Protocol for overseas customers

Re-establishment of suspension cultures of tobacco BY-2 and related cell lines

Cell lines

- rpc00001: BY-2
- rpc00039: GV7
- rpc00040: GF11
- rpc00041: GT16
- rpc00062: BY-TIPG
- rpc00091: TBY2-31/ST
- rpc00092: TBY2-31/ST(E)
- rpc00093: TBY2-41/ST
- rpc00095: TBY2-R31
- rpc00097: TBY2-31/41
- rpc00109: BY-HR

Culture medium

- medium no. 1: modified Linsmaier and Skoog (mLS) medium, 0.2 mg/L 2,4-dichlorophenoxyacetic acid, pH 5.8

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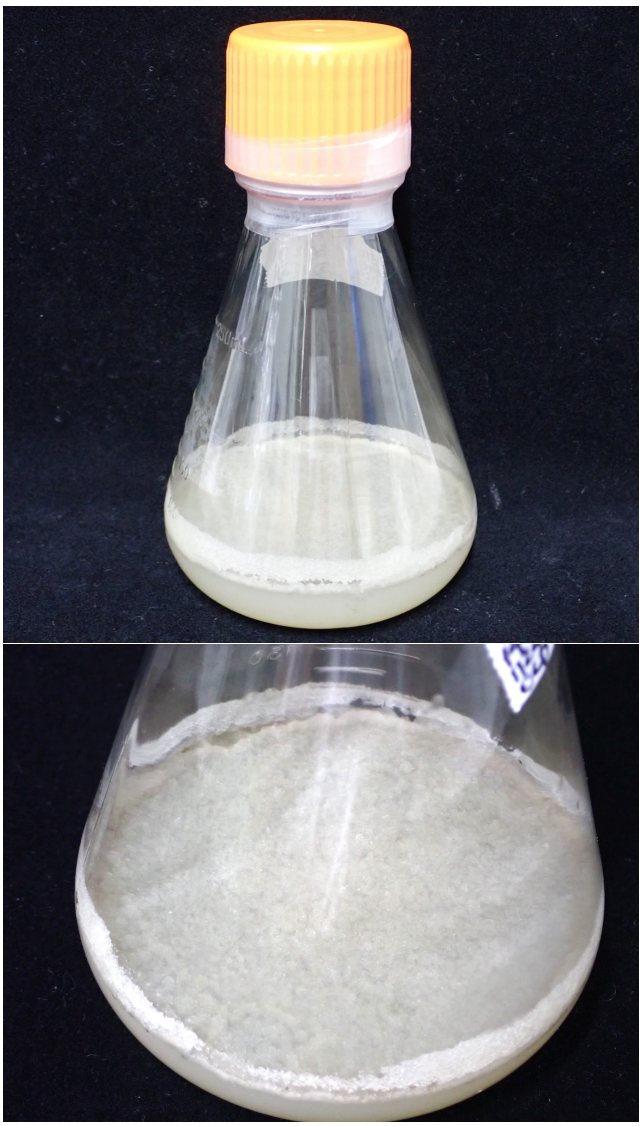
Methods

Regrowth of BY-2 cells

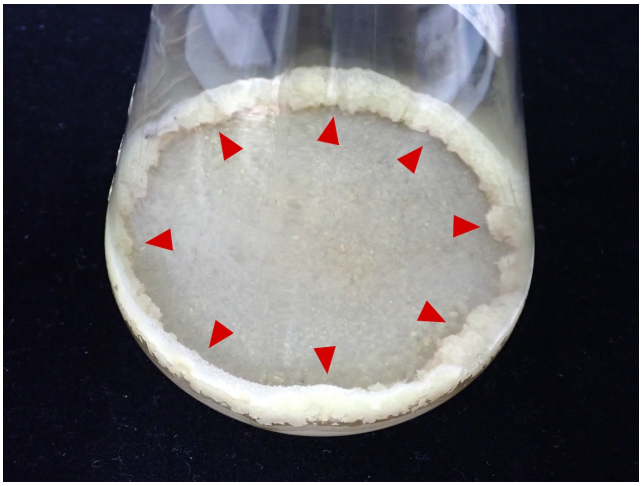
1. Recieve an 250-mL Erlenmeyer flask containing BY-2 cells.



