Competent cells

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Introduction

This protocol provides information on how to make Asaia competent cells for approximately 10 transformations. This process takes 3 days.

Remarks: Be careful with the competent cells: Defrost them only when you need them and always keep the cells on ice. Do not shake or pipette competent cells too vigorously. We recommend you to use microtubes for each transformation, so you don't have to refreeze the competent Asaia after each use.

Material

To make competent cells, you need :

- *Asaia liquid culture*
- *An optical density machine*
- *An –80°C freezer*
- *A centrifuge*
- *Liquid nitrogen*
- *10% cold Glycerol*
- *1mM HEPES at pH5*

Protocol

Day 1

1. Pickup a colony from a plate and make a overnight preculture in 2ml of Glycerol medium (GLY medium)

Day 2

2. Transfer 1ml of the preculture in 49ml of GLY medium in a 500ml flask. Put it at 30°C overnight.

Day 3

3. Dilute liquid culture with the ratio 1:11 in GLY medium. e.g. 20ml of culture with 200ml of GLY medium.
4. Incubate with aeration until cells reach early log phase (optical density at 550nm between 0.5 and 0.8).
5. Transfer culture into an 15ml centrifuge tube
6. Incubate them on ice for 15 minutes. *After this point it's very important to keep the cells cold!*
7. Sediment them at 2'700xg for 10 minutes at 4°C
8. Throw away the supernatant.
9. Re-suspend the pellet with 10ml of 1mM HEPES at pH5
10. Sediment them at 2'700xg for 10 minutes at 4°C
11. Throw away the supernatant
12. Redo step 8 to 10 once
13. Re-suspend the cells in 5ml of cold 10% Glycerol
14. Sediment them at 2'700xg for 10 minutes at 4°C
15. Throw away the supernatant
16. Re-suspend the cells in 0.65 ml of cold 10% Glycerol
17. Fill microtubes with 65 µl of competent cells
18. Snap freeze the tubes in liquid nitrogen to freeze them
19. Put all tubes in the –80°C fridge