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Growing Arabidopsis on nutrient-rich media

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We use this protocol and it's working

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Abstract

Notes on growing Arabidopsis seedlings on nutrient-rich media in plates.

Prepare media

1 Select and prepare autoclaved media of choice (see recipes below). Make sure to pH before adding gelling agent.

	A	B
	MS basal salts	2.15 – 4.33 g/L
	Sucrose	10 - 30 g/L
	Vitamins (1000X) (optional)	1 mL/L
	MES (optional)	0.1% (w/v)
	KOH	pH 5.8
	Agar/Phytoblend	8 g/L

MS Media (Murashige & Skoog, 1962)

	A	B
	Gamborg's B-5 basal medium	3.21 g/L
	Sucrose	10 - 30 g/L
	KOH	pH 5.8
	Agar/Phytoblend	8 g/L

Gamborg B-5 Media (Gamborg et al 1968)

	A	B
	Kanamycin	30 - 50 uM
	Basta (Glufosinate Ammonium)	20 - 50 uM

Supplement for transgenic selection - add to cooled autoclaved media immediately prior to pouring plates.

2 Pour room temperature media into plates in a biosafety cabinet - keep everything sterile! Allow media to cool and solidify, then package and store at 4 °C.

Surface sterilize seed

3 Collect small aliquots of desired Arabidopsis seeds into clean microfuge tubes.

4 Add 500 uL of a solution made of 40% (v/v) household bleach, and 0.01% (v/v) Tween20 dissolved in distilled water and incubate for 5 minutes.

5 Remove supernatant, then rinse seeds with 700 uL of 70% ethanol incubating for 5-10 minutes. If you did not perform bleach sterilization step, repeat ethanol wash an additional 1-2 times.

6 Remove supernatant then rinse with distilled water at least 3 times.

7 After final rinse, plate seeds out on media plates in sterile environment (e.g. BioSafety cabinet). Seeds can be stored at 4 °C for up to 1 month (maximum).