

Cloning and Tissue Culture



What is it?

- Tissue culture is the term used for “the process of growing cells artificially in the laboratory” (*OSMS.otago.ac.nz/main/bursary*)
- Tissue culture involves both plant and animal cells
- Tissue culture produces **clones**, in which all product cells have the same genotype (unless affected by mutation during culture)

What's the Background?



Haberlandt



Carrel

- Tissue culture had its origins at the beginning of the 20th century with the work of Gottlieb Haberlandt (plants) and Alexis Carrel (animals)

The Background, II

- The first commercial use of plant clonal propagation on artificial media was in the germination and growth of orchid plants, in the 1920's
- In the 1950's and 60's there was a great deal of research, but it was only after the development of a reliable **artificial medium** (Murashige & Skoog, 1962) that **plant tissue culture** really 'took off' commercially



Young cymbidium orchids

The Background, III

- A more recent advance is the use of plant and animal tissue culture along with genetic modification using viral and bacterial **vectors** and **gene guns** to create genetically engineered organisms

What is needed?

Tissue culture, both plant and animal has several critical requirements:

- **Appropriate tissue** (some tissues culture better than others)
- **A suitable growth medium** containing energy sources and inorganic salts to supply cell growth needs. This can be liquid or semisolid
- **Aseptic (sterile) conditions**, as microorganisms grow much more quickly than plant and animal tissue and can over run a culture

What is Needed, II

- **Growth regulators** - in plants, both auxins & cytokinins. In animals, this is not as well defined and the growth substances are provided in serum from the cell types of interest
- **Frequent subculturing** to ensure adequate nutrition and to avoid the build up of waste metabolites

Culturing (micropropagating) Plant Tissue - the steps



- Selection of the plant tissue (**explant**) from a healthy vigorous 'mother plant' - this is often the apical bud, but can be other tissue
- This tissue must be sterilized to remove microbial contaminants

The Steps, II

- **Establishment** of the explant in a culture medium. The medium sustains the plant cells and encourages cell division. It can be solid or liquid
- **Each plant species** (and sometimes the variety within a species) has particular medium requirements that must be established by trial and error



The Steps, III



Dividing shoots



- **Multiplication**- The explant gives rise to a **callus** (a mass of loosely arranged cells) which is manipulated by varying sugar concentrations and the **auxin (low): cytokinin (high)** ratios to form multiple shoots
- The callus may be subdivided a number of times

Warmth and good light are essential

The Steps, IV

- **Root formation** - The shoots are transferred to a growth medium with relatively **higher** auxin: cytokinin ratios



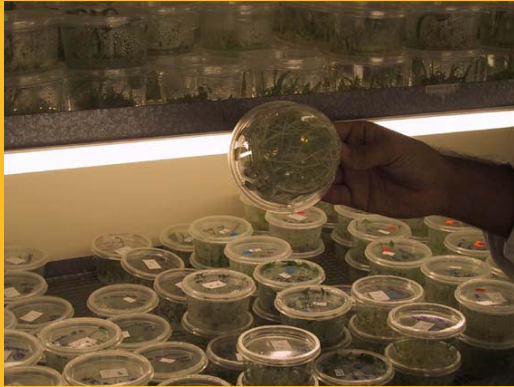
The pottles on these racks are young banana plants and are growing roots

The Steps, V



- The rooted shoots are potted up (deflasked) and 'hardened off' by gradually decreasing the humidity
- This is necessary as many young tissue culture plants have no waxy cuticle to prevent water loss

Tissue culture plants sold to a nursery & then potted up



Why do Plant Tissue Culture?

- A single explant can be multiplied into several thousand plants in less than a year - this allows fast commercial propagation of new cultivars
- Taking an explant does not usually destroy the mother plant, so rare and endangered plants can be cloned safely
- Once established, a plant tissue culture line can give a continuous supply of young plants throughout the year

Why do Plant Tissue Culture, II

- In plants prone to virus diseases, virus free explants (new meristem tissue is usually virus free) can be cultivated to provide virus free plants
- Plant 'tissue banks' can be frozen, then regenerated through tissue culture
- Plant cultures in approved media are easier to export than are soil-grown plants, as they are pathogen free and take up little space (**most current plant export is now done in this manner**)

Why do Plant Tissue Culture, III

- Tissue culture allows fast selection for crop improvement - explants are chosen from superior plants, then cloned
- Tissue culture clones are 'true to type' as compared with seedlings, which show greater variability

