Cultivation and

Operations Manual For

Pharming Humboldt Dreams

APN: 223-241-006

Proposed Cannabis Microbusiness Facilities For Pharming Humboldt Dreams (PHD)

Lead Agency:

Humboldt County Planning Department

3015 H Street

Eureka, CA 95501

Prepared By:

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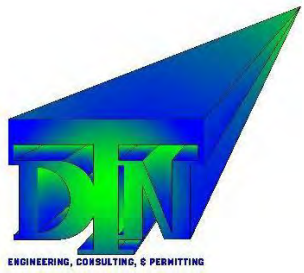

In Consultation with:

Pharming Humboldt Dreams 3295 Alderpoint RD Garberville CA

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1.0 PROJECT SUMMARY

1.1 PROJECT OBJECTIVE

Pharming Humboldt Dreams (Client). is proposing to permit a microbusiness in accordance with the County of Humboldt' s (County) *Commercial Cannabis Land Use Ordinance* (CLUO). The project requires a Special Permit for the microbusiness that includes Distribution, Commercial Nursery, and Manufacturing.

1.2 SITE DESCRIPTION

The Project is located at (APN 223-241-006) 3295 Alderpoint Rd Garberville, CA. The subject parcel is approximately 38 acres in size (per the County of Humboldt's WebGIS). The property is primarily grassland, and has some development including two existing single-family residences, approximately, one building that will be proposed for use as storage and a processing / drying facility that appears to be within the setback of the SMA. None of the existing on-site structures have been permitted. The property currently has a permitted 10,000 S.F. mixed light cultivation.

1.3 LAND USE

The subject property has a General Plan designation of Agricultural Exclusive (AE) as identified by the Humboldt County Genera l Plan and is zoned Residential Agriculture (RA40). Land uses surrounding the parcel are comprised of residential, timber and agriculture. The surrounding parcels are zoned Residential Agriculture (RA40).

1.4 STATE AND LOCAL COMPLIANCE

1.4.1 STATE OF CALIFORNIA COMMERCIAL CANNABIS ACTIVITY LICENSE

The client will obtain a Microbusiness license from the State of California at time such a license becomes available.

1.4.2 STATE WATER RESOURCES CONTROL BOARD

Water for cultivation use is provided by a rainwater catchment utilizing a proposed twenty-seven 5,000-gallon tanks at the greenhouse location for a total of 135,000 gallons of water storage.

1.4.3 NORTH COAST REGIONAL WATER QUALITY CONTROL BOARD

Client. has enrolled with the North Coast Regional Water Quality Control Board (NCRWQCB) for coverage under Tier 1 of Order No. 2015-0023 Waiver of Waste Discharge Requirements and General Water Quality Certification for Discharges of Waste Resulting from Cannabis Cultivation and Associated Activities or Operations with Similar Environmental Effects in the North Coast Region (WDID Number 1_12CC439002).

1.4.4 HUMBOLDT COUNTY BUILDING DEPARTMENT

Building permits have been obtained from the Humboldt County Building Department for all existing and proposed structures and supporting infrastructure as part of Cannabis permit PLN-2020-16677.

1.4.5 CALFIRE

The subject property is located within a State Responsibility Area (SRA) for fire protection. Several improvements have been met and completed for SRA requirements, including designating a fire turn

around and pull-out area for emergency vehicles, and management of trees and vegetation around existing structures to maintain the required 100-foot defensible space. All structures on the property meet the 30-foot SRA setback requirement from property lines.

1.4.6 CALIFORNIA DEPARTMENT OF FISH AND WILDLIFE

A Lake and Stream bed Alteration Agreement (LSAA) from the Department of Fish and Wildlife (DFW) has been obtained for culvert replacement, pond spillways, and points of diversion.

2.0 MICROBUSINESS ACTIVITIES (NURSERY/DISTRIBUTION/MANUFACTURING)

All Microbusiness activity will occur within the approved processing and drying building constructed under permit BLD-2022-56256. See attached Plot Plan and Building Floor Plan in **Exhibit A**. The description of the Microbusiness activities is detailed below:

2.1 COMMERCIAL NURSERY

Plant Tissue culture can be referred to as micropropagation, and as the name suggests, the process to introduce a few plant tissues on artificial media under controlled conditions for propagating plants. In summary tissue cloning involves the following four processes:

In simple terms, here are the steps of tissue culturing cannabis.

1. **Extraction**: The grower cuts small plant shoot tips from their selected plant. Under a laminar flow hood, they dissect the shoots in desired lengths. The shoot tips are then surface sterilized to clean any microorganisms with different chemicals.
2. **Incubation**: Each sample is either placed in its own test tube or shares space in a larger culture vessel. The culture vessel is filled 1/5-full with tissue culture media — a sterile gelling agent containing nutrients and plant hormones. The grower seals each container and places it in an incubator under specific light conditions.
3. **Plantlet development**: With the right media containing micro and macro elements, an iron source, vitamins, and other additives and hormones, plantlets will eventually develop. These plantlets are transferred (known as subculturing) into a medium with root growth-promoting hormones to stimulate root growth.
4. **Hardening**: Once the plantlets have well-established roots, the grower hardens them under reduced light and high humidity. This allows the plantlets to survive harsh environments outside a climate-controlled lab. The grower then transfers them to an appropriate growing medium in the greenhouse.

In the process, plant tissue (referred to as the explant) is gathered from the mother plant and placed in a sterile environment or container containing gelling media rich with nutrients and vitamins to ensure healthy growth. The media provides the developing plantlets with the necessary nutrients and hormones required for healthy root and shoot development.

Traditional cloning can take up a fair amount of space and account for the mother plant space required as well. With tissue culture, the space required is significantly less, and your strain genetics are still preserved. Once the plant media has grown and is ready to be transplanted, it is transferred into a different medium that helps to manage growth.

With Tissue Cloning juvenile plants are propagated on site from 'mother plants' that demonstrate the desired genetics for the specific cannabis strain. Mother plants remain in the vegetative stage solely for propagation. The method of propagation that will be used in the nursery is tissue cloning. Tissue cloning can be referred to as micropropagation and is the process to introduce a few plant tissues on artificial media under controlled conditions for propagating plants. See Appendix A for a full detailed description of the tissue cloning process.

In the process, plant tissue (referred to as the explant) is gathered from the mother plant and placed in a sterile environment or container containing gelling media rich with nutrients and vitamins to ensure healthy growth. The media provides the developing plantlets with the necessary nutrients and hormones required for healthy root and shoot development.

Traditional cloning can take up a fair amount of space accounting for mother plants as well. With tissue culture, the space required is significantly less, and the strain genetics are still preserved. Once the plant media has grown and is ready to be transplanted, it is transferred into a different medium that helps to manage growth.

The clones are placed into the clone room, and once fully rooted they are transplanted directly into one (1) gallon plastic containers and moved to a 1000 ft2 nursery greenhouse (see Appendix A for nursery location). The juvenile plants are irrigated using hand watering methods. After 2-4 weeks the clones are then transplanted into 20-gallon smart pots with a living soil medium and moved into the propagation greenhouse where they continue their 'vegetative' cycle.

2.1.1 NURSERY EQUIPMENT / CHEMICAL LIST

Permanent Markers	Black	12pc.
pH Meter	N/A	1
Pipette Tips	10mL	100pc.
Pipettor	10mL	1
Pipettor Set	Set	1
Plastic Mason Jar Lids	N/A	32pc.
Plastic Spray Bottles	N/A	4
Pressure Cooker	27.3 Quarts	2
Refrigerator	N/A	1
Ring Light	N/A	1
Root Riot Plugs in trays	N/A	400ct.

Scale	0.000g	1
Scalpel Blades #10	#10	100ct.
Scalpel Handle (No. 3) with #11 Blades	Kit	3 Kits
Specimen Lighting	4ft	4
Specimen Shelving	N/A	1
Stereo Microscope	N/A	1
Surgical Gloves	N/A	5
Syringe Filters	13mm	50ct.
Syringes	10mL	100ct.
Thermal Cycler	N/A	1
Tissue Culture Vessel Lids	N/A	500ct.
Tissue Culture Vessels	N/A	500ct.
Tool Sterilizer	N/A	1
Vortex	N/A	1
Weigh Boats	N/A	1000ct.
Wide-Mouth Mason Jars	16oz	32pc.
YouPCR Starter Bundle	Reactions	50

Consumables		
Item	**Size**	**Quantity**
70% Isopropyl Alcohol	5 Gallon	2
99% Isopropyl Alcohol	1 Gallon	1
Aluminum Foil	1000ft Roll	1
BAP B130	500mL	1
Bleach	1 Gallon	5
Buret, Stopcock Dropper type and stand clamp	1	1
Calcium Chloride	2lbs	1
Carbenicillin C346	5g	1
Dish Soap	N/A	As Needed
Distilled Water	1 Gallon	20
DMSO D241	500mL	1
GA3 G198	25mL	1
Gamborg's Vitamins G210	500mL	1
IBA I460	100mL	2
Kinetin K483	500mL	1
mT T7885	25mL	1
Murashige & Skoog Medium M519	50L	2
NAA N605	500mL	1
Nuclease Free Water	500mL	1
Nystatin N581	5g	1
Paper Towels	N/A	20
Polysorbate 20	4oz Bottle	2

PPM™ Biocide	250mL	1

Ribavirin R795	1g	2

Sodium Alginate A108	250g	1
Sodium Hydroxide S835	1L	1
Sugar/Sucrose	5lbs.	1
Sulfuric Acid S804	1L	1
Supreme Tissue Culture-Grade Agar A1000	1kg	1

A detailed description of tissue cloning is attached in **Exhibit B**.

