

rpc00055

## ***Arabidopsis thaliana* gnom 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: mMS medium, 1 mg/L 2,4-D, 0.8% (w/v) agar, pH 5.8 (medium no. 43) [[Materials III](#)]
- Culture conditions: 23°C, dark [[Methods II](#)]
- Subculture: 28-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: “*Arabidopsis thaliana* gnom cell line (rpc00055) was provided by the RIKEN BRC through the National BioResource Project of the MEXT, Japan.”

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## Introduction

*Arabidopsis gnom* cell line was established from a root of *Arabidopsis thaliana* (L.) Heynh. *gnom* mutant (Ueda *et al.* 2004). *GNOM* encodes a GDP/GTP exchange factor for small G-proteins of the ADP ribosylation factor class (Geldner *et al.* 2003). The *gnom* mutant plants show severe growth arrest, but the *gnom* cell culture proliferate normally like wild-type cells (Ueda *et al.* 2004). The *gnom* cells are grown on a modified Murashige and Skoog (mMS) medium supplemented with 1 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D), and solidified with 0.8% (w/v) agar, pH 5.8. Our *gnom* cell culture has been maintained in the dark at 23°C and subcultured at 28-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) KH<sub>2</sub>PO<sub>4</sub> (100 mg/mL)

KH <sub>2</sub> PO <sub>4</sub>	100 mg/mL
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D) B5\_VT

Nicotinic acid	0.4 mg/mL
Pyridoxine·HCl	0.4 mg/mL
Thiamine·HCl	4 mg/mL
<i>myo</i> -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) Agar, powder

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 mMS medium (medium no. 43)

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

MS salt mix	1 bag (1 L)
Sucrose	20 g

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

KH <sub>2</sub> PO <sub>4</sub> (100 mg/mL)	3.4 mL
B5_VT	5 mL
2,4-D (0.2 mg/mL)	5 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 8 g of agar to the medium.
5. Autoclave the medium at 121°C for 20 min.
6. 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-day-old culture with a forceps and place the cells onto fresh mMS medium.
2. Seal the Petri dishes using two rounds of surgical tape.
3. Incubate cell cultures under the dark condition at 23°C.

## Notes

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

- In order to maintain gnom callus culture stably, it is essential to observe the growth of cells carefully. Because proliferation of gnom 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 seven pieces of gnom callus (about 5-mm in diameter) on 30 mL of mMS medium in a 9-cm Petri dish, and culture them for 28 days.

## References

- Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G (2003) The *Arabidopsis* GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth. *Cell* 112: 219–230. DOI: [10.1016/S0092-8674\(03\)00003-5](https://doi.org/10.1016/S0092-8674(03)00003-5)
- Ueda T, Uemura T, Sato MH, Nakano A (2004) Functional differentiation of endosomes in *Arabidopsis* cells. *Plant Journal* 40: 783–789. DOI: [10.1111/j.1365-313X.2004.02249.x](https://doi.org/10.1111/j.1365-313X.2004.02249.x)

## Appendix A: Formulation of culture medium

Table A.1. modified Murashige and Skoog medium  
(medium no. 43)

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>	510
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
Nicotinic acid	2
Pyridoxine·HCl	2
Thiamine·HCl	20
<i>myo</i> -Inositol	200
Sucrose	20000
2,4-D sodium monohydrate	1.18
Agar	8000