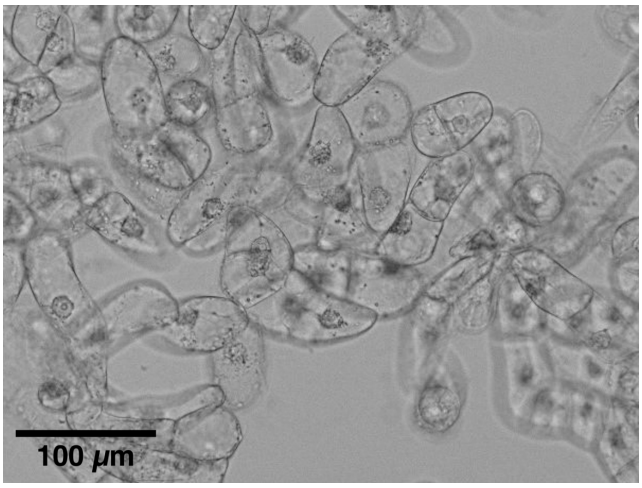
2. Loosen the screw cap slightly to keep good aeration.

   CAUTION Avoid microbial contamination.

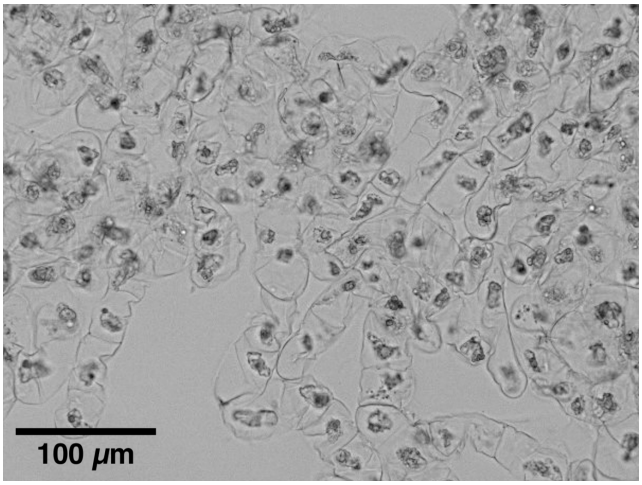
3. Incubate the BY-2 cells in the dark at 27℃ for 1–2 weeks.



After 8 days.
The red arrowheads indicate the growing BY-2 cells.



BY-2 cells around the edge are alive.



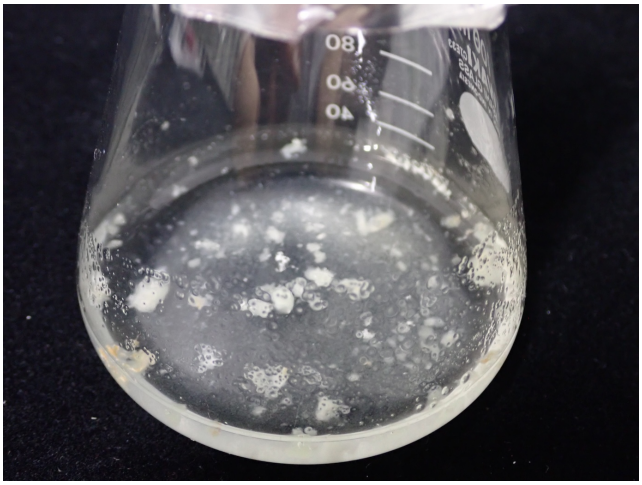
BY-2 cells around the center are dead.

4. Use the growing BY-2 cells for the induction of suspension cell cultures and for the maintenance of stock agar cultures.

Induction of suspension cell cultures

1. Transfer the BY-2 cells into a 100-mL Erlenmeyer flask containing 20 mL of a liquid culture medium.

   CAUTION Use at least the amount of BY-2 cells shown below.