2.2 DISTRIBUTION

The distributor may transport cannabis goods to any licensee or license type to which Distributors are authorized to distribute cannabis goods pursuant to state law and regulations. The Distributor will transport cannabis goods from its licensed premises at 3295 Alderpoint Rd, Garberville, CA, to any address in the State of California. Overnight transportation of cannabis goods may occur. The applicant will be obtaining a motor carrier permit.

Cannabis goods will be transported in a metal toolbox bolted to the bed of the pickup truck or cannabis goods will be transported in accompanying trailer. The trailer will be secured by locked door and is windowless. Cannabis goods will be stored within the trailer.

Vehicle: 2008 Ford F350; VIN 1FTWW31R88EC55971

Trailer: 2021 Forest River Trailer; VIN: 5NHUCH01XMB481737

Vehicle - Registration is temporary at this time; to be provided when obtained via US Mail.

Vehicle Insurance - State Farm Policy No. 05-6469-R41, effective January 14, 2022 - March 14,

2022, Trailer Registration - Title Issued 1/14/2021 (attached)

Trailer Insurance - Pharming Humboldt Dreams, LLC's Sole Owner Elliott David Morris was advised by State Farm Insurance Agency that the size of the trailer exempts it from being required to have its own insurance policy/coverage, specifically that the trailer will be covered by the insured vehicle that will be pulling it (insurance evidence attached).

Applicant will be transporting cannabis goods from the parcel to licensed retailers, distributors, or licensed manufacturers, by motor vehicle. Applicant will be transporting cannabis goods from the cultivation licensee's distribution location (see Plot Plan), keeping appropriate inventory and transportation records.

Cannabis goods will be transported in a metal toolbox bolted to the bed of the pickup truck or

cannabis goods will be transported in accompanying trailer. The trailer will be secured by locked door and is windowless. The number of round trips on a daily basis will range from 0-5 and 0-25 per week.

Cannabis goods will be stored within the trailer of the vehicle(s) authorized to transport cannabis goods pursuant to 16 CCR 5311. Cannabis goods will be locked in a fully enclosed lock box and/or cage (both installed in each vehicle) that is secured to the inside of the vehicle. The trailer will not comprise any portion of the lock box or cage used to secure the cannabis goods. No cannabis goods will be visible from outside the trailer. No cannabis goods will be transported in the vehicle. In the event that it is necessary to enclose the cannabis goods in a secondary container for protection during shipping, the cannabis goods will not be removed from the containers or packaging form in which they were received at the shipping premises.

2.3 MANUFACTURING

The Operator is proposing to manufacture products using differing methods to produce cannabis extracts. Cannabis extracts shall be produced with solventless nonflammable methods. Systems shall include presses, and trimmers. All development shall be fully engineered, equipment CL- Certified, food or laboratory grade, and operations licensed as required by law. The facility complex shall feature the following equipment:

1. Vacuum Ovens
2. Chillers
3. Rosin Presses
4. Freeze Dryer
5. Kief Tumblers
6. Preroll Machines
7. Stainless Steel Shelves and Tables
8. Cleaning Area
9. Measuring Devices
10. Packaging Equipment

The Operator shall, regardless of the method of the category of concentrate or packaged cannabis being produced, do the following:

A. Ensure that the space in which any cannabis product is to be produced within a fully enclosed room and clearly designated on the site map.

B. Ensure that all applicable sanitary rules are followed.

C. Ensure that the standard operating procedures for each extraction method includes, but not limited to, step-by-step instructions on how to safely and appropriately:

    1. Extract cannabinoids and other essential components.
    2. Purgco

3. e any unwanted components from extractions.
4. Clean all equipment, counters, and surfaces thoroughly meeting sanitary food standards; and
5. Dispose of any waste produced with all applicable local, state, and federal laws, rules and regulations.

D. Establish written and documentable quality control procedures designed to maximize safety for employees and minimize potential product contamination.

E. Establish written contingency plans to be followed by employees in case of a fire, chemical spill, or other emergency.

F. Have a comprehensive training manual that provides step-by-step. The training manual shall include, but not limited to, the following:

1. All standard operating procedures for each method of concentrating production.
2. Quality control procedures.
3. Emergency procedures.
4. Appropriate use of any necessary safety or sanitary equipment.
5. The hazards presented by all solvents used in the material safety data sheet.
6. Clear instructions on the safe use of all equipment in accordance with manufacturer's instructions; and,
7. Periodic cleaning is required to comply with all applicable sanitary rules.

G. Provide adequate training for every employee prior to that individual undertaking any process involving equipment.

1. Adequate training shall include, but not limited to, providing a copy of the training manual and live, in-person instruction and hands on instruction.
2. The trainer shall sign and date a document attesting that all required aspects of training were conducted and that he or she is confident that the trainee can safely operate within the facility. The trainee shall also sign the said document so that they feel confident to operate safely within the facility.

H. Maintain clear, comprehensive records with the name and signature of every individual who engaged in any step related to the creation of a production batch and the procedure that individual executed.

3.0 Production Quality Control, Consumer Safety, and Processes

The Operator shall implement quality control and consumer safety control processes, procedures, and documentation that meets all state and local requirements, including but not limited to State and Humboldt County Code:

3.1 QUALITY CONTROL

A. Samples of all raw materials shall be screened and tested by an independent State licensed and/or locally permitted licensed laboratory.

B. All raw materials shall meet state requirements for pesticides, mold, and other undesirable qualities prior to extraction.

C. Samples from each batch of finished product shall be screened and tested by a State licensed and/or locally permitted licensed independent laboratory.

The facilities inventory control process includes tracking of all incoming raw and processed materials, including the name and state license number of the cultivator, and the testing lab data (as applicable). the strain, the supplier's product tracking identification data, and' bill of lading from the transport company.

A. All incoming raw materials shall be assigned a batch number that can be cross- referenced to the above referenced data and stays with the product through the manufacturing process and to final sale to distributors and/or retailers.

B. All finished products shall meet state requirements for pesticides, mold, and other undesirable qualities prior to release for sale to wholesalers and retailers.

C. All outgoing products shall be tracked by SKU, batch number, invoice, and shipping documents; unless the product is not for sale, which shall be destroyed and documented.

D. Documentation of all lab test results shall be kept on file.

3.2 PACKAGING

A. All packaging shall meet state requirements.

B. Labeling shall include a warning if nuts or other known allergens are used.

C. Labeling shall include the total weight in grams of cannabis or milligrams of THC in the package.

D. A warning that the item is cannabis and not a food shall be distinctly and clearly legible on the front of the package.

E. The package label shall have a warning that's clearly legible and emphasizes that the product is to be kept away from children.

F. The label shall also state that the product contains cannabis.

G. The label shall specify the date of manufacture and batch number.

H. Packaging that makes the product attractive to children shall not be used.

I. The methodologies for tracking and inventory of cannabis and cannabis extracts may be subject to requirements imposed by the State Licensing Authority and Humboldt County which shall be adjusted accordingly as required.

3.3 SANITARY PRACTICE

All reasonable measures and precautions shall be taken to ensure the following sanitary conditions:

A. Any person who, by medical examination or supervisory observation, is shown to have, or appears to have, an illness, open lesion, including boils, sores or infected wounds, or any other abnormal source of microbial contamination for whom there is a reasonable possibility of contact with cannabis or cannabis extracts shall be excluded from any operations which may be expected to result in contamination until the condition is corrected.

B. Hand-washing facilities shall be adequate and convenient and be furnished with running water at a suitable temperature throughout the facility.

C. Sanitary practices shall require employees to wash or sanitize their hands and provide effective hand-cleaning and sanitizing preparations and sanitary towel service or suitable drying devices.

D. Nitrile gloves shall be donned after sanitizing hands.

E. All persons working in direct contact with cannabis and cannabis extracts sha11 conform to hygienic practices while on duty, including but not limited to:

    1. Maintaining adequate personal cleanliness.

    2. Wash hands thoroughly before starting work and at any other time wl1en the hands may have become soiled or contaminated; and

    3. Refrain from having. direct contact with cannabis and cannabis -extracts if the person has or may have an illness, open lesion, including boils, sores, or infected wounds, or any other abnormal source of microbial contamination, until such condition is corrected.

3.4 WASTEMANAGEMENT

A. Litter and waste are properly removed.

B. Operating systems for waste disposal are maintained in an adequate manner so that they do not constitute a source of contamination in areas where cannabis and cannabis extracts are exposed.

C. Floors, walls, and ceilings are constructed in such a manner that they may be adequately cleaned, and each is kept clean and in good repair.

D. That there is adequate lighting in all areas where cannabis and cannabis extracts are produced or stored or sold and where equipment or utensils are cleaned.

E. That there is adequate screening or other protection against the entry of pests.

F. Rubbish shall be disposed of to minimize the development of odor and minimize the potential for the waste becoming an attractant, harborage, or breeding place for pests.

G. That fixtures and other facilities are maintained in a sanitary condition.

H. That toxic cleaning compounds, sanitizing agents, and other chemicals shall be identified, held, stored and disposed of in a manner that protects against contamination of cannabis and cannabis extracts and in a manner that is in accordance with any applicable local, state or federal law, rule, regulation or ordinance.

I. That all operations in the receiving, inspecting, transporting, segregating, preparing, manufacturing, packaging, and storing of cannabis and cannabis extracts shall be conducted in

accordance with adequate sanitation principles.

J. That employees are provided with adequate and readily ADA accessible toilet facilities that are maintained in a sanitary condition and good repair; and that cannabis and cannabis extracts that can support the rapid growth of undesirable microorganisms are held in a manner that prevents the growth of these microorganisms.

