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Version 2

Agrobacterium-Mediated Transformation of Fluorescent Constructs in *Caenorhabditis elegans* V.2

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

Standard protocol used for attempted genetic transformation of *C. elegans* by *Agrobacterium*

Materials

Yeast Extract Peptone (YEP) agar:

1% Bacto peptone, 1% Yeast extract 0.5% NaCl, 1.5% BioAgar

Yeast Extract Peptone (YEP) media:

1% Bacto peptone, 1% Yeast extract 0.5% NaCl

Induction Media

1% NH₄Cl, 0.145% MgSO₄, 0.15 KCl, 0.01% CaCl₂, 0.0025% FeSO₄-7H₂O, 2 mM NaPO₄ (pH 5.6), 50 mM 2-(4-morpholino)-ethane sulfonic acid (MES), 0.5% glucose

LB-Nematode Grown Media (NGM) Agar:

1% tryptone, 0.5% yeast extract, 1% NaCl, 1mM CaCl₂, 1mM MgSO₄, 1 mM KPO₄ (dx.doi.org/10.17504/protocols.io.bnh2mb8e), 1.7% BioAgar, 5 µg/mL Cholesterol



Safety warnings

! *Agrobacterium* have the potential for transformation into Human cells. Wear gloves and lab coat to ensure you don't come into contact with the *Agroabcterium*. In case of contact of *Agrobacterium* with skin wash the exposed area, In case of contact of *Agrobacterium* with eyes, flush eyes with water.



- 1 Streak *Agrobacterium* (expressing T-binary vector of interest) to Yeast Extract Peptone (YEP) agar plates containing appropriate antibiotics to maintain T-binary and Vir helper plasmids. Incubate for 3 days at 28°C. 3d 
- 2 Pick a single colony of *Agrobacterium* into 20 ml YEP media (with appropriate antibiotics). Incubate overnight at 28°C and 250-rpm. 1d
- 3 Dilute the overnight culture 1:100 and incubate at 28°C and 250-rpm until the *Agrobacterium* reach an OD600 of 0.3. 6h
- 4 Pellet the *Agrobacterium* by centrifugation (5 min 4000g). 5m
- 5 Resuspend the pellet in 1 ml induction medium containing acetosyringone (100 µM), and shake at 50-rpm at room temperature for 23 hours. 23h
- 6 Add 0.5 ml of *Agrobacterium* in induction medium to LB-NGM plates with appropriate antibiotics and allow to dry. 1h
- 7 Wash a mixed population of *C. elegans* from a plate using M9 buffer, and wash three times by centrifuging (2 minutes at 1500 rpm), and resuspending in M9. 10m
- 8 Add 15 *C. elegans* worms to LB-NGM plates with *Agrobacterium*, and incubate at 20°C for 4 days before begining to observe fluorecence using widefield fluorescence microscopy. 4d