

Cauliflower cloning

Technical & Teaching Notes

Introduction

This method of cauliflower cloning uses the sterilising agent Sodium Dichloroisocyanurate (SDICN) to sterilise the cauliflower explants. This bleaching agent is commonly used to sterilise babies' bottles and for emergency drinking water purification. Due to its gentle action the sterilant does not need to be rinsed off the plant material before adding the explants to the media. The agar plant growth media in this method also contains low levels of the SDICN to help maintain a clean culture. The combination of no rinsing and a background level of sterilant greatly reduces contamination over previous methods of plant cloning in schools.

The technique is based on a protocol developed by plant scientists at the Royal Botanic Gardens, Kew, and is used in their conservation programmes, allowing critically endangered plant material to be cloned in the field, rather than waiting until a 'clean lab' can be found to rescue plant material.

Demo videos

Our demo videos show how best to use this in the classroom:

www.youtube.com/watch?v=cEbGnJnVuLI

and how to prepare the growth medium:

<https://www.youtube.com/watch?v=Nw3ZQGBnsWc&t=2s>

Safety Notes

Risk assessments should be prepared for students and also for staff involved with media preparation

Students

- Safety Glasses to be worn at all times
- Sodium Dichloroisocyanurate is toxic and a bleach that removes colour from clothing
- Wear protective apron /lab coat and gloves when handling bottles containing the sterilant as caps may leak.
- Do not inhale chlorine vapours from SDICN.
- Beware sharp instruments.

Technician / teacher

- In addition to above, when preparing media beware hot liquids when using microwave
- Follow CLEAPSS / SSERC guidelines when weighing chemicals: wear a mask and gloves
- Ensure heated medium is cooled to 45° C before adding stock solution of Sodium Dichloroisocyanurate, ideally in a fume cupboard, and avoid inhaling vapours released.
- Pour medium into vials in well ventilate area, although avoid draughts to reduce contamination of agar whilst pouring.
- Dispose of scalpel blades in sharps bin or equivalent.

Apparatus

Note that you can purchase a kit from the NCBE, containing everything necessary for the protocol. For further details, see:

<http://www.ncbe.reading.ac.uk/NCBE/MATERIALS/METABOLISM/ptc.html>

Each student/pair requires:

- 'Diluvials' or small sterilised glass jars containing medium (MS, 20g/l sucrose, 2.5mg/l Kinetin, 0.032% SDICN – see media prep notes)
- White ceramic tiles / chopping board
- Forceps
- Scalpel
- 0.5% Solution Sodium Dichloroisocyanurate (SDICN) in small glass jar with cap (for sterilising forceps)
- 10ml 0.5% SDICN solution in Universal bottle (28ml glass bottle) with screw cap. (1 x 4g Milton tablet in 160ml DI water, 2 in 320ml, 4 in 640ml or 5 in 800ml - see media prep notes)
- Petri dish
- Safety glasses and disposable gloves
- Lab coat

Students/pairs require access to:

- 70% ethanol for wiping down surfaces
- paper towels
- Cauliflower curd (the white 'floret' part) cut into 10mm³ pieces. Curd should be taken from a fresh, whole cauliflower, not ready-prepared cauliflower pieces.
- Glass or plastic beaker for waste solutions

Aseptic Technique

- All glassware should be kept scrupulously clean and, after washing with hot, soapy water, double-rinsed with distilled water and allowed to dry.
- Wash hands and arms thoroughly before procedures
- Wear a lab coat or a similar protective garment.
- Wipe the bench/ tiles with 70% alcohol
- Don't bend over the plant material or the containers you are working with.
- Keep movements as smooth as possible to prevent the creation of eddies
- Instruments should be kept sterile by standing in the SDICN solution. It's advisable to have sterilised the class set of forceps and scalpels in advance in a SDICN solution for at least 20 minutes.

Media Preparation

Plant tissue culture medium is normally autoclaved under pressure (15 psi at 121°C for 15 minutes) in order to sterilise. This practical is based on a method developed at the Conservation Biotechnology Unit, Royal Botanic Gardens, Kew for media made without an autoclave, using just a microwave and water sterilising tablets (active ingredient sodium dichloroisocyanurate). This sterilant is added to the media which has the benefit both of simplifying media preparation and substantially reducing contamination of cultures as it persists in the medium.

- **'Milton' sterilising tablets** (not the ready made 'Milton' solution available in bottles) must be used for this practical. The tablets contain a different bleaching agent to the ready made solution. Unbranded water sterilising tablets are also available from supermarkets and chemists and are suitable for this medium. Milton tablets are commonly sold in packs of 28 tablets (112g). Each tablet is 4g, but contains 19.5% m/m (by mass) of the sterilant SDICN. Therefore each tablet contains ~800mg (0.8g) SDICN. Check your pack if using own brand alternatives, which are often sold in smaller tablets. The 0.5% solution we are using is stronger than that advised on the pack. Adding 20ml of 0.5% SDICN solution to your Agar gives an end content of 0.032%.
- **Plain Agar powder** should be used, not Nutrient Agar powder, to make up this medium.