1. Inspections may result in additional specific standards to meet local jurisdiction restrictions.
2. An annual fire safety inspection may result in the required installation of fire suppression devices, or other means necessary for adequate fire safety.
3. Cannabis and cannabis extract waste shall be rendered unusable and unrecognizable prior to leaving the facility through one of the following methods unless another method is prescribed by the County of Humboldt or the State of California:
4. Grinding and incorporating the cannabis waste with non-consumable, solid wastes listed below such that the resulting mixture is at least 50 percent non-cannabis waste:
    i. Paper waste.
    ii. Plastic waste.
    iii. Cardboard waste.
    iv. Food waste.
    v. Grease or other compostable oil waste.
    vi. Bokashi, or other compost activators.
    vii. Other wastes approved by the State Licensing Authority that shall render the cannabis and cannabis extracts waste unusable and unrecognizable as cannabis.

A. For destroying and disposing of cannabis and cannabis extracts shall be subject to requirements imposed by the State Licensing Authority and shall be adjusted accordingly as required.

B. Records of destroyed raw materials and product shall be kept and cross- referenced by batch number and SKU. The weight or volume, as appropriate, shall be recorded along with the method of disposal.

C. The methodology for recording destroyed cannabis and cannabis extracts shall be subject to requirements imposed by the State Licensing Authority and shall be adjusted accordingly as required.

4.0 SECURITY AND HOURS OF OPERATION

The facility is for the purpose of the distribution and manufacturing of cannabis products according to State and Humboldt County Code standards. All products shall be sold to State licensed facilities on a wholesale basis. The facility shall not be open to the public and shall not accept visitors without a specific pre- authorized business purpose.

Visitors of the facilities shall be monitored by video at all times. Only authorized representatives of state licensed wholesalers and licensed vendors shall be -allowed to enter the facility, be in close proximity to products and raw materials, and shall be supervised at all times. Any other vendors or maintenance workers allowed in the facility shall at all times be escorted and separated from finished products and raw materials.

The Operator shall protect against diversion of cannabis by theft of not only intruders, but also from staff members, and visitors. This shall be achieved by limiting access both into and within different areas of the facility as necessary. Surveillance monitoring of personnel and visitors shall occur at all times. Strict inventory control measures shall also be engaged to prevent and detect diversion. The security measures located on the premises shall include the following:

A. Indoor and outdoor lighting controlled by photocell switching, timers, infrared motion sensors and/or other state-of-the-art control systems to maintain an adequate light level at the interior and exterior of the- facilities to ensure that personnel and the video surveillance system can effectively monitor the space in and around the facility at all times. Exterior lighting shall be directed so as to not pose a nuisance to neighboring properties.

B. An independently monitored third party security and fire alarm system shall be installed and operated at all appropriate times within the facility.

C. Communications between the facilities alarm system and the central control station shall be uninterruptible by power outage and/or disability of the telephone system. Communications shall be powered by an uninterruptible power supply, and transmission shall either be by cellular or radio.

D. All entrances to the occupied building space of the facility shall be restricted by an access control system capable of identifying authorized personnel. The system may also be cap-able of limiting personnel access to the appropriate locations within the facility depending on the person's job and responsibilities and also limit facility access to certain times and days as appropriate.

E. All cannabis raw material deliveries shall be received at the facility from a State licensed and/or locally permitted licensed transport company or individual.

F. All cannabis product delivers shall be transported to State licensed and/or locally permitted licensed Wholesale/Distribution companies by a State licensed and/or locally permitted licensed transport company.

G. 24 hour access to the facility by emergency responders shall be provided via a KNOX Box.

5.0 MICROBUSINESS FACILITY (NURSERY/DISTRIBUTION/MANUFACTURING)

As part of Cannabis permit PLN-2020-16677 a processing building was approved and building permit BLD-2022-56256 permitted the construction of the processing building and for drying and trimming. The use of some rooms in the processing building will be modified (see Plot Plan) to accommodate the proposed Microbusiness.

5.1 EMPLOYEE PLAN

There will be up to two temporary seasonal workers on site to trim Cannabis for permit PLN-2020-

16677, and two full time employees for Microbusiness.

The CLIENT will be an " agricultural employer " as defined in the Alatorre-Zenovich- Dunlap-Berman Agricultural Labor Relations Act of 1975 (Part 3.5 (commencing with Section 1140) of Division 2 of the Labor Code), and complies with all applicable federal, state, and local laws and regulations governing California Agricultural Employers and shall have 3 seasonal employees with 2 permanent employees.

5.2 JOB DESCRIPTIONS AND EMPLOYEE SUMMARY

The Client will conduct business oversight and management of the cultivation. Responsibilities include, but are not limited to inventory and tracking, personnel management, record keeping, budget, and liaison with State and County inspectors as needed.

5.3 STAFFING REQUIREMENTS

The number of seasonal laborers varies based on the needs of the farm during the cultivation, harvest, and processing seasons. During the peak harvest and processing season, there are an estimated total of four (4) employees on site including temporary workers.

5.4 EMPLOYEE T R A I N I N G AND SAFETY

On site cultivation, harvesting, and drying is performed by employees and principals trained on each aspect of the procedure including cultivation and harvesting techniques and use of pruning tools; proper application and storage of pesticides and fertilizers; trim machine use and cleaning; and correct hand trimming methods. All cultivation and processing staff are provided with proper hand, eye, body, and respiratory Personal Protective Equipment (PPE). Access to the onsite cultivation, drying and processing facilities are limited to authorized and trained staff.

All employees are trained on proper safety procedure including fire safety; use of rubber gloves and respirators; proper hand washing guidelines; and protocol in the event of an emergency. Contact information for the local fire department, CAL FIRE, Humboldt County Sheriff and Poison Control as well as the Agent in Charge will be posted. Each employee is provided with a written copy of emergency procedures and contact information. The material safety data sheets (MSDS) are kept on site and accessible to employees.

5.5 TOILET AND HANDWASHING FACILITIES

The constructed processing building has an ADA compliant restroom.

5.6 ON SITE HOUSING

The Client and employees live off site and commute daily to the cultivation site. No new residential structures are proposed as a part of this project.

6.0 SECURITY PLAN AND HOURS OF OPERATION

6.1 FACILITY INGRESS / EGRESS

The cultivation facilities, including greenhouses and processing building that will be enclosed with two (2) entry gates. The primary entry gate is located off Upper Sawmill along the PGE access road and leads to the proposed processing building and mixed light greenhouses, and the twenty-seven 5,000 gallon water storage tanks. The entry gates will always remain locked and access to the cultivation area is limited. Restricted access signs are posted conspicuously at the entry gates. The existing Stream Management Areas will not be fenced to allow for animal migration through the riparian areas.

The secondary entry gate is located off Alderpoint Rd

6.2 HOURS OF OPERATION

Microbusiness activities will be conducted Monday through Friday 8 AM to 5 PM or as required during harvest season.

7.0 ENVIRONMENT

7.1 WATER SOURCE AND PROJECTED WATER USE

Water for the microbusiness / processing facility is provided entirely with rainwater catchment, and no other diversions of surface waters are proposed.

The projected water demand for the microbusiness / processing facility will be approximately 500 gallons per day for all uses within the facility.

7.2 WATER STORAGE

The Microbusiness / processing facility will have one 5000 gallon water tank. The total amount of tanks on site for irrigating the cultivation is sixty-one 5000 gallon water tanks. The water tank will be equipped with a UV disinfection system and filter.

Water storage for fire use is provided in the form of a plastic water storage tank. A total of 5,000 gallons of water fire storage exists on the site.

7.3 SITE DRAINAGE, RUNOFF, AND EROSION CONTROL

CLIENT is enrolled with the California Regional Water Quality Control Board for Tier 1 coverage and a Site Management Plan (SMP)has been developed utilizing the Waterboard's recommendations.

7.3.1 DRAINAGE AND RUNOFF

The site is mostly flat with surface flow in the wet season generally draining from the center of the parcel to the west and northeast. Drainage to the east is directed by a ditch that disperses the water into an unnamed tributary.

Site investigation for the development of the Site Management Plan (SMP) showed no evidence of surface runoff with the associated cultivation. To further prevent runoff to riparian areas, water conservation and containment measures will be implemented including the use of hand irrigation to prevent excessive water use, and the maintenance of a stable, vegetated buffer between the cultivation area and riparian zone.

7.3.2 EROSION CONTROL

The Client will utilize best management practices including but not limited to:

1. Maintenance of roads, including rocking and armoring.
2. Proper management of solid, liquid and cultivation waste (see section 3.8)
3. Regulated products will be safely stored with secondary containment (see section 3.7)

7.3.3 WATERSHED AND HABITAT PROTECTION

Adherence to the proposed best management practices ensures that the watershed and surrounding habitat are protected. Site development and maintenance activities utilize BMPs in accordance with the Waterboard's recommendations. Any grading and earthwork activities will be conducted by a licensed contractor in accordance with approved grading permits. The stream setbacks and wildlife

corridors on the property will not be fenced as to allow for animal migration through riparian areas.

7.4 MONITORING AND REPORTING

Monitoring will be conducted to confirm the effectiveness of corrected measures listed in the SMP and determine if the site meets all Standard Conditions. Inspections will include photographic documentation of any controllable sediment discharge sites as identified on the site map. Visual inspection will occur at those locations on the site where pollutants or waste, if uncontained, could be transported into receiving waters, and those locations where runoff from roads or developed areas drain into or towards surface water. The inspection will also document the progress of any plan element subject to a time schedule, or in the process of implementation.

Onsite monitoring shall occur before and after any significant alteration or upgrade to a given stream crossing, road segment, or other controllable sediment discharge site. Inspection should include:

A. photographic documentation, with photo records to be kept on site.

B. Prior to October 15 and December 15 to evaluate site preparedness for storm events and stormwater runoff. Following any rainfall event with an intensity of 3 inches precipitation in 24 hours.

