Botrytis cinerea transformation protocol

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Oct 08, 2021

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Botrytis cinerea transformation protocol. Please, acknowledge the work of others.

Paulo Canessa 2021. Botrytis cinerea transformation protocol. **protocols.io** https://protocols.io/view/botrytis-cinerea-transformation-protocol-bywcpxaw

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Day 1: Fungal growth 18h

1 Add 10 ml sterile MiliQ water on top of a well-sporulated Botrytis cinerea plate (7-day old PDA-bean plate). 3m

2 Disaggregate conidia. 2m

3 Collect conidia and filter using Nytex attached to a flask. You can use the spatula to speed up the process. 2m

4 Inoculate all collected spores in one flask containing 100 ml of maltose medium (1.5%) for 18 hours. 1m

5 Incubate at 20º Celsius and 120 rpm for 18 hours. 18h

Day 2: Protoplast generation 2h 22m

6 Filtrate de ON culture using Nytex and one 250 ml flask. You can use a sterile spoon to speed up the process. 3m

7 Wash 4-5 times with KC buffer until completing 50 ml (at room temperature). It is important to remove the media. Collect approximately 2.0 gr. of half-wet mycelium or 1.5 gr. if it is really dry. Transfer to a 100 ml sterile flask in order to prepare protoplasts as indicated below. 5m

8 Place KC buffer on ice and add filtered-sterilize enzyme mixture solution described below to the “half-wet mycelium”. 5m

9 Incubate for 2 hours at 28 Celsius and 95 RPM. 2h

10 Filtrate using Nytex on top of a small 100 ml flask. Wash with KC buffer (on ice). For this procedure, use a total of 50 ml KC buffer. 4m

11 Centrifuge the filtrate at 4000 rpm (50 ml swinging bucket rotor; Eppendorf 5804 R centrifuge) and 5 min, 4 Celsius. Adjust the speed-up and the speed-down setting of the centrifuge to the minimum. 5m

12 Wash with KC buffer (50 ml) and centrifuge again as mentioned. Resuspend protoplast in the backflow. Alternatively, remove all KC and then add 500 µl KC.

13 Take a 1:50 aliquot (4µl:200µl) in order to quantify protoplasts in a Thoma chamber. Adjust the protoplasts to the desire $1x10^8$/ml stock solution.

Day 2: Transformation 48m

14 Add 70 µl KC to the genetic construct (three 50 µl purified PCRs in 30 µl total). 1m

15 Add 100 µl protoplasts to each transformation (from the solution prepared in step 13). Incubate 5 min in ice. 6m

16 Add 100 µl PEG solution and incubate at RT for 20 min. 20m

17 Add 500 µl PEG solution and incubate on ice for 10 min. 10m

18 Add 200 µl KC solution for a final volume of 1ml. 1m

19 Add 500 µl of protoplasts to 50 ml 45 Celsius SH agar (liquid; using 50 ml sterile disposable tubes). Transfer 10 ml to five Petri dishes. Repeat with the remaining 500 µl of protoplasts to end up with 10 plates per transformation. 10m

20 Incubate the plates at 20-22 Celsius (12:12 h. photoperiod) for 20 hours.

Day 3: Selection 4d 0h 15m

21 Add 10 ml SH agar with the selection marker: **Hygromycin B** (70 µg/ml) or **Nourseothricin** (140 µg/ml). 15m

22 Approximately, between 3-7 days, thin (and flat) growing colonies will emerge. Transfer each individual colony to B5-medium + 2% Glucose Petri plates with selection: Hygromycin B 70 µg/ml. 4d