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Tomato Transformation

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is slightly modified from the citatation below. All credit for this protocol should be directed toward those authors.

This protocol details how to transform tomato (Solanum lyycopersicum) with Agrobacterium and to regerneate the plants via tissue culture.

Materials

MATERIALS

- Difco Bacto Agar **Carolina Catalog #**156783B
- Sucrose
- Luria broth powder
- Glycine **Bio Basic Inc. Catalog #**GB0235.SIZE.2.5Kg
- Nicotinic acid (NIACIN) **Bio Basic Inc. Catalog #**NB0660.SIZE.250g
- Myo-Inositol **Gold Biotechnology Catalog #**I-525
- Kinetin **Gold Biotechnology Catalog #**K-100
- Pyridoxine HCl (Vitamin B6) **Gold Biotechnology Catalog #**P-780
- Timentin™ Ticarcillin/Clavulanate (15/1) **Gold Biotechnology Catalog #**T-104
- Thiamine HCl **Gold Biotechnology Catalog #**T-260
- trans-Zeatin **Gold Biotechnology Catalog #**Z-105
- Murashige and Skoog with Nitsch Vitamins **Caisson Labs Catalog #**MSP29-50LT
- Murashige & Skoog Basal Salts **Caisson Labs Catalog #**MSP01-50LT
- Agargel **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A3301

100mm x 15mm petri dishes (Fisher #FB0875713)
100mm x 20mm petri dishes (VWR #82050-918)
16oz soup containers with lids (Fabri-Kal #9501034 and #9501070)
Micropore Tape (Fisher #19-061655)

Before start

- Prepare 100mm x 15mm plates of MSO and KCMS
- Prepare 100mm x 20mm plates of 2Z
- Prepare 16oz soup containers of 1Z and rooting media
- Prepare liquid LB and MSO-2% media

Sterilize and Sow Seeds

1 Surface sterilize seeds with chlorine gas according to the following protocol

Protocol

NAME

Seed Sterilization

CREATED BY

Alex Rajewski

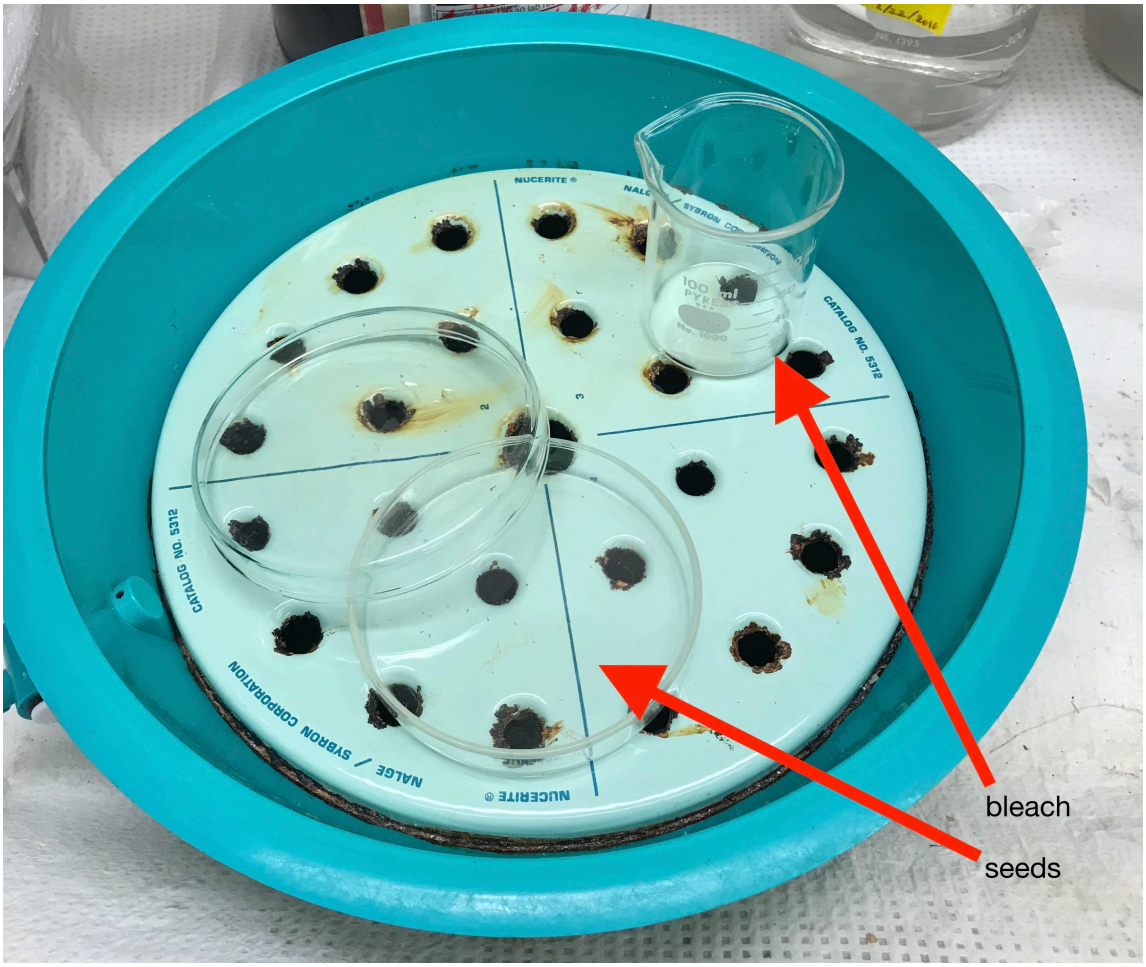
PREVIEW

1.1 In a fume hood, place an empty dessication chamber with a petri dish of the seeds you wish to surface sterilize. Leave the lid of the petri dish halfway on, so that you can quickly close it later.

