

Transformation of Tobacco

Supply and Reagents:

Tobacco Germination Medium (TGM)

Final Concentration	1L	500ml	250ml
0.5XMS	50ml	25ml	12.5ml of 10X MS
1XB5 Vitamin	2ml	1ml	500ul of 500X
2% Sucrose	20g	10g	5g
7.5% Agar	7.5g	3.75g	1.875g

Adjust pH to 5.7 with 1N KOH. Autoclave for 20mins.

Co-Culture Medium (LDMI):

Final Concentration:	1L	500ml	250ml
1X MS	50ml	25ml	12.5ml of 10X MS
1X B5 Vitamin	2ml	1ml	500ul of 500X
2% Sucrose	20g	10g	5g
1 ug/ml BA (BAP)	1ml	500ul	250ul of 1mg/ml
0.1 ug/ml NAA	100ul	50ul	25ul of 1mg/ml
0.27.5% Agar	7.5g	3.75g	1.875g

Adjust pH to 5.7

Selection Medium (LDMII)

Prepare 1Liter **LDMI**.

After autoclave, cool down, before pouring medium onto petri dishes, add Kanamycin (50-100 ul of 100mg/ml) and Cefotaxime (2.5ml of 200mg/ml) or add Kanamycin (50-100ul of 100mg/ml) and Timentin (667ul of 300mg/ml).

Rooting medium (LDMIII)

Prepare 1liter **TGM**.

After autoclave, cool down, before pouring medium onto petri dishes, add Cefotaxime (2.5ml of 200mg/ml) and Kanamycin (50ul of 100mg/ml) or add Timentin (667ul of 300mg/ml) and Kanamycin (50ul of 100mg/ml)

1X MS medium: autoclave

Protocols:

1. Preparation of plant materials

See germination: sterilize seeds in 10% clorox for 10mins, wash 4X with sterile water, and put on TGM plates, incubate onto a petri dish, and after germination, transfer to a Magenta box in the tissue culture room (25°C, 16h/8h light/dark).

Subculture: When plant is 1-3 month old, their shoot can be cut and transferred into a new Magenta box.

2. Grow Agrobacterium C58, which harboring your construct, in LB medium with Kanamycin (50ug/ml) and Gentomycin (50ug/ml) at 28°C overnight in 5ml. Inoculate 100ul into 25ml LB medium with same antibiotics, and culture until they are ready for transformation (about 12h).
3. Harvest the young leaves, use scalpel to cut the middle part of leaf (contain vein) in 1X0.5cm pieces, and put in petri dish containing bacterial suspension (2ml LB culture in 20ml 1XMS) for less than 5mins.
4. Used paper towels to remove excess medium, and transfer the explant onto LDM1 medium. Incubate for 2 days.
5. Transfer onto LDMII medium. You can use paper towel to remove excess solution. If agrobacterim grow too much, wash the explant 3X with sterile water.
6. Subculture every 1-2 weeks.
7. After 3-5 weeks. Shoot grow. When shoot are big enough, cut the shoot and transfer onto LDMIII plate.
8. Once rooting, subculture on LDMIII medium in Magenta box, or move to the greenhouse.