NZY5α Transformation Intein Notebook

Benchling

HORMONIC
UPF_Barcelona

NZY5α competent cells transformation

MARTES, 11/8/2020

NZY5α competent cells transformed with the plasmid (IMT3_eGFP_pUC-Kan) from IDT following the following protocol:

NZY5α competent cells transformation protocol

Left in the incubator at 37 ºC (without shaking) overnight.

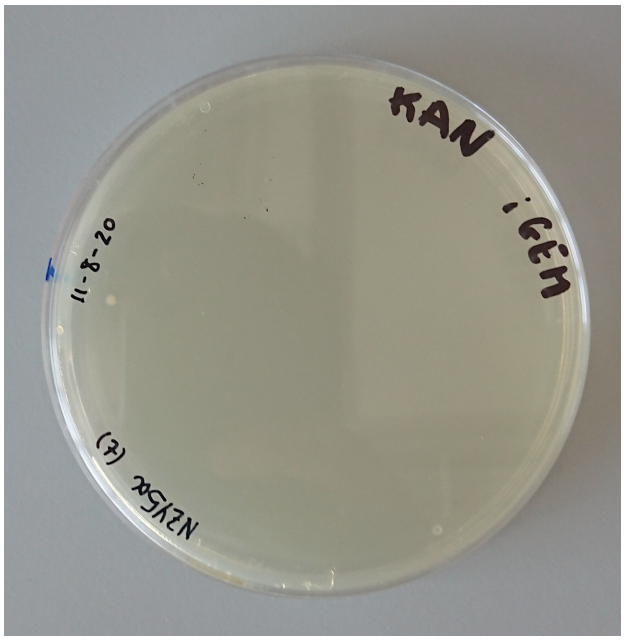
Protocol followed:

1. Thaw competent cells on ice. Gently mix cells. Do not mix cells by pipetting.
2. Add 10 µL of the reaction to the NZY5α competent cells. Gently tap tubes to mix.
3. Incubate cells on ice for 30 minutes.
4. Heat-shock cells for 40 seconds in a 42 °C water bath; do not shake.
5. Place on ice for 2 minutes.
6. Add 0.9 mL room temperature SOC Medium.
7. Shake at 225 rpm (37 °C) for 1 hour.
8. Spread 250 µL of the cells transformed with the ligation reaction on LB agar plates. To obtain the maximum number of colonies, spin the 1000 µL cell culture for 1 min at 5000 rpm, remove 750 µL of media and spread cells after re-suspending in the remaining buffer.
9. Incubate overnight at 37 °C.

MIÉRCOLES, 12/8/2020

After overnight incubation it seems that there is only one colony. We will let it incubate for another day to see if there is any population growing.

image.png



MIÉRCOLES, 19/8/2020

Repeat the transformation. During all the process we work with a population that will be transformed with pINT and a negative control.

NZY5α competent cells transformation protocol

Place 100 µL of DH5α competent cells for defrosting and two eppendorfs on ice (below 4 ºC). Divide 50 µL of the cells for the transformation and 50 µL for the control. Dilute pINT 1:10 with 9 µL of sterile water and 1 µL of diluted DNA (concentration?) to have (X?) ng of DNA. Add 1.5 µL of the diluted DNA to the competent cells for transformation, and 1.5 µL of the sterile water to the control ones. All this process must be done with all the components in ice.

Take the tubes to 42 ºC (in the heat-block or the water-bath) for 30 seconds. Put the cells back on ice for 2 minutes and add 120 µL of SOC medium. Place the cells on 37 ºC with 350 rpm shaking in the heat-block or water-bath for 1 hour.

Plate 1/3 of the cells (≃ 55 µL) to a kanamicine resistant plate and incubate at 37 ºC overnight.

JUEVES, 20/8/2020

4 colonies where found in the plasmid plate. Each one was inoculated in cultures tubes with 5 mL of Lb + 5uL KAN. placed incubating overnight shaking at 37ºC. Plates where kept in the incubator.

VIERNES, 21/8/2020

Miniprep (NYZminiprep) was performed on 4mL of the cultures from the colonies 2 and 4. Remaining 1mL of culture and the other colonies were kept in cold room.

Observations about the miniprep:

- *Elution buffer was warmed to 37 ºC before usage*
- *For clarification of lysate centrifugation at room temperature was done for 8 minutes.*

Final DNA yeld was:

- #2: 349,6 ng/uL
- #4: 316,2 ng/uL

Sent to sequence. ID references:

- #2 + VF2: 02663818 (good)
- #2 + VR: 02663819 (good)
- #4 + VF2: 02663816 (good)
- #4 + VR: 02663817 (good)

Plasmid from colony #2 was saved to transform. (IMT3_eGFP_pUC-Kan)