



Mar 31, 2020

Version 1

Transformation of *Diplonema papillatum* by electroporation V.1

DOI

<https://dx.doi.org/10.17504/protocols.io.bedxja7n>

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Protocol Citation: Matus Valach 2020. Transformation of *Diplonema papillatum* by electroporation. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.bedxja7n>

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Protocol status: Working

We use this protocol and it's working

Created: March 31, 2020



Last Modified: March 31, 2020

Protocol Integer ID: 34967

Keywords: transformation of diplomema papillatum, diplomema papillatum by electroporation, electroporation variant protocol for transformation, diplomema papillatum, electroporation, transformation buffer, transformation, procedure

Abstract

Variant protocol for transformation of *Diplonema papillatum* by electroporation using a "home-made" transformation buffer. The procedure was devised based on previously published protocols by Kaur *et al.* (DOI: 10.1111/1462-2920.14041) and Dyer *et al.* (DOI: 10.3791/54342).

Materials

MATERIALS

- ☒ Sodium Phosphate monobasic
- ☒ Glucose
- ☒ KCl
- ☒ CaCl₂
- ☒ MgCl₂
- ☒ BSA
- ☒ Sucrose
- ☒ HEPES
- ☒ EDTA
- ☒ Inosine triphosphate
- ☒ G418 (geneticin sulfate) **Bioshop Catalog #GEN418**

Before start

Perform a simple test of antibiotic resistance of wild-type cells in the chosen culture conditions, e.g., temperature (16 vs 20 vs 27 °C), medium composition (e.g., horse serum vs fetal bovine serum) or antibiotic supplier. Into a 24-well plate, distribute 1.5 mL medium per well and add the antibiotic at several different concentrations (e.g., for G418, choose 0, 50, 75, 100, 150, and 200 µg/µL). This arrangement (6 columns, each with a different antibiotic concentration) allows to perform three WT replicates together with one positive control resistant to the antibiotic of choice. Inoculate $1-5 \times 10^5$ cells per well and let the cells grow for 3–4 days, then examine the extent of growth. The lowest antibiotic concentration at which the WT cells do not grow is then used for the selection. For example, when cultivating *Diplonema papillatum* in a horse serum-based medium and using G418 (*Bioshop*; potency min. 650 µg/mg), 100 µg/µL is the threshold value at 20 °C, but >125 µg/µL is needed for efficient selection at 16 °C.

1 Prepare the transformation (cytomix-like) buffer.

	Com pon ent	Final con cent ratio n
	HEP ES pH7. 5	25 mM
	KCl	25 mM
	CaCl 2	0.15
	NaH 2PO 4 pH7. 5	10
	MgC l2	2.5
	EDT A	1
	gluc ose	30 mM (0.5 %)
	sucr ose	145 mM (4.3 5%)
	bovi ne seru m albu min (BSA)	0.1 mg/ mL
	inosi ne triph osph ate (ITP) [or	1 mM



	hypo xanthine]	
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Note

The addition of ITP (or hypoxanthine) is optional. If preparing a large volume of the buffer, make aliquots and store them at -70°C until further use.

- 2 Inoculate *Diplonema* cells at 2×10^5 /mL into 45 mL OSS medium and let them grow for 2–3 days.

Note

The resulting amount of cells is usually sufficient for 3–4 transformations. Therefore, if performing additional transformations, scale-up the cultivation volume.

Note

If performing the selection at 20°C , pre-culture is done at this same temperature.

- 3 Harvest the cells while they are in the exponential phase (optimal density $2\text{--}4 \times 10^6$ /mL). Wash twice with OS (i.e., medium without the serum) and aliquot the cells into tubes, so that after the final centrifugation, each pellet contains 10^7 cells. Remove as much OS buffer as possible. Keep the cells on ice.
- 4 Resuspend the pellet in ice-cold 200 μL transformation buffer (see the recipe above), immediately centrifuge (4°C , $1,000 \times g$, 2 min), and discard the supernatant.
- 5 Resuspend the pellet in ice-cold 100 μL transformation buffer supplemented with 2 μg linearized DNA.

Note

Optimally, add the DNA in a volume of 5 μL or less. To the negative control, add the same volume of the buffer used to solubilize the linearized DNA (e.g., 10 mM Tris pH8.0).



- 6 Immediately transfer the cell suspension into an electroporation cuvette (0.2 mm), which has been pre-cooled on ice.
- 7 Quickly transfer the cuvette into an electroporation apparatus (e.g., *Gene Pulser Xcell* from *Bio-Rad*) and apply the pulse.
Pulse parameters: **1**) 1,500 V, 0.3 ms; or **2**) 140 V, 1,400 μ F.

Note

Both conditions work. Cell survival is more substantial in the option **2** (may be preferred when numerous clones are required), but clone selection is more straightforward and clear-cut for the option **1**.

- 8 Immediately after the pulse, put the cuvette back on ice, add 1 mL cold (5–10 °C) OSS and resuspend the cells.
- 9 Transfer the cell suspension into a well of a 24-well plate, add additional 0.8 mL OSS, and cultivate for 5–8 h without selection.
- 10 Distribute the cell suspension into additional (5–10) wells and add OSS with the antibiotic of choice to the final volume of 1.8 mL. Keep one well without antibiotic as a control of cell recovery (i.e., only add OSS).

Note

Make sure that the final concentration of the antibiotic is as determined by the resistance test.

- 11 Let the cells grow for 2–3 days, then perform 10 $\times$ serial dilutions of cells from each selection well of the 24-well plate into a 48-well plate to select individual clones.

Note

This phase usually takes up to 2 weeks.