

## Maintenance of Tobacco GF11 Cell Suspension Culture

### Introduction

The Tobacco GF11 cell line is a transgenic BY-2 cell line expressing Green Fluorescent Protein (GFP) fused with AtFim1 (Sano et al. 2005). Because actin microfilaments can be visualized by using a fluorescence microscope, the GF11 cell line is used for studying detailed structures of plant cells. A parent cell line BY-2 was derived from a callus induced from a stem of *Nicotiana tabacum* L. cultivar Bright Yellow 2 (Nagata et al. 1992). Our GF11 cells have been maintained in a modified Linsmaier and Skoog (mLS) medium in dark at 27°C and subcultured at one-week intervals.

### Materials

#### I. Stock Solution and Chemicals

- |   |       |
|---|-------|
| A) Modified LS_VT (100 ml)  |       |
| Thiamine•HCl  | 40 mg |
| <i>myo</i> -Inositol  | 4 g   |
| B) BY2_P (100 ml)   |       |
| KH <sub>2</sub> PO <sub>4</sub>   | 8 g   |
| C) MS salt mix.   |       |
| Murashige and Skoog Plant Salt Mixture, Wako Pure<br>Chemical Industries (catalog #392-00591) |       |
| D) Sucrose  |       |
| E) 0.2 mg/ml 2,4-D (100 ml)   |       |
| 2,4-Dichlorophenoxyacetic acid  | 20 mg |

#### II. Glassware

(All are sterilized by autoclaving at 121°C for 20 min)

- A) Erlenmeyer flask (300 ml), capped with two layers of aluminum foil
- B) Pipette, large tip opening (10 ml), and a bulb.

#### III. Preparation of mLS Medium

- A) Dissolve one bag (1 L) of the MS salt mixture and 30 g of sucrose in approximately 800 ml of distilled water.
- B) Add following stock solutions to A), and fill up to approximately 950 ml with distilled water.

Modified LS_VT	2.5 ml
BY2_P	2.5 ml
0.2 mg/ml 2,4-D	1 ml
- C) Adjust the pH of the solution to 5.8 with 0.2 N KOH, and fill up to 1 L with distilled water.
- D) Pour 95 ml of mLS medium into a flask, and autoclave the flask at 121°C for 20 min.

## Methods

- I. Agitate a one-week-old culture well and transfer 2.8–3.4 ml of cell suspension to 95 ml of fresh mLS medium with a pipette.
- II. Incubate cell cultures on a rotary shaker at 130 rpm under dark condition at 25–27°C.

## Notes

- I. We transport the GF11 cells on semi-solid mLS medium in 250-ml disposable flasks. The cells should be transferred to fresh mLS medium immediately after arrival. Cell suspension cultures should be established using small fractions of the original culture because some cells may have been damaged during transport. Collect the light yellow cells from the medium with a spatula and transfer them to Erlenmeyer flasks containing fresh liquid medium (*e.g.*, 20 ml of medium in a 100-ml flask). Brown cells should not be used because they are damaged.
- II. To maintain the GF11 cell suspension culture stably, an adequate amount of cell suspension should be transferred to fresh mLS medium for every subculturing. The amount of cell suspension may vary from one lab to another, because proliferation of GF11 cells is affected by culture conditions, such as a room temperature, rotation speed of the rotary shaker, and aeration conditions of the culture. GF11 cell suspension culture should reach a stationary phase around 7 days after culturing. In addition, extreme dilution of GF11 cell suspension culture below 1:100 results in suppression of cell division.
- III. A low growth rate of the parent BY-2 cell line is sometimes caused by poor aeration of suspension cultures (Kumagai-Sano *et al.* 2007). The aluminum foil caps should be closed loosely to obtain good aeration. Silicone caps may be used instead of the aluminum foil caps.

## References

- Kumagai-Sano F, Hayashi T, Sano T, Hasezawa S (2007) Cell cycle synchronization of tobacco BY-2 cells. *Nature Protocols* 1: 2621-2627
- 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
- Sano T, Higaki T, Oda Y, Hayashi T, Hasezawa S (2005) Appearance of actin microfilament ‘twin peaks’ in mitosis and their function in cell plate formation, as visualized in tobacco BY-2 cells expressing GFP-Fimbrin. *Plant Journal* 44: 595-605

Appendix 1 Composition of modified Linsmaier and Skoog medium

Chemical	Concentration (mg/L)
KNO <sub>3</sub>	1900
NH <sub>4</sub> NO <sub>3</sub>	1650
CaCl <sub>2</sub> •2H <sub>2</sub> O	440
MgSO <sub>4</sub> •7H <sub>2</sub> O	370
KH <sub>2</sub> PO <sub>4</sub>	370
H <sub>3</sub> BO <sub>3</sub>	6.2
MnSO <sub>4</sub> •4H <sub>2</sub> O	22.3
ZnSO <sub>4</sub> •7H <sub>2</sub> O	8.6
KI	0.83
Na <sub>2</sub> MoO <sub>4</sub> •2H <sub>2</sub> O	0.25
CuSO <sub>4</sub> •5H <sub>2</sub> O	0.025
CoCl <sub>2</sub> •6H <sub>2</sub> O	0.025
FeSO <sub>4</sub> •7H <sub>2</sub> O	27.8
Na <sub>2</sub> •EDTA	37.3
Thiamine•HCl	1
<i>myo</i> -Inositol	100
Sucrose	30000
2,4-Dichlorophenoxyacetic acid	0.2