IGEM

UANL MTY-MEXICO

P R O T O C O L S

PROTOCOLS

1. Handling of lyophilized DNA's plates.

This step is for searching and obtaining the desired piece.

Procedure:
1.- Search for the BioBrick in the Parts Registry
2.- Drill the plate, and add 10ul of mQ water
3.- Mix very well, and take the resusupended DNA to an Eppendorf tuve

2. Preparation for competent cells and their transformation

This is to prepare the plasmid in the bacterium with the thermal shock

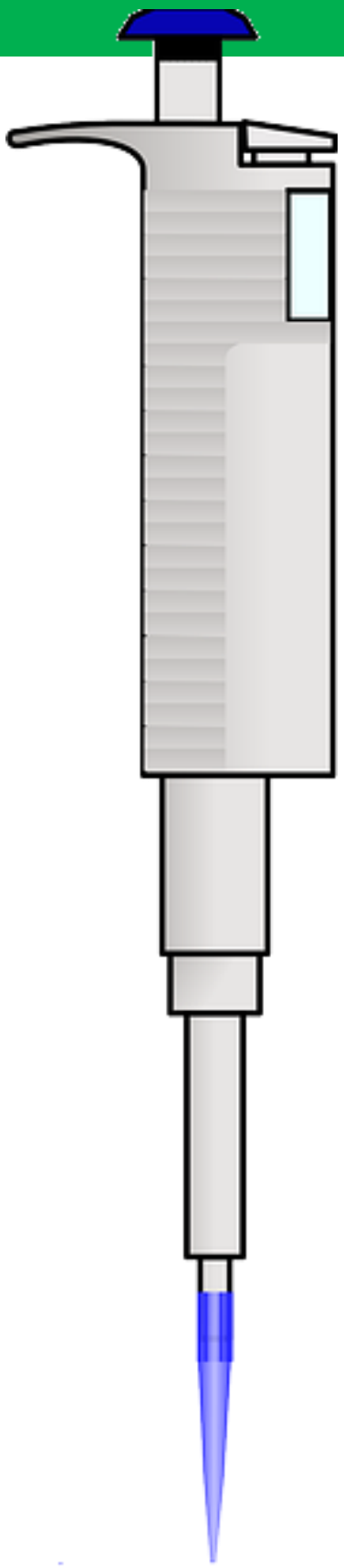
Transformation Procedure:
1.- Take 100ul of the bacteria to an Eppendorf tube
2.- Add 2ul of DNA and mix them
3.- Let in ice from 20 to 30 minutes
4.- Dive the Eppendorfs with 42°C water for 1 minute
5.- Take it to ice for 2 minutes
6.- Add LB and incubate them for 20 min, then put them into petri dish at 37°C all the night.

3. Inoculation in Petri Dish and Test Tube

Inoculate bacteria in dishes with agar and medium tubes with their antibiotics

Procedure:
For planting
1.- Take the colonies in a test tube with LB and the antibiotic
2.- Take the handle sterilized and spread the colonies in a Petri-dish.
3.- Incubate for 37°C FOR 16 hours

Reseeding
1.- Add the antibiotic to a test tube previously half filled with Lb medium
2.- With a handle take a colony
3.- Then agitate the handle in the Tube, finally incubate at 37°C



4. Plasmid DNA miniprep

This is to obtain the DNA plasmid of a colony

Procedure:
1.- Add 1.5ml of colonies to an Eppendorf, centrifuge 30 seconds and throw the liquid in the trash with soap.
2.- Add 200ul of solution I, II and III in times of 5 minutes respectively and then centrifuge the new solution.
3.- Pass the liquid to and Eppendorf with 1ml of alcohol 100%.
4.- Incubate at -20°C for 10 minutes
5.- Centrifuge and throw the liquid.
6.- Add 200ul of Ethanol 70% and mix very well
7.- Centrifuge again and throw the liquid
8.- Let the precipitate incubate at 37°C, then add 200ul of mQ water with RNase and mix it
9.- Check in the gel or save it at 4°C

5. DNA quantification by UV spectrophotometer

This is for quantify the plasmid DNA extracted

Procedure:
1.- Take 1ml of mQ water in an Eppendorf
2.- Add 1ul of plasmid DNA
3.- Prepare the spectrophotometer
4.- Pass the DNA to the spectrophotometer
5.- Select the option (DNA or RNA)
6.- Write the lecture of the spectro at 260, 280 and 320, relation 260/280 and the concentration.

6. Plasmid DNA characterization

This is for identify the piece we have

Procedure:
1.- Prepare the mix
2.- Deal the mix in equal parts
3.- Add the DNA and mix
4.- Incubate the reactions at 37°C for 1 hour
5.- Use the gel for check the result

6. PCR

It used the New England Biolabs Q5 High Fidelity PCR kit, carry out the following protocol:

1) Preparation of the master mix to 25 µL reaction:

a. Q5 High-Fidelity Mix: 12.5 µL
b. 10 µM first forward: 1.25 µL
c. 10 µM first reverse: 1.25 µL
d. DNA: 1 µL
e. Nuclease-free water: 9 µl

2) Conditions of the PCR

	Time	Temperature
Initial denaturation	**30 seg**	**98˚C**
25 cycles	**5 seg** **30 seg** **2 min**	**98˚C** **64˚C ±4** **72˚C**
Final extension	**2 min**	**72˚C**
Maintenanace	**-**	**10 ˚C**

7. Flourescent measurement

- Cultivate the selected clones in microcentrifuge tubes during 15 hours with 600 µL of LB media. Remember that the tubes must have a hole in the cap.
- Take 200 µL of every tube to a black 96-Wells plate (of COSTAR). Also, include a negative control (LB media).
- Measure in a Plate reader (Biotech Synergy HT).*
- Process the data in Excel. The Optical density (OD) and fluorescence of the media is subtracted to the data. From results, divide the highest OD to every data to obtain a correction factor. Then multiply that factor to the fluorescence to obtain the relative fluorescence.
- Graph the resulting data.

For GFP measurement set the excitation filter of 460±40nm, emission filter of 528±20nm and sensibility to 60. For RFP set the excitation filter of 530±25nm, emission filter of 590±35nm and sensibility to 60.

8. Bacteriophage production and usage

1. Transform *E. coli with any plasmid containing the part* ***BBa_J72152***.
2. Plate the transformed cells in LB plates and incubate overnight at 37°C
3. Pick a colony and grow it in liquid culture until the stationary phase.
4. Dilute the culture 100 times in Phage Lysis Medium (PLM*) and grow it 1 h at 37°C
5. Add 13 mM arabinose and incubate 4 hours at 37°C
6. Add chloroform (2.5% v/v) and vortex for 30 s
7. Centrifuge the culture (12,100 g / 90 s) and recover the supernatant
8. Prepare the Bacteriophage Solution by mixing the supernatant recovered with saline solution as needed. Lyophilization or freeze-drying can be used for long term storage of the bacteriophage.
9. Calculate the efficiency of transduction of the resulting bacteriophage by using as a target organism a Preparation of Target Cells (PTCs**) in the appropriate media (e.g., if the organism to be infected is in minimal media, use the same to grow the PTCs).

*PLM = LB + 100 mM MgCl2 + 5 mM CaCl2
**PTC = cells grown to the stationary phase, isolated by centrifugation and resuspended to an OD600 of 2 in the appropriate media.

We recommend to use the Bacteriophage Solution in excess according to the results observed when calculating the efficiency of transduction and according to the volume of the media containing the organism to be hacked.

SYNBIO WORKSHOP