PROLUNG

BIOCONTAINMENT

GENOME INTEGRATION

LAB BOOK 1

iGEM Stockholm

Plating bacteria

A plasmid (pTNS2) carrying the transposase gene was received from Addgene. Bacteria from the provided bacterial stab were plated on agar and left for overnight growth according to the following protocol.

Obtain two plates with ampicillin; use aluminum foil for storage in fridge. Label the bottom of the plate with the plasmid name, the date, the antibiotic resistance, and your initials.

Keep your lab bench area sterile by cleaning with EtOH before beginning work and then working near a flame or bunsen burner.

Using a sterile toothpick or pipette tip, touch the bacteria growing within the punctured area of the stab culture in the vial from Addgene.

Run this tip lightly over a section of the plate to spread the bacteria over approximately one-third of the surface area of the plate, to create streak #1.

Using a fresh sterile pipette tip, pass through streak #1 and spread the bacteria over the next one-third section of the plate, to create streak #2.

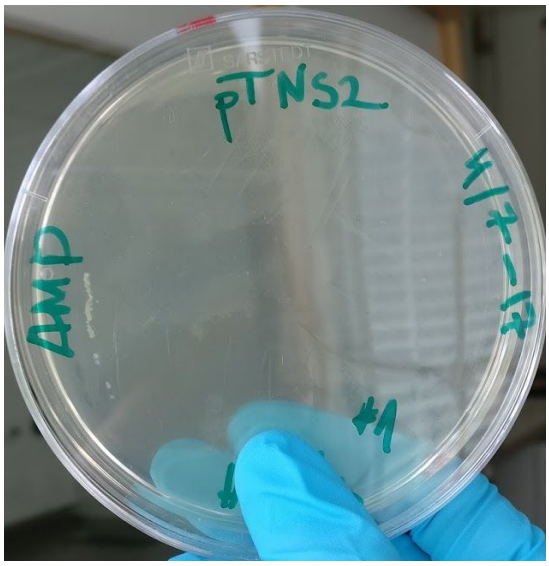
Using a third sterile pipette tip, pass through streak #2 and spread the bacteria over the last one-third section of the plate, to create streak #3.

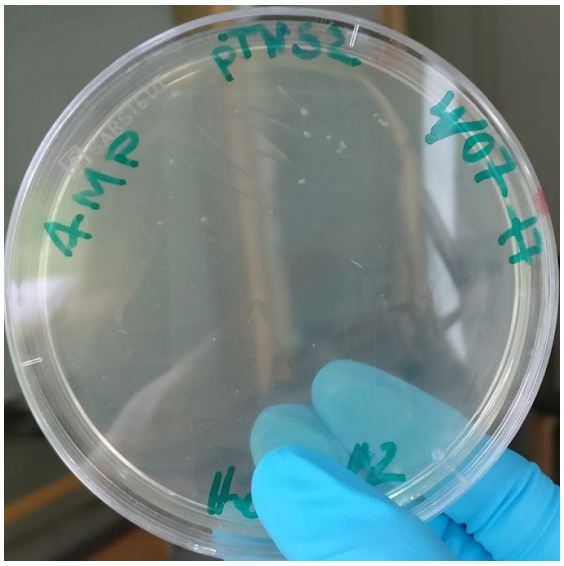
(Note: the same pipette tip was used for all three streaks. The tip was not submerged into the stab after the first streak. For future reference: use different sterile tips to create the new streaks.)

Incubate the plate overnight (12-18 hrs) at the designated growth temperature (37C).

On the next day, check for single colonies. A single colony should appear as a white dot growing on the solid medium.

Note: If the bacterial growth is too dense to locate a single colony, re-streak onto a new agar plate to obtain single colonies.





