Low-cost DNA extraction for recalcitrant plant species

Version 1.4, Plant Breeding and Genetics Laboratory, February 7,2013, developed by Bernhard Hofinger and Bradley Till

1. OBJECTIVE

The PBGL has developed a low cost DNA extraction protocol that obviates the need for toxic organic phase separation common to many non-kit plant genomic DNA extraction methods. This is based on DNA binding to silica in the presence of a chaotropic salt. While this has worked well for many crop species, quality and/or yield can be dramatically reduced in some recalcitrant species. One common factor is the presence of secondary metabolites.

We have developed and tested modified lysis buffers, which include additional components facilitating DNA extraction from recalcitrant plant species (addition of PVP, sodium sulphite, NaCl, increased detergent), but avoiding very toxic substances, like β-Mercaptoethanol or CTAB. Because inhibitory componenents can vary in type and amount between species and potentially even gentoytpes, we advise testing all extraction buffers listed in this protocol on a small subset of samples and choosing the one that works best for your materials.

2. MATERIALS

	Company
MATERIALS FOR LOW-COST DNA EXTRACTIONS	
Celite 545 silica powder (Celite 545-AW reagent grade)	Supelco 20199-U
SDS (Sodium dodecyl sulfate) for mol biol approx 99%	Sigma L-4390-250G
Sodium acetate anhydrous	Sigma S-2889 (MW=82.03g/mol)
NaCl (Sodium chloride)	Sigma S-1314-1KG (MW=58.44g/mol)
PVP (Polyvinylpyrrolidone)	Sigma PVP-40-500G
Sodium sulphite extra pure	Merck 6652.1000 (MW=125g/mol)
N-Laurylsarcosine sodium salt	Sigma L-5125
RNase A	Qiagen RNase A (100 mg/ml)
Ethanol	Ethanol absolut for analysis (Merck 1.00983.2500)
Nuclease-free H2O	Gibco ultrapure distilled water (DNase, RNase-free)
Guanidine thiocyanate	Sigma G9277 (MW=118.2g/mol)
Microcentrifuge tubes (1.5mL, 2.0mL)	From any general laboratory supplier
Micropipettes (1000uL, 200uL, 20uL)	From any general laboratory supplier
Microcentrifuge	Eppendorf Centrifuge 5415D
Optional: Shaker for tubes	Eppendorf Thermomixer comfort for 1.5mL tubes
Optional: 65^{0}C incubator (heat block or water bath)	
MATERIALS FOR GRINDING OF LEAF MATERIAL (depending	

on grinding method)	
Liquid nitrogen	
Mortar and pistil, TissueLyser, …	e.g. Qiagen TissueLyser II
Metal beads (tungsten carbide beads, 3mm)	Qiagen Cat.No. 69997
EVALUATION OF DNA YIELD AND QUALITY	
DNA concentration	ND-NanoDrop 1000 Spectrophotometer
Agarose gel equipment	For horizontal electrophoresis from any general laboratory supplier

SOLUTIONS TO PREPARE:

Buffer	**Receipt**		**Comments**
STOCK SOLUTIONS			
5M NaCl stock solution	MW=58.44g/mol 29.22g / 100mL		Do not use if percipitate forms. Either heat to get fully back into solution or discard and make fresh.
3M Sodium acetate (pH = 5.2)	MW=82.03g/mol 24.61g / 100mL		Adjust pH value with glacial acetic acid
95% (v/v) Ethanol	95 mL ethanol abs + 5 mL H_2O		Use fresh. Ethanol absorbs water and the % will drop over time.
Tris-EDTA (TE) buffer (10x)	Composition: - 100mM Tris-Cl, pH=8.0 - 10 mM EDTA	For 100mL (10x): - 10mL of 1M Tris-Cl stock - 2mL of 0.5M EDTA stock	Can make Tris and EDTA from powders. This may be less costsly. However, note that the pH of Tris changes with temperature.
LYSIS BUFFER – LB1	Composition 0.5% SDS (w/v) in 10x TE	For 100mL 0.5g SDS /100mL in 10x TE	
LYSIS BUFFER – LB2	Composition - 0.5% SDS (w/v) - 0.5M NaCl - 3% PVP (w/v) in 10x TE	For 100mL - 0.5g SDS /100mL - 10mL of 5M NaCl - 3g / 100mL in 10x TE	
LYSIS BUFFER – LB3	Composition - 0.5% SDS (w/v) - 0.5M NaCl - 3% PVP (w/v) - 1% sodium sulphite in 10x TE	For 100mL - 0.5g SDS /100mL - 10mL of 5M NaCl - 3g / 100mL - 1g / 100mL in 10x TE	
LYSIS BUFFER – LB4	Composition - 0.5% SDS (w/v) - 0.5M NaCl - 3% PVP (w/v) - 1% sodium sulphite - 2% N-lauryl-sarcosyl sodium salt	For 100mL - 0.5g SDS /100mL - 10mL of 5M NaCl - 3g / 100mL - 1g / 100mL - 2g / 100mL	

