BTI Tomato Transformation Protocol

last updated August 18, 2021

A) Preparation of Plant Material

1) Sterilize seed
   a. Immerse 0.9 - 1.0g of seed in 25 ml 20% bleach with 2 drops Tween (100 seeds~350mg). Shake on a rotary shaker at 250 rpm for 20 min.
   b. Rinse 3 times with sterile Milli-Q water.

2) Sow seed in Magenta boxes containing 1/2 MSO (approximately 25 - 30 seeds/box).

3) One day prior to inoculation with *Agrobacterium*
   a) Cut cotyledons from 6 - 8-day-old seedlings or the appropriate germination time for your material. It is very important that the first true leaves have not enlarged or opened. Often they will be visible, but barely. These are good to use.
      i. Place seedling on a sterile paper towel moistened with sterile water.
      ii. Excise cotyledon at petiole and cut tips off. Cut in half if size of cotyledon is > 1 cm. Use curved scalpel blades because they cause less damage than straight blades.
      iii. Place explants on moistened sterile Whatman paper on plates of 2Z - - medium, 50 per plate, adaxial side down.
      iv. Seal plates with Parafilm.
      v. Include 10 explants on one plate for controls.
      vi. Culture 24C ± 2C, 16 hr photoperiod.

B) Agrobacterium

1) Streak AGL1 *Agrobacterium* onto MG/L Carb selective medium. Incubate 36 - 48 hrs at 28C until well-formed colonies have developed.

2) Select 4, single, well-formed colonies from the plate and transfer to 50 ml of LB selective medium with antibiotic selection appropriate for the vector. Culture in a shaking incubator 250 rpm at 28C overnight.

3) Check the OD_{600}. Optimum OD_{600} = 0.6. If OD_{600} > 0.65, dilute the culture until the OD_{600} reading is below 0.5 and grow for another 30 minutes. Check the OD_{600} reading periodically.

4) Centrifuge at 8000 rpm (Sorvall centrifuge, SS34 rotor) for 10 minutes at 20C.

5) Note and record the volume of the supernatant. Discard the supernatant. Add 10-30 ml of MS-0, 2% liquid medium.

6) Resuspend the pellet by vortexing 3 – 4 sec, 3 separate times. Then bring the volume up to the recorded volume in step #5. Inoculum is ready to use.

C) Transformation

1) Incubate explants in *Agrobacterium* culture/MS-O,2%
   a. Pipette 25 ml of *Agrobacterium* culture into a sterile Magenta box.
   b. Transfer 100 explants to 25 ml inoculum in Magenta box.
   c. Incubate for 5 min with occasional shaking.
   d. Remove explants to a sterile paper towel.
   e. Return explants to plates containing 2Z - - medium, adaxial side down.
   f. Seal plates with Parafilm.

2) Cocultivate explants in the dark at 19C or 25C if 19C not available, for 48 hrs.

3) Transfer 25 explants to each plate of 2Z selection media, adaxial side up. Seal plates with Micropore tape. Culture at 24C ± 2C, 16-hr photoperiod, for two weeks.

4) After 2 weeks, transfer to 1Z selection medium (15 explants per plate).

5) Transfer explants to new 1Z selection medium plates every 2 weeks. When shoots begin to appear and touch the lid of the plate, transfer explants to 1Z selection medium in Magenta boxes (5 explants per box).

D) Regeneration and Rooting

1) Initial shoots should appear within 4 - 6 weeks.

2) Excise shoots from explants when shoots are at least 2 cm and include at least 1 node. Place in Magenta boxes containing Tomato Rooting Media with selective agent and Timentin. Place no more than 4 shoot cuttings per box.

3) Roots should begin to appear in 5 – 9 days.

MEDIA

1/2 MSO

	Per liter
MS salts	2.15 g
Myoinositol	100 mg
Thiamine HCl stock (1 mg/ml)	2 ml
Pyridoxine HCl stock (0.5 mg/ml)	1 ml
Nicotinic acid stock (0.5 mg/ml)	1 ml
Sucrose	10 g
pH to 5.8 ± 0.03	
Sigma Agar	8 g

2Z- preculture[1]

	Per liter
MS salts	4.3 g
Myoinositol	100 mg
Modified Nitsch Vitamins stock (1000X)	1 ml
Sucrose	20 g
pH to 6.0 ± 0.03	
Sigma Agargel	5.2 g
Zeatin trans-isomer (1 mg/ml)[2]	2 ml

LB

	Per liter
Bacto Tryptone	10 g
Bacto Yeast Extract	5 g
NaCl	10 g

MGL

	Per liter
Tryptone	5 g
Yeast Extract	2.5 g
NaCl	5 g
Mannitol	5 g
MgSO4	100 mg
K2HPO4	250 mg
Glutamic Acid	1.2 g
Sucrose	15 g
pH to 7.2 ± 0.3	
Bactoagar	15 g
Carbenicillin (100 mg/ml)[2]	0.5 ml
Selective agent[2]	

For selective agent, we have used kanamycin and spectinomycin both at 50 mg/l.