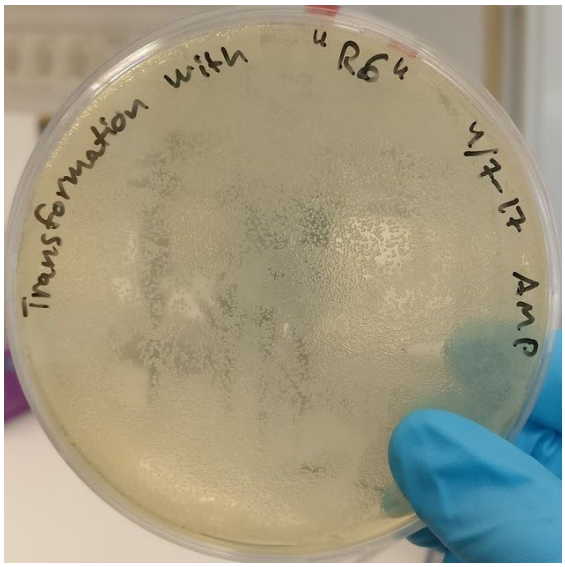
Transformation of Tn7R/Tn7L plasmids

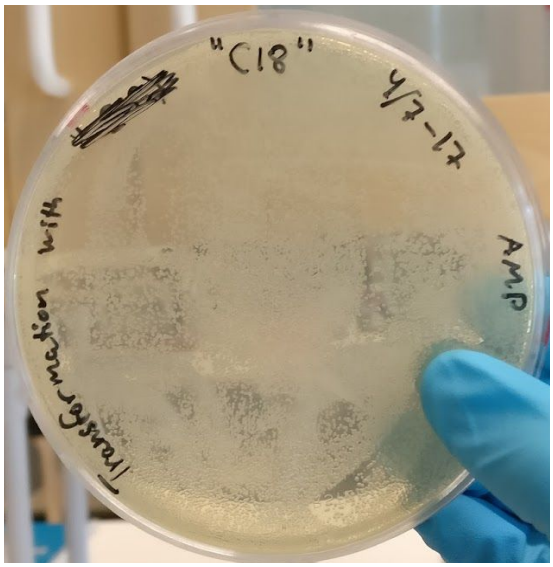
Objective

Transform cells with plasmids carrying the transposition cassette for subsequent integration of RFP.

Procedure

Performed according to standard transformation protocol.
The plates were checked for colonies the following morning (5/07/17). The bacteria had spread over the whole plate and it was impossible to locate a single colony.
Two new plates were streaked with the bacteria and left for overnight incubation in the same conditions.

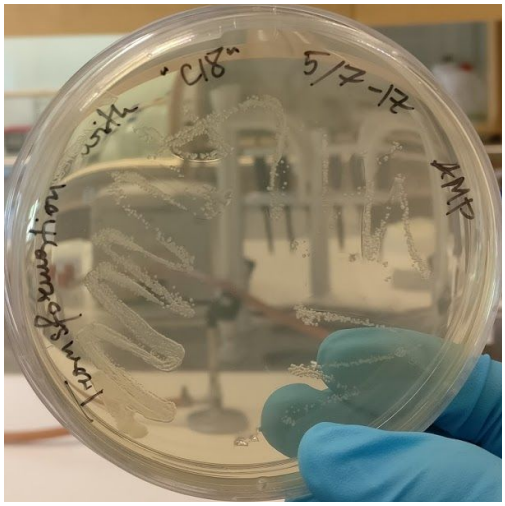


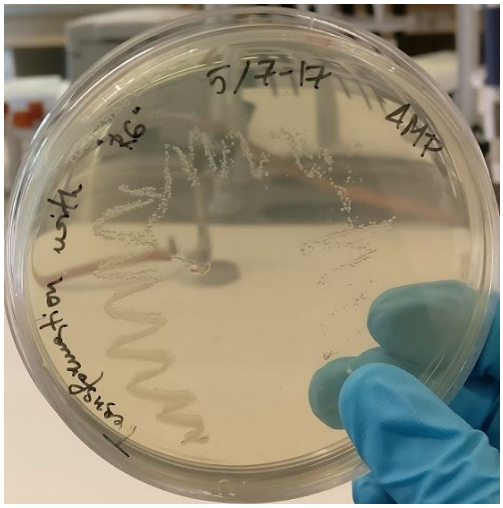


Inoculation of liquid bacterial culture

Since single colonies were located only on the pTNS2 plates, only those colonies were inoculated. The Tn7 plates had overgrown, therefore new plates were streaked and left for overnight incubation (15hrs/37C).

New plates with C18 and R6





Inoculation

Add 10mL of nutrient broth to a 100mL Erlenmeyer flask.
Add 10uL of the appropriate antibiotic (ampicillin; c=?)
Using a sterile pipette tip, pick a single colony from each agar plate and drop the tip in the flask.
Incubate overnight (16C) at 37C/200rpm.

MiniPrep and glycerol stocks

Spin the cell culture (4mL) in a centrifuge to pellet the cells, empty the supernatant (media) into a waste collection container. (Liquid cell culture from 5/07/17 - pTNS2)
Performed according to the QIAprep Spin Miniprep Kit protocol provided by Qiagen.
Measuring concentration using NanoDrop:

	pTNS2 1	pTNS2 2
Abs	1.475	1.283
A-260 10mm path	2.882	2.773
A-280 10mm path	1.581	1.515
260/280	1.82	1.83
260/230	1.95	2.16
ng/ul	144.1	138.7

Glycerol stocks

Add 0.5 ml of 40% glycerol in H2O to a cryogenic vial.
Add 0.5 ml sample from the culture of bacteria to be stored.
Gently pipette up and down ensure the culture and glycerol is well-mixed. Alternatively, vortex.
Use a tough spot to put the name of the strain or some useful identifier on the top of the vial.
On the side of the vial list all relevant information - part, vector, strain, date, researcher, etc.
Store in a freezer box in a -80C freezer. Remember to record where the vial is stored for fast retrieval later.
Store the rest of the samples in -20C freezer.

MiniPrep and glycerol stocks for genome integration plasmids from iGEM HQ

Performed according to the QIAprep Spin Miniprep Kit protocol provided by Qiagen.
Measuring concentration using NanoDrop:

	C18	R6
Abs	0.599	0.544
A-260 10mm path	1.439	1.327
A-280 10mm path	0.778	0.733
260/280	1.85	1.81
260/230	2.40	2.44
ng/ul	71.9	66.3

Glycerol stocks

Stocks prepared according to protocol mentioned above.