Note

- A dessication chamber per se is not necessary; you simply need some container to hold the gas for a few hours. It does not need to be thoroughly airtight, but it helps.
- If you plan to sterilize multiple genotypes of seeds, be aware that the gas produced will erase most markers. Pencil and paper labels work well though.

1.2 Place a 100mL beaker containing approximately 50 mL bleach in the chaber next to the petri dish of seeds.



1.3 Prepare approximately 3 mL HCl in a transfer pipet or similar.

1.4 The following steps must occur quickly to prevent the escape of the gas. Hold the lid to the chamber in one hand. With the other hand squirt the HCl into the beaker of bleach and quickly close the chamber to seal in the gas.

Note

The HCl and the bleach will react violently to produce the gas, do not be startled. The larger beaker should prevent any bubbles of bleach or HCl from contacting the rest of the set up.

1.5 Allow the seeds to be sterilized for approximately 3 hours. This time works for tomato, tobacco, and Arabidopsis seeds. The seeds may become whiter in color, this is also normal and does not seem to affect germination rates.

1.6 Once the time is up, quickly open the chamber and slide the lid completely onto the petri dish to seal it. The seeds can be stored like this for several weeks or transfered to a laminar flow hood for further use. Allow the chmaber to air out for an hour or so.

1.7 If the reaction of the bleach and HCl went to perfect completion, the beaker should only contain salt water. In reality it is likely acidic. Dispose of this according to your local regulations.

2 Sow the seeds on plates 1/2 MSO media and place in the growth room in the light. They can be sown fairly densely because they won't spend long on this media.

Prepare Agrobacterium

3 About two days before the transformation (Step 8) and one day before the cotyledons are to be dissected (Step 4) inoculate a 25mL broth of selective LB with Agrobacteria and incubate for ~36 hours at 28ºC.

Note

Selection will depend on the Agro strain and plasmid you are using. We find that when incopulating from a glycerol stock of Agrobacterium and using kanamycin selection, a 25mL broth will take approximately 36 hours to grow to the correct density. It is best to know how fast your Agro will likely grow beforehand so you can time the transformation well.

Prepare Cotyledons

4 One day before the transformation (step 8), after the cotyledons have emerged but before the first true leaves appear (about 6-8 days after sowing for tomato), take the plates sterilely into the hood and (one at a time) place each seedling onto the lid of a new sterile petri dish. Using an autoclaved scalpel cut off each cotyledon at its petiole. Also cut off the tip of the cotyledon, and, if the cotyledon is larger than 1cm, cut it in half.

Note

- When dissecting a cotyledon, make sure to keep the lid of the seedling plate closed to prevent dessication, which will make dissection much harder.
- For other species (ie tobacco) the cotyledons will not be large enough to remove the tip without destroying the cotyledon.
- In tobacco, the petiole is often so short that the apical meristem is captured along with the cotyledon. Including this tissue leads to faster regeneration, but we have not throughly evaluated if this leads to untransformed plants in tomato.

5 Place the excised cotyledons adaxial (top) side-down onto plates containing KCMS media, seal with micropore tape, and allow to grow for one day at 25ºC, 16-hour days.

Prepare Agrobacterium

6 Early on the day of the transformation, check the OD_{600}. The optimum is 0.6-07. If the OD is above 1.0, dilute it to below 0.5 and allow to grow for another hour. Once the optimum OD is reached, transfer the broth into a 50mL falcon tube and centrifuge at 8000rpm for 10 minutes.

7 Observe the volume of the supernatant, discard it, and resuspend the pellet with the same volume of liquid MS0-2% media. The inoculum is now ready to use.

Transformation

8 In the sterile hood, transfer the explants to the Falcon tube of Agrobacteria and incubate for 5 min with occasional shaking.

9 Pour the suspension of agrobacterium and cotyledons into an empty, sterile petri dish for better access. Transfer the cotyledons from this suspension to new KCMS plates (adaxial side-down again), seal with micropore tape, and culture for 48 hours in the dark.

Note

- For tomato, you can fit ~20 cotyledons on a single plate.
- Alternatively, you can pour the suspension onto sterile paper towels and then transfer the cotyledons onto KCMS plates. We have found that this infrequently introduces yeast contamination.

Regeneration

10 In the sterile hood, transfer the explants to selective 2Z media with their adaxial side up, seal with micropore tape, and culture at 25ºC and 16-hour days. Transfer the explants to new 2Z media twice weekly for two weeks.

Note

- Plant selection and concentration here will depend on the T-DNA being used for transformation.
- This media should also include timentin (300mg/L) in order to kill the Agrobacterium.
- We are currently experimenting with adding indole-3-butyric acid (1mg/L) to this media as well.

11 After two weeks, transfer the explants to selective 1Z media (~15 per container) and refresh on new selective 1Z media once every two weeks until shoots begin to form. Once shoots have formed (4-6 weeks), reduce the explants to 5 per container and continue to refresh as usual. (See note above on selection.)

12 Once shoots are at least 2cm long and have at least one node, excise them and place them singly into containers with selective Rooting Media. For tomatoes, roots should form within about 2 weeks.

13 For propagation of confirmed lines, transfer to nonselective Rooting Media. This allows the plants to grow faster and will let you check if Agrobacterium is still present. However, do not transfer to antibiotic-free media until the shoots have undergone at least 3 transfers onto media with antibiotics and selection.