The following describes some general considerations in the setup of a tissue culture laboratory at an academic institution where one is usually restricted to making the best use of existing laboratories. Kyte and Kleyn (1996) describe a commercial tissue culture laboratory design. Determining the location of the tissue culture laboratory is an important decision. Avoid locating it adjacent to laboratories that handle microorganisms or insects or facilities that are used to store seeds or other plant materials. Contamination from air vents and high foot traffic can be a problem. Foot traffic scuffs up the wax on floors as well as dust which help spread contaminants.

The tissue culture area should be kept clean at all times. This is important to ensure clean cultures and reproducible results. Avoid having potted plants in this area because they can be a source of mites and other contaminating organisms. Avoid field or greenhouse work immediately before entering the laboratory because mites and insects can be carried into the laboratory on hair and clothing. Personnel should shower and change clothes before entering the laboratory from the field or greenhouse.

In designing a laboratory for tissue culture use, arrange the work areas (media preparation/culture evaluation/record-keeping area, aseptic transfer area, and environmentally controlled culture area) so that there is a smooth traffic flow. The following is an outline of the major equipment and activity in each of the work areas of the laboratory.
WORK AREAS

Media Preparation/Culture Evaluation/Record-Keeping Area

1. Bench
2. Gas outlet
3. Hot plate and magnetic stirrer
4. Analytical and top-loading balances
5. pH meter
6. Refrigerator, freezer
7. Water purification and storage system
8. Dish-washing area
9. Storage facilities—glassware, chemicals
10. Autoclave (pressure-cooker will work for small media volume)
11. Low bench with inverted light and dissecting microscopes (avoid locating next to autoclaves or other high-humidity areas)
12. Fume hood
13. Desk and file cabinets
14. Desktop centrifuge, spectrophotometer, microwave (transformation studies and protoplast isolation)

Culture media may be conveniently prepared on a laboratory chemical bench with a pH meter, balances, and a sink in close proximity. The reagents and stock solutions should be located on shelves or in a refrigerator adjacent to the bench.

Glassware cleaning is a constant process because the turnover is usually very high. Culture tubes containing spent medium should be autoclaved at least 30 min, and the contents disposed of before washing. Autoclaved glassware should be promptly washed. Glassware should be scrubbed in warm, soapy water, rinsed three times with tap water, rinsed three times with distilled water, and placed in a clean area to dry. Generally dishwashers do not effectively clean culture vessels, and test tubes should be hand scrubbed (Table 2.1).

A low bench, table, file cabinet, and a desk are essential for culture evaluation and record-keeping. A desktop computer is very desirable for writing up reports.

Aseptic Transfer Area

1. Laminar air flow transfer hood and comfortable chair
2. Dissecting microscope
3. Gas outlet
4. Vacuum lines
5. Forceps, spatulas, scalpel, and disposable blades

A separate room for the transfer hood is ideal. This room should be designed so that there is positive-pressure air flow and good ventilation. It is also desirable to have a window to the outside or into the laboratory so that an individual spending long hours working in the hood may occasionally relieve eye strain.
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A laminar airflow hood can be expensive, and an alternative is to build one. One can improvise if a laminar air flow hood is not available and use a fume hood which has been thoroughly scrubbed down, sprayed with a hospital disinfectant, and has had the glass lowered to allow only enough room for the worker’s hands and arms to do the transfers. When using a fume hood, do not turn it on as it will draw contaminated air from the room over the cultures and try to avoid having foot traffic in the area while transfers are under way. Cardboard boxes lined with aluminum foil or plastic containers can also function as clean, dead air spaces to do transfers. A HEPA filter can also be attached to a plastic storage box and used as a transfer hood. An article by Joe Kish in *Mushroom—The Journal of Wild Mushrooming* (Winter, 1997) describes the construction of a small hood (back issues are available for $5. Make checks to Maggie Rodgers at Fungal Cave Books, 1943 SE Locust, Portland, OR 97214; rogersm@aol.com). Complete plans including construction details for a 2- to 4-ft. hood can be found at [http://envhort.ucdavis.edu/dwb/lamflohd.pdf](http://envhort.ucdavis.edu/dwb/lamflohd.pdf).

### Environmentally Controlled Culture Area

1. Shelves with lighting on a timer and controlled temperature
2. Incubators—with controlled temperature and light
3. Orbital shakers
High humidity in the culture room should be avoided because it increases contamination. Some culture rooms have dehumidifiers and air scrubbers.

Most cultures can be incubated in a temperature range of 25–27°C under a 16:8-h light:dark photoperiod controlled by clock timers. Experiments described in this manual use this as a standard culture condition. Illumination is from Gro-Lux or cool white 4-ft. long fluorescent lamps mounted 8 inches above the culture shelf and 12 inches apart. Light intensity varies depending on the age of the lights and whether the cultures are directly under them or off to one side. The light can be measured in foot-candles (fc; full sun is approximately 10,000 fc) or microeinsteins (µE) per second per square meter (1 µE·sec⁻¹·m⁻² = 6.02 × 10¹⁷ photons⁻¹·m⁻² = µmol·sec⁻¹·m⁻²; full sun is approximately 2000 µE·sec⁻¹·m⁻²). The range of light readings can be 40–200 fc or 20–100 µE·sec⁻¹·m⁻². A meter to measure foot-candles and a quantum radiometer–photometer light meter to measure microeinsteins per second per square meter may be used to measure the light level.

**BIBLIOGRAPHY**

**Books**

An extensive list of books on plant tissue culture and related topics is available from Agritech Consultants, Inc., P.O. Box 255, Shrub Oak, NY 10588, Fax/Phone: (914) 528–3469; e-mail: agricell@aol.com; HTTP://AgritechPublications.com/


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**Journals**

Acta Horticulturae

Agricell Report

American Journal of Botany

Biologia Plantarum

Biotechnology Letters

Biotechnology Advances

Biotechnology and Applied Biochemistry

Bio/Technology
BioTechniques
Botanical Gazette
Canadian Journal of Botany
Critical Reviews in Plant Sciences
Crop Science
Current Science
Electronic Journal of Biotechnology (http://www.ejb.org)
Engineering in Life Sciences
Genetics and Molecular Research
HortScience
In Vitro Cellular & Developmental Biology, Plant
Journal of Horticultural Science
Journal of Plant Physiology
Korean Journal Medicinal Crop Science
Molecular Breeding
Nature
Nature Biotechnology
Physiologia Plantarum
Plant Biology
Plant Biotechnology Reports
Plant and Cell Physiology
Plant Cell Reports
Plant Cell, Tissue and Organ Culture
Plant Growth Regulation
Plant Journal
Plant Molecular Biology
Plant Physiology
Plant Science
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Proceedings of the American Society for Horticultural Science
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Propagation of Ornamental Plants
Protoplasma
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International Association for Plant Tissue Culture and Biotechnology
International Society for Horticulture Science
Society for In Vitro Biology