7.5 INVASIVE SPECIES

There are Species of Concern within this project site, some of which have state and federal protection, including Threatened and Endangered Species status. Operations can have an impact to these species at any time, due to noncompliance with environmental regulations. These species can be vegetation, aquatic lifeforms, and terrestrial wildlife including the Northern Spotted Owl (Strix occidentalis caurina). Species of most concern regarding operations and direct impacts are salmon found within local rivers and streams, and avian species can also be affected by activities. Aquatic species are most at risk because they require clean, cool, and flowing rivers for survival.

Activities can potentially have a direct impact upon the water quality of the Class III stream. This in turn affects the salmonid fishery of the Eel River. Impacts to the Eel River can be created through several ways including:

A. Diversion during forbearance period which directly reduces in-stream flows and increases temperatures for juvenile rearing habitat.

B. Diversion rates are higher than allowed, which reduces instream flows needs for seasonal migration and spawning.

C. Infiltration of nutrients beyond the crop root zone into the stream which contributes to in-stream algae blooms and reduced dissolved oxygen levels.

D. Improper implementation of sediment and erosion control measures can result in increased water turbidity.

The impacts listed above are a number of ways those operations can have an impact upon the environment. Water use for seasonal cannabis operations is in direct opposition to the salmonid fishery life cycle which is protected by state and federal law.

It is a primary concern that project operations minimize and mitigate impacts to the local environment. All requirements shall be adhered to as found within permits, including discharge and water forbearance periods, development of environmentally responsive business policies and procedures, and regular monitoring with recording of on-site conditions.

7.6 LIGHTING, ENERGY, AND GENERATOR USE

The power for the microbusiness will be sourced from grid power from PGE, an emergency backup generator in the event of power disruption from PGE. The generator is housed in a shed. The generator is located away from the property line to ensure that noise from cultivation and related activities shall not result in an increase of more than three decibels of continuous noise above existing ambient noise levels at any property line of the site. A noise study will be conducted as part of the application process. The only cultivation lights that will be used is for the nursery and they will just be LED's and T-5's.

8.0 USE AND STORAGE OF REGULATED PRODUCTS

8.1 BEST MANAGEMENT PRACTICES

Best Management Practices (BMP's) are employed when storing, handling, mixing, application and disposal of all fertilizers, pesticides, and fungicides. All nutrients, pesticides and fungicides are located in a locked storage room, and contained within watertight, locked and labeled containers in accordance with manufactures instruction. Application rates will be tracked and reported with the end of the year monitoring report required in the SMP. The persons responsible for application are trained to handle, mix, apply or dispose of pesticides/fungicides with proper hand, eye body and respiratory protection in accordance with the manufacturer's recommendations. See the SMP for complete BMP specifications for the use and storage of regulated products.

9.0 WASTE MANAGEMENT PLAN

9.1 SOLID WASTE MANAGEMENT

Trash and recycling containers are located near the residence. The trash containers are enclosed within a fenced area to prevent animal intrusion. Solid waste and recycling are hauled off-site via a trailer to the Redway Transfer Station, at least once per week.

9.1.1 CULTIVATION WASTE AND SOIL MANAGEMENT

Cultivation vegetative matter such as root balls, branches, and leaves are composted or burned at a designated area. Spent potting soil is used in orchard/vegetable garden. The soil containment area is lined to prevent any soil erosion or nutrient seepage. The soil will be re-amended onsite and reused, new soil will be brought onsite every couple of years and tilled into the beds. All packaging from soil amendments and fertilizers will be collected and disposed of at an appropriate facility.

9.2 WASTEWATER MANAGEMENT

An unpermitted septic system currently serves the residential buildings. A portable restroom will be utilized when seasonal workers are present, and it will be serviced as needed until the processing building with ADA bathroom is completed.

10.0 PRODUCT MANAGEMENT

10.1 PRODUCT TESTING AND LABELING

Samples are selected from individual harvested cannabis strains and are tested by a licensed third-party lab in accordance with State and local standards and will include tracking ID's provided by the County of Humboldt and/or Statewide tracking systems once they become available.

10.2 PRODUCT INVENTORY AND TRACKING

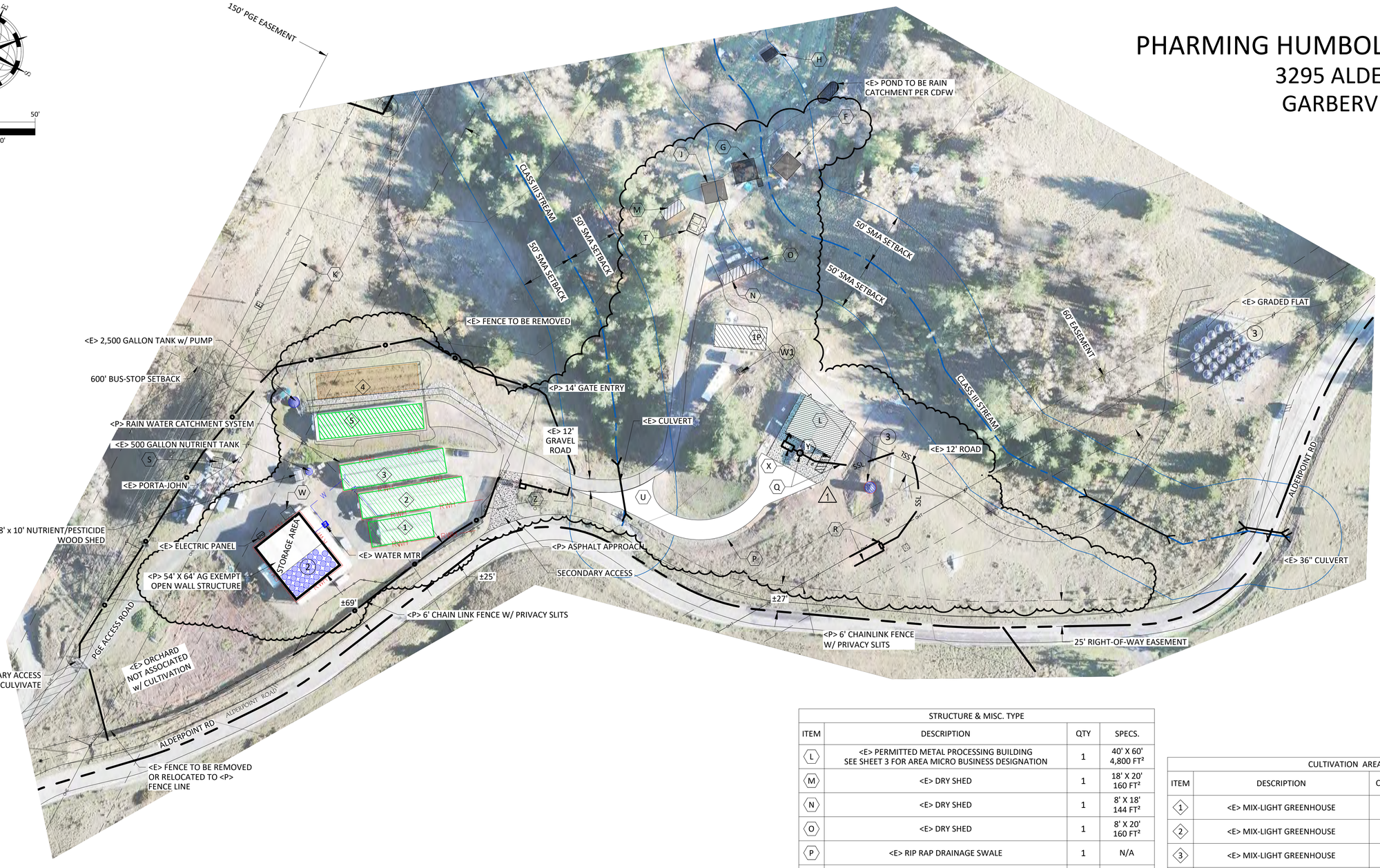
Until such time as either a County or Statewide cannabis product and inventory tracking system becomes available, an internally developed system of inventory and tracking is utilized. The Agent in Charge and Lead Cultivator ensure all medical cannabis from clone to packaged product is tracked, accounted for, and inventoried. Records are kept at each phase of the harvest and processing operation for reporting and compliance with State and Local regulations. The information recorded for each harvest includes:

- Cultivation canopy area
- Weight of flowers, by-product, and trim waste after drying and separation
- Weight of buds after trimming
- Product ID numbers and product weight.
- Staff identification (at each step).
- Physical location of the plant material at all times.

