

SUPPLEMENTAL INFORMATION.

APPENDIX 1. GRINDERS

**Fluid Management paintshaker**

Model: Skandex SO-10M (110 or 220 V)

Supplier: Fluid Management Europe

Hub van Doorneweg 31

2171 KZ,

Sassenheim, The Netherlands

Ph: 31 252 240800

**Model: Genogrinder 2000**

Supplier: SPEX CertiPrep

203 Norcross Avenue

Metuchen, NJ 08840

Phone: 1-800-LAB-SPEX or (732) 549-7144

Web: <http://www.spexcsp.com/spex-dataentry/spexcertiprep/index.aspx>

**Model: Retsch MM300 Mix Mill**

Supplier: Retsch or Qiagen (part#85110)

Website: [www.retsch.de](http://www.retsch.de) or [www.qiagen.com](http://www.qiagen.com)

Table 1S. Comparison of grinding methods for bulked vegetable seeds in 96-well format. The same results were obtained for single seeds. A minimum of five seeds was used for lettuce and carrot.

Crop	Paintshaker			MM300		Genogrinder	
	# seeds	time (minutes)	grind	time (minutes)	grind	time (minutes)	grind
broccoli	10	4	+	4	+	6	+
carrot	25	4	+	4	+	6	+
lettuce	25	4	+	4	+	6	+
melon	3	4	+	4	+	6	-
onion	10	4	+	4	+	6	+
pepper	10	4	+	4	+	6	+
radish	10	4	+	4	+	6	+
squash <sup>1</sup>	1	4	+	4	+	6	-
sweet corn <sup>2</sup>	1	4	+	4	+	6	-
tomato	10	4	+	4	+	6	+
watermelon <sup>1</sup>	1-2	4	+	4	+	6	-
Arabidopsis	.05-.1 g	4	+	NA		NA	

<sup>1</sup>Cracking the seed may be necessary for some varieties.

<sup>2</sup>Three of four varieties were ground adequately.

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## APPENDIX 2. PROTOCOLS

### 1. Sodium bisulphite

**Cost/sample:** \$US 0.30

**Time for 192 samples:** 1.5 hour

*Prepare lysis buffer. Add 5.0 g/l of sodium bisulfite and 0.1% ascorbic acid **immediately prior** to use. Buffer can be prepared and stored ahead of time without sodium bisulfite and ascorbic acid.*

1. Add seeds and ¼ inch stainless steel beads/tumblers to each well. Make or buy bead dispenser. Seal plates with foil sealer or rubber plate covers and secure lid. Secure plate(s) on grinder/shaker and shake for recommended time (see Table 1) depending on grinder/shaker.
2. Add 600 ul preheated lysis buffer by puncturing thru foil.
3. Seal again with adhesive aluminum foil, vortex and incubate at 65°C for 30 minutes.
4. Spin at 4000 rpm on swing out centrifuge for 20 minutes.
5. Transfer 400 ul supernatant to 96 well striptube racks.
6. Add 40 ul 3 M sodium acetate pH 5.2 and 400 ul cold isopropanol to plate (can be done while samples are centrifuging after lysis).
7. Invert several times and centrifuge at 4000 rpm 10 minutes.
8. Pour out isopropanol and add 400 ul cold 70% ethanol.
9. Invert or pipette gently several times and pour out ethanol.
10. Aspirate under flowhood to dry.
11. Add 100 ul TE and store at 4 C.

### **Modifications:**

#### **A. High Salt Precipitation:** Fang et al. (1992)

After Step 8,

9. Add 800 ul 2 M NaCl dissolved in 100% ethanol.
10. Invert several times and centrifuge at 4000 rpm 10 minutes.
11. Pour out ethanol and add 400 ul cold 70% ethanol.
12. Invert or pipette gently several times and pour out ethanol.
13. Repeat steps 11-12.
14. Aspirate under flowhood to dry
15. Add 100 ul TE and store at 4°C.

#### **B. Proteinase K:**

Proteinase K was added at 100 mg/ml working concentration to the lysate after Step 3 followed by incubation at 37°C for 30 minutes and 5 minutes at 65°C.

#### **Lysis Buffer:**

500 mM NaCl

100 mM Tris pH 7.2

50 mM EDTA  
2% PVP  
0.1% diethyldithiocarbamic acid (DIECA)

*Add 5g/l of sodium bisulphite and 0.1% ascorbic acid on the day of use.*

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**2. SDS** – Edwards et al. (1991)

([http://www.unizh.ch/botinst/Cyto\\_Website/schneitzLab/Methods/DNAIsolation/genomicQuickprepPCR2.html](http://www.unizh.ch/botinst/Cyto_Website/schneitzLab/Methods/DNAIsolation/genomicQuickprepPCR2.html))

**Cost/sample:** \$US 0.30

**Time for 192 samples:** 1.5-2 hours

This is the same procedure as for sodium bisulphite except the following buffer is used:

**Extraction Buffer:**

250 mM NaCl  
200 mM Tris pH 7.2  
25 mM EDTA  
0.5% SDS

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**3. Sodium Hydroxide:** (ZhangGuo et al. 2003).\*\*

**Cost/sample:** \$US 0.15

**Time for 192 samples:** ½ hour

1. Add seeds and ¼ inch stainless steel beads/tumblers to each well. Make or buy bead dispenser. Seal plates with foil sealer or rubber plate covers and secure lid. Secure plate(s) on paint shaker and shake for recommended time (See Table 1) depending on grinder/shaker.
2. Add 200 ul buffer A (made fresh) and incubate for 10 minutes at 95°C
3. Add 200 ul buffer and mix add moderate speed
4. Transfer 1 ul mixture to reaction tubes for PCR

Buffer A

100 mM NaOH  
2% Tween 20

Buffer B

100 mM Tris-HCl  
2 mM EDTA

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**4. CTAB** (modified from Bernatzsky and Tanksley, 1986)

**Cost/sample:** \$US 0.30

**Time for 192 samples:** 1.5 hours

1. Add seeds and ¼ inch stainless steel beads/tumblers to each well. Make or buy bead dispenser. Seal plates with foil sealer or rubber plate covers and secure lid. Secure plate(s) on grinder/shaker and shake for recommended time (see Table 1) depending on grinder/shaker.
2. Add 600 ul of Buffer Mix to each sample. Seal again with adhesive aluminum foil. Vortex and incubate at 65C for 