rpc00004

# *Vitis* VW 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, 0.05 mg/L 2,4-D, 0.2 mg/L kinetin, 1.2% (w/v) agar, pH 6.1 (medium no. 3) [Materials III]
- Culture conditions: 27℃, dark [Methods II]
- Subculture: 56-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: "*Vitis* VW cell line (rpc00004) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan."

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## Introduction

Grape VW cell line was established from anthers of *Vitis* interspecific hybrid cultivar Bailey Alicante A (Yamakawa *et al.* 1983a, b). The VW callus cells accumulate lower amount of anthocyanins than VR callus cells (rpc00003). The VW cells are grown on a modified Linsmaier and Skoog (mLS) medium supplemented with 0.05 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.2 mg/L kinetin, and solidified with 1.2% (w/v) agar, pH 6.1. Our VW cell culture has been maintained in the dark at 27℃ and subcultured at 56-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) LS_VT_modified

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

D) 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)

E) Kinetin (0.2 mg/mL)

Kinetin 0.2 mg/mL
Dissolve kinetin in small volume of KOH (1 N), and fill up with distilled water

F) Agar, powder

Agar, powder, Junsei Chemical (#24440-1201)

G) KOH (1 N)

### Glassware and equipment

A) Erlenmeyer flask (300 mL), capped with two layers of aluminum foil

B) Forceps, sterilized before use

**Preparation of mLS medium (medium no. 3)**

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.

| | |
|---|---|
| LS_VT_modified | 2.5 mL |
| 2,4-D (0.2 mg/mL) | 0.25 mL |
| Kinetin (0.2 mg/mL) | 1 mL |

3. Adjust the pH of the solution to 6.1 with KOH (1 N), and fill up to 1 L with distilled water.

4. Pour 100 mL of the medium into a 300-mL flask containing 1.2 g of agar.

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

## Methods

1. Pick up an appropriate amount of callus cells from a 56-day-old culture with a forceps and place the cells onto fresh mLS medium.

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

## Notes

- We send VW 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 VW callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of VW 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 one to two pieces of VW callus (about 8-mm in diameter) on 100 mL of mLS medium in a 300-mL flask, and culture them for 56 days.

- It is important to subculture good healthy cells. Yellow VW cells are the most suitable. White and brown cells should not be used.

## References

Yamakawa T, Ishida K, Kato S, kodama T, Minoda Y (1983a) Formation and identification of anthocyanins in cultured cells of *Vitis* sp. Agricultural and Biological Chemistry 47: 997–1001. DOI: 10.1080/00021369.1983.10865764

Yamakawa T, Kato S, Ishida K, Kodama T, Minoda Y (1983b) Production of anthocyanins by *Vitis* cells in suspension culture. Agricultural and Biological Chemistry 47: 2185–2191. DOI: 10.1080/00021369.1983.10865938

## Appendix A: Formulation of culture medium

Table A.1. modified Linsmaier and Skoog medium (medium no. 3)

| Chemical | Concentration (mg/L) |
|---|---|
| $KNO_3$ | 1900 |
| $NH_4NO_3$ | 1650 |
| $CaCl_2 \cdot 2H_2O$ | 440 |
| $MgSO_4 \cdot 7H_2O$ | 370 |
| $KH_2PO_4$ | 170 |
| $H_3BO_3$ | 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 | 0.059 |
| Kinetin | 0.2 |
| Agar | 12000 |