Exhibit **A**

PLOT PLAN
PHARMING HUMBOLDT DREAMS
3295 ALDERPOINT ROAD
GARBERVILLE, CA 95542

50' 0' 50'
SCALE: 1" = 50'



NOTE: RAIN FALL CATCHMENTS WILL BE PRIMARY IRRIGATION WATER SOURCE

WATER STORAGE						
ITEM	DESCRIPTION	QTY	USAGE	TOTAL CAPACITY	LATITUDE	LONGITUDE
2	<E> 5,000 GALLON PE WATER TANK	31	IRRIGATION	155,000 GALLONS	40.11389167°	-123.7541667°
2	<P> 5,000 GALLON PE WATER TANK	28	IRRIGATION	140,000 GALLONS	40.11163594°	-123.75338882°
3	<P> 5,000 GALLON PE WATER TANK 2,500 GALLON FIRE PROTECTION 2,500 GALLON FOR BUILDING	1	FIRE PROTECTION & BUILDING	400,000 GALLONS	40.11151111°	-123.75361111°
W1	<E> WELL WITH PUMP (BACK UP IRRIGATION)	1	IRRIGATION	N/A	40.11288889°	-123.75361111°

STRUCTURE & MISC. TYPE ***NOT ASSOCIATED WITH CULTIVATION* OR MICRO BUSINESS**			
ITEM	DESCRIPTION	QTY	SPECS.
F	<E> TWO-STORY BUILDING	1	20 X 24' ± 960 FT²
G	<E> OUTDOOR LIVING AREA / DECKING	1	20 X 24' ± 960 FT²
H	<E> BUILDING *TO BE REMOVED*	1	10' X 15' ± 150 FT²
J	<E> METAL POD (8'x20') <E> WOOD SHED (8'x22')	1	TOTAL 336 FT²
K	<P> PGE ACCESS ROAD (BY OTHERS)	1	N/A

STRUCTURE & MISC. TYPE			
ITEM	DESCRIPTION	QTY	SPECS.
L	<E> PERMITTED METAL PROCESSING BUILDING SEE SHEET 3 FOR AREA MICRO BUSINESS DESIGNATION	1	40' X 60' 4,800 FT²
M	<E> DRY SHED	1	18' X 20' 160 FT²
N	<E> DRY SHED	1	8' X 18' 144 FT²
O	<E> DRY SHED	1	8' X 20' 160 FT²
P	<E> RIP RAP DRAINAGE SWALE	1	N/A
Q	<E> SEPTIC DOSING TANK & PUMP w/ 2" PE FM	1	N/A
R	<E> SEPTIC DIST-BOX AND LEACH LINES	1	N/A
S	<E> COMPOST AREA	1	N/A
T	<E> SECURE TRASH ENCLOSURE	1	N/A
U	<E> SRA TURN-A-ROUND	1	N/A
W	<E> 500 GALLON PROPANE TANK WITH 4 TIER BACKUP GENERATOR	1	N/A
X	<E> 1,500 GALLON SEPTIC TANK	1	N/A
Y	<E> (1) ADA VAN ACCESSIBLE AND (4) STANDARD PARKING	5	N/A
Z	<E> ROLLING GATE - SECONDARY ACCESS	1	14'

CULTIVATION AREA				
ITEM	DESCRIPTION	QTY	SPECS.	CANOPY AREA
1	<E> MIX-LIGHT GREENHOUSE	1	24' X 50' 1,200 FT²	1,200 FT²
2	<E> MIX-LIGHT GREENHOUSE	1	24' X 100' 2,400 FT²	2,400 FT²
3	<E> MIX-LIGHT GREENHOUSE	1	24' X 100' 2,400 FT²	2,400 FT²
4	<E> OUTDOOR GARDEN	1	20' X 80' 1,600 FT²	1,600 FT²
5	<E> 24' X 100' MIXED LIGHT GREENHOUSE	1	24' X 100' 2,400 FT²	2,400 FT²
TOTAL CULTIVATION AREA				10,000 FT²

PROPAGATION			
ITEM	DESCRIPTION	QTY	SPECS.
1P	<E> 24' X 100' GREENHOUSE 20' X 50' USED FOR PROPAGATION (SEE GREENHOUSE LAYOUT ON SHEET 3)	1	20' X 50' 1,000 FT²

DTN ENGINEERING&CONSULTING
dnicoletti@dtnengineering.com

NO.	HISTORY / REVISIONS	BY	CHCK	DATE
1	REVISED BUILDINGS, SEPTIC SYSTEM, DRIVEWAY, TABLES & ADDED TABLE			

PLOT PLAN
PHARMING HUMBOLDT DREAMS
3295 ALDERPOINT ROAD
GARBERVILLE, CA 95542

APN: 223-241-006-000
DRAWN BY: RGN - UDD
DATE: 3/18/2025

PLOT PLAN ENLARGED

SHEET NO.
2
OF 3

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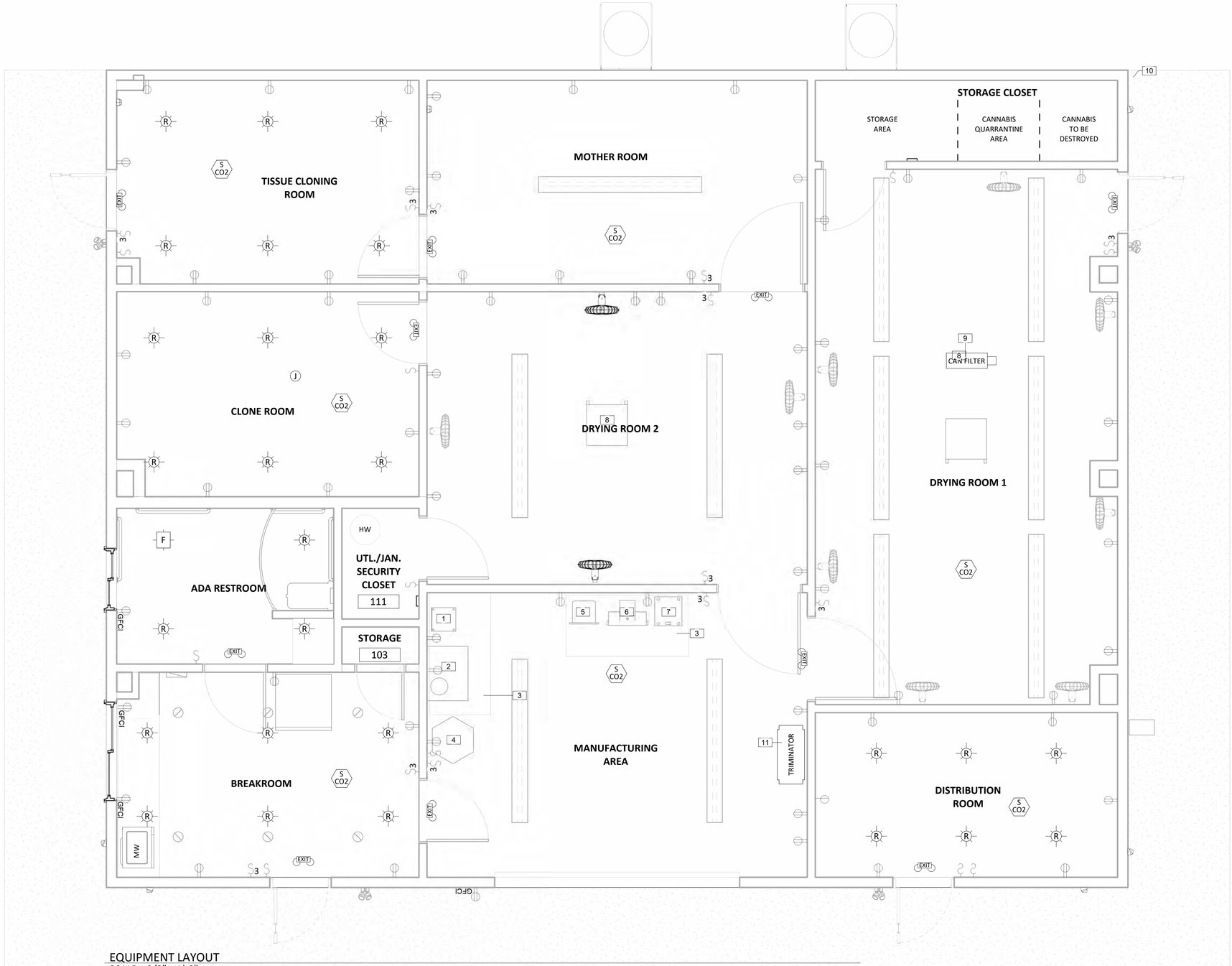

EQUIPMENT LAYOUT
SCALE: 3/8" = 1'-0"

Equipment	
Key	Equipment Name
1	Rosin Press
2	Freeze Dryer
3	3' x 6' Work Table - Stainless Steel
4	Preroll Machine
5	Vacuum Oven
6	Vacuum Sealer
7	Scale
8	Dehumidifier
9	Carbon Filter and Fan
10	Security Camera
11	Triminator

DTN ENGINEERING&CONSULTING
dnicoletti@dtnengineering.com

REGISTERED PROFESSIONAL ENGINEER * DANIEL NICOLETTI * 68414 * CIVIL * STATE OF CALIFORNIA

NO.	HISTORY / REVISION	DATE

PHARMING HUMBOLDT DREAMS
COMMERCIAL BUILDING - APN: 223-241-006
3295 ALDERPOINT ROAD
GARBERVILLE, CA

APN: 223-241-006
DRAWN BY: RKH
DATE: 1/4/2024 8:29:08 AM

EQUIPMENT PLAN

SHEET NO.
A1.5
OF27

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Exhibit **B**

Media Preparation

Ingredients to make 500ml Establishment/Multiplication medium

- Two liters of purified water, RO or deionized
- 15g sucrose (ordinary white sugar)
- 2.22g of Murashige & Skoog Basal Salts with Vitamins (M519)
- 0.5ml of 1 mg/ml Benzyl Amino Purine (BAP) phytohormone
- 4.4g of TC -grade agar
- 1mL of PPM™ (PCT's broad-spectrum biocide/fungicide for plant tissue culture - the ultimate solution to the never-ending struggle against microbial airborne, waterborne and endogenous contamination.)

Materials

- Pint Mason Jars with lids for sterile water
- 500+ ml beaker for mixing/dispensing media
- Scale
- Gloves
- Laminar Flow Hood
- Magnetic Stirrer/bar/retriever
- Measuring papers or boats
- Micropipetters and 1 ml tips
- PH meter
- Dilute Potassium Hydroxide solution (0.1N KOH) pH up
- Dilute Phosphoric Acid solution (0.1N H2PO4) pH down
- Spray Bottle of 70 - 95% alcohol
- Pressure Cooker
- Measuring/dispensing syringe
- Timer
- Vessels - We will use (20)

Procedures

Prepare Sterile Water

- Fill Pint Mason jars 80% full with purified water.
- Fit lid loosely enough to allow even pressure inside of jar and set aside to sterilize.

