

Transformation



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Introduction

Now that you have competent cells, you can transform them. We use the technique of electroporation. So you need an electroporator in the lab.

MATERIAL

To transform the competent cells, you need :

- ◆ An electroporator machine
- ◆ 100 μm diameter cuvette for the electroporator
- ◆ 65 μl of competent *Asaia* for each transformation
- ◆ The plasmid you want to transform
- ◆ 1 ml of Glycerol for each transformation



Figure 1. 1 mm diameter cuvette

PROTOCOL

1. Put all 1 mm diameter cuvettes on ice to cool them down.
2. Mix 0.2 μg of plasmid and 65 μl of competent *Asaia*. Don't pipet up and down too much and keep on ice.
3. Transfer the solution at the bottom of a 1 mm diameter cuvette.
4. Ensure that all the liquid is at the bottom of cuvette by gently tapping the tube on the bench twice.
5. Dry the cuvette to prevent electric arc in the electroporator.
6. Put the cuvette in the electroporator.
7. Make a 2'000V pulse. If you hear an electric arc, all your cells are dead. Restart the transformation.
8. Mix the transformed cells immediately with 1 ml of GLY medium.
9. Transfer the solution into a 1.5 ml microtube.
10. Incubate at 30°C for 3 hours (shaking).
11. Also put the GLY agar plate with your antibiotic into the incubator to prewarm.
12. Plate the cells and incubate them at 30°C

INCUBATION TIME

If the plate contains some antibiotic, e.g. Tetracycline, you will start to see colonies after 2—3 days.

Here are the different concentration we use for Tetracycline and Kanamycin

- ◆ Tetracycline : 50 $\mu\text{g}/\text{ml}$
- ◆ Kanamycin : 100 $\mu\text{g}/\text{ml}$



Figure 2. Electroporator