Nicotiana tabacum TBY2-31/ST transgenic cell suspension culture

Components

- Domestic delivery: A 50-mL plastic conical centrifuge tube, containing cell suspension
- Overseas delivery: A 250-mL plastic Erlenmeyer flask, containing cells placed on semi-solid medium

Notice

- Subculture the cells to fresh medium immediately after arrival [Notes I, II].
- 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, pH 5.8 (medium no. 1) [Materials III]
- Culture conditions: 27°C, dark, 110 rpm [Methods II]
- Subculture: 7-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* TBY2-31/ST cell line (rpc00091) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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Introduction

Tobacco TBY2-31/ST cell line is a transgenic BY-2 cell line expressing Green Fluorescent Protein (GFP) fused with Arabidopsis SYP31 and monomeric Red Fluorescent Protein (mRFP) fused with rat sialyltransferase (Ito *et al.* 2012). GFP fluorescence is observed in *cis*-Golgi, and mRFP fluorescence is in *trans*-Golgi, 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 TBY2-31/ST cells are grown in a modified Linsmaier and Skoog (mLS) medium supplemented with 0.2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), pH 5.8. Our TBY2-31/ST cell culture has been maintained in the dark at 27°C with rotary shaking at 110 rpm and subcultured at 7-day intervals.

Materials

Chemicals and stock solutions

(All stock solutions are stored at 4°C)

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 myo-Inositol 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) KOH (1 N)

Glassware

A) Erlenmeyer flask (100 mL), capped with two layers of aluminum foil

B) Pipette (2 mL; large tip opening) and a bulb, sterilized by autoclaving at 121°C for 20 min

Preparation of mLS medium (medium no. 1)

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. Pour 30 mL of the medium into a 100-mL flask.
- 5. Autoclave the flask at 121°C for 20 min.

Methods

- 1. Agitate a 7-day-old culture well and transfer 0.4 mL of cell suspension to 30 mL of fresh mLS medium with a pipette.
- 2. Incubate cell cultures on a rotary shaker at 110 rpm under the dark condition at 27° C.

Notes

- For domestic customers: We send TBY2-31/ST cell suspension in a 50-mL disposable conical centrifuge tube. The cells should be transferred to fresh mLS medium immediately after arrival.
- For overseas customers: We send TBY2-31/ST cells placed on semi-solid mLS medium in a 250-mL disposable Erlenmeyer flask. The cells should be transferred to fresh mLS medium immediately after arrival. Collect the cells from the semi-solid medium with a spatula and transfer them to Erlenmeyer flasks containing fresh liquid medium.
- In order to maintain TBY2-31/ST cell suspension cultures stably, it is essential to transfer an adequate amount of cells to fresh mLS medium in every subculture. The

- amount of cells may vary from one lab to another, because proliferation of TBY2-31/ST cells is affected by culture conditions, such as a room temperature, rotation speed of a rotary shaker, and aeration condition of the culture.
- A low growth rate of the parent BY-2 cells is sometimes caused by poor aeration (Kumagai-Sano *et al.* 2007). In order to obtain good aeration of a suspension culture, a silicone sponge plug may be used instead of the aluminum foil cap (*e.g.*, cap-type Silicosen; Shin-Etsu Polymer, Tokyo, Japan; https://www.shinpoly.co.jp/en/product/product/medical/plugs.html).

References

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- 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: $\underline{1}$ 0.1016/S0074-7696(08)62452-3

Appendix A: Formulation of culture medium

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

Chemical	Concentration (mg/L)
KNO ₃	1900
NH_4NO_3	1650
CaCl ₂ ·2H ₂ O	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 ·5H ₂ O	0.025
$CoCl_2 \cdot 6H_2O$	0.025
FeSO ₄ ·7H ₂ O	27.8
Na ₂ -EDTA	37.3
Thiamine·HCl	1
myo-Inositol	100
Sucrose	30000
2,4-D sodium monohydrate	0.236