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. Thaw them only when you need them and always keep the cells on ice. Do not shake or pipette competent cells too vigorously. We recommend 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 reader*
- *A –80°C freezer*
- *A centrifuge*
- *Liquid nitrogen*
- *10% cold Glycerol*
- *1mM HEPES at pH5*

Protocol

Day 1

1. Pick-up a colony from a plate and make an overnight pre-culture in 2 ml of Glycerol medium (GLY medium).

Day 2

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

Day 3

3. Dilute liquid culture with the ratio 1:11 in GLY medium, e.g. 20 ml of culture with 200 ml of GLY medium.
4. Incubate with aeration until cells reach early log phase (optical density at 550 nm between 0.5 and 0.8).
5. Transfer culture into a 15 ml 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. Remove the supernatant.
9. Re-suspend the pellet with 10ml of 1mM HEPES at pH5.
10. Pellet the bacteria at 2'700xg for 10 min at 4°C.
11. Remove the supernatant.
12. Redo step 8 to 10 once
13. Re-suspend the cells in 5 ml of cold 10% Glycerol.
14. Sediment them at 2'700xg for 10 minutes at 4°C.
15. Remove 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.