Culturing Animal Tissue- the Steps

- Animal tissue is obtained either from a particular specimen, or from a 'tissue bank' of **cryo-preserved** (cryo = frozen at very low temperatures in a special medium)
- Establishment of the tissue is accomplished in the required medium under aseptic conditions



Culture vessels and medium for animal cell culture

Culturing Animal Tissue, II



- Growing the cells / tissue requires an optimum temperature, and subculturing when required
- Human cells, for example are grown at 37degrees and 5% CO₂

Incubator

Animal tissue/cell culture - differences from plant tissue culture

- Animal cell lines have limited numbers of cell cycles before they begin to degrade
- Animal cells need frequent subculturing to remain viable
- Tissue culture media is not as fully defined as that of plants - in addition to inorganic salts, energy sources, amino acids, vitamins, etc., they require the addition of serum (bovine serum is very common, but others are used)

Animal tissue/cell culture - differences from plant tissue culture

II



Gloves and labcoat are
always worn

- Animal tissue cultures can pose **biohazard** concerns, and cultures require special inactivation with hypochlorite (e.g. Janola, Chlorox, etc.) and then incineration

The pipettes are disposable

Uses of Animal Tissue Culture

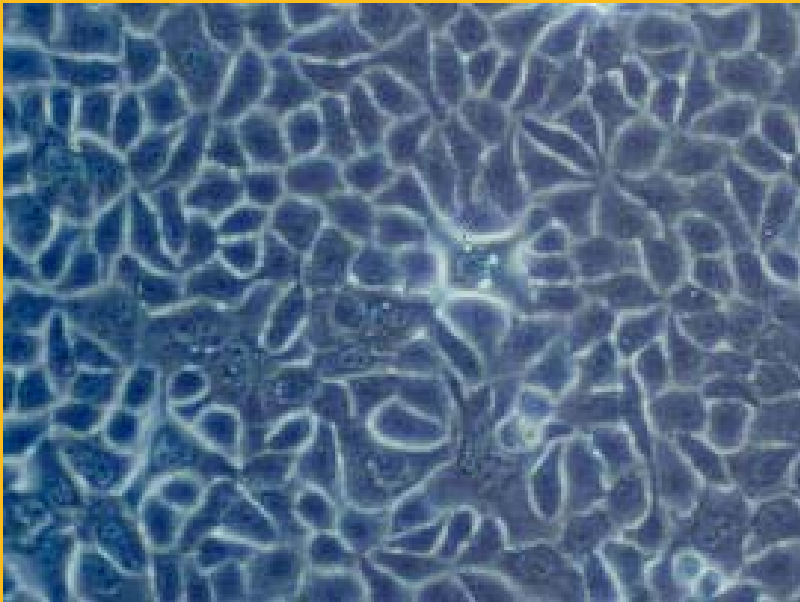


Photo courtesy of Sigma Aldrich

- Growing viruses - these require living host cells
- Making **monoclonal antibodies**, used for diagnosis and research
- Studying basic cell processes
- Genetic modification & analysis

Uses of Animal Tissue Culture

II

- 'Knockout' technology - inactivating certain genes and tracing their effects
- Providing DNA for the **Human Genome Project** (and other species' genome projects)

Bibliography

- Dodds, J.H., Roberts, L.W., 1995, Experiments in Plant Tissue Culture, 3rd ed., Cambridge University Press
- Hartmann, H., Kester, D., et.al., 1997, Plant Propagation, 6th ed., Prentice Hall International
- <http://www.une.edu.au/agronomy/AgSrHortTCinfo.html>
- <http://aggie-horticulture.tamu.edu/tisscult/pltissue/pltissue.html>
- <http://www.liv.ac.uk/~sd21/tisscult/what.htm>
- <http://user.school.net.th/~anoparp/bptc1.htm>

- <http://www-plb.ucdavis.edu/courses/s99/plb111I/TCMedium.html>
- <http://members.aol.com/mrDJReed/private/PTC.html>
- <http://www.accessexcellence.org/LC/ST/st2bgplantprep.html>
- www.osms.otago.ac.nz/main/bursary
- <http://www.kitchenculturekit.com/historyTC.htm>
- http://www.sigmaaldrich.com/Area_of_interest/Life_Science/Cell_Culture/Helpful_Resources/Cell_Culture_handbook.htm
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