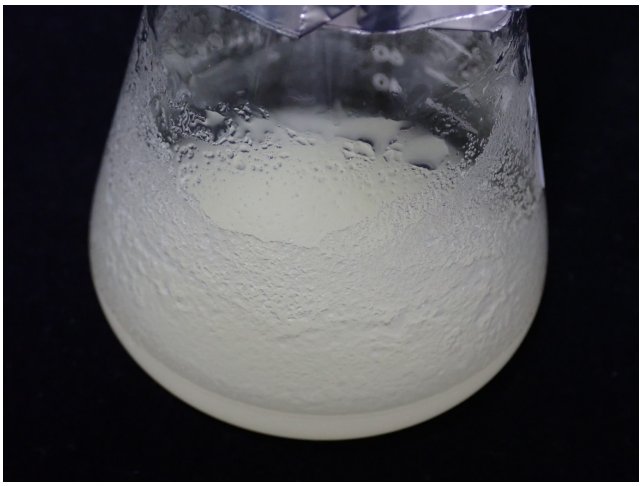


2. Incubate the BY-2 cells in the dark at 27℃ with rotary shaking at 130 rpm for 5–10 days.

   CAUTION Avoid microbial contamination.

   CAUTION Cover the flask mouth loosely with an aluminum foil cap for good aeration.

   NOTE A silicone sponge plug can be used instead of an aluminum foil cap (e.g., Cap-type Silicosen; Shin-Etsu Polymer, Tokyo, Japan).

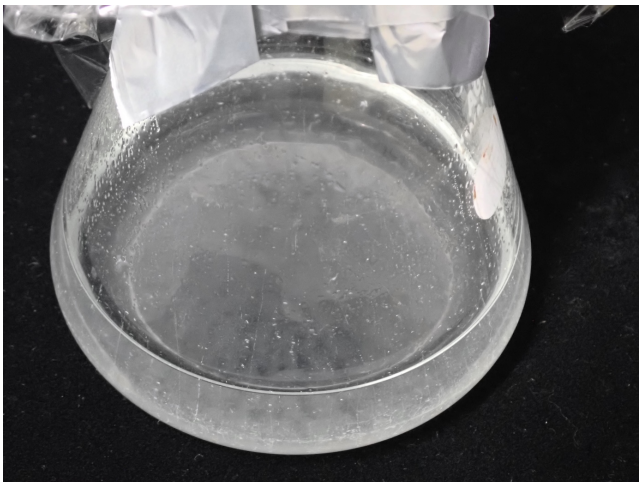


After 7 days

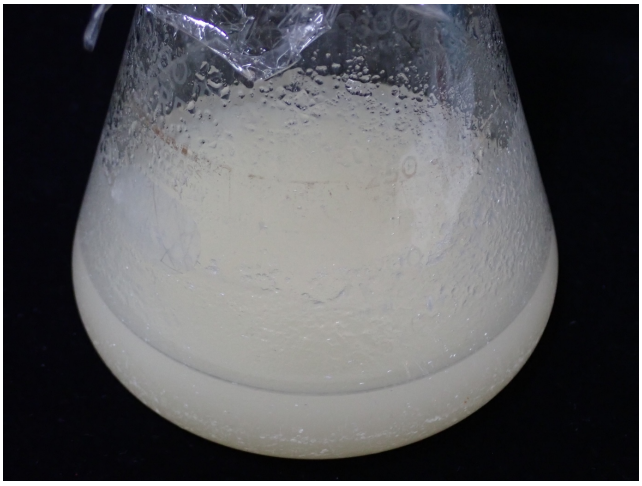
3. Allow the Erlenmeyer flask to stand for a few minutes just before the first subculture of suspension cell culture.

4. Transfer 1–2 mL of the sedimented cells into 95 mL of a fresh culture medium in a 300-mL Erlenmeyer flask.

   NOTE See Appendix A for the BY-2-related cell lines.



