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700 - Infection Medium V.3

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Transformation of B104 m…

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We use this protocol and it's working

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Disclaimer

Abstract

This is part of the Leiboff Lab maize transformation protocol for somatic embryogenesis of B104 immature embryos. This protocol is a combination of Chen et al. 2022 and Kang et al. 2022 with some modifications based on material availability. This protocol is intended for the GRF-GIF/BBM somatic embryogenesis transformation strategy with the LBA4404 Met- auxotrophic *Agrobacterium* strain.

Embryos will dissected and transferred to 700 prior to infection with 700 + L-Methionine (50mg/L) and Acetosyringone (100uM) with Agrobacterium at 0.35-0.4 OD600. Infection Medium 700 is used for 5 minutes before transferring infected embryos to 562V-MSM Co-cultivation medium. Infection Medium contains added synthetic auxin (2,4-D), a high level of sucrose, and glucose to encourage rapid plant growth. 700AMet is supplemented with Acetosyringone tpB contains 5 mg/L of bialaphos (preferred for basta selection in maize over glufosinate) as a plant selective agent, and uses both Cefotaxime and Timentin to control *Agrobacterium* contamination. The antibiotic concentrations used here are sufficient to control the LBA4404 Met- auxotrophic strain, but were not sufficient to control wild-type LBA4404 in 3 prior trials.

700 liquid media should be prepared in standard glass bottles, planning for 50 mL per construct. Pelleted Agrobacterium will be resuspended in 700 + L-Methionine (50mg/L) and Acetosyringone (100uM) at 0.35-0.4 OD600 and shaken in the dark for 2-6 hours at RT. Dissected embryos will be briefly stored in 700, rinsed in 700, then combined with infection media with Agrobacerium. Material grown on 605CefTB will be sealed with micropore tape and be incubated at 28C in the dark. Embryos are ready to move off 605CefTB after 1 week. There should be noticeable growth on the scutellum side of the embryo at this time and somatic embryos may be established, but do not be alarmed if this is not obvious.

Planning

1 Estimate the volume of 700 you will need based on the following:

$$Volume = 50mL * NumberConstructs$$

Make sure to round up! Check the table below to plan your media
Do not forget to add L-Methionine or Acetosyringone prior to transformation

Mixing Heat-Stable Ingredients

2 Retrieve the following heat-stable ingredients:
1. Murashige and Skoog (MS) Basal Salts - Stored in Main Lab, Chemical Shelf 'M'
2. 2,4-D (5 mg/mL) - Stored in Main Lab, -20C Freezer, Bottom drawer 'Tissue Culture 1'
3. Sucrose - Stored in Main Lab, Chemical shelf 'S', use Fowler refillable container
4. D-Glucose - Stored in Main Lab, Chemical shelf 'G'

3 Retrieve a graduated cylinder for measuring your final solution
Place a stir bar at the bottom on a beaker that is ~1.5x the volume of your solution
Rinse stir bar+beaker and graduated cylinder with MQ H2O, discard rinse water in sink
NOTE: Any soap or detergent residue will interfere with the tissue culture process; if you see suds, rinse again or find different glassware

4 Add approximately 90% of your final media volume in MQ H2O to your beaker
Place beaker on a magnetic stir plate
Turn stir plate on to generate a vigorous stir

5 Using a fresh weigh paper and dry spatula/scoopula/pipette tip for each ingredient, add the following to your beaker:

	A	B	C	D	E
	Ingredient	50 mL	100 mL	150 mL	200 mL
	MS Salts	0.22 g	0.44 g	0.66 g	0.88 g
	2,4-D	15 uL	30 uL	45 uL	60 uL
	Sucrose	3.425 g	6.85 g	10.275 g	13.70 g
	D-Glucose	1.80 g	3.60 g	4.40 g	7.20 g

6 Thoroughly rinse all used tools with running water
Place clean tools in drying rack
Return chemical reagents to their original storage location

Adjust solution pH to 5.7 with 0.1 M KOH

7 Turn on the Hanna Instruments pH meter
Unscrew and remove the small green pH probe exchange cover and set cap aside
Gently remove the probe from the storage tube and set storage tube aside
Using squeeze bottle, rinse the glass probe with H2O, catch rinse water in a waste beaker
Gently blot probe with laboratory tissue paper to dry

8 Using adjustable arm, lower the pH probe into the beaker with stir plate on
Ensure that the stir bar does not strike the probe
Electrode at the base of the probe must be fully submerged

9 Using a plastic transfer pipette, add 0.1M KOH to your solution until you measure pH 5.7
NOTE: KOH can be added rapidly until pH 5.4, then add one drop at a time to reach pH 5.7
Solution pH between 5.6 - 5.8 is acceptable

10 Using the adjustable arm, remove the pH probe from the beaker
Using squeeze bottle, rinse the glass probe with H2O, catch rinse water in a waste beaker
Gently blot probe with laboratory tissue paper to dry
Return the probe to the storage tube -- Ensure the electrode bulb is fully submerged in storage solution
Return and secure the small probe exchange cover
Turn off the pH meter

Bring solution to target volume and autoclave

11 Turn off the stir plate and remove your beaker
Hold a large stir bar in your hand to stabilize the one in your beaker
Pour your solution into the graduated cylinder -- Do not include the stir bar
Add a small amount (50-100 mL) of water to your beaker
Carefully add water from the beaker to the graduated cylinder until your solution reaches the target volume -- Do not include the stir bar

12 Loosely place the cap over the bottle
Add a small piece of autoclave tape on the cap and bottle
Place the bottle in an autoclave-safe bin
Autoclave 20-25 min using the 'Liquid' setting
NOTE: Recommended autoclaves are in Cord 3112 and 4112. Complete cycle will take ~1 hr.

13 Rinse all used tools and glassware in running water
Place clean items on drying rack
Return chemical reagents to their original storage location

14 Return to the autoclave to pick up your solution -- Be prompt, sucrose can degrade if left too long
Using autoclave gauntlets, gently seal the cap of the bottle
Swirl the autoclaved solution to evenly mix
Carefully return to the lab with autoclave bin and sealed bottle
Discard any liquid remaining in the autoclave bin and return to bin storage
NOTE: Your solution must cool completely to room temperature before it can be used

15 Sealed, cooled bottles of 700 can be stored at 4C for 1-2 weeks
Check for contamination prior to use
Remember to add L-methionine and acetosyringone for Agro resuspension and transformation steps (700 -> 700A)

Protocol references

Chen, Zongliang, Juan M. Debernardi, Jorge Dubcovsky, and Andrea Gallavotti. 2022. “The Combination of Morphogenic Regulators BABY BOOM and GRF-GIF Improves Maize Transformation Efficiency.” *bioRxiv*. https://doi.org/10.1101/2022.09.02.506370.

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