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# Agrobacterium-Mediated Transformation of Fluorescent Constructs in Caenorhabditis elegans V.1

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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, and 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, 1mM KPO4, 1.5% bioagar

## 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 at 28°C and 250-rpm until the *Agrobacterium* reach an OD600 of 0.3. 6h

3 Pellet the *Agrobacterium* by centrifugation (5 min 4000g). 5m

4 Resuspend the pellet in 1 ml induction medium containing acetosyringone (100 µM), and shake at 50-rpm at room temperature for 23 hours. 23h

5 Add 0.5 ml of *Agrobacterium* in induction medium to LB-NGM plates with appropriate antibiotics and allow to dry. 1h

6 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

7 Add 15 *C. elegans* worms to LB-NGM plates with *Agrobacterium,* and incubate at 20°C for 4 days before begining to observe fluorescence using widefield fluorescence microscopy. 4d