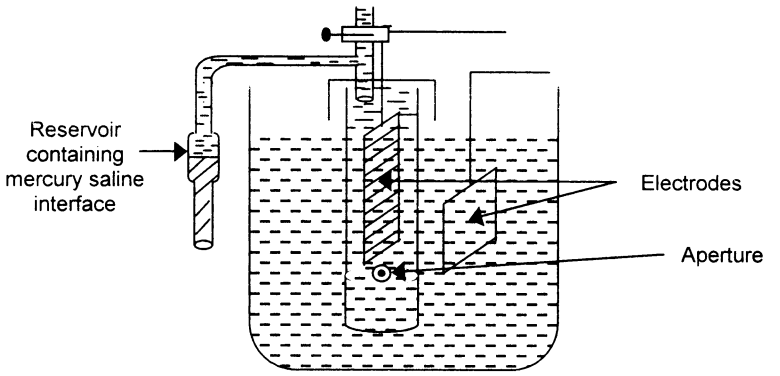

Fig. 1.7. Schematic diagram showing electronic cell counter

- Maintain the pH around 7.8 and incubate at 37°C for 5 to 10 minutes. The enzyme action dislodges the cells from inert surface.
- Gently triturate the suspension to disperse the cells.
- Add 1/10 volume of foetal calf serum of total volume suspension. This will stop the future activity of enzyme.
- Transfer the cell suspension to a conical flask and centrifuge at 200g for 5 minutes. This will pelletize the cells.
- Decant the trypsin solution and disperse the pellet in the same volume of complete growth medium.
- Count the cells and add the addition media so as to produce 2-3 $x10^5$ cells/ml.
- Inoculate the suspended cells in to clean sterilized culture vessel and keep the vessels in an incubator maintained at 37°C.

PROTOCOL 1.1

LYMPH NODE CELL CULTURE

Materials and Equipment

Lymph node, BSS medium, Tris-ammonium chloride buffer, Centrifuge, Haemocytometer, CO_2 incubator

Procedure

- Collect a lymph node from a young animal from which culture is desired, e.g., bovine, swine, ovine, etc. For this purpose, collect a precapsular lymph node or a mesenteric lymph node without any damage to it.
- Transfer the lymph node to a Petri dish containing BSS. Remove the fibrous tissues, fat and other connective tissues attached. Then remove the capsule.
- Transfer the lymph node to a fresh Petri dish and wash it.

Growing

- Place the test tubes in the planter tray (or other appropriate holder) and place the tray on a shelf under fluorescent light, which is 8-10 inches above the top of the tubes. Room temperature and continuous light is acceptable, but 16 hours light/ 8 hours darkness is standard. Check daily for contaminants. If any are found, sterilize the tube and contents before discarding the contents.
- Transfer the explant every two weeks or so until it is actively growing. In one to two months the culture can be divided into two pieces, each of which is about 0.5 cm in diameter. Continue to divide and transfer until enough plantlets are obtained. The plantlets should be singulated as they are transferred to prerooting medium, which has no hormones (or only IAA).

Transplanting

- When the plantlets begin to root, perhaps two to four weeks, transplant them to a light artificial soil mix, such as peat/ pearlite, in a seedling tray.
- Cover with clear plastic and place on a lighted shelf or in a shaded greenhouse. After two or three weeks begin leaving the plastic off for a period of time each day. The time the plantlets are left uncovered should get longer each day, until after about a week, the cover can be left off completely.

Types of Plant Tissue Cultures

Plant tissue cultures are broadly divided into three categories.

- Callus culture
- Organ culture
- Meristem culture

PROTOCOL 1.9

CALLUS CULTURE

In vivo, callus is frequently formed as a result of wounding at the cut edge of a root or stem, following invasion by microorganisms or damage resulting from insect feeding. Endogenous auxin and cytokinin control its formation. By incorporation of these plant growth regulators into a growth medium, callus can be induced to form *in vitro* on explants of parent tissue. The initiation of callus material from angiosperms, gymnosperms, ferns, mosses and liverworts can be achieved in this way.

Materials

The medium components are enlisted in Table 1.3.