	in 10x TE	in 10x TE	
DNA BINDING BUFFER	6M Guanidine thiocyanate MW = 118.2 g/mol 70.92 g / 100mL (6M)		**!!!** it takes several hours until dissolved (leave it approx. 4-5 hours)
WASH BUFFER	1mL of 5M NaCl + 99mL of 95% EtOH		**!!!** PREPARE FRESH, because the salt precipitates during storage
DNA ELUTION BUFFER	depending on application (e.g. TE-buffer; Tris-HCl buffer)		

3. METHODS (method for centrifuge tubes):

PREPARATION OF SILICA POWDER-DNA BINDING-SOLUTION

- Fill silica powder (Celite 545 silica) into 50 mL-Falcon-tube (to about 2.5-mL = approx. 800mg)
- Add 30 mL dH2O
- Shake vigorously (vortex and invert)
- Let slurry settle for approx. 15 min
- Remove (pipette off) the liquid
- Repeat 2 times (a total of 3 washes)
- After last washing step: resuspend the silica powder in about the same amout of water (up to about 5 mL)
- STORE the silica solution at RT until further use (silica : H2O = 1 : 1)

Immediately before use:

- suspend stored silica solution (silica : H2O = 1 : 1) by vortexing
- Transfer ~50 uL of silica solution to 2mL-tubes (prepare 1 tube per sample)
  !! try to keep the silica suspended during pipetting to ensure an equal distribution
- Add 1mL H2O (a final wash step)
- Mix by vortexing
- Centrifuge: full speed (13.200rpm) for 10-20 sec
- Pipette off liquid
- Add 700 uL DNA binding buffer (6M Guanidine thiocyanate)
- Suspend the silica powder in DNA binding buffer
- The silica binding solution is now ready for further use in the protocol (see below)

PREPARATIONS

- Prepare 2 mL-tubes (1 per sample): add 3 metal beads (Tungsten Carbide beads) per tube
- Harvest leaf material (starting amount of material: about 100 mg fresh weight)
- **OPTIONAL:** water bath or thermo mixer at 65^0C

GRINDING

Use appropriate / available grinding protocol (Mortar&Pistill, Qiagen TissueLyser, …)
e.g. grinding using Qiagen TissueLyser:

- Freeze 2-mL tubes containing leaf material and 3 metal beads in liquid nitrogen
- Grind in TissueLyser by shaking (10 sec at 1/30 speed)
- Re-freeze in liquid nitrogen (>30 sec)
- Grind again in TissueLyser by shaking (10 sec at 1/30 speed)
- Re-freeze in liquid nitrogen (>30 sec)
- Store in liquid nitrogen until lysis buffer is added

LYSIS

- Add 800 uL **Lysis buffer**
- Add 4 uL **RNase A** (100 mg/ml)
- Vortex (~2 min until the powder is dissolved in the buffer)
- Incubate (optional): **10min at room temperature** or **10min at 65ºC**
- Add 200 uL **3M Sodium Acetate (pH = 5.2)**
- Mix by inversion of tubes
- Incubate on ice for 5 min
- Centrifuge 13.200 rpm / 5 min / RT (pellet the leaf material)

