Chemical Standard Operating Procedure

All work involving materials classified as Particularly Hazardous requires the completion of Section 6.

Procedure Name	Plant DNA Extraction				
Procedure Author					
Name of Responsible Person					
Location to be Performed					
Creation Date		**Review Date(s)**		**Revision Date(s)**	

1. THIS STANDARD OPERATING PROCEDURE (SOP) IS FOR A:

☒Specific laboratory procedure or experiment
- Examples: synthesis of chemiluminescent esters

☐Generic laboratory procedure that covers several chemicals
- Examples: distillation, chromatography

☐Generic use of a specific chemical or class of chemicals with similar hazards
- Examples: Organic azides, mineral acids, hydrofluoric acid

2. DESCRIPTION: *Briefly describe how the chemical will be used.*

Cell lysis required for DNA extraction

3. RISK IDENTIFICATION: *Identify potential safety hazards – refer to Section 2 of the SDS.*

☐Explosive	☐Carcinogen
☐Pyrophoric	☒Sensitizer (respiratory and/or skin)
☒Flammable (liquid, solid, gas or aerosol)	☒Irritant (skin and/or eye)
☐Self-Reactive	☒Corrosive (skin and/or eye damage)
☐Peroxide Forming	☒Acute Toxicity (oral, dermal and/or inhalation)
☐Organic Peroxide	☐Germ Cell Mutagen
☐Oxidizing (liquid, solid or gas)	☐Reproductive Toxicity
☐Water-Reactive	☒Specific Target Organ Toxicity: Single Exposure
☐Compressed Gases	☒Specific Target Organ Toxicity: Repeated Exposure
☐Cryogen	☐Other: Click or tap here to enter text.
☐Corrosion to Metals	
☐Radionuclides	
☐Other: Click or tap here to enter text.	

Notes (include hazardous chemicals that will be used, additional cautions, permissible exposure limits, etc.):
- Hexadecyltrimethylammonium bromide
  - harmful if swallowed, causes skin irritation, causes serious eye damage, may cause respiratory irritation, may cause damage to organs

	(gastrointestinal tract) through prolonged or repeated exposure if swallowed)
	• β-mercaptoethanol – **particularly hazardous (acute toxicity, dermal)**
	  ○ Combustible liquid, toxic if swallowed or if inhaled, fatal in contact with skin, causes skin irritation, may cause an allergic skin reaction, causes serious eye damage, may cause damage to organs (Liver, Heart) through prolonged or repeated exposure if swallowed.
	• EDTA
	  ○ causes serious eye irritation, harmful if inhaled, may cause damage to organs through prolonged or repeated exposure)
	• Ethanol, PEL = 100 – 1000 ppm (check SDS)
	  ○ Highly flammable liquid and vapor, causes serious eye irritation, causes damage to organs, causes damage to organs through prolonged or repeated exposure
	• Isopropanol, PEL = 400 ppm
	  ○ Highly flammable liquid and vapor, causes serious eye irritation, may cause drowsiness or dizziness.
	• Sodium dodecyl sulfate
	  ○ Flammable solid, harmful if swallowed or if inhaled, causes skin irritation, causes serious eye damage, may cause respiratory irritation.

4.	**WHAT ENGINEERING CONTROLS WILL BE USED TO MINIMIZE EXPOSURES TO THESE HAZARDS?** *select all that apply*
	☒Fume Hood ☐Snorkel ☐Glove Box ☐Clean Room ☐Explosion Shielding ☐Splash Shielding ☐Beta Shielding ☒Safety Storage Cabinet (for flammables) ☐Flammable Storage Refrigerator ☐Other: Click or tap here to enter text.
5.	**WHAT PERSONAL PROTECTIVE EQUIPMENT IS REQUIRED TO MINIMIZE THESE HAZARDS?** *select all that apply*
	☒Safety Glasses ☒Lab Coat ☐Fire-Resistant Lab Coat ☒Gloves - specify type: nitrile ☐Acid Resistant Gloves ☐Acid Resistant Apron ☐Face shield

	☐Other: Click or tap here to enter text.
6.	**STEP-BY-STEP OPERATING PROCEDURE** *Provide a sequential description of work, including as much detail as possible such as designated work area(s), chemical concentrations ranges and amount used (mass, volume) and when special safety equipment is to be utilized. Include temperature, pressure, and other experimental conditions if possible. Pictures and schematics are recommended for complex setups.* ***Highlight the steps with the highest hazards.***

1. Preparation of Extraction Buffer A (EBA) starts in the weighing area at the east side of Rm 181. Lab coats, gloves and safety glasses should be worn prior to starting.
2. To prepare 100 ml EBA:

2% (w/v) hexadecyltrimethylammonium bromide (CTAB)	2.0 g
100 mM Tris (pH 8.0) (Use 1 M stock)	10 mL
20 mM EDTA (Use 0.5 M stock)	1 mL
1.4 M NaCl	8.2 g
4% (w/v) polyvinylpyrrolidone (PVP)	4.0 g
0.1% (w/v) ascorbic acid	0.1 g
10 mM β-mercaptoethanol (BME) (Use 14.3 M stock)	70 µL
DI water	

