Standard Operating Procedure
for
Hazardous Chemicals

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Building and rooms: Davison Life Sciences Building, Lab B310

Chemical(s)	Tris-HCl, EDTA (ethylenediaminetetraacetic acid), βME (β-mercaptoethanol), SDS (Sodium Dodecyl Sulfate), Ethanol, KOAc (potassium acetate), Isopropanol, liquid nitrogen (LN), sodium chloride (NaCl).
Process	**Plant Genomic DNA extraction - miniprep**
Specific Hazards *referred to MSDSs for more detailed information*	Isopropanol, Ethanol: Flammable. βME: flammable, and may be harmful upon skin/eye contact, ingestion or inhalation. LN: frostbite hazard. KOAc: Hazardous in case of eye contact (irritant).
Personal protective equipment	☒ 3-5 mil nitrile gloves ☒ double gloves (w/ concentrated stock) ☒ lab coat (except when used in microcentrifuge tubes) ☒ chemical safety goggles (when splash potential exists)
Engineering/ventilation controls	All operations involving βME must be done in a chemical fume hood.
Special handling procedures and storage requirements	Store isopropanol, βME in the flammable cabinets under the hood B. LN: Store and use with adequate ventilation. Under normal conditions these containers will periodically vent product. Do not plug, remove, or tamper with pressure relief device.
Spill and accident procedures	Skin exposure: Rinse affected skin with plenty of water while removing contaminated clothing/shoes. Rinse for > 15 minutes. Eye exposure: Wash eyes for > 15 minutes. For both cases, seek medical attention immediately.
for hazardous chemicals only	Small (< 2L): Absorb with vermiculite or spill pads and transfer absorbed material to a closed container. Label and date as hazardous waste for disposal. Notify PIs.
	Large (> 2L): Evacuate the room, notify PIs and call 2-5801 to request emergency spill assistance from the Environmental Safety Division.
Waste disposal	Dispose waste as regular disposing method.
Special approval	No special authorization needed after SOP training & reading MSDSs.
Prepared by	Name/date: Kate Tay, 9/2/2009; updated by CJ Tsai 6/27/2013
Reviewed by	Name/date: C-J Tsai, 9/4/2009; 6/27/2013

Plant Genomic DNA (gDNA) extraction miniprep v2.2

Reagents and Buffers:

gDNA Extraction Buffer:	[Stock]	[Final]	For 50 ml
Tris-HCl (pH 8)	1.0 M	50 mM	2.5 ml
EDTA (pH 8)	0.5 M	10 mM	1.0 ml
NaCl	5.0 M	100 mM	1.0 ml
SDS	20%	1%	2.5 ml
ddH_2O	--	--	43.0 ml
β-mercaptoethanol	--	10 mM	34 µl **(add right before use)**

Potassium acetate (KoAc) pH4.8:	For 50 ml
ddH_2O	15 ml
Acetic acid glacial	15 ml *** Always add acid into water, NOT water into acid**
KOH pellet	to bring pH up to pH = 4.8, then bring volume to 50 ml.

*** Maintain the bottle in a cold water bath to dissipate the heat during the prep and pH measurement.**

Equipment: 65°C water bath

Cleaning Micro-pestle:

Sonicate in 70% EtOH for 20 min, rinse with plenty of ddH_2O, dry and (optional) autoclaved.

Procedures:

1. Collect tissue (e.g., a single punch, a small apical leaf or ~ 50 mg) into a 1.5 ml microcentrifuge tube and keep on ice if multiple samples are to be collected. Snap-freeze in LN immediately after return to the lab. Store at -80°C until use.
2. Grind tissue to a fine powder using a micro-pestle under liquid nitrogen (fill to one-third of the tube). Proceed to the extraction step immediately or store samples at -80 °C until use.
3. Add 750 µl gDNA extraction buffer to each tube of frozen powder. Mix well by vortexing.
4. Incubate @ 65°C for 20 min. Invert the tubes to mix every 5 min.
5. Add 200 µl **ice-cold** 5M KOAC and Invert 5 times to mix. Incubate on ice for 20 min.
6. Centrifuge at max speed for 10 min at room temperature.
7. While waiting, add 480 µl (or 0.6 Vol. of DNA extract) isopropanol into new 1.5ml tubes.
8. Transfer 800 µl of the extract to the tube containing isopropanol. Invert 5 times to mix.
9. Centrifuge at max speed for 10 min at room temperature to pellet DNA.
10. Wash with 500 µl of 70% EtOH, invert the tube several times to mix, and centrifuge for 5 min. Repeat the wash once more.
11. Vacuum dry using centrivap for ~10min.
12. Resuspend DNA in 50 µl ddH_2O. If the level of co-purifying RNA is high, resuspend DNA in ddH_2O containing 10 µg/ml RNase A (make a master mix, just enough for your samples) and incubate at 37°C for 20 min.
13. QC 1 µl on a 1% agarose gel. Some amount of RNA usually co-purify with the gDNA, therefore, QC by Nanodrop is not necessary/meaningful (unless RNaseA treatment & EtOH ppt are done). Estimate gDNA concentration based on the ladders. You need 1-5 ng for each PCR reaction. If yield is high, dilute gDNA 10X and check on a gel again.

References:

Stephen L. Dellaporta, Jonathan Wood, James B. Hicks. (1983) A plant DNA minipreparation: Version II. Plant Molecular Biology Reporter, Vol.1, 4:19-21