Preparing Media

1. Premeasure the dry ingredients-sugar, agar and nutrient salts.
2. Pre-set your micropipette to 0.5mL.
3. Set your mixing flask on the magnetic stirrer and add a magnetic stir bar.
4. Add **About 400mL of purified water** to the mixing flask.
5. Power the magnetic stirrer until the bar is stirring quickly but not bouncing.
6. Gradually add **Sucrose** to the mixing flask, rinsing weigh boat into the mix.
7. Slowly add **2.22g of MS Basal Salts with Vitamins** to avoid clumping of dry powder.
8. **Micropipette** to measure the exact hormone amount.
9. Gradually add **TC-Grade Agar**, also avoiding clumping.
10. **Add PPM™** using the micropipette.
11. Slow the magnetic stirrer to allow the level to be read.
12. Add purified water to the final volume.
13. Speed up the magnetic stirrer to suspend the agar powder.

Adjusting pH

1. Start the pH meter. Ensure it has been calibrated to pH 7 and pH 4.
2. Remove the probe cap, rinse it with water, shake off or pat dry, and place it into the mixing beaker.
3. Use solutions to adjust pH to 6.2. Note: agar typically lowers pH by about 0.2 as it solidifies.
4. Rinse the probe and cap it. Store in a few drops of 4.0 buffer.

Preparing the Tissue Culture Vessels

1. Remove lids of media vessels, and line them up.
2. Keep agar powder evenly suspended when pouring or using measuring baster. 3. Fill each GA7 vessel with approximately **50mL of media**. (=250ml for 5 vessels)
4. Cap lids loosely and stack in the pressure cooker.

Prepare Sterile Rinse Water and Paper Towels

Fill two Pint Mason jars with RO water, loose lids and stack in the pressure cooker as Sterile Rinse water.

Cut commercial paper towels in half and fold loosely in aluminum foil. Stack in pressure cooker. with tools. Similarly, wrap metal tools in foil for sterilizing.

Autoclaving - Using the Pressure Cooker

Note that we are using an All American brand sterilizer with an outer pot that boils water and seals the entire unit, a thin inner pot that holds the sterilizing items, and a venting tube that runs to the bottom of the inside pot. The inner pot and vent tube are designed to allow steam from the outer pot to move up and around the inner pot and purge the air from top down.

Modify the venting so water can be added to the inner pot to boil and mix the agar for proper melting and gel set. We will add ¾" purified water to the inner pot and move the tube to the space above the sterilized items. We will also use a home pressure cooker with automatic venting built into the lid for faster sterilizing of small loads.

5. Set pressure cooker to **15PSI**.
6. **Load vessels and sterile water jars** into the pressure cooker to be sterilized.
7. Place tools on top of the vessels and jars.
8. Place the pressure cooker lid on the pot without sealing it and let the air escape.
    i. Note: You will hear the steam bath bubbling as this is happening.
9. **After a few minutes** steam will be escaping under the lid and it will be ready to seal. Lock down the lid of the pressure cooker. The meter will be in the *green zone*.
10. Ensure that the pressure cooker pressure meter stays in the green zone the entire time and start the **20-minute timer**.

Prepare the laminar hood

11. **Spray** the Laminar Hood filter screen and all inside surfaces and edges with alcohol.
12. Start the Laminar Flow Hood and watch the speed increase.
13. Turn off the autoclave after **20 minutes** and it to cool naturally.
14. In the case of the home pressure cooker, it can be moved into the hood to cool
15. Remove the lid when pressure is zero and lock drops.
16. Using the **autoclave gloves**, take the removable inside pot and place it inside the Laminar Flow Hood. The clean air removes the heat without threat of recontamination
17. Items can be unloaded if faster cooling is needed. Use autoclave gloves for hot items.

18. Store cooled sterile vessels on upper grow racks, out of direct light

(clean room slide)

(prep room slide)

Cannabis cultures are initiated from meristem tissues, the growing points at the tips and nodes where growth originates, not from leaves or stems. Some plants can be cultured from those tissues but for cannabis at this time those tissue are too inefficient and complicated for production culture. We will start with 4-5cm tip cuttings and work down to smaller single node cuttings, dissected meristems and even bud cluster florets as we go through the workshop.

Start with the best vegetative donor plants such as young Veg plants and avoid tired mothers. The tips that are pruned from veg plants to force strong side shoots and distributed growth such as for indoor grows under artificial lights are ideal tissue culture starts. The most vigorous plants from cloning can be easily identified and the tips must be removed anyway. One inch prunings make poor cuttings but are ideal tissue clones. As the veg plants push out more side growth after the pruning, the best plants can be tissue cloned again. Best plants should be marked, such as with a blue dot, to insure they make up the new veg stock and tissue culture mother/donor plants if Multiplication Is to be used.

Other tips and nodes that are removed can also be tissue cloned for backup and clone sales. A vertical farm can produce all of their veg stock from best-plant tissue culture, and supply another farm or two with quality A- grade, just from the material necessarily pruned from the veg plants, ***without ever keeping another mother plant***.

Cut eight two-inch tip cuttings and trim off the leaves with only ¼" of leaf stem (petiole) remaining, per cutting protocol. We will plant these in Root media for fastest rooting and take-homes. Then take another 20 one-inch tip and node cuttings that we will plant in the Multiplication medium we made in section 1. Clean the 2-inch cuttings using the Dichlor. They clean slower and do not need to be rinsed which gives us time to work and learn the new tools and techniques.

When those are in tubes and labeled, the smaller cuttings can be cleaned using the Clorox protocol (10% for 15 min) plus the sterile water rinse.

Plant Collection and Cleaning/Tissue Culture

Ingredients

- Purified (RO/Deionized) Water
- 150mL of Household Bleach (between 3.5-5%)
- 2 Drops of Polysorbate 20 (Tween 20) or Dawn detergent
- Optional: Dichlor granular chlorine cleaner-2g/liter or equivalent

Materials

- Two beaker, jars or pitchers of 1000mL or more
- Clean spring-assisted snips
- Plant waste collection tray
- Magnetic Stirrer with stir bar or orbital shaker
- Medium-neck pint or quart mason jar with lid
- Plants
- Timer
- Spray bottle of 70% isopropyl alcohol (alcohol spray)
- Laminar Flow Hood (Hood)
- Tool sterilizer such as alcohol Lamp or glass bead sterilizer ● Tool rest for hot instruments ● TC tools:
    - 8” and 12” forceps
    - Scalpel and new blade, #10, #11
    - SS surgical scissors
    - Sterile paper towels/paper plates/glass plates
- GA-7 Vessels with sterile semisolid media
- Small trash can

Procedures

Make Sterilization Solution

1. Make 10% bleach by adding **100mL of Household Bleach** to 900ml purified water in the beaker.
2. **Add 2 Drops of Polysorbate 20 (Tween 20)** or Dawn detergent to break surface tension.
3. Set aside for plant cleaning.
4. Optional: Make Dichlor solution of 1 gram Dichlor to one liter purified water, plus Dawn

Taking Plant Cuttings

5. Add purified water to the Mason jar to about ⅔ full plus two drops of detergent.
6. Evaluate the plant for cutting locations, limbs near the top with nodes and tips that will be just over an inch. Review cutting location illustration.
7. Using your spring-assisted scissors, trim the limb off ¼" above any node being left on the donor plant.
8. Trim off the leaves leaving **about ¼ inch** of the leaf stems (petioles).
9. Divide the limb into 1-½ to 2 inch sections for cleaning. Sections will be trimmed once more before culture.
10. Continue until you have 20 node and tip cuttings.
11. Place the plant cuttings in the mason jar, cap the jar and **swirl it around** to eliminate any topical contaminants.
12. Replace the lid with a wash screen and pour off the water.

Plant Cuttings Sterilization

13. Wash hands with soap up to elbows.
14. Spray off your hands and arms with alcohol.
15. Set a **14-minute** timer on the magnetic stirrer.
16. Carefully add the magnetic stir bar to the mason jar with the plant cuttings.
17. Pour the prepared bleach solution into the mason jar with the plant cuttings.
18. Run the jar on the magnetic stirrer at slow/medium speed.
    a. Alternatively, an orbital shaker is easier on the plant cuttings.
19. After the set time, **spray your hands and the outside of the mason jar** with alcohol and place in the hood.
20. **Pour off the bleach** solution into a large beaker.
21. **Rinse the plant cuttings** in the jar with Sterile Rinse water for about 30-45 seconds.
22. Pour off and repeat two more times.
    i. Note: After the rinses, you will notice some bleach damage. This is normal.
23. Sterilize all tools with an alcohol spray. An alcohol lamp or bead sterilizer is a plus..
24. Set up your **sterilized workstation**, including your sterilized paper towels, forceps, scalpel and surgical scissors.
25. Using sterile forceps, take four cuttings from the mason jar and place them on a **sterile paper towel**.

26. Using the 8" forceps and scalpel, or scissors, trim the petioles back to ⅛" and remove any bleached leaf tips. Trim off ¼" from the base of the cutting.
    i. Single node cuttings only need ⅛" stem above the node.
    ii. Bleach is absorbed deepest at the base.
27. Sterilize your tools again.
28. Open the lid of a clear square vessel with sterile solid media
29. With a sterilized pair of forceps, place a trimmed cutting in the center of a quadrant of the media. Replace the cover if working slowly.
30. Repeat for the other three node or tip cuttings.
31. **Close the lid** and set it aside.
32. Label vessel w vital info-Strain, date, initials, etc.
33. Sterilize tools and place on Tool Rest.
34. Wrap up litter in paper towel and place in compost
35. Repeat with four cuttings at a time until all cuttings are in vessels.

