

# Competent cells



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## Introduction

In this document, you will learn how to make competent *Asaia*. When the cells are competent, be very careful. Defrost the cells only when you need them and always keep them on ice. It is important to : not shake or

pipette competent cells too vigorously. If you follow this protocol, one microtube is used for one transformation, so you don't have to defreeze *Asaia* after usage. With this protocol you will make enough com-

petent cells to do 10 transformation. We suggest to do 5 culture in parallel.

Keep in mind that to make cells competent, it will take 3 days.

## MATERIAL

To make competent cells, you need :

- ◆ *Asaia* liquid culture
- ◆ An optical density machine
- ◆ An  $-80^{\circ}\text{C}$  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 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  $30^{\circ}\text{C}$  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  $2'700\times g$  for 10 minutes at  $4^{\circ}\text{C}$
8. Throw away the supernatant.
9. Resuspend the pellet with 10ml of 1mM HEPES at pH5
10. Sediment them at  $2'700\times g$  for 10 minutes at  $4^{\circ}\text{C}$
11. Throw away the supernatant
12. Redo step 8 to 10 once
13. Resuspend the cells in 5ml of cold 10% Glycerol
14. Sediment them at  $2'700\times g$  for 10 minutes at  $4^{\circ}\text{C}$
15. Throw away the supernatant
16. Resuspend the cells in 0.65 ml of cold 10% Glycerol
17. Fill microtubes with 65  $\mu\text{l}$  of competent cells
18. Snap freeze the tubes in liquid nitrogen to freeze them
19. Put all tubes in the  $-80^{\circ}\text{C}$  fridge