Shoot and Tuber cultures

Before beginning an Agrobacterium-mediated transformation of potato disks, it is necessary to establish axenic shoot and tuber cultures of the potato variety that will be used in the experiment.

In our lab we grow most cultures in Magenta Vessels, but other vessels such as glass tubes and jars may be substituted. All work is done under aseptic conditions.

Shoot propagation:
Meristems (usually apical) from previously established shoot cultures are transferred in 4-week intervals to fresh shoot media. Meristems are propagated four or five per vessel. Magenta vessels are adapted with couplers to achieve double the height (7.75in / 20cm) of a standard vessel. Cultures are incubated in growth rooms on laminar flow bench tops under fluorescent light supplemented with incandescent bulbs--16hr light/ 8 hr dark, 23°C, ambient humidity.

Tuber propagation:
Tuber cultures are initiated from existing shoot cultures. Nodal cuttings containing meristems are placed in magenta boxes containing tuber induction media (9 meristems per box). Leaf tissue may be removed (without disturbing meristem) to eliminate plant debris in final tuber culture. Cultures are incubated at 18°C in total darkness (ambient humidity) for 2-3 months depending on cultivar. For use in Agrobacterium infection, young and expanding, yet as large as possible. Autoclave times are for 500ml. Less media may take less time to autoclave. Autoclaving aliquots > 500ml is not recommended.

MS Salts = Caisson Labs #MSP001 = Sigma #5524 = Phytotechnology Labs #M524
LS Salts = Caisson Labs #LSP001 = Sigma #6899

Shoot Multiplication Medium (1 Liter)

LS Salts	4.4 grams
Sucrose	30.0 grams
MSMO vitamin stock	1ml of stock (freezer)
Adjust pH to 5.6	
Phytagel (GelRite)	2.0 grams

Autoclave for 20 minutes. Cool to 55°C.
Dispense to sterile Magenta boxes
Alternatively---melt in microwave, pour into tall boxes, and autoclave filled boxes

Tuberization Medium (1 liter)

LS Salts	4.4 grams
Sucrose	60.0 grams
Kinetin (cytokinin)	2.5 ml of 1mg/ml stock
Adjust pH to 5.6	
Phytagel (GelRite)	2.0 grams

Microwave to melt Phytagel.
Dispense into short boxes.
Autoclave filled boxes for 15 minutes.

MSMO Vitamin Stock (1000X)	mg/100 ml	Conc. in medium
Nicotinic Acid	100	1 mg/L
Pyridoxine HCl	100	1 mg/L

Filter sterilize after dissolving vitamins. Aliquot to sterile tubes and freeze at -20°C.