

Agrobacterium mediated transformation of the liverwort, *Marchantia polymorpha*

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1) Liquid culture of the liverwort cells from spores

- > Prepare surface-sterilized sporandisporangiums
- > Add 100 μL sH_2O to 1 sporandisporangium (i.e. 700 μL to 7 sporandisporangiums) and suspend the spores by pipetting.
- > Transfer the 100 μL spore suspension to 25 mL 0M51C^{a)} media in 100 mL flask.
- > Culture the cells under continuous white light ($60 \mu\text{mol m}^{-2}\text{s}^{-1}$) at 130 rpm, and 22 °C for 7 days.

2) Preparation of *Agrobacterium*

- > Prepare streak plates of *Agrobacterium*^{b)} carrying binary vector^{c)} onto LB medium containing antibiotic for plasmid of interest plus Rifampicin (100 $\mu\text{g} / \text{mL}$) for *Agrobacterium*. Incubate for 2 days at 28 °C. The plates can be stored for 1 month at 4 °C.
- > Pick a single colony off the plate into a 5 mL LB medium containing antibiotic for plasmid of interest plus Rifampicin (100 $\mu\text{g} / \text{mL}$). Culture for 2 days at 28 °C.
- > Centrifuge cultures for 15 minutes at 2000 \times g.
- > Pour off supernatant and add to a 10 mL 0M51C medium containing 100 μM Acetosyringone^{d)} to resuspend cells.
- > Culture the *Agrobacterium* for ~6 hours at 28 °C with shaking.

3) Co-cultivation of the liverwort cells and *Agrobacterium*

- > Add 1 mL of *Agrobacterium* suspension prepared in step 2 (above) into the 7-day culture of liverwort cells.
- > Add Acetosyringone to the final concentration of 100 μM .
- > Culture the liverwort for 2 further days under continuous white light $60 \mu\text{mol m}^{-2}\text{s}^{-1}$ at 130 rpm and 22 °C.

4) Selection of transformants

- > Collect the liverwort cells with a 20- μm square grid nylon mesh attached to one side of a 3 cm diameter, 4cm long glass tube.
- > Wash the cells 3 times with ~ 25 mL 0M51C medium.
- > Recover the cells with spatula and place them onto 0M51C agar medium containing appropriate antibiotics, i.e. 10 $\mu\text{g} / \text{mL}$ hygromycin, plus 100 $\mu\text{g} / \text{mL}$ Claforan^{e)}.
- > Incubate the liverwort cells under continuous white light $60 \mu\text{mol m}^{-2}\text{s}^{-1}$ at 22 °C. Transformants will be visible after 1~2 weeks.