5. Culture the BY-2 cells by the usual method.



After 7 days

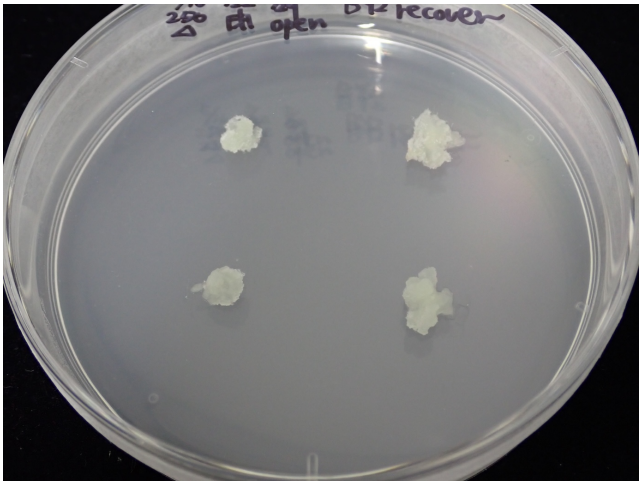
6. After 3–4 subculturing, the BY-2 suspension cell cultures grow stably.

Maintenance of stock agar cultures

1. Prepare cell culture dishes containing 30 mL of a culture medium solidified with 0.8% (w/v) agar.

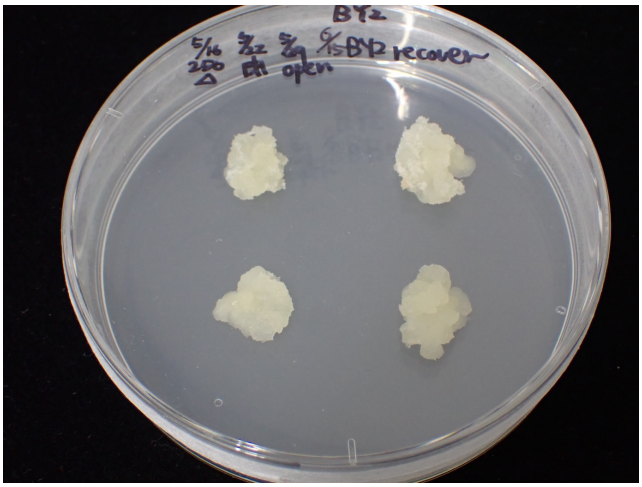
   NOTE BY-2 cells can also be cultured on the media solidified with gellan gum.

2. Transfer small pieces (3–5 mm in diameter) of BY-2 cells onto agar culture medium.



3. Seal culture dishes by using surgical tape to keep good aeration.

4. Incubate the BY-2 cells in the dark at 27℃ for 2–4 weeks.



   After 4 weeks

5. Subculture the BY-2 cells on fresh agar medium at 2–4-week intervals.

Appendix A: The first subculture of suspension cell cultures of BY-2-related cell lines

BRC No.	Cell line	Transfer volume* (mL)	Culture medium (mL)	Erlenmeyer flask (mL)
rpc00001	BY-2	1–2	95	300
rpc00039	GV7	1–3	95	300
rpc00040	GF11	3–6	95	300
rpc00041	GT16	3–6	95	300
rpc00062	BY-TIPG	1–3	95	300
rpc00091	TBY2-31/ST	0.4–0.8	30	100
rpc00092	TBY2-31/ST(E)	0.4–0.8	30	100
rpc00093	TBY2-41/ST	0.4–0.8	30	100
rpc00095	TBY2-R31	0.4–0.8	30	100
rpc00097	TBY2-31/41	0.4–0.8	30	100
rpc00109	BY-HR	2–4	95	300

* Subculture sedimented cells to a fresh culture medium.

CAUTION For the transgenic BY-2 cell lines, check the expression of fluorescent proteins in re-established suspension cells before use.

Appendix B: Preparation of BY-2 cell cultures for transportation

Preparation of a cell culture

1. An agar-solidified culture medium was prepared in a 250-mL disposable Erlenmeyer flask.

   NOTE mLS medium (medium no. 1) solidified with 1.4% (w/v) agar, 80 mL

2. BY-2 cell suspension culture was prepared on day 7 of the culture.

3. BY-2 cells were spread on the agar culture medium in the 250-mL disposable Erlenmeyer flask.

4. The screw cap was loosely closed to keep good aeration.

5. The BY-2 cells were pre-cultured in the dark at 27℃ for 6 days.

6. The screw cap was tightly closed and sealed with thermoplastic sealing film.

Transportation test

1. The BY-2 cells was stored in the dark at 27℃ for 7 days (simulating transport).

2. The BY-2 cells were tested for regrowth.