For 1 litre of plant tissue culture media (makes around 100 vials of media):

Chemicals:

- 4.44g Murashige and Skoog (MS) medium (Melford Labs M0222 - Store in Fridge)
- 20g Granulated cane sugar
- 7g Agar (2 x 3.5g)

- Kinetin stock solution (10mg (0.01g) dissolved in 10ml 70% ethanol)
- Sodium Dichloroisocyanurate (1 x 'Milton' (800mg SDICN) tablet in 100ml distilled water)

Equipment:

- 0.1M Hydrochloric acid and 0.1M potassium hydroxide solution (for adjusting pH)
- Microwave
- pH meter
- Chemical balance
- Diluvials (see supplier info following)
- Pipettes for Kinetin stock solution and SDICN solution
- A large glass beaker or jug (suitable for use in a microwave)
- Smaller glass beaker or jug for pouring media
- A stirring rod
- Safety glasses, gloves

Making up Media

1. Weigh the individual components of the medium carefully
2. Measure 960ml water into a large beaker or jug (liquid should fill the vessel no more than half-full)
3. Add the powdered medium stirring constantly until it has dissolved completely
4. Once the mineral salts have dissolved, add the sucrose and stir until it has dissolved.
5. Add 2.5ml kinetin using pipette (2.5mg/l kinetin from stock solution of 10mg/10ml, dissolved in ethanol).
6. Measure the pH either using pH paper or a pH meter
7. Adjust pH to pH 5.7 using 0.1M Potassium hydroxide
8. Add the agar and stir well.
9. Divide media into 500ml batches
10. Heat medium to dissolve agar:
 - Cover the jug with cling-film, pierced to prevent build-up of steam.
 - Cook on high power for half of the recommended time, stir, re-cover and heat again for the remaining time. Recommended times for heating will vary with the power of the microwave (e.g. 8 minutes per litre for a 850kw microwave).
 - When the agar is completely dissolved, allow to cool to around 45°C (leave hot enough to pour before setting but cool enough to reduce chlorine release).

11. Add sterilant

- In a well ventilated area or fume hood if available add 20ml of 800mg/100ml stock solution of sodium dichloroisocyanurate (SDICN) to 500 ml heated and cooled medium, stir well (this gives a concentration of 0.032%) Do not breathe in chlorine vapours released
- Dispense medium immediately:

12. Dispense medium

- Wipe down lab surface or tray with 70% alcohol or a bleach solution
- Remove lids from Diluvials and place inner side downwards on surface.
- Pour media into Diluvials (approximately 10ml per vial) , and leave to cool with lids ajar (about 1 hour)
- When medium is cooled and set (about 1 hour) replace lids and label vials

Store in fridge until required (will last about 6 weeks)

Suppliers

Sterile Containers

Alpha Laboratories supply polystyrene Coulter counter cups (manufacturer Aptaca, Italy). These 'Diluvials,' because they are manufactured under clean room conditions, in effect are sterile although not sold as such. At £65.00 per 1000 (as of 2011) plus postage they are considerably cheaper than vials sold as sterile. You may want to consider sharing a load with a nearby school.

40 Parham Drive, Eastleigh, SO50 4NU UK

Telephone: 0800 387732

<http://www.alphalabs.co.uk/product.aspx?p=1715>

Pre-sterilised Universal glass jars (28ml) and lids could be used as an alternative to these, although inserting the explants is more difficult.

MS Medium (Product code - M0222) & Kinetin (Product code - K0905)

Melford Laboratories Ltd are the UK distributor for a wide range of plant tissue culture media components and products from Duchefa (www.duchefa.com)

Bildeston Road, Chelsworth, Ipswich, Suffolk, IP7 7LE, UK



Freephone 0800 085 27401

Tel 01449 741178

MS medium (M0222):

<http://www.melford.co.uk/index.php?t=details&sid=396401522&&id=77>

Kinetin (K0905):

<http://www.melford.co.uk/index.php?t=details&sid=396401522&&id=177>

MS Media and Kinetin are also available through some standard school suppliers.

Plain Agar

Plain agar is available from various suppliers, including:

- Timstar (http://www.timstar.co.uk/Item/NA/AG1092/AGAR_FINE_POWDER.html)
Agar fine powder, stockcode AG1092, £7.91 / 100g
- Philip Harris (<http://www.philipharris.co.uk/product/Agar-powder-B8A65560S>)
Agar powder, order number : B8A65560S, £7.50 / £9.00 inc VAT
- Griffin Education
(https://extranet.fisher.co.uk/insight2_griffin/getProduct.do?productCode=A/1080/48&resultSetPosition=0), Agar powder, product code: A/1080/48
- Scientific and Chemical
(<http://www.scichem.com/productinfo.aspx?kw=agar&tier1=Microbiological+Media&tier2=Miscellaneous&catref=MBM010190>)
Agar powder, catalogue number MBM010190