Date: 2017/07/19
Operators: Gabriel, Ersin, Alexis, Diane, Karima

Dephosphorylation of plasmid or DNA: pET43.1a (X-B)

Aim: To remove the phosphate group at the 5' end of vector DNA to prevent self-ligation

Equipment:

- rSAP enzyme (stored at -20°C) (rSAP: recombinant Shrimp Alkaline phosphatase)
- Plasmid or DNA :pET43.1aC162 XbaI-BamHI (stored at -20°C)
- Buffer 10X (stored at -20°C)
- Deionized Water
- Pipettes p10, p20, p200, p1000 and associated cones
- Eppendorf tubes (1.5 ml)

Plasmid size: 5.6 kb
Plasmid concentration: 69.5 ng/µl

Protocole

1 pmol DNA ends	1 µg	3 kb
1 pmol DNA ends	1.8 µg	5.6 kb (plasmid…….)
0.269 pmol DNA ends	0.5 µg	……. kb (plasmid ……)

rSAP Dilution 10X	
rSAP	1 µl
H20 sterile	8 µl
Buffer 10X	1 µl

Plasmid:…… Concentration	
69.5 ng	1 µl
500 ng	7.2 µl

Mix :

DNA pET43.1a XbaI-BamHI (69.742 µg/ml)	7.20 µl = 500 ng
rSAP Buffer 10X	2 µl
deionized Water	8.1 µl
Diluted rSAP (10X) (add last)	2.7 µl
Total Volume	20 µl

1. Mix gently by pipetting up and down
2. Micro-centrifuge briefly
3. Incubate at 37°C for 30 minutes
4. Stop reaction by heat inactivation at 65°C for 5 minutes

Stored at -20°C:
pET43.1a. D.G.
BamHI-XbaI
De-P 500 ng
2017-07-19 V=20µl

Date: 2017/07/19
Operators: Ersin, Alexis

Solubilization of Eurofins Plasmid E2 gene: pEXA128-E2

Aim: Reception and handling of our Gene synthesis products from Eurofins Genomics

Equipment:

- $TE_{0.1}$ 10X
- Pipettes p10, p20, p200, p1000 and associated cones
- Electric pipetman and plastic 5 ml or 20 ml or 50 ml pipettes
- Microcentrifuge Eppendorf Tubes (1.50 ml)
- Deionized Water
- Lyophilised pEXA128-E2

Protocole

Dilute $TE_{0.1}$ 10X, 10 times to obtain $TE_{0.1}$ 1X
Centrifuge lyophilized DNA (3.4 µg) at 14 800 rpm, for 2 minutes
Add 340 µl of $TE_{0.1}$ 1X
Vortex and centrifuge again at 14 800 rpm, for 2 minutes
Measure concentration with UV5 machine (Mettler Toledo):

Sample of: pEXA128-E2 Concentrations (ng/µl)	Average concentration (ng/µl) of: pEXA128-E2
64.2	62.3
53.6	
69.0	

Prepare a solution of 10 ng/µl, by diluting the solution of 62.3 ng/µl
Store at -20°C

Date : 20170719
Operators: Diane, Ersin, Gabriel, Alexis, Karima

Protocol for Bacteria DH5α Transformation with pEXA128-E2

Aim: Transform competent bacteria with plasmids

Equipment:

- 500 µL tube of competent Bacteria (competent cells stored at -80°C) :
- Plasmid to transform
- 1,5 mL Eppendorf tubes
- P1000, P200, P10 pipettes + paired cones
- Petri Dish with LB agar/CARB for Carbenicillin (an equivalent of amp, resistant to temperature) → Petri Dish (LB/CARB)
- 42°C Water-bath
- Incubator 37°C with or without a stirrer/agitator
- Sterile rake/scraper/comb
- Timer

Plasmid transformed
- pEXA128-E2 10ng/µl

Protocol:
Competent cells are extremely sensitive**; all manipulations must take place on ice**; never handle the tube containing the competent cells with your hands, never take the tubes in your hands or remove them from the ice,
You must **operate in the vicinity of the Bunsen burner** when manipulating bacterial cultures.
Name the tubes with the transformed cells: Cell type/name, plasmid (vector and composition) initials of the operator: First Name/Last Name.

1. Split the tube of competent cells:DH5α in aliquots of 50µL, in 1.5 Eppendorf tubes placed on ice.
2. Add 1 µl = 10 ng of plasmid pEXA128-E2 to transform
3. Mix by gently taping the bottom of the tubes with soft end of your finger.
4. Let it rest for 30 minutes on ice.

In the meantime check the water-bath at 42°C, and place the SOC media at 37°C for heating

5. Put the tubes in the floats
6. Place the floats in the 42°C water-bath for 40 seconds, after 38 seconds open the water-bath's lid as to remove the floats after 40'' precisely, remove the floats quickly.
7. Place the tubes on ice for 3 minutes
8. Add 750µL of SOC media per tube
9. Incubate and mix the tubes at 150 rpm at 37°C for 40 minutes on a holder placed on it's side and containing the attached (with tape) tubes.

In the meantime place the LB/AMP petri dishes at 37°C and name the dishes.

10. Generate 2 Petri dishes with each tube: one dish containing 200µL and the other 500µL.
11. Spread the bacteria using an inoculator (2 rotations)
12. Wait for the dishes to dry (5 min maximum)
13. Store the dishes upside down in the incubator at 37°C for 16 hours (ON)