

Production of competent Asaia

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July 28, 2010

Where	On iGEM wet-bench	
	Quantity	What
Material needed	25-50 ml	Asaia liquid culture
	20 ml	cold HEPES medium (1mM, pH 7)
	5.5 ml	cold glycerol 10% (already in fridge)
		Ice Liquid Nitrogen (ask Carine)

HEPES and glycerol must be at *fridge temperature* (4°C) when used.

Steps

1. Grow the Asaia culture until OD 0.5-0.8. The bacteria have to be in the early exponential phase,
2. transfert the liquid in centrifuge tubes,
3. incubate on ice for 15 min. Asaia should be kept cold for the rest of the procedure,
4. sediment by centrifugation : $2700\times g$, 10min, 4°C,
5. discard supernatant,
6. resuspend with 10 ml of **cold** HEPES,
7. repeat once points 4 and 5,
8. resuspend with 5 ml of **cold** glycerol,
9. sediment by centrifugation : $2700\times g$, 10min, 4°C,
10. resuspend with 0.5 ml of **cold** glycerol,
11. make tubes containing 150 μ l of suspended cells,
12. shock-froze the cells in liquid nitrogen and stock them -80°C. ¹

Warnings

- Do not forget to set the incubator at 4°C before as it takes time to cool to 4°C
- keep Asaia, HEPES and glycerol always on ice.

¹use a artigianal spoon made with aluminium paper to take the tubes out of the liquid nitrogen. bath