iGEM2015 – Microbiology – BMB – SDU	
Title: Deletion **SOP number:** SOP0002_v01 **Version number:** v01	**Date issued:** 2015.07.16 **Review date:** 2015.07.16 **Written by:** EMT, KBS

Purpose

To delete a gene from BW25113/pKD46.

Area of application

Inducing cells with plasmid DNA.

Apparatus and equipment

Apparatus/equipment	Location (Room number)	Check points	Criteria for approval/rejection
1 Sterile Glass Flask	Micro Storage	● ●	
Eppendorf Tubes	Micro Storage	● ●	
Plastic centrifuge tubes (50 mL)	Micro Storage	● ●	
Ice Bucket (Filled with Ice)	Laboratory 1. Floor	● ●	
Centrifuge	Laboratory 1. Floor	● ●	
Incubator	Incubation Room 1. Floor	● ●	
Cuvettes	Micro Storage	● ●	
PCR machine	Laboratory 1. Floor	● ●	

Materials and reagents – their shelf life and risk labelling

Name	Components	Supplier / Cat. #	Room (hallway storage)	Safety considerations
20 % Arabinose (500 µL)		Contact lab manager	Basement hallway	
LB (50 mL)			Basement hallway	
Prewarmed LB (1 mL)		Contact lab manager	Basement hallway	
DNA (varying amount)		Contact Lab manager	Basement hallway	
Cold Water (42,3 mL)		Contact Lab manager	Laboratory 1. Floor	
Ampicillin		Contact Lab manager	Basement hallway	
BioRad Gene Pulse		BioRad	Laboratory 1. Floor	
BioRad Pulse Controller		BioRad	Laboratory 1. Floor	
Plasmids pDK3 and pDK4		Contact Lab manager	Freezer 1. Floor	
Primers (Forward and Reverse)		Contact Lab manager	Freezer 1. Floor	
dNTP (4 uL)		Contact Lab manager	Freezer 1. Floor	
Forward Primer (2 uL)		Contact Lab manager	Freezer 1. Floor	
Reverse Primer (2 uL)		Contact Lab manager	Freezer 1. Floor	
CG PCR Buffer (10 uL)		Contact Lab manager	Freezer 1. Floor	
Water (28 uL)		Contact lab manager	Laboratory 1. Floor	
Phusion DNA polymerase (0,5 uL)		Contact Lab manager	Freezer 1. Floor	
$MgCl_2$ (50 mM) (2 uL)		Contact Lab manager	Basement hallway	

QC – Quality Control

List of other SOPs relevant to this SOP

- iGEM 2014 SOP0010 v01 - Phusion PCR

- iGEM 2014 SOP0014 v01 - Gelpurification

Environmental conditions required

Procedure for PCR amplification and purification

1. - **PCR**
    a. Mix fallowing in a PCR tube: 50 uL total volume.
        i. 10 ul CG PCR Buffer
        ii. 4 uL dNTP (2,5 mM of each deoxynucleotide)
        iii. 2 uL Forward Primer
        iv. 2 uL Reverse Primer
        v. 2 uL $MgCl_2$ (50 mM)
        vi. 1 uL Template DNA (pDK3 or pDK4)
        vii. 28 uL Water
        viii. 0,5 uL Phusion DNA Polymerase
    b. Run the following program on the PCR machine:
        i. Run for 5 cycles x
            1. Initial denaturation 1 min., 98°C.
            2. Denaturation 20 sec., 98°C.
            3. Annealing 20 sec., 55°C.
            4. Extension 1 min., 72°C.
        ii. Run for 30 cycles x
            1. Denaturation 20 sec., 98°C.
            2. Annealing 20 sec., 65°C.
            3. Extension 1 min., 72°C.
            4. Final extension 5 min., 72°C.

2. - **Purification of the PCR product**

Add agarose loading buffer (10x) to the PCR reaction and the DNA on an 1% agarose gel. After this the DNA need to be cut out from the gel and purified. This is done following the iGEM 2014 SOP0014.

Procedure for Electoporation

1. - **Preperation.**
    a. Place a bottle of sterile water on ice.

b. Dilute an overnight culture 100-fold in 50 mL LB in a steile glass flask.
c. Add 50 ug/mL ampicillin and incubate at bacterial specific temperature until OD_{450} is around 0,4-0,6.
d. When desired OD_{450} is reached induce culture wit 0,2 % arabinose (500 µL of 20 % arabinose).
e. Shake and induce culture for 30 min.
f. Prepare bucket of ice.
g. Cool centrifuge down to 4°C.
h. IMMEDIATELY after induction rapidly cool flask in ice water.
i. Leave on ice for 5-10 min.

2. - **Washing to make competent cells.**
   a. Transfer cells to sterile 50 mL plastic centrifuge tubes.
   b. Spin at 6500 rpm for 10 min.
   c. Discard/decant supernatant.
   d. Add 1 mL of cold water to tube and GENTLY resuspend cells.
   e. Add 40 mL of cold water to tube and invert several times to mix.
   f. Spin as before - <u>Pellet will be soft</u>.
   g. AS SOON AS the centrifuge comes down, remove tubes and discard/decant supernatant.
   h. Add 1 mL cold water and transfer to eppendorf tubes.
   i. Spin for 1 min.
   j. Aspirate off supernatant with pipette.
   k. Carefully resuspend pellet in 300 µL cold water.
3. - **Electroporation.**
   a. Mark and place cuvette(s) on ice (BioRad 0,1 cm gap).
   b. Add DNA to cold cuvette(s).
   c. Add 50-100 µL[1] cells to each cold cuvette.

[1]

d. Prepare ‘BioRad Gene Pulse’ on 1,8 kV and 25 µFD (small cuvettes) 2,5 kV and 23 µFD (big cuvettes) and ‘BioRad Pulse Controller’ on 200 Ω.
e. Wipe ice of cuvettes and zap with ‘BioRad Gene Pulse’.
f. Add 1 mL prewarmed LB to cuvettes.
g. Transfer to clean eppendorf tubes.
h. Place tubes for phenotypic expression at 37°C for 1-2 h.
i. Spin cells at 3500 rpm for 5 min.
j. Spread cells on selection plates and incubate plates overnight at 37°C.

Waste handling

Chemical name	Concentration	Type of waste (C, Z...)	Remarks
One use Plastics		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
2015.07.16/CEM	01	The SOP has been written

Appendices