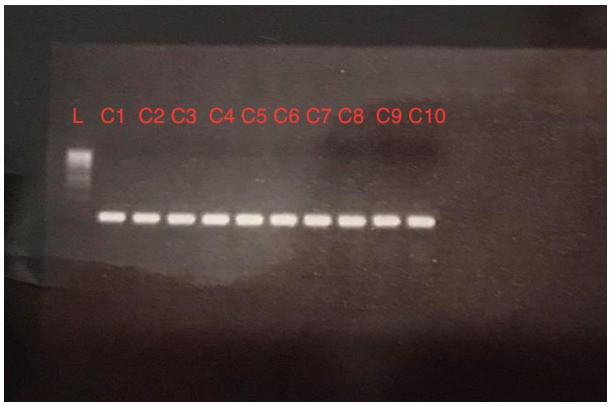
Cell Transformation with BBa_K731520 part.

1. Thaw 100 µL of XL1 Blue competent bacteria on ice
2. Add 1 µL of plasmid containing 100 ng of plasmid
3. Put on ice for 30 minutes
4. Heatshock at 42°C for 45 seconds
5. Return on ice for 5 minutes
6. Add liquid LB to 500 µL
7. Regenerate at 37°C for 30 minutes
8. Plate cells and resuspend in 100 µL of liquid LB
9. Let grow at 37°C, rotation 220 rpm, 5% CO2 overnight

24/09/2019

Select 10 colonies for BBa_K731520 part PCR check.

1. Mix together
    - 10 µl of primer 1 (forward)
    - 10 µl of primer 2 (reverse)
    - 230 µl of MilliQ
    - 250 µl Taq'Ozyme HS Mix
2. Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate and rub down PCR tube.
3. Use the the PCR machine with adapted cycles
4. To prepare 2% agar gel: heat 2g of agar with 100 ml of TBE. Keep cool and add 5 µL of Ethidium bromide.
5. For the gel verification mix together:
    - 10 µl of PCR product
    - 2 µl of Loading buffer (6x)
6. 130 watts for 30 minutes
7. Observe under UV



The colonies 2,3,5 for selected for GFP expression test. No transformed cell was selected as negative control.

25/09/2019

1. Prepare 15 ml of liquid LB with 13 µL of Chloramphenicol and 0,2 g (2%) of D-glucose for promoter inhibiting.
2. Add 3 ml of prepared liquid LB to a tube.
3. Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate.
4. Drop the tip or toothpick into the liquid LB + antibiotic and swirl.
5. Incubate bacterial culture at 37°C for 18 hrs in a shaking incubator.

26/09/2019

For bacteria and IPTG:

1. Measure bacterial OD_{600}
2. Adjust with OD_{600} water to OD = 0.1.
3. For prepare 0,5 mM IPTG solution: mix 0.119 g of IPTG with 1 ml of LB medium.
4. Vortex to dissolve IPTG.
5. Dilute in cascade 1/5 to 0,005 mM.

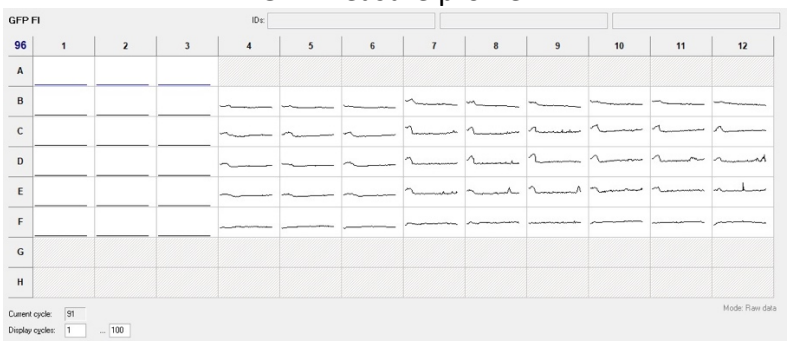
Plate preparation:

1. Mix on non-transparent (black) 96-well plate bacteria with different PTG solutions.
2. Add LB media for blanc.
3. Add positive fluorescent sample for adjust measurement.

These parameters were used for was used for GFP measure by PheraStar Plus BM6 LABTECH.

Basic settings	
Measurement type:	Fluorescence (FI)
Microplate name:	GREINER 96 F-BOTTOM
Plate mode	
No. of cycles:	90
Cycle time [s]:	120
No. of flashes per well:	200
Optic settings	
Optic module:	FI 485 520
Excitation:	485
Emission:	520
Gain:	178
Focal height [mm]:	5
Shaking settings	
Shaking width [mm]:	1
Shaking mode:	double orbital
Additional shaking time:	1s before each cycle
General settings	
Settling time [s]:	0,5
Reading direction:	bidirectional, horizontal left to right, top to bottom
Target temperature [°C]:	37

GFP measure profile:



Cell incubation:

1. Prepare 15 ml of liquid LB with 13 µL of Chloramphenicol and 0,2 g (2%) of D-glucose for promoter inhibiting.
2. Add 3 ml of prepared liquid LB to a tube.
3. Using a sterile pipette tip or toothpick, select a single colony from your LB agar plate.
4. Drop the tip or toothpick into the liquid LB + antibiotic and swirl.
5. Incubate bacterial culture at 37°C for 18 hrs in a shaking incubator.

27/09/2019

For bacteria and IPTG:

6. Measure bacterial OD_{600}
7. Adjust with OD_{600} water to OD = 0.1.
8. To prepare 0,5 mM IPTG solution: mix 0.119 g of IPTG with 1 ml of LB medium.
9. Vortex to dissolve IPTG.
10. Dilute in cascade 1/5 to 0,005 mM.

Plate preparation:

4. Mix on non-transparent (black) 96-well plate bacteria with different PTG solutions.
5. Add LB media for blanc.
6. Add positive fluorescent sample for adjust measurement.

The previously described parameter was used for GFP measure by PheraStar Plus BM6 LABTECH.

GFP measure profile: GFP measure profile: