Arabidopsis Germination Media (Raizada Lab) for 1L

ddH20	up to 1L
MS+B5 (phytotech M404)	2.2 g/L
MES	0.97g/L
Sucrose (1% final)	10g/L
---	---
	1L (final)

pH to 5.7 with 1M KOH while stirring (make sure you calibrated the machine that day using pH 4 and pH7 standard solutions).

Volume up to 1L final with ddH20

Place into 2L flasks

Add Phytagel 3g/L
(swirl immediately)

Cover flasks with tinfoil and add autoclave tape as an indicator.

Autoclave on liquid cycle. Turn on large water baths (2L per bath).

Cool to 58-60C 45min. Use red weight rings to stabilize flasks in water. Turn on tissue culture hood and sterilize.

Could prelabel dishes with date and media type at edge of plate using price gun.

In sterile hood, pour into deep Petri dishes (100mm x 25mm). Usually ~25-30/L.

Dry ~90 minutes with lids partially opened in hood OR leave in hood overnight to dry (with lids on completely and hood left on).

Store at 4C packaged back into original sleeve and labelled on the outside.

Arabidopsis CIM (Callus Induction) Media (Raizada Lab)

ddH20	up to 1L
Gamborg's B5 with vitamins pkg (Sigma G5893)	1pkg
MES	0.5 g
Glucose/Dextrose (2% final)	20g
---	---
	1L (final)

pH to 5.8 with 1M KOH while stirring (make sure you calibrated the machine that day using pH 4 and pH7 standard solutions).

Volume up to 1L final with ddH20

Pour into 2L flasks. Include a stir bar. Wrap top of flask with tin foil and piece of autoclave tape.

Add Phytagel 3g/L (if expect a lot of root growth, decrease this to 2.5g/L to make it easier to remove the roots later for weighing)

Autoclave on liquid cycle. Turn on large water baths (2L per bath).

Cool to 58-60C 45min. Use red weight rings to stabilize flasks in water. Turn on tissue culture hood and sterilize.

Could pre-label dishes with date and media type at edge of plate using price gun.

In sterile hood, add hormones (kept in fridge/freezer in Room 312):

Kinetin (cytokinin)(1mg/ml stock)	100uL per 1L media (0.1mg/L final)
2,4-D (auxin)(1mg/ml stock)	500uL per 1L media (0.5mg/L final)

Cover flask again and stir for 1 minute.

In sterile hood, pour into deep Petri dishes (100mm x 25mm). Usually ~25-30/L.

Dry ~90 minutes with lids partially opened in hood OR leave in hood overnight to dry (with lids on completely and hood left on).

Store at 4C packaged back into original sleeve and labelled on the outside.

Arabidopsis SIM (Shoot Induction) Media (Raizada Lab)

ddH20	up to 1L
Gamborg's B5 with vitamins pkg (Sigma G5893)	1pkg
MES	0.5 g
Glucose/Dextrose (2% final)	20g
	1L (final)

pH to 5.8 with 1M KOH while stirring (make sure you calibrated the machine that day using pH 4 and pH7 standard solutions).

Volume up to 1L final with ddH20

Pour into 2L flasks. Include a stir bar. Wrap top of flask with tin foil and piece of autoclave tape.

Add Phytagel 3g/L (if expect a lot of root growth, decrease this to 2.5g/L to make it easier to remove the roots later for weighing)

Autoclave on liquid cycle. Turn on large water baths (2L per bath).

Cool to 58-60C 45min. Use red weight rings to stabilize flasks in water. Turn on tissue culture hood and sterilize.

Could pre-label dishes with date and media type at edge of plate using price gun.

In sterile hood, add hormones (kept in fridge/freezer in Room 312):
2ip (cytokinin) (1mg/ml stock) 894uL per 1L media (4.4 uM final)
NAA (free acid; if Ksalt see below) (auxin)(1mg/ml stock) 93uL per 1L media

Cover flask again and stir for 1 minute.

In sterile hood, pour into deep Petri dishes (100mm x 25mm). Usually ~25-30/L.

Dry ~90 minutes with lids partially opened in hood OR leave in hood overnight to dry (with lids on completely and hood left on).

Store at 4C packaged back into original sleeve and labelled on the outside.

New regeneration media B (all unchanged except 2-iP):
2iP = 6-(gamma-gamma-dimethyallylamino)purine
FW = 203.2 g/mol
n=cv=4.4uM x 1L=0.0000044 mol x 1L=0.0000044moles
m=Mn=203.2g/mol x 0.0000044 moles = 0.000894 g/L = 0.894 mg/L
Final amount = 0.894 mg/L of 2iP

And just to double-check, the NAA amount added:
If NAA (free acid) FW = 186.2 g/Mol
n=CV = 0.5 uM x 1L = 0.0000005 moles
m=Mn = 0.0000005 mol x 186.2 g/mol = 0.093 g/L = **0.093 mg/L**

If NAA is Potassium salt, then FW = 224.3 g/mol
n=CV=0.5uM x 1L = 0.0000005 moles
m=Mn = 0.0000005 mol x 224.3 g/mol = **0.11 mg/L**

Arabidopsis RIM (Root Induction) Media (Raizada Lab)

ddH20	up to 1L
Gamborg's B5 with vitamins pkg (Sigma G5893)	1pkg
MES	0.5 g
Glucose/Dextrose (2% final)	20g
---	---
	1L (final)

pH to 5.8 with 1M KOH while stirring (make sure you calibrated the machine that day using pH 4 and pH7 standard solutions).

Volume up to 1L final with ddH20

Pour into 2L flasks. Include a stir bar. Wrap top of flask with tin foil and piece of autoclave tape.

Add low% Phytagel 2g/L

Autoclave on liquid cycle. Turn on large water baths (2L per bath).

Cool to 58-60C 45min. Use red weight rings to stabilize flasks in water. Turn on tissue culture hood and sterilize.

Could pre-label dishes with date and media type at edge of plate using price gun.

In sterile hood, add hormones (kept in fridge/freezer in Room 312):
No Cytokinin (2-iP) added
NAA (auxin)(1mg/ml stock) 93uL per 1L media

Cover flask again and stir for 1 minute.

In sterile hood, pour into deep Petri dishes (100mm x 25mm). Usually ~25-30/L.

Dry ~90 minutes with lids partially opened in hood OR leave in hood overnight to dry (with lids on completely and hood left on).

Store at 4C packaged back into original sleeve and labelled on the outside.

Arabidopsis Basal Media (Raizada Lab)

ddH20	up to 1L
Gamborg's B5 with vitaminspkg (Sigma G5893)	1pkg
MES	0.5 g
Glucose/Dextrose (2% final)	20g
---	---
	1L (final)

pH to 5.8 with 1M KOH while stirring (make sure you calibrated the machine that day using pH 4 and pH7 standard solutions).

Volume up to 1L final with ddH20

Pour into 2L flask

Add Phytagel 3g/L (if expect a lot of root growth, decrease this to 2.5g/L to make it easier to remove the roots later for weighing)

Cover flask with tinfoil and add autoclave tape as an indicator.

Autoclave on liquid cycle. Turn on large water baths (2L per bath).

Cool to 58-60C 45min. Use red weight rings to stabilize flasks in water. Turn on tissue culture hood and sterilize.

Could prelabel dishes with date and media type at edge of plate using price gun.

Swirl the flask to mix any undissolved Phytagel.
In sterile hood, pour into deep Petri dishes (100mm x 25mm). Usually ~25-30/L.

Dry ~90 minutes with lids partially opened in hood OR leave in hood overnight to dry (with lids on completely and hood left on).

Store at 4C packaged back into original sleeve and labelled on the outside.