iGEM2015 – Microbiology – BMB – SDU	
Title: P1 Phage Tansduction. **SOP number:** SOP0004_v01 **Version number:** v01	**Date issued:** 2015.07.20 **Review date:** 2015.07.20 **Written by:** CEM

Purpose

To move a set of genes from one bacteria to another, using P1 phages.

Area of application

Selection for transposition of genes.

Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
2x 15 mL Plastic Screw cap Tube	Micro Storage	● ●	
25 mL Flask	Micro Storage	● ●	
Storage unit 4°C	Basement Hallway	● ●	
Centrifuge	Laboratory 1. Floor	● ●	
Eppendorf tubes	Micro Storage	● ●	
Selection plates	Laboratory 1. Floor	● ●	
Incubator	Incubation Room 1. Floor	● ●	

Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
LB medium		Contact lab manager	Basement hallway	
$MgCl_2$		Contact lab manager	Basement hallway	
$CaCl_2$		Contact lab manager	Basement hallway	
P1 Phage stock		Contact lab manager	Basement hallway	
400 uL Chloroform		Contact lab manager	Basement hallway	
Overnight Culture (desired mutant)		Contact lab manager	Incubation room 1. Floor	
Overnight Culture (resipient)		Contact lab manager	Incubation room 1. Floor	
$MgSO_4$		Contact lab manager	Basement hallway	
500 mM Sodium citrate.		Contact lab manager	Basement hallway	
LB containing 100 mM Sodium Citrate.		Contact lab manager	Basement hallway	

QC – Quality Control

List of other SOPs relevant to this SOP

Environmental conditions required

Procedure

1. **Making P1 Phages**
    a. An overnight culture of desired mutant is diluted 1:100 in LB and incubated at 37°C to an OD_{450} of 0,5.
    b. Take 1 mL and mix with 10 mL LB + 10 mM $MgCl_2$ + 5 mM $CaCl_2$.

c. Add 100 uL P1 phage stock.
d. Grow at 37°C until lysis occurs (1-4 hours).[1]
e. Transfer lysate to a 15 mL screw cap bottle.
f. Add 200 uL chloroform and vortex for 1 min.
g. Spin at 3500 rpm for 5 min. and transfer supernatant to a new 15 mL tube.
h. Add 200 uL chloroform and vortex for 1 min.
i. Store Lysate at 4°C.

2. P1 Phage Transduction

a. Centrifuge 5 mL overnight culture of recipient bacteria at 3500-5500 rpm for 5 min.
b. Resuspend pellet in 2 mL of 10 mM $MgSO_4$, containing 5 mM $CaCl_2$.
c. Repeat wash!
d. Resuspend the cells in 2 mL of 10 mM $MgSO_4$, containing 5 mM $CaCl_2$.
e. Transfer recipient cells to 4 sterile eppendorf tubes.
f. Add P1 lysate as follows:

Tube nr.	**Recipient cells**	**P1 lysate**
1	100 uL	-
2	100 uL	20 uL
3	100 uL	100 uL
4	-	100 uL

g. Incubate the tubes for 30 min. at 37°C without shaking.
h. Add 0,1 mL of 500 mM sodium citrate to each tube and mix.

[1] Note: If cells do not lyse after 3-4 hours, proceed to step e. Cultures that do not clear often yield usable lysates. The most common mistake when making P1 Lysates is to start with a cultre that is too dense. Ca^{2+} is required for absorption of P1 phages and P1 phages are sensitive to chloroform in the absence of Ca^{2+}.

i. Add 1 mL LB to each tube and incubate for 1 hour at 37°C without shaking.
j. Spin cells at 3500 rpm for 5 min.
k. Remove supernatant.
l. Suspend cells in 150 uL LB containing 100 mM sodium citrate.
m. Spread cells on selection plates and incubate plates at 37°C overnight.[2]

Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
One used Plastic		GMO	Yellow GMO Trash

Time consumption

- Total-time 3,5 hours.
- Hands-on-time 1 hour.

Scheme of development

Date / Initials	Version No.	Description of changes
2015.07.20 /CEM	01	The SOP has been written

Appendices

[2] Note: Sodium citrate is added to chelate $Ca^{2+}$and prevent reinfection of the cells overnight. Transductants should appear on the plates from tube 1 and 4.