

ALGINATE TRANSFORMATION

Follow your favorite protocol for treatment of spheroplasts with DNA, including the incubation in PEG. Or you can use the following protocol, which is a recipe for a transformation on a total of 10 100 X 15mm plates.

For 10 plates:

1. Grow yeast culture to saturation.
2. Inoculate 50 ml of YPD with 5-10 ul saturated culture, and grow overnight.
3. Harvest 50 ml cells at $A_{600} = 2$ to 4 by centrifugation (3 krpm for 5 min)
4. Wash 2 times with 25 ml of 1M Sorbitol by sedimentation and resuspension (3 krpm for 2 min).
5. Resuspend in 5 ml of 1M Sorbitol, 50mM NaPO₄, pH 7.5 containing 5 ul B-mercaptoethanol and 0.5 mg Zymolase-100T (spin out insolubles before addition to cells).
6. Incubate culture with gentle shaking at 30⁰C for 1 hr.
7. Check cells in microscope for spheroplasting.
8. Sediment cells (3 krpm for 2 min).
9. Wash 3 times with 10 ml of 1M Sorbitol.
10. Resuspend in 0.5 ml of 1M Sorbitol, 10mM Tris, 10mM CaCl₂, pH 7.5.
11. Prepare transforming DNA by adding 1-2000 ng of DNA to 100 ul of 1M Sorbitol, 10mM Tris, pH 7.5.
12. Add DNA to cells.
13. Add 5 ml of 40% PEG (filter-sterilized) containing 10mM Tris, 10mM CaCl₂. Incubate 5-10 min at room temperature.

Now, recover spheroplast transformants in alginate as follows:

1. Spin cells out of PEG solution (5 min spin in tabletop). Carefully remove the PEG from tube.
2. Resuspend in 1.2M Sorbitol (45 ul/plate, so 450 ul in this case).
3. You can place the tubes on ice till ready to add the alginate.
4. Remove 45 ul cells and place into small sterile glass tube. Add an equal volume of 4% alginate, 1.2M Sorbitol (45 ul), and mix well.
5. Using a p200 pipetman, transfer the alginate cell suspension to the surface of an osmotically balanced (1.2M Sorbitol) selective plate containing 50mM CaCl₂. **Immediately** spread over plate using a bent glass rod. Be careful as the alginate will rapidly dry, and do not continue to work the spreader over the plate once the alginate has been somewhat distributed since this will create clumps on the plate.
6. Incubate the plates as usual, and score colonies within a few days.

SOLUTIONS AND MEDIA:

1. 1M Sorbitol
2. 1M Sorbitol, 50mM NaPO₄, pH 7.5
3. 1M Sorbitol, 10mM Tris, 10mM CaCl₂, pH 7.5
4. 4% Alginate solution: Add 0.2g of alginic acid (Sigma) to a test tube. Then add 1.1g of sorbitol and vortex into a uniform powder. Add 4.5ml of water, vortex thoroughly, and autoclave 20 min, slow exhaust. This solution is best if made fresh the day of use, though can store at -20C.
5. Plates: Good results obtained using relatively dry (i.e. not freshly poured) plates with two layers of agar, though if only minimal medium needed for selection you can mix everything together and pour on plates. The bottom layer (~25 ml/plate) is yeast minimal medium. The top agar (10-20 ml/plate) is synthetic complete medium, minus nutrients used for selection, containing 1.2M Sorbitol and 50mM CaCl₂ (best if calcium chloride added from a 1M stock after autoclaving the media).

