SCHNABLE LAB
Plant Genomics

2035C Roy J. Carver Co-Laboratory
Plant Sciences Institute
Iowa State University
Ames, Iowa 50011-3650
515 294-7209
FAX 515 294-5256

96-well Format DNA Extraction Protocol for Freeze-dried Maize Seedling Leaves

(Last revised August 2014)

Protocol developed by Drs. Jin Li and Patrick S. Schnable, please contact Dr. Schnable at schnable@iastate.edu regarding questions or corrections.

Written with the editorial assistance of Drs. Marna Yandeau and Dave Skibbe. Using this protocol (a modification of one provided by Rogers and Bendich, 1985) it is possible to extract DNA from 8 x 96 maize samples/day. Additional modification (provided by Smith and Wilkening, 2011) includes an RNase A step to increase purity of DNA extracted.

The citation for this protocol is:
Dietrich CR, F Cui, ML Packila, J Li, DA Ashlock, BJ Nikolau, PS Schnable (2002). *Mu* transposon insertions are targeted to the 5' UTR of the maize *gl8* gene. Genetics, 160: 697-716

Reagents & Recipes:

CTAB extraction buffer (stored at bench)

For 1L:	Addition
1M Tris pH 7.5	100mL
5M NaCl	140mL
0.5M EDTA (see below)	20mL
CTAB	10g
dH_2O	730mL
*(BME)	(10mL) 1% **(added before use)**

*Once BME is added, CTAB should be kept in the hood

Chloroform/Octanol (24:1) (store under fume hood)

For 800mL: (Week's supply)	Addition
Chloroform	768mL
Octanol	32mL

Isopropanol (2-propanol) (stored under fume hood)

0.5M EDTA (stored at bench)

For 1L:	Addition
Na_2-EDTA (MW 292.25)	146.1g
NaOH	~50g
dH_20	up to 1L
Adjust to pH 8.0 with NaOH	

SCHNABLE LAB
Plant Genomics

2035C Roy J. Carver Co-Laboratory
Plant Sciences Institute
Iowa State University
Ames, Iowa 50011-3650
515 294-7209
FAX 515 294-5256

70% Ethanol (stored under fume hood)

RNase A PureLink TM Invitrogen Cat# 12091-021 10µL

Procedure

1. Preparing samples
    a. Prepare a 96-well rack (**1.2 mL microdilution tube in strips of 8 tubes, USA Scientific Cat#1212-8400**) with disposable tubes (**Strips x 8 Plug Caps for Cluster Tubes Simport Cat# T100-30**). Add ~ 1 cM of glass beads* or three 3 mm metal beads (**3 mm Tungsten Carbide Beads Qiagen Cat# 69997**) to each tube. Collect tissue and place in tubes - usually 1-1.5 inches of the tip of the youngest leaf.
    * Note that glass beads are not compatible all extraction methods. Metal beads are necessary for MagAttract. If tissue may be used for other methods, use metal beads.
    b. Cover tubes with air pore tape and lyopholize tissue using a freeze drier immediately after collection. Depending on the amount of tissue, samples are typically dried for 2-3 days.
    c. Briefly spin down samples. Remove airpore tape and put strip caps on tubes, making sure the caps are oriented in the same direction. Grind the tissue in a tissue grinder (paint shaker), making sure shaker is balanced. May require changing position of plate to fully grind plate (10-30 minutes). Samples can be frozen at -20 or -80C for later use.
2. If samples have been frozen, take samples out of the freezer and let stand at room temperature for 10 minutes. In the meantime, prepare CTAB extraction buffer (Step 3a).
3. Gently tap racks on bench-top and do a **quick spin** for 2 minutes at 3000 rpm.

4. CTAB extraction: Total time ~45 minutes
    a. Add 4mL BME (betamercaptoethanol) to 396mL CTAB buffer (1mL BME/100mL buffer.) This is enough CTAB for the day (8 plates / day). For fewer plates, prepare less CTAB with BME.
    b. For RNase A treatment (**RNase A: Invitrogen PureLink Cat#12091-021**)): Add 1µL/well, e.g. 400 µL per 400 ml CTAB to the prepared CTAB solution.
    c. *Carefully* remove and place strip caps on a clean paper towel, and place a clean Kimwipe over each well while removing caps. Remove strip caps from every *second row* (i.e. odd rows). Lay the strips on the bench in such a way that you can place them back exactly the same position to avoid contamination.
    d. Add 500µL CTAB extraction buffer to each well using a 1250µL 8-channel automated pipette. Use CTAB dispensing boat in hood. Remember not to contaminate the samples, so dispense the pipette **without touching the wells**.
        → Fill: 1050µL; Dispense: 500µL

SCHNABLE LAB
Plant Genomics

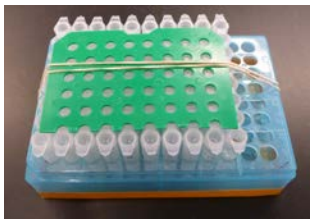
2035C Roy J. Carver Co-Laboratory
Plant Sciences Institute
Iowa State University
Ames, Iowa 50011-3650
515 294-7209
FAX 515 294-5256

→ Dispense 500µL x 2 rows at a time, 6 times per plate

e. After putting the strip caps back on tightly, gently invert the plates to mix.
f. **Incubate** for 2 x 15 min minutes (a total of 30 minutes) in the 58°C oven, place weights on top of the plates so that the strip caps do not pop off.
g. **Invert** the plates ~10 times after the first 15 minutes, then return to the incubator. Close oven door firmly!
h. Remove samples from oven and **cool** in the hood for ~10 minutes.
i. **Balance plates** and **quick spin** the plates up to **2500 rpm**.