DNA BINDING

- prepare **700 uL silica binding solution** (see above)
- transfer 800 uL of the supernatant to the tubes containing silica binding solution)
  !! Do not transfer leaf material
- Completely resuspend the silica powder by vortexing and inversion of tubes (approx. 20 sec)
- incubate 15 min at RT (on a shaker at 400 rpm and/or invert tubes from time to time)
- Centrifuge 13.200 rpm / 3 min / RT (pellet the silica)
- Remove the supernatant (with pipette)

WASHING (2 times)

- Add 500 mL **wash buffer** (prepared fresh)
- Completely resuspend the silica powder by vortexing and inversion of tubes (approx. 20 sec)
- Centrifuge 13.200 rpm / 3 min / RT (pellet the silica)
- Repeat the washing step (optional: a third washing step)
- Remove the supernatant with pipette (as complete as possible)
- optional: short spin and remove residual liquid with pipette
- After last washing step: dry the silica in the hood up to 1 hour at RT (make sure there is no wash buffer left)

RESUSPENSION

- Add 200uL **TE buffer** or **10mM Tris buffer**
- Completely resuspend the silica powder by vortexing and inversion of tubes (approx. 20 sec)
- Incubate: 20 min / RT / with gentle agitation (on a shaker at 400 rpm and/or invert tubes from time to time)
- Centrifuge: 13.200 rpm / 5 min / RT (pellet the silica)
- transfer 180 uL supernatant to new tube (avoid transferring silica powder!)
- optional: if there is still silica powder in the preps – repeat the centrifugation
- check for concentration (NanoDrop) and integrity of DNA (agarose gel)
- store the genomic DNA at -20^{0}C for long-term storage or 4^{0}C for short-term storage

4. RESULTS:

DNA CONCENTRATIONS AND YIELDS

Table 1. Sorghum and grapevine genomic DNA extractions using the silica powder method with 4 different lysis buffers LB1, LB2, LB3 or LB4

	SORGHUM								**GRAPEVINE**							
Sample	S1a	S1b	S2a	S2b	S3a	S3b	S4a	S4b	G1a	G1b	G2a	G2b	G3a	G3b	G4a	G4b
Lysis buffer	**Lysis buffer LB 1**		**Lysis buffer LB 2**		**Lysis buffer LB 3**		**Lysis buffer LB 4**		**Lysis buffer LB 1**		**Lysis buffer LB 2**		**Lysis buffer LB 3**		**Lysis buffer LB 4**	
Incubation temperature (for lysis)	RT	65^{o}C	RT	65^{o}C	RT	65^{o}C	RT	65^{o}C	RT	65^{o}C	RT	65^{o}C	RT	65^{o}C	RT	65^{o}C
DNA concentration (ng/uL)	**11**	**15**	**2**	**3**	**6**	**5**	**7**	**7**	**22**	**32**	**7**	**3**	**11**	**6**	**4**	**9**
Total yield (ug)	2.0	2.6	0.4	0.6	1.0	0.9	1.3	1.3	3.9	5.7	1.3	0.6	2.1	1.1	0.6	1.5

