Maintenance and Cryopreservation of PSB-D and PSB-L suspension cultures

Protocol tested by ABRC

Immediately upon arrival store cells in liquid nitrogen or begin re-initiation protocol. This is especially important for PSB-L.

Cell suspension (CS/MSMO) medium (1 L)

4.43 g MSMO (minimal organics Sigma #M6899)

30 g Sucrose

900 ml dH_2O

50 µl kinetin (1 mg/ml stock solution)

500 µl NAA (1 mg/ml stock solution)

Adjust pH to 5.7 with 1 M KOH

Adjust volume to 1000 ml

Aliquot to 25 ml and 125 ml flasks

Autoclave for 20 minutes, allow to cool

For plates add 1% agar before autoclaving

Re-initiation from cryostock

1. Thaw 1 aliquot each PSB-D and PSB-L in water bath at 45°C for 2 minutes.
2. In laminar flow hood, pour aliquot on plate and spread with spatula. Seal with surgical tape, wrap PSB-D plate in foil and keep at 25°C. Place PSB-L plate in 16 h light 8 h dark cycle at 21°C.
3. In approximately 14 days, after you notice good callus growth, scrape cells from surface of agar and chop into small pieces with cell scraper. Divide cells into two 25 ml flasks containing 10 ml CS liquid medium. Wrap PSB-D in foil and place in incubator at 25°C with shaking at 130 rpm. Place PSB-L in 16 h light 8 h dark cycle at 21°C with shaking at 130 rpm.
4. After 7 days, sub-culture 7 ml of small culture in 43 ml CS medium. Use each 10 ml culture to inoculate one larger culture. If cultures are difficult to pipette, use a sterile graduated tube to

measure 7 ml. Wrap PSB-D in foil and place in incubator at 25°C with shaking at 130 rpm. Place PSB-L in 16 h light 8 h dark cycle at 21°C with shaking at 130 rpm.

Maintenance

Every 7 days, sub-culture 7 ml culture in 43 ml CS medium. Wrap PSB-D in foil and place in incubator at 25°C with shaking at 130 rpm. Place PSB-L in 16 h light 8 h dark cycle at 21°C with shaking at 130 rpm.

Cryopreservation

Pre-freeze (PF) medium (250 ml) recipe is the same as CS except with 0.6 mol/L mannitol

1.1 g MSMO (minimal organics Sigma #M6899)

7.5 g sucrose

225 ml dH_2O

12.5 µl kinetin (1 mg/ml stock solution)

125 µl NAA (1 mg/ml stock solution)

27 g mannitol

Adjust pH to 5.7 with 1 M KOH

Adjust volume to 250 ml

Aliquot to 100 ml flasks (43 ml each)

Autoclave for 20 minutes and allow to cool

Cryoprotective medium (250 ml)

1.1 g MSMO (minimal organics Sigma #M6899)

85.6 g sucrose

200 ml dH_2O

12.5 µl kinetin (1 mg/ml stock solution)

125 µl NAA (1 mg/ml stock solution)

11.5 g glycerol (use about 20 ml water to rinse out container used to weigh glycerol)

2.5 g proline

Adjust pH to 7 with 1 M KOH

Filter sterilize

Add 9 ml filter sterilized DMSO

1. Sub-culture 7 ml culture in 43 ml CS medium. Wrap PSB-D in foil and place in incubator at 25°C with shaking at 130 rpm. Place PSB-L in 16 h light 8 h dark cycle at 21°C with shaking at 130 rpm.
2. After 7 days, sub-culture 10 ml culture in 40 ml PF medium. Wrap PSB-D in foil and place in incubator at 25°C with shaking at 130 rpm. Place PSB-L in 16 h light 8 h dark cycle at 21°C with shaking at 130 rpm. Also continue sub-culturing in CS media as described in maintenance to maintain a back-up culture in case cryopreservation fails.
3. After 2 days, transfer PF cultures to 50 ml Falcon tubes and centrifuge for 1 minute at 133 g
4. Aspirate off the supernatant leaving 2-2.5 ml liquid with most of the cells in bottom of tube.
5. Add 6 ml of cryoprotective media to each tube and gently shake to re-suspend then place in ice water for 1 hour.
6. Aliquot 2 ml of cells in cryoprotective media to cold 2 ml Nalgene tubes (store tubes in fridge before use and keep on ice while aliquotting).
7. Place tubes in Mr Frosty filled with isopropanol that has been cooled in fridge.
8. Place Mr Frosty on bottom shelf of -80°C freezer for approximately 1 hour 40 min, follow the temperature using an electronic thermometer until it reaches -50°C
9. Transfer tubes to liquid nitrogen storage.

For Agrobacterium-mediated transformation please refer to the protocol from VIB.