** **Be very careful** when balancing the plates. The high speed can break rubber bands and cause the centrifuge to unbalance. Wrap them the "long way" around the plate.**

Correct



Incorrect

5. Chloroform/Octanol addition: Total time ~30 minutes
   a. Remove strip-caps, and carefully place in order on new paper in the hood. Add 200µL 24:1 Chloroform:Octanol solution using the manual 200µL multi-channel pipette (green) **without touching wells**. Use a heavy plastic dispensing boat labeled for chloroform use (the other boats will leak). Replace caps.
   b. Sandwich the plates between two pieces of **plexiglass** and clamp (the strip caps like to pop off because of pressure built-up by chloroform addition so beware!) **Shake vigorously for 30 seconds.**
   c. Let the plates sit for 5 minutes, with the plexiglass still clamped on.
   d. **Balance plates** and spin at **4K for** 10 minutes (see step 5a).

6. Isopropanol Addition: Total time ~40 minutes
   a. During step 4d), **label** 0.6mL V-bottom 96-well Costar plates (Costar 3957 available from Fisher as 07-200-724. (Optional: in order to better see the well and row indicators**, color** in the numbering and lettering with a black sharpie, or write row numbers on the bottom row.)
   b. After 4d), remove the top 200µL **of the top aqueous layer** using the manual 200µL 8-channel pipette (green) and transfer to labeled plate prepared in 5a). Change tips after every row. **See NOTES below in step 5c.**

SCHNABLE LAB
Plant Genomics

2035C Roy J. Carver Co-Laboratory
Plant Sciences Institute
Iowa State University
Ames, Iowa 50011-3650
515 294-7209
FAX 515 294-5256

c. Add 200µL Isopropanol using a plastic boat. Do not touch wells!

> **NOTES**: In order to prevent sucking up any junk it is best to remove the strip rows from the rack as you work and use the 8-channel pipette (vs. the 12-channel). **Change tips after every row!** Also, do not suck up the supernatant from wells that do not have plant tissue (the pure chloroform:octanol solution does not react well with the sealing tape in the next step). Remove tips from these "empty" positions and pipette as usual.

d. Our DNA has now been transferred to the Costar isopropanol plate. Cover the Costar plate firmly with tape and **invert** 1-2 times.
e. Incubate 1 hour in the -20˚C freezer.
f. **Balance plates** and spin for 15 minutes **at 4000 rpm** to pellet DNA.
g. Remove tape and quickly invert plates over sink (45 degree angle) to discard isopropanol and then blot the plate on a paper towel.

7. Ethanol rinse: Total time ~45 minutes
   a. Add 150µL 70% ethanol to each well to wash the pellets. Use ethanol dispensing boat in hood.
   b. Cover with tape, **balance plates** and spin for 5 minutes **at 4000 rpm**.
   c. Remove tape and *gently* **invert** plates over the sink to discard ethanol and then blot the plate on a paper towel.
   d. Allow plates to **air dry** for at least 15 minutes (ideally 30 minutes). Or place in speed-vacuum dryer at 40–45 ˚C for approximately 15 minutes. Caution: Check often so that pellets don't dry excessively.
   e. Examine the pellets in each well, there should not be any remaining solution, if there is, the plates should continue to dry until all solution has evaporated.

8. Pellet re-hydration: Total time ~20 minutes
   a. Add 50 - 100µL millipore water. Use water dispensing boat in hood.
   b. Cover firmly with **tape**.
   c. Incubate plates at **58°C for** 10 minutes**.**
   d. **Vortex** for 30 seconds.
   e. Cool DNA and then place samples in the freezer for storage.
   f. Before use, thaw and then centrifuge for 5 minutes at 4000 rpm.

9. Clean-up: **We must clean up every day after DNA isolation!!!**
   a. Wrap a few rubber bands around both the width and length of the blue collection plate to secure the tubes in the rack.
   b. Invert the rubber banded rack over a large 4 liter plastic beaker and shake to pour off the liquid and the glass beads.
   c. Dispose of the empty tubes and rubber bands in the large garbage bag-lined solid waste bucket in the fume hood.
   d. The liquid waste *cannot* remain in the large beaker. Place a funnel in the labeled waste bottle in the hood and carefully pour the liquid contents of the beaker into

2035C Roy J. Carver Co-Laboratory
Plant Sciences Institute
Iowa State University
Ames, Iowa 50011-3650
515 294-7209
FAX 515 294-5256

the labeled wasted bottle. Do not worry if a few glass beads inadvertently drop into the bottle. However, once the liquid is disposed, the bulk of the glass beads should be thrown in the solid waste container in the hood.

e. Make sure that wet solids have evaporated and there is no longer an odor before disposing of a full solid waste bag to the regular trash bins.
f. Please promptly remove empty blue racks from the hood after disposing of the contents. Rinse off the blue racks with DI water and let dry.

Modifications:

- August 19, 2014 - Added description of sample preparation including freeze drying and grinding. Changed name to "96wellformat.2014.08.19.detailed.doc"
- June 26, 2013 – Added item description and catalog number for Costar plates. Changed name from "96wellformat.2012.08.16.detailed.doc" to 96wellformat.2013.06.26.detailed.doc
- Aug 16, 2012 – Added warning about balancing plates. Clarified wording for a few steps. Changed name from "96wellformat.2011.08.12.detailed" to "96wellformat.2012.08.16.detailed.doc"
- Aug 12, 2011 – Renamed protocol to "96wellformat.2011.08.12.detailed.doc" and made formatting changes to match "96wellformat.2011.06.23.doc"
- Aug 4, 2011 – Updated & renamed protocol "DNA Isolation-2011.doc" and added RNase A steps. Previous version: "DNA Isolation-2007.doc"