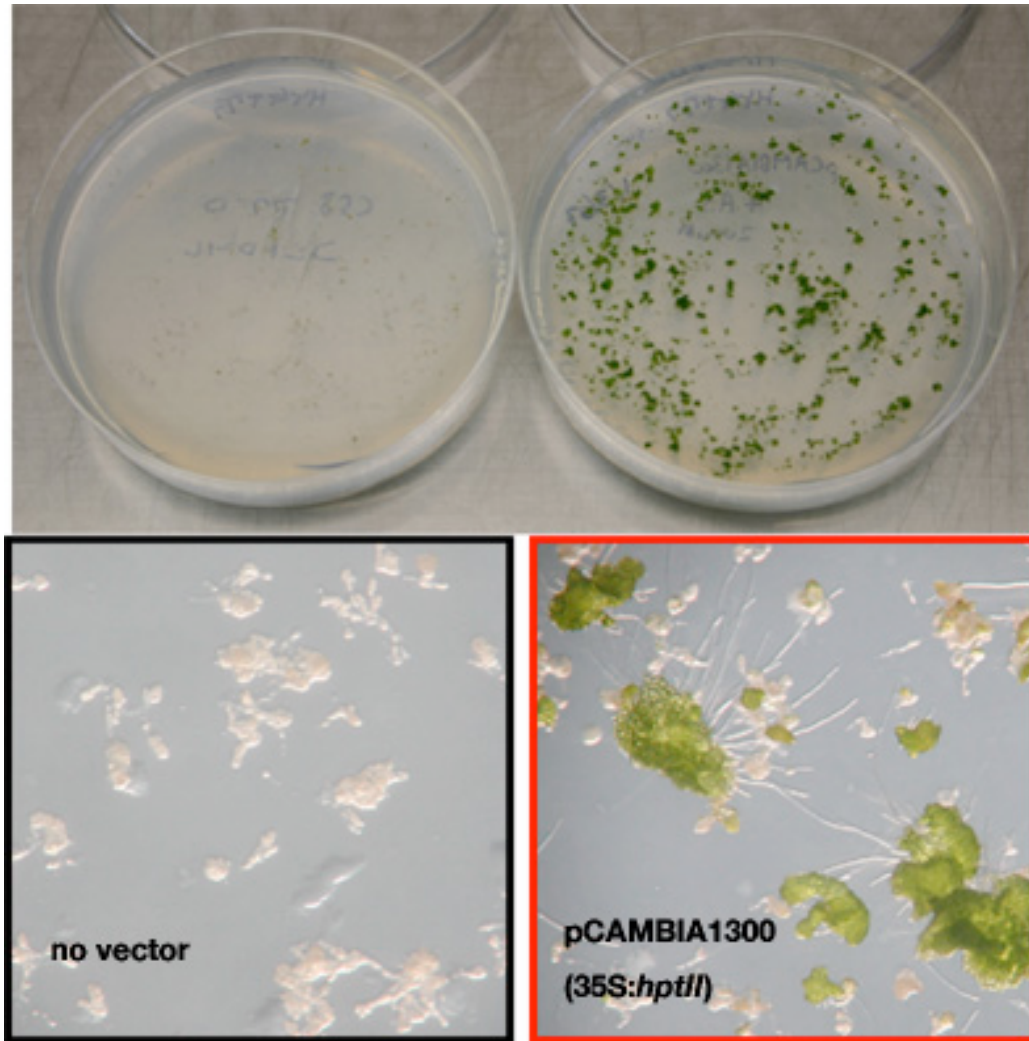


Fig.1 An example of successful *Agrobacterium* transformation of the liverwort, *Marchantia polymorpha*. Transformants grew on selective 0M51C medium containing 10 µg / ml hygromycin.

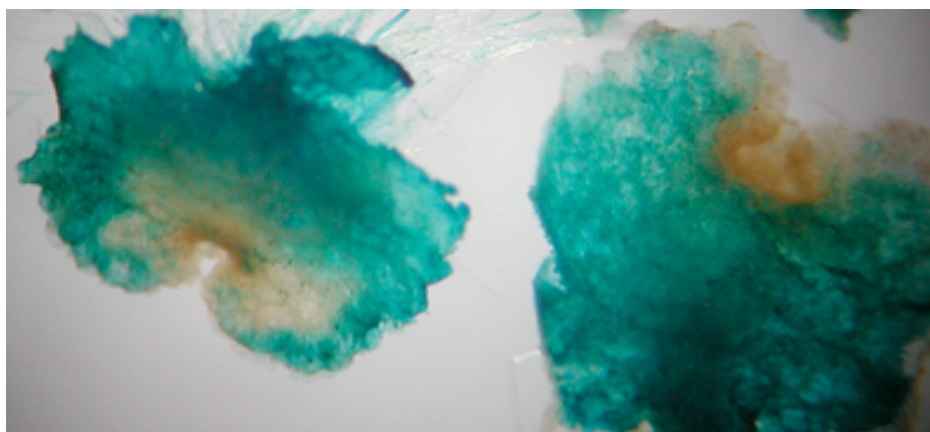


Fig. 2 GUS staining of pIG121-Hm transformants. pIG121-Hm is a binary vector containing CaMV35S:intron-gusA (pIG121-Hm was kindly provided by Prof. Kenzo Nakamura, Nagoya University)

a) 0M51C medium (for 1 litre)

10×OM51C stock	100 mL
2% Sucrose	20 g
0.03% L-glutamine	0.3 g
<u>0.1% Casamino acids</u>	<u>1.0 g</u>
(pH5.5 with 1N KOH)	
Agar 14 g / litre	

<10 X 0M51C stock for 4 litre (store at -30°C)>

KNO ₃	80 g
NH ₄ NO ₃	16 g
MgSO ₄ 7H ₂ O	14.8 g
CaCl ₂ 2H ₂ O	12 g
KH ₂ PO ₄	11 g
EDTA-NaFe(III)	1.6 g
B5-micronutrient	40 mL
B5-vitamin	40 mL
<u>KI solution (750 mg / 100 mL)</u>	<u>4 mL</u>
	4L

<B5-Vitamin for 100 mL (store at -30 °C)>

Inositol	10 g
Nicotinic acid	100 mg
Pyridoxine-HCl	100 mg
<u>Thiamine-HCl</u>	<u>1 g</u>
	100 mL

<B5-micronutrient for 100 mL (store at -30 °C)>

NaMoO ₄ 2H ₂ O	25 mg
CuSO ₄ 5H ₂ O	2.5 mg
CoCl ₂ 6H ₂ O	2.5 mg
ZnSO ₄ 7H ₂ O	200 mg
MnSO ₄ 7H ₂ O	1 g
<u>H₃BO₃</u>	<u>300 mg</u>
	100 mL

b) *Agrobacterium* C58 C1 pGV2260 has been tested for transformation of the liverwort in our lab.

c) CaMV35S:*hpt* works fine as a marker and the transformants can grow on 0M51C medium containing 10 µg / mL hygromycin. nosP: *hpt* works but not as efficiently as CaMV35S:*hpt*.

d) 3',5'-Dimethoxy-4'-hydroxy-acetophenone. 100 mM stock = 19.2 mg / ml in DMSO

e) Cefotaxime sodium: Cefem antibiotic to prevent *Agrobacterium* growth.