Plant growth regulator stock solutions (1 mg/ml).

Naphthalene acetic acid, indole acetic acid and 2,4-dichlorophenoxyacetic acid present in the solution should be titrated with NaOH. Kinetin, gelatin and benzylaminopurine can be dissolved in dilute NaOH or 95% aqueous ethanol. Store at 4°C.

- Leave for 3-5 days until 3 or 4 small, study roots approximately 5 mm long are visible.
- Remove each plantlet carefully from the jar.
- Remove as much agar as possible without harming the root structure and transfer to damp compost in 5 mm pots.
- Keep in high humidity conditions for 12-24 hours (propagator, plastic bag or misting).
- Transfer to the glass house, preferably placing on top of capillary matting. Shade from direct sunlight.
- Transfer the plants (when approximately) 70 mm high and have begun to loose their juvenile characteristics to large pots or to the field.

Notes

The test for infections by known viruses should be done before initiating the culture, viral pathogens, once in culture are rapidly spread by axillary bud cultures.

PROTOCOL 1.13

CLONED CAULIFLOWER

Various steps involved in the procedure are shown in figure 1.6.

Material

White part of Cauliflower
Sterile distilled water, 100 ml
70% Ethanol, 50 ml
20% Domestos solution (Chlorate solution with added detergent)
Test tubes, containing 2-3 ml of plant tissue growth medium
Sterile Petri dish
Metal forceps and scalpel
Non-adsorbent cotton wool and aluminium foil

Preparation of Plant Tissue Growth Medium (1 litre)

Granulated sugar, 20 g
Agar, 10 g
Murashige and Skoog's (M S) medium, 4.7 g
Kinetin stock solution, 25 ml

- Prepare the kinetin stock solution using 0.1 g kinetin in 1 litre of distilled water. As kinetin is not readily soluble in water, add one or two pellets of sodium hydroxide to solubilize it. Store the stock solution at 4°C.
- In order to prepare the growth medium, dissolve the sugar, MS medium and agar in 725 ml distilled water.
- Mix kinetin in the stock solution, then dispense into test tubes and take 2-3 ml per tube. Plug the tubes with non-absorbent cotton wool and cap them with aluminum foil.

Table 2.2. Concentration and grade of agarose

Agarose grade	Size of DNA (base pairs)	Final agarose concentration % (W/V)	
		1X TAE buffer	1X TBE buffer
NuSieve® 3:1 Agarose	500-1000	3.0	2.0
	100-500	4.0	3.0
	10-100	6.0	5.0
NuSieve® GTG Agarose	500-1000	2.5	2.0
	150-700	3.0	2.5
	100-450	3.5	3.0
	70-300	4.0	3.5
	10-100	4.5	4.0
	8-50	5.0	4.5
MetaPhor® Agarose	150-800	2.0	1.8
	100-600	3.0	2.0
	50-250	4.0	3.0
	20-130	5.0	4.0
	<80	---	5.0
SeaPlaque® and SeaPlaque GTG Agarose	500-25000	0.75	0.70
	300-20,000	1.0	0.85
	200-12000	1.25	1.0
	150-6000	1.5	1.25
	100-3000	1.75	1.5
	50-2000	2.0	1.75
SeaKem® GTG and SeaKem LE Agarose	1000-23000	0.60	0.50
	80-10,000	0.80	0.70
	400-8000	1.0	0.85
	300-7000	1.2	1.0
	200-4000	1.5	1.25
	100-3000	2.0	1.75

Table 2.3. Optimal voltage for agarose gel systems

Size	Voltage	Recovery	Buffer analytical
< or = 1kb	5 Vcm^{-1}	TAE	TBE
1 to 2kb	4-10 Vcm^{-1}	TAE	TAE, TBE
> 12kb	1-2 Vcm^{-1}	TAE	TAE

The problem is exacerbated by the fact that the agarose is not being agitated to help dilute the highly concentrated solution around each particle and dissolution is slowed. The final stage in dissolving the agarose is the melting and dissolution of a high concentration gel. Melting can be done in either a microwave oven or on a hot plate. As the particles hydrate, they become small, highly concentrated gels. Since the melting temperature of a standard