MS 0, 2% Liquid Medium

	Per liter
MS salts	4.3 g
Myoinositol	100 mg
Thiamine HCl	0.4 mg
Sucrose	20 g
pH to 5.8 ± 0.3	

2Z Selection Medium[1]

	Per liter
MS salts	4.3 g
Myoinositol	100 mg
Modified Nitsch Vitamins stock (1000X)	1 ml
Sucrose	20 g
pH to 6.0 ± 0.3	
Agargel	5.2 g
Zeatin trans-isomer (1 mg/ml)[2]	2 ml
Timentin (100 mg/ml)[2]	3.5 ml
Selective agent[2,3]	

1Z Selection Medium[1]

	Per liter
MS salts	4.3 g
Myoinositol	100 mg
Modified Nitsch Vitamins stock (1000X)	1 ml
Sucrose	20 g
Agargel	5.2 g
pH to 6.0 ± .03	
Zeatin trans-isomer (1mg/ml)[2]	1 ml
Timentin (100mg/ml)[2]	3.5 ml
Selective agent[2,3]	

Selective Rooting Medium

	Per liter
MS salts	4.3 g
Modified Nitsch Vitamins stock (1000x)	1 ml
Sucrose	30 g
pH to 6.0 ± 0.03	
Difco Bacto Agar	8 g
Timentin (100 mg/ml)[2]	3.5 ml
Selective agent[2,3]	

[1] **For Petri plates, use the 100 x 20 mm size**, which are deeper than the standard 100 x 15 mm used for bacterial cultures.

[2] These media components should be added after autoclaving, when medium has cooled to the point that it can be comfortably held while pouring (~55C). We cool our autoclaved medium for one hour in a water bath to 55C.

[3] Use a concentration of selective agent appropriate for your tomato line. Selection agents we have used include: kanamycin (50, 75, 100, 200mg/l) and hygromycin (6 mg/l). We recently modified the kanamycin concentration and increased it to 200 mg/l.

MEDIA COMPONENTS AND STOCK SOLUTIONS

Carbenicillin (100 mg/ml)
Dissolve 5 g carbenicillin in 50 ml ddH_2O. Filter sterilize and store in 1 ml aliquots at -20C. Add after autoclaving.

Kanamycin (100 mg/ml)
Dissolve 5 g kanamycin in 50 ml ddH2O. Filter sterilize and store in 1 ml aliquots at -20C. Add after autoclaving.

Modified Nitsch Vitamins Stock (1000x)	**50 ml**
Glycine	0.1 g
Nicotinic acid	0.5 g
Pyridoxine HCl	0.025 g
Thiamine HCl	0.025 g
Folic acid	0.025 g
d-biotin	0.002 g

pH to 7.00 ± 0.03. Store in 1 ml aliquots at -20C.

Nicotinic Acid (0.5 mg/ml)
Dissolve 25 mg nicotinic acid in 50 ml ddH_2O. Store in 1 ml aliquots at -20C.

Pyridoxine HCl (0.5 mg/ml)
Dissolve 25 mg pyridoxine HCl in 50 ml ddH_2O. Store in 1 ml aliquots at -20C.

Spectinomycin (50 mg/ml)
Dissolve 2.5 g spectinomycin in 50 ml ddH_2O. Filter sterilize and store in 1 ml aliquots at -20C. Add after autoclaving.

Thiamine HCl (1 mg/ml)
Dissolve 50 mg thiamine HCl in 50 ml ddH_2O. Wrap in foil and store at 4C.

Timentin (100 mg/ml)
Timentin is based on ticarcillin content. Dissolve 5.17 g timentin (GoldBio #T-104) in 50 ml ddH_2O. Filter sterilize and store in aliquots at -20C. Add after autoclaving.

Zeatin trans-isomer (1 mg/ml)
Dissolve 50 mg zeatin trans-isomer in a few drops of 0.5M HCl. Add ddH_2O to a total volume of 50 ml. Filter sterilize and store in 1 ml aliquots at -20C. Add after autoclaving.

CATALOG NUMBERS

Component	Company	Catalog #
Agar	Sigma	A1296
Agargel	Sigma	A3301
Carbenicillin	Phytotechnology	C346
Difco Bacto Agar	Fisher Scientific	DF0140-15-4
d-Biotin	Phytotechnology	B140
Folic Acid	Phytotechnology	F430
Glycine	Phytotechnology	G503
Hygromycin	Phytotechnology	H385
Kanamycin	Phytotechnology	K378
MS Salts	Caisson Labs	MSP0501
Nicotinic Acid	Phytotechnology	N765
Pyridoxine HCl	Phytotechnology	P866
Spectinomycin	Phytotechnology	S742
Sucrose	Phytotechnology	S 391
Thiamine HCl	Phytotechnology	T390
Timentin	Gold Bio	T-104
Zeatin trans-isomer	Caisson Labs	Z007
Micropore Tape 1/2"	VWR	S6222-182