VERSION 1

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Tomato Transformation Media V.1

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ABSTRACT

This protocol is simply the media recipes for use with my tomato transformation protocol.

OPEN ACCESS

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

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PROTOCOL integer ID:
20391

MATERIALS

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- ✕ Difco Bacto Agar Carolina Catalog #156783B
- ✕ Sucrose Contributed by users
- ✕ Luria broth powder Contributed by users
- ✕ Glycine Bio Basic Inc. Catalog #GB0235.SIZE.500g
- ✕ 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 Millipore Sigma 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**)

BEFORE START INSTRUCTIONS

It helps to make up stock solutions of the vitamins, hormones, and antibiotics ahead of time and freeze them.

- Since the concentration of phytohormones and vitamins can vary across medias, we make them both up to a standard concentration of 1mg/mL for easy math.
- Antibiotics are typically used at a single concentration in all of our medias, so they are made up as a 1000x stock. That is, their actual concentration varies depending on the antibiotic, but in every case you will add 1µL of stock for every 1mL of media.

- 1** To make 1L, for each media, dissolve the following components to ~800mL of MilliQ water in a 1L beaker

Component:	1/2MSO	MS0-2%	KCMS	2Z	1Z	Rooting
MS Basal Salts	2.15g	4.3g	4.3g	--	--	--
MS Salts with Nitsch Vitamins	--	--	--	4.3g	4.3g	4.3g
Sucrose	10g	20g	30g	20g	20g	30g
myo-Inositol	100mg	100mg	100mg	100mg	100mg	--
KH ₂ PO ₄	--	--	200mg	--	--	--
Thiamine HCl (1mg/mL)	2mL	400μL	1.3mL	--	--	--
Pyridoxine HCl (1mg/mL)	500μL	500μL	--	--	--	--
Nicotinic Acid (1mg/mL)	500μL	500μL	--	--	--	--
Glycine	--	2mg	--	--	--	--
2,4-D (1mg/mL)	--	--	200μL	--	--	--
Kinetin (1mg/mL)	--	--	100μL	--	--	--

- 2** Bring volume to 1L and adjust pH (±0.03) according to the table below:

	1/2MSO	MS0-2%	KCMS	2Z	1Z	Rooting
pH	5.8	5.6	5.5	6.0	6.0	6.0

- 3**

Divide the volume into two screw-top jars and add the following gelling agent. Note that not all medias require a gelling agent, and that this mass is the *TOTAL* mass added for 1L. It is not necessary to dissolve this powder; it will dissolve in the autoclave.

	1/2MSO	MS0-2%	KCMS	2Z	1Z	Rooting
Agar	8g	--	--	--	--	--
Agargel	--	--	5.2g	5.2g	5.2g	--
Difco Bacto Agar	--	--	--	--	--	8g

Only half this mass should go into each screw-top jar.

- 4** Autoclave the media, and allow to cool to ~50°. Add any antibiotics and phytohormones (filter-sterile) according to the table below:

	1/2MSO	MSO-2%	KCMS	2Z	1Z	Rooting
Zeatin (1mg/mL)	--	--	--	2mL	1mL	--
Timentin (300mg/mL)	--	--	--	1mL	1mL	--
Antibiotics	No	No	No	Yes	Yes	Maybe