rpc00098

Nicotiana tabacum topless3-GFP 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, 0.5% (w/v) gellan gum, pH 5.8 (medium no. 59) [Materials III]
- Culture conditions: 27℃, dark [Methods II]
- Subculture: 21-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* topless3-GFP cell line (rpc00098) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

Experimental Plant Division
RIKEN BioResource Research Center (BRC)
Koyadai 3-1-1, Tsukuba, Ibaraki 305-0074
Japan
FAX: +81 29 836 9053
E-mail: plant.brc@riken.jp
http://epd.brc.riken.jp/en/

Introduction

Tobacco topless3-GFP cell line is a transgenic BY-2 cell line expressing Green Fluorescent Protein (GFP) fused with NtTPL3 (Nagashima *et al.* 2019). GFP fluorescence is observed in nuclei 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 topless3-GFP cells are grown on a modified Linsmaier and Skoog (mLS) medium supplemented with 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), and solidified with 0.5% (w/v) gellan gum, pH 5.8. Our topless3-GFP cell culture has been maintained in the dark at 27℃ and subcultured at 21-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) Gellan gum

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

G) 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. 59)

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 5 g of gellan gum to the medium.

5. Autoclave the medium at 121℃ for 20 min.

6. Pour 35 mL of the medium into a 9-cm Petri dish.

Methods

1. Pick up an appropriate amount of callus cells from a 21-day-old culture with a forceps and place the cells onto fresh mLS medium.

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

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

Notes

- We send topless3-GFP 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 topless3-GFP callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of topless3-GFP 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 sixteen pieces of topless3-GFP callus (about 1–2-mm in diameter) on 35 mL of mLS medium in a 9-cm Petri dish, and culture them for 21 days.

References

Nagashima A, Higaki T, Koeduka T, Ishigami K, Hosokawa S, Watanabe H, Matsui K, Hasezawa S, Touhara K (2019) Transcriptional regulators involved in responses to volatile organic compounds in plants. Journal of Biological Chemistry 294: 2256–2266. DOI: 10.1074/jbc.RA118.005843

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

Appendix A: Formulation of culture medium

Table A.1. modified Linsmaier and Skoog medium (medium no. 59)

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
Gellan gum	5000