Together, we will make an agar-free multiplication media to use in Day 2 bioreactor trials. Follow Multiplication media protocol, adjusted to one liter, and leave out agar.

Together, prepare sterile coir tubes for Day 2 Sterile Seed Germination trials. Add coir to 2.5 to 3 cm in narrow vessels such as 25mm glass culture vessels or centrifuge tubes and use cork plunger to press gently. Add drops of water to moisten, leaving visible air pockets. Sterilize for 20 min and allow to cool in a sterile hood. Be sure to have some sterile water on hand to hydrate seeds after surface sterilizing.

Meristem Trials

Introduction

To eliminate viroids by tissue culture, a piece of new growth that the viroid cannot reach is cut and cultured. That piece is the apical meristem and can be sensitive to different tissue culture media. Removing it requires a powerful microscope with lenses for both eyes and steady hands to remove it carefully.

The extreme meristem tip of a shoot is the apical (topmost) portion of the shoot usually equal to or less than 0.5 mm in size. For the most consistent chance of viroid and virus remediation, excised tips should only have one to two sets of leaf primordia. The apical portion of the shoot segment comprises meristem tissue. The meristematic tip can be excised using a scalpel under the dissecting microscope.

The method can further comprise transferring the meristematic tip into a culturing plate comprising a supplemented suitable plant growth medium for further culturing. The meristematic tip can comprise the apical dome and a limited number of young leaf primordia. The meristematic tip excludes any differentiated provascular tissues or vascular tissues. Shoot segments including procambium, xylem, or phloem may contain viroids.

After induction, leave meristems for 4-6 weeks to grow in Petri dishes on hormone-free media, then subculture individuals into separate vessels for stage 2 multiplication phytohormone trials. Testing of plants for different viruses and viroids should be performed before induction and after plants are recovered several times and tested before being declared pathogen free for your in-house clean plant program.

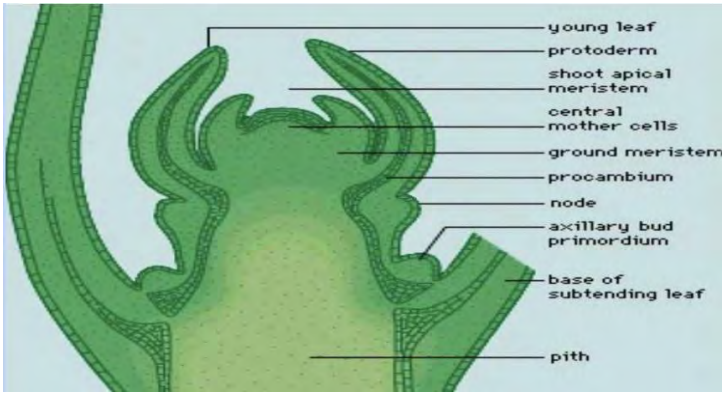

Figure 1. Meristem

Ingredients

- Prepared media (Video #1)
- Cleaned Plants (completed sterilization process from video #2)

Materials

- 50 Tissue Culture Plastic Test Tubes with Lids
- Test tube rack
- Micropipette
- Prepared media container (Video #1)
- Sterilized TC Tools
- Laminar Flow Hood
- GA-7 Vessel
- Microscope
- Petri Dish
- Sterilized Paper Towels
- Scalpel

Procedures

Preparation of the Test Tubes and Petri dishes

1. Prepare media on magnetic stirrer per earlier instructions.
2. Wash hands.
3. **Set up your sterilize handling station** under the hood with all your materials.
4. Arrange test tubes in the rack in a location where some spilling is ok.
5. Dispense 15 ml of media with kitchen baster into the first tube.
6. Repeat for all tubes making sure each tube gets an even amount of suspended agar gel powder.
7. Cap the tubes, but not completely tight.
8. Similarly, fill the lower half (narrower) of the Petri dishes with 6mm of media and cover.
9. Load the Pressure Cooker with tools and paper towels as before.
10. Move items to Filter Hood to cool after autoclave cycle. Stack for use.

Meristem Dissection

1. If you have not already done so, complete the process in *video #2* of **sterilizing your plant cuttings**.
2. Rinsing your plant material in water in the wash jar.
3. Using sterile forceps, **take only one** of your apical meristems and place it on the sterilized paper towel.
4. Make your first major cuts outside of the microscope, then place them on the petri dish **under the microscope** to perform the minor cuts.

i. Note: You will focus on removing the **leaf primordia** and leaving the **apical meristem**. (*See Figure 1*).
ii. We recommend you watch video #3 for this part of the process.

5. After removing all of the leaves and are only left with the apical meristem, use a scalpel blade tip and place each apical meristem in a test tube.
    i. Note: Complete this process one at a time to avoid contamination.
6. **Subculture every 21 days** until it is regenerated.
    i. Tip: We like to do these in batches because not all of them will survive.

Alternate Shooting Protocols

1. Hormone Free, Induce Node allowing endogenous auxins generated at the meristem to root plantlet, then resulting cytokinins being generated in the roots drive shoot growth and development.
2. Lata Plus, 0.4mg mT(Optional: 0.1mg(100ul) GA3)c
3. Phytoax, 0.5ml/L or 0.25ml/L (prefers DKW)
4. Moonshine Formula, 0.3mg(300ul) BAP, 0.05mg(50ul) GA3, and 0.2mg(200ul) IBA per lt.
5. Perpetual Formula, 1mg KIN & 0.5mg NAA

Rooting Phytohormone Trials

When ready to root plantlets, either leave plantlets hormone-free until rooted or subculture into one of the following. Consider ex vitro plug rooting or running rooting in vitro trials at ½ MS and ½ Sucrose.

1. Auxin Dip, dip node into Clonex rooting gel or Synergy rooting solution for 5-10 seconds, then induce into the hormone-free gel or go to plug rooting model.
2. Auxin In Media 1, 0.5mg IBA
3. Auxin In Media 2, 1.0mg IBA

Synergy Rooting Solution

- 10.5ml 99% Iso
- 1.3ml (1,300ul) IBA (1mg/ml)
- 0.65ml (650ul) NAA (1mg/ml)
- 87ml Water
- 0.55ml (550ul) Food Dye (Green)
- = 100ml Total
- Aliquot into 2ml microcentrifuge tubes and store in the freezer until needed.

Explant Preparation

The typical induction of plantlets from a room that is known to be clear of bugs and with or without known mold issues goes:

1. Place cuttings into a jar with **10% bleach solution with 2 drops of tween 20 per 1500mL**.
2. Agitate for **13.5 - 15 minutes** on an orbital shaker, by hand, or with a magnetic stirrer.
3. Pour out bleach water and **rinse explants 2 times** with sterilized water.
4. Trim branched shoots are to be at **least 1.0 cm in length** and contain **at least one node**.
    i. Example: Stem containing the leaf junction approximately **½ to ¾** the way up the length of the stem.
5. Remove the shoot **apical meristem**.
6. Cut all the petioles halfway between the leaf and stem on each node.
7. Place nodes on the appropriate basal media fo**r 14 - 21 days** in the growth room.
8. Subculture growing stem tissue into nodes so that leaves are removed at petiole, shoot apical meristem is removed, and tissue is at least **1.0 cm in length**.
9. If plants have known bugs consider adding an isopropyl wash using **70% before the bleach wash.**
    i. Remember that not all phenotypes will tolerate an aggressive sterilizer the same way.

Seed Germinating Protocol

The Seed Starter kit provides seeds with the greatest possible advantages for germination. The kit should be ideal for those attempting to germinate old seeds, valuable seeds, and difficult to germinate seeds. Tissue culture provides a microbe-free environment containing sugar, nutrients, and vitamins that provide developing plants with energy and mineral nutrition. This kit will decontaminate the surface of seeds, hydrate them with a sterile solution containing uM sugar, nutrient and vitamins,, and germinate them on a sterile medium.

When plants are actively growing, they are inoculated with beneficial microorganisms and planted in larger containers and raised as normal. Seeds can be cleaned and added in batches such as when mixing varieties or doing a trial run. We recommend that with any new technique, the user should dry run to make sure all the parts are nearby when they are needed and that reaching them is fast and convenient. And of course it will be unfamiliar the first time through so maybe washing six healthy young seeds will give good practice and demonstrate how the cleaning and growing stages will go. Leave enough of the solutions for both batches.

