rpc00042

Nicotiana tabacum TBY2-AtRER1B transgenic callus culture

Components

- A 9-cm plastic Petri dish, containing cells placed on semi-solid medium

Notice

- Subculture the cells to fresh medium immediately after arrival [Notes I].
- Do not store the cell culture in a refrigerator and a freezer.
- Maintain aseptic conditions of the cell culture, and work in a laminar flow cabinet.

Method

- Culture medium: mLS medium, 0.2 mg/L 2,4-D, 200 mg/L kanamycin, 500 mg/L carbenicillin, 0.4% (w/v) gellan gum, pH 5.8 (medium no. 51) [Materials III]
- Culture conditions: 27℃, dark [Methods II]
- Subculture: 28–42-day intervals [Methods I]

Citation of cell line

When results obtained by using this cell line are published in a scientific journal, it should be cited in the following manner: "*Nicotiana tabacum* TBY2-AtRER1B cell line (rpc00042) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Tobacco TBY2-AtRER1B cell line is a transgenic BY-2 cell line expressing Green Fluorescent Protein (GFP) fused with AtRer1B (Takeuchi *et al*. 2000, 2002). GFP fluorescence is observed in the Golgi apparatus by using a fluorescence microscope. The parent cell line BY-2 (rpc00001) was established from a callus induced from a seedling of *Nicotiana tabacum* L. cultivar Bright Yellow 2 (Nagata *et al*. 1992). The TBY2-AtRER1B cells are grown on a modified Linsmaier and Skoog (mLS) medium supplemented with 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), 200 mg/L kanamycin and 500 mg/L carbenicillin, and solidified with 0.4% (w/v) gellan gum, pH 5.8. Our TBY2-AtRER1B cell culture has been maintained in the dark at 27℃ and subcultured at 28–42-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4℃)

A) MS salt mix

Murashige and Skoog Plant Salt Mixture, FUJIFILM Wako Pure Chemical Corporation (#392-00591)

B) Sucrose

C) BY2_P

KH_2PO_4	80 mg/mL

D) LS_VT_modified

Thiamine·HCl	0.4 mg/mL
myo-Inositol	40 mg/mL

E) 2,4-D (0.2 mg/mL)

2,4-D sodium monohydrate	0.236 mg/mL

(2,4-Dichlorophenoxy)acetic acid sodium salt monohydrate, Sigma-Aldrich (D6679)

F) Kanamycin (200 mg/mL)

Kanamycin sulfate	200 mg/mL

Sterilize the solution by membrane filtration

G) Carbenicillin (500 mg/mL)

Carbenicillin disodium salt	500 mg/mL

Sterilize the solution by membrane filtration

H) Gellan gum

Gellan gum, FUJIFILM Wako Pure Chemical Corporation (#073-03071)

I) KOH (1 N)

Glassware and equipment

A) Petri dish (9 cm diameter, 2 cm height), sterile

B) Forceps, sterilized before use

C) Surgical tape

3M™ Micropore™ Surgical Tape, 12.5 mm × 9.1 m, 3M Japan Limited (#1530-0)

Preparation of mLS medium (medium no. 51)

1. Dissolve the following chemicals in approximately 800 mL of distilled water.

MS salt mix	1 bag (1 L)
Sucrose	30 g

2. Add following stock solutions, and fill up to approximately 950 mL with distilled water.

BY2_P	2.5 mL
LS_VT_modified	2.5 mL
2,4-D (0.2 mg/mL)	1 mL

3. Adjust the pH of the solution to 5.8 with KOH (1 N), and fill up to 1 L with distilled water.

4. Add 4 g of gellan gum to the medium.

5. Autoclave the medium at 121°C for 20 min.

6. Add the following antibiotics to the medium just before agar solidification and swirl the medium gently to mix.

Kanamycin (200 mg/mL)	1 mL
Carbenicillin (500 mg/mL)	1 mL

7. Pour 30 mL of the medium into a 9-cm Petri dish.

Methods

1. Pick up an appropriate amount of callus cells from a 28–42-day-old culture with a forceps and place the cells onto fresh mLS medium.

2. Seal the Petri dishes using two rounds of surgical tape.

3. Incubate cell cultures under the dark condition at 27℃.

Notes

- We send TBY2-AtRER1B cells on semi-solid mLS medium in a 9-cm disposable Petri dish. The cells should be subcultured to fresh mLS medium immediately after arrival.

- In order to maintain TBY2-AtRER1B callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of TBY2-AtRER1B cells is affected by culture conditions, such as a room temperature, aeration conditions of the culture and so on, an amount of cells transferred to fresh medium and the subculture intervals may vary from one lab to another. We usually inoculate nine pieces of TBY2-AtRER1B callus (about 3-mm in diameter) on 30 mL of mLS medium in a 9-cm Petri dish, and culture them for 28–42 days.

- GFP fluorescence of TBY2-AtRER1B cells can be weakened during long-term maintenance.

References

Nagata T, Nemoto Y, Hasezawa S (1992) Tobacco BY-2 cell line as the "HeLa" cell in the cell biology of higher plants. International Review of Cytology 132: 1–30. DOI: 10.1016/S0074-7696(08)62452-3

Takeuchi M, Ueda T, Sato K, Abe H, Nagata T, Nakano A (2000) A dominant negative mutant of sar1 GTPase inhibits protein transport from the endoplasmic reticulum to the Golgi apparatus in tobacco and *Arabidopsis* cultured cells. Plant Journal 23: 517–525. DOI: 10.1046/j.1365-313x.2000.00823.x

Takeuchi M, Ueda T, Yahara N, Nakano A (2002) Arf1 GTPase plays roles in the protein traffic between the endoplasmic reticulum and the Golgi apparatus in tobacco and *Arabidopsis* cultured cells. Plant Journal 31: 499–515. DOI: 10.1046/j.1365-313X.2002.01372.x

Appendix A: Formulation of culture medium

Table A.1. modified Linsmaier and Skoog medium (medium no. 51)

Chemical	Concentration (mg/L)
KNO_3	1900
NH_4NO_3	1650
$CaCl_2 \cdot 2H_2O$	440
$MgSO_4 \cdot 7H_2O$	370
KH_2PO_4	370
H_3BO_3	6.2
$MnSO_4 \cdot 4H_2O$	22.3
$ZnSO_4 \cdot 7H_2O$	8.6
KI	0.83
$Na_2MoO_4 \cdot 2H_2O$	0.25
$CuSO_4 \cdot 5H_2O$	0.025
$CoCl_2 \cdot 6H_2O$	0.025
$FeSO_4 \cdot 7H_2O$	27.8
Na_2-EDTA	37.3
Thiamine·HCl	1
myo-Inositol	100
Sucrose	30000
2,4-D sodium monohydrate	0.236
Kanamycin sulfate	200
Carbenicillin disodium salt	500
Gellan gum	4000