Heat-shock transformation of NYZ5α cells Protocol

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Introduction

To perform a more accurate and **interference-free purification** of the components of the biosensors, a single plasmid will be transformed into each *E.Coli* colony. Meaning that a plasmid for Cas12a synthesis will be **cloned** into a separate cell culture, as well as each plasmid for gRNA synthesis. We first **transform** these cells, because our first objective is not expressing those plasmids but instead **conserving** them in cells to use later in other experiments.

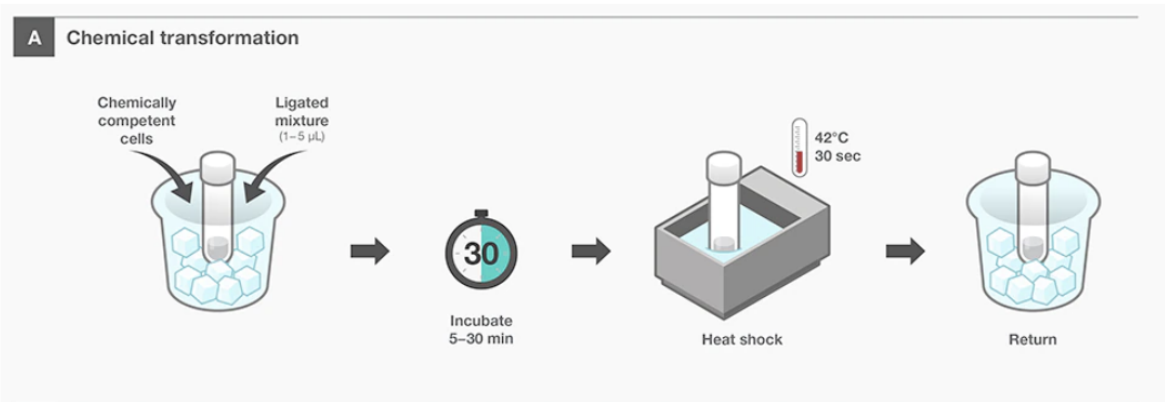
Materials

- NZY5alpha cells (glycerol stock)
- Cloning reaction
- SOC at RT
- Ice

Procedure

Heat-shock transformation of NYZ5α cells

1. The NZY5α Chemically Competent Escherichia coli cells are shipped on dry ice. Upon receipt, store at -80 °C.
2. For each assembly, thaw a 25μl of the cell stock in a 1,5mL Eppendorf tube.
3. Add 1 μl of the assembly reaction; gently mix by flicking the tube 4-5 times.
4. Incubate on ice for 20 min aprox.
5. **Heat shock** at 42°C for 30-40 sec.
6. Place back on ice for 2 min.



7. Add 300 μl of room temperature Outgrowth Medium.

8. Incubate at 37°C for 60 min, shaking vigorously (250 rpm) or using a rotation device [1].

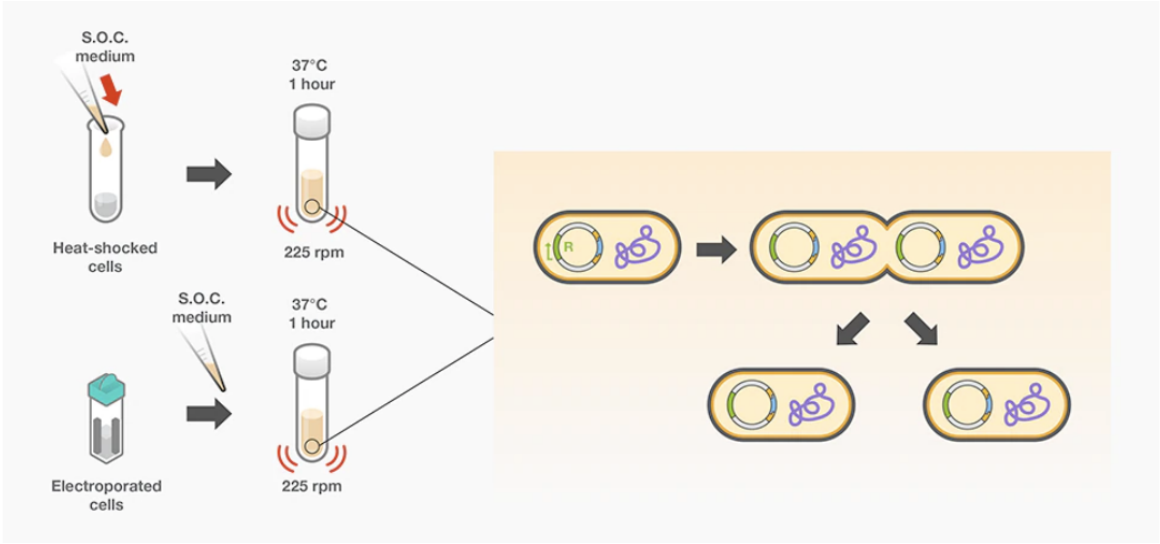


Figure 5. Cell growth during the recovery step.

** The mentionated protocol is a mix of Thermofisher and Lab protocol.**

Bibliography

1. *Bacterial transformation workflow–4 main steps - UK*. (n.d.). Retrieved from https://www.thermofisher.com/es/es/home/life-science/cloning/cloning-learning-center/invitrogen-school-of-molecular-biology/molecular-cloning/transformation/bacterial-transformation-workflow.html