The materials in the kit are: 1) Seed wash vial with thermometer and drainage holes 2) Seed surface sterilizing solution powder (A) 3) Seed hydrating solution (B) 4) Sterile seed germination media-ten tubes and tray 5) Forceps 8" 6) Sealing tape 7) Two tubes of tissue culture medium for seed rescue

Instructions:

1. Wash hands before using kit. Hydrate Seed Sterilizing pill (A) in 60ml water, about ½ full in Wash Vial.
2. Find location that will maintain at 26 degrees C. Good warm locations are bright indirectly lighted windows, above the refrigerator, on top of a nightlight or similar. Use cardboard as an insulating spacer if needed.
3. Clean seeds of any surface debris and place in wash vial. Swirl occasionally and roll in vial to wash seed surface and inside of vial. You may want to cover holes with small pieces of tape but covering with clean fingers is OK.
4. Soak seeds for one hour. The Sterilizing Solution may be saved in a separate container for using again within a few days.
5. Drain Sterilizing Solution and add Hydrating Solution (B) and soak in warm location until seeds sink. The root radical may not yet appear. Usually about 24 hours. The ideal temperature for soaking is 26-28 degrees C. It is a good idea to test your warm location before using real seeds.
6. Setup a clean handling area: Seeds can be planted in tubes on any clean surface, however, a clean hood helps prevent contaminating organisms from getting into the sterile planting tubes when planting. It is simply a plastic storage container turned on its side to prevent air from drafting over the open containers.(see picture)
7. Set up in a clean kitchen or bathroom, if possible. HEPA air filtering is always recommended in any growing environment and especially in a clean handling environment.
8. Clean forceps with an alcohol spray before using. 70% or 91% is fine. Wash and spray hands for added protection. Spray inside area of forceps too. A tall narrow tube of alcohol is a good place to clean tools (see setup in the picture). Forceps are used to pick up and plant seeds.
9. Have the planting tubes within reach and work quickly.
10. Drain sugar solution into a small cup in the clean hood.
11. Loosen the lid of one of the planting tubes.
12. Pick up your first seed , using the forceps, with the root end pointed away from the forceps. The point on the seed end should be like the pointy end of a spear, the best way to handle and plant a decontaminated seed.
13. Stab into media about ¼" deep along the edge of the tube. The progress of the germination can be observed through the plastic.

14. Grow the tube plants under a small fluorescent light in a cool area not exceeding 76 degrees. The closed tube is like a greenhouse and will be a few degrees warmer inside the tube. Provide ten watts per sq foot of light, same as clone lighting.
15. When the seedling stands up and the leaves are open to the sky, the seedling is ready to be planted. Use the forceps to remove it along with attached soil and plant in a small pot for continued growth under the fluorescent light.
16. Add root microbes if you have them.
17. Cover with a dome if dry air is a concern. Seedlings adapt to new soil within a few days and will grow as normal.

Tissue Culture Seed Embryo Rescue

1. Partially germinated seeds can be moved to TC Multiplication media to feed the hypocotyl, cotyledons, and apical meristem of extracted seed embryos.
2. Follow Sterile Hood technique to move seedling to fresh Dichlor Sterilizing Solution (A) in a shallow dip cup to rinse off sterile coco.
3. Move to a clean saucer to attempt to remove the seed shell. Only remove if easy. Any forcing will damage delicate seed leaf tissue.
4. Plant in agar tube off center with seed leaves and root radicle pointed up, in U-shape.
5. Cap tube and repeat. Label the tubes with details.
6. TC seedlings will either stand up as expected and planted in soil or be maintained in tissue culture environments until viable shoots can be produced.
7. Embryos may also be encapsulated in artificial seeds and/or cryopreserved.

Artificial Seed Protocol

Synthetic tissue culture seeds (Synth Seeds) are made from tiny meristem tissues and embryos that are encapsulated with media and a soft alginate agar skin. Synth Seeds are valuable for storage and transportation, including cryopreservation. Cannabis Synth Seeds are not yet ready to plant in soil pots and are returned to tissue culture to grow. Best practice is make Synth Seeds with 5mm nodal meristems and excised seed embryos.

We will load our ***largest pipettes*** with nutrient media containing thick agar alginate and make seeds by inserting them into the controlled drops falling into the CaCl solution. The calcium in the beaker reacts with the alginate on the surface of the sticky drop to form a containing skin. Leave drops in solution and swirl gently to react. Decant off excess solution and store "seeds" in a sterile tube with lid.

We begin by making a media with a special gel. **Ingredients**

- Sodium alginate
- Calcium chloride
- Purified water
- Media nutrient salts with vitamins
- Phytohormones
- Sugar
- PPM™

Materials

- Beaker greater than 100ml and glass rod stirrer
- Heated magnetic stirrer and bar
- Scale
- pH meter and correction solutions
- Micropipette and tips
- Centrifuge tubes and lids, 30ml+
- Optional microwave oven

Procedures

1. Prepare and sterilize 100 ml 0.5uM TDZ media in a beaker on stirrer:
    i. 3g sugar
    ii. 0.44g MS with vitamins
    iii. 0.5uM TDZ (=0.11ml of 1mg/ml TDZ solution) iv. 0.2ml PPM

v. 5g sodium alginate

2. Add sugar, nutrients, vitamins, phytohormone and preservative to purified water.
3. pH to 6.2.
4. Bring to a boil in the microwave or on stirrer
5. Dissolve alginate by adding slowly while stirring with a glass rod
6. Prepare and sterilize 100ml 50mM Calcium Chloride
    a. 0.74g (CaCl2 x 2H2o) or 0.55g (CaCl2 anhydrous) in 100ml water
7. Sterilize a weigh spatula and dissecting forceps.
8. Sterilize pipette tips cut to 5mm opening
9. Sterilize centrifuge tubes synth seeds will be stored in

Making Synthetic Seeds in the Sterile Hood

1. Arrange within reach in hood
    a. 5mm sterilized shoot tips and excised embryos in sterile petri dish
    b. Pipetter and pipettes
    c. Sterile instruments and paper towels
    d. CaCl solution in sterile beaker
    e. Centrifuge tubes
2. Using forceps, place one meristem tip on surface of alginate solution
3. Attach sterile pipette tip to pipetter
4. Using the pipetter, gently lift a few ml or medium into the opening of the tip and then the meristem . The tiny plant should have a protective coating and enough gel above it to fall into the CaCl by gravity.
5. Drop the meristem and alginate into the CaCl and the shell will form in about ten minutes
6. The spatula or forceps may be used to dislodge stuck meristems. Alternatively, the spatula and forceps can be used to retrieve and drop meristems, with sufficient gel attached.
7. After ten minutes, drain off CaCl and transfer synth seeds into centrifuge tube and label

Equipment List

All items are clickable links. If you do not own the virtual copy of this document, please email info@plantcelltechnology.com, and we will be happy to assist you.

Lab Equipment		
Item	**Size**	**Quantity**
1080p Monitor	N/A	1
Autoclave Gloves	1 Set	1
Beaker Set (50mL, 100mL, & 250mL)	Set	1
Blue Glass Droppers	1oz.	12
Camera Mounts	N/A	1
Castroviejo Scissors	5.5	3pc.
Dissection Tweezers	5.5”	3
Filter Patches 0.3 Micron	N/A	512ct.
Forceps	8”	6
Forceps	12”	6
Glass Beaker	2000mL	2
Glass Rods	1 Pack	1
HDMI Cord	N/A	1
Infrared Thermometer	N/A	1
Instrument Rest	N/A	1

Laminar Flow Hood	N/A	1
Light Timer	N/A	2
Magnetic Stir Bar Retriever	N/A	1
Magnetic Stir Bars	Set	1
Magnetic Stirrer	3000mL	1
Measuring Cup	32oz.	2
Media Bottle	100mL	2
Media Bottle	1 Liter	4
Media Bottle	2 Liter	4
Microcentrifuge	N/A	1
Microcentrifuge Tube Rack	N/A	3
Microcentrifuge Tubes	15mL	100pc.
Microcentrifuge Tubes	2.0mL	1000ct.
Microcentrifuge Tubes	0.2mL	1000ct.
Micropore Tape	N/A	48pc.
Microscope Camera	N/A	1
Microwave	N/A	1
Orbital Shaker	N/A	1

Permanent Markers	Black	12pc.
pH Meter	N/A	1
Pipette Tips	10mL	100pc.
Pipettor	10mL	1
Pipettor Set	Set	1
Plastic Mason Jar Lids	N/A	32pc.
Plastic Spray Bottles	N/A	4
Pressure Cooker	27.3 Quarts	2
Refrigerator	N/A	1
Ring Light	N/A	1
Root Riot Plugs in trays	N/A	400ct.

Scale	0.000g	1
Scalpel Blades #10	#10	100ct.
Scalpel Handle (No. 3) with #11 Blades	Kit	3 Kits
Specimen Lighting	4ft	4
Specimen Shelving	N/A	1
Stereo Microscope	N/A	1
Surgical Gloves	N/A	5

Syringe Filters	13mm	50ct.
Syringes	10mL	100ct.
Thermal Cycler	N/A	1
Tissue Culture Vessel Lids	N/A	500ct.
Tissue Culture Vessels	N/A	500ct.
Tool Sterilizer	N/A	1
Vortex	N/A	1
Weigh Boats	N/A	1000ct.
Wide-Mouth Mason Jars	16oz	32pc.
YouPCR Starter Bundle	Reactions	50

Consumables		
Item	**Size**	**Quantity**
70% Isopropyl Alcohol	5 Gallon	2
99% Isopropyl Alcohol	1 Gallon	1
Aluminum Foil	1000ft Roll	1
BAP B130	500mL	1
Bleach	1 Gallon	5
Buret, Stopcock Dropper type and stand clamp	1	1

Calcium Chloride	2lbs	1
Carbenicillin C346	5g	1
Dish Soap	N/A	As Needed
Distilled Water	1 Gallon	20
DMSO D241	500mL	1
GA3 G198	25mL	1
Gamborg's Vitamins G210	500mL	1
IBA I460	100mL	2
Kinetin K483	500mL	1
mT T7885	25mL	1
Murashige & Skoog Medium M519	50L	2
NAA N605	500mL	1
Nuclease Free Water	500mL	1
Nystatin N581	5g	1
Paper Towels	N/A	20
Polysorbate 20	4oz Bottle	2
PPM™ Biocide	250mL	1

Ribavirin R795	1g	2

Sodium Alginate A108	250g	1
Sodium Hydroxide S835	1L	1
Sugar/Sucrose	5lbs.	1
Sulfuric Acid S804	1L	1
Supreme Tissue Culture-Grade Agar A1000	1kg	1