3. To prepare 100 mL of Extraction Buffer B (EBB):

100 mM Tris-HCL (pH 8.0) (Use 1M stock)	10 mL
50 mM EDTA (use 0.5 M stock)	2.5 mL
100 mM NaCl	0.6 g
10 mM 10 mM β-mercaptoethanol (BME) (Use 14.3 M stock)	70 µ
DI water	

4. All chemicals are mixed together in a clean capped glass bottle except for β-mercaptoethanol (BME)
5. After all the components are well mixed, transport the glass bottle to the fume hood in Rm 181B using a secondary container. **Add the BME in the fume hood** prior to starting DNA extraction. Mix well.
6. Prepare 0.3 g of plant tissue by chopping into a paste with a single edge razor blade. **Handle the razor with caution**.
7. Add 300 µL EBA, 900 µL EBB, and 100 µL 20% (w/v) sodium dodecyl sulfate (SDS) to the homogenized tissue.
8. Vortex and incubate at 65°C for 10 min using water bath.
9. Add 410 µL cold potassium acetate. Mix by inversion and incubate on ice for 3 min.
10. Centrifuge at 13,200 rpm, 4°C, for 15 min.
11. Transfer 1 mL of the supernatant to a new 1.5 mL tube, add 540 µL of ice cold absolute isopropanol, and mix by inversion.
12. Incubate in ice for 20 min.
13. Centrifuge at 10,200 RPM for 10 min. Discard the supernatant.
14. Wash the pellet once in 500 µL 70% ethanol and let dry in an air-flow clean bench.
15. To prepare 100 mL of TE Buffer:

	10 mM Tris (pH 8.0) (use 1 M stock) 1.0 mL 1 mM EDTA (use 0.5 M stock) 50 µL DI water 16. Resuspend the dry pellet in 600 µL of TE Buffer. Add 60 µL 3M sodium acetate (pH 5.2) and 360 µL ice cold absolute isopropanol. Incubate on ice for 20 min. 17. Repeat steps 13-15 twice. 18. Resuspend the pellet in 50 µL TE and carry out agarose gel QC.
7.	**TRANSPORT, RECEIVING AND STORAGE REQUIREMENTS** *Describe transport, receiving and storage requirements. Include secondary containment, transport devices (carts, carriers, etc.), segregation requirements, any special temperature or atmospheric requirements, and container compatibility requirements. Information may be included in Section 6.*

Chemical name	*Storage location/requirement*
2% (w/v) hexadecyltrimethylammonium bromide (CTAB)	Chemical storage cabinet, Rm181
100 mM Tris (pH 8.0) (Use 1 M stock)	North lab bench, Rm 181
20 mM EDTA (Use 0.5 M stock)	North lab bench, Rm 181
1.4 M NaCl	Chemical storage cabinet, Rm181
4% (w/v) polyvinylpyrrolidone (PVP)	Chemical storage cabinet, Rm181
0.1% (w/v) ascorbic acid	Chemical storage cabinet, Rm181
10 mM β-mercaptoethanol (BME)	Cabinet below fume hood, Rm 181B

8. **WASTE DISPOSAL**

Type of waste generated by this procedure/process (*check all that apply*):
☒Solid ☒Liquid

Waste hazard determination (*check all that apply*):

Type of Waste	*Hazard Determination*
Solid	☐Flammable ☐Oxidizer ☐Corrossive ☐Reactive ☒Toxic
Liquid	☒Flammable ☐Oxidizer ☐Corrossive ☐Reactive ☐Toxic

Expected waste generation per experiemntal run (mass/volume): ~150 uL per tube sample

Disposal procedure and location of Satellite Accumulation Area: DNA extraction wastes are stored in closed containers in the Satellite Accumlation Area next to the fume hood - spill resistant waste containers with yellow sticker labeled “Hazardous Waste”. The label will include all constituents of the waste and percentage. EHS Hazard Materials Management will pick up all hazadous wastes.

9.	**EMERGENCY PROCEDURES** *Indicate how spills, personnel exposure/injury, and other accidents should be handled and by whom.*
	Refer to Emergency Information Sheet **Spills:** Small spills that are not hazardous should be cleaned with paper towels or other absorbent material and disposed of in the trash. Hazardous chemicals should be cleaned up with the spill kit, located under the sink in room 181 or in the cabinet in room 181B. Absorbent waste should be placed in a container properly label as hazardous waste. Report spill to lab manager. **Personnel exposure (refer to SDS):** • Contact with skin: Remove contaminated clothing if necessary and wash affected area with plenty of soap and water. The emergency shower is located along the hallway next to Rm 177 (Equipment Room). If skin irritation or rash occur get medical advice. • Contact with eyes: Flush with water for 15 min in eyewash station located along the hallway next to Rm 177. Irrigate eyes thoroughly while lifting eyelids. Seek medical advice if necessary. • Ingestion: Rinse mouth with water (do not swallow). Never make an unconscious person vomit or drink fluids. Call poison control center and obtain immediate medical assistance if needed. If medical advice is needed, have product container, label or SDS at hand. • Inhalation: If breathing is difficult, remove victim to fresh air and keep at rest in a position comfortable for breathing. Call a poison control center or doctor/physician if you feel unwell. Emergency contact numbers: Lab manager xxx-xxx-xxxx Building Manager xxx-xxx-xxxx Principal Investigator xxx-xxx-xxxx Poison Control Center 800-222-1222 Emergency 911 EHS 352-392-1591