Transformation

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July 16, 2010

Where

On the bench

Material needed

Quantity	What
30 μl	Competent cells (from the -80°C freezer)
2-3 μl	Plasmid (ask Henrike again about amount!)
300 μl	SOC medium (quantity: 10 times the amount of cells used)
	Water bath
	Ice box

Steps

1. Defreeze the competent cells on ice!
2. When deefrozen pipette the cells and the plasmids into the tube. Avoid pipetting up and down since the cells are very sensitive to stress. Flick gently to mix.
3. Incubate the tubes on ice for 20 minutes.
4. Heat shock: Put the tubes in the waterbath at 42 °C for 30 seconds.
5. Transfer the tubes QUICKLY back on the ice.
6. Work at the flame: Add SOC medium to the tubes. Do NOT touch the wall of the SOC bottle with the pipette → your medium can be contaminated.
7. Place the tubes in the incubator (for E.Coli at 37 °C) for on hour. If you use plasmids containing a strong anti-biotic like Cm then incubate longer e.g. for 1.5 hours. Place the tubes slightly tilted in the incubator.
8. If you plan to plate your transformation product also put the plates in the incubator.
9. Take the cells and the plates out. Add autoclaved beads to the plates. Then add 100 μl of the product to the plates. Shake horizontally to spread the cells on the plate. Put the plates into the incubator O/N.

Warnings

- When you get the cells from the freezer get them with the ice box → put them immediately on ice!
- Preheat the water bath early enough and check that it really reached the required temperature!! To set the temperature keep the "SET" button pressed and adjust the temperature with the "UP" and "DOWN" arrows.