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Hypocotyl test

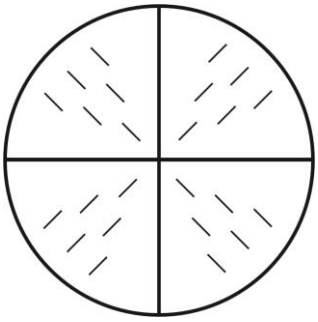
Procedure:

1. Sterilize seeds by 70% EtOH.
2. Sow seeds on square Petri dishes with GM medium and 1% saccharose.

GM medium	**final c**	**400 ml**	**1000 ml**
MS medium including Gamborg B5 vitamins		1,76 g	4,4 g
MES; pH 5,7	2,35 mM	0,2 g	0,5 g
1M KOH		0,5 ml	1,25 ml
saccharose	1%	4 g	10 g
Phytagel (Sigma)	0,3 %	1,2 g	3 g

MES = 2-(N-morpholino) ethanesulphonic acid

3. Cover plates by tape.
4. Put plates into fridge (4°C) overnight.
5. Transfer plates on light in the morning for one day. Temperature: 21 °C, humidity 70-80%.
6. Next day morning pack plates into two layers of aluminium foil a growth seedling for 5 days in the dark to etiolate hypocotyls. Temperature: 21 °C, humidity 70-80%.
7. Sterilize appropriate amount of GM medium aliquoted in flasks, cool down medium at 60°C or lower.
8. Add phytohormones, 100xDMSO and biotin into 100 ml GM medium in flow-box.
9. Cut hypocotyls after 5 days of cultivation in the dark. Cut off the shoot part, cut off the hypocotyl (+-1 cm) above the root-shoot junction and arrange it on the plate.



10. Cover plates by tape.
11. Cultivate hypocotyl explants appropriate time (1-21 days) and conditions (LD/CL, 100/150 $\mu Em^{-2}s^{-1}$), 21 °C, humidity 50-70%.
12. Imaging.