QUALITY OF GENOMIC DNA

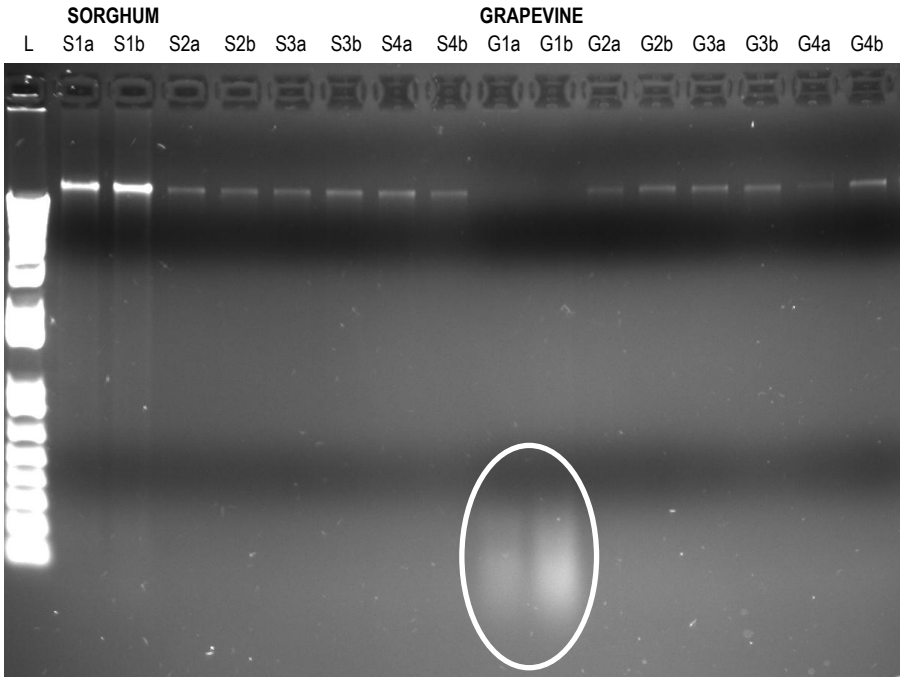

Figure 1. Quality of genomic DNA extracted from Sorghum and grapevine using the silica powder method with four different lysis buffers. 8 uL of each genomic DNA preparation were separated on a 0.7% agarose gel. S and G indicate sorghum or grapevine, Numbers 1-4 represent lysis buffers LB1 -4 respectively. a=incubation in lysis buffer for 10 min at room temperature, b=incubation in lysis buffer for 10 min at 65^0C ,L: size standard (1 kB Plus DNA ladder - Invitrogen)

The NanoDrop measurements of DNA concentrations (Table 1) and the agarose gel of the genomic DNA preparations (Figure 1) from Sorghum and grapevine leaves showed that the isolation protocol with the modified compositions of lysis buffers generally works.

However, the Sorghum leaf samples showed the highest yield with the standard lysis buffer (LB1). DNA extractions with the stronger lysis buffers (LB2, LB3 and LB4) also provided genomic DNA from Sorghum, but at a clearly lower yield. These results indicate that for Sorghum samples the standard lysis buffer from the low-cost DNA isolation protocol is the optimal choice.

In contrast, the DNA preparations from grapevine with the standard buffer (LB1) developed for the low-cost method did not work at all. Although the samples isolated with the standard buffer LB1 showed a relatively high DNA concentration value in the NanoDrop measurements, there is no genomic DNA band visible at the expected position on the agarose gel. Instead, there is a smear present at a lower molecular weight indicating sample degradation. Grapevine is known to be a plant with a high level of secondary metabolites (e.g. polysaccharides and polyphenoles), which makes DNA isolation difficult. We added substances to the lysis buffers, which help to

improve DNA isolation from difficult sources. All of the improved buffers had PVP and NaCl as additional ingredients. Buffers LB3 and LB4 also contained sodium sulphite. Buffer LB4 has in addition a relatively high content of detergent (2% N-Laurylsarcosyl sodium salt). We tried each of the lysis buffers with two incubation temperatures, at room temperature and at 65^0C. However, the modified buffers (LB2, LB3 and LB4) allowed reproducible recovery of intact genomic DNA from grapevine leaves.

The concentration and yield of DNA, however, is relatively low. The average concentration of a total of 6 extractions with buffers LB2, LB3 and LB4 from grapevine were 6.7 ng/uL and the average concentration from Sorghum were 5ng/uL. All of the modified buffers showed relatively similar results (in DNA yield and quality) indicating that the addition of PVP (common to all modified buffers) causes the main improvement for the preparations. There is no significant correlation between DNA yield/ quality and the incubation temperature during lysis (room temperature or 65^0C).

5. CONCLUSIONS

A modified version of the silica-based low-cost DNA isolation method for plants with a high content of secondary metabolites has been tested with the plant species grapevine and Sorghum. While DNA extractions from Sorghum worked with all four buffers, the extractions from grapevine did not work with the standard lysis buffer, but could be facilitated with the three modified buffers. However, the yields of the DNA extractions using the modified buffers were relatively low suggesting that modifications could be incorporated to improve yields. However, the yields achived with this method aresufficient for some molecular studies such as TILLING with a limited number of amplicons, and so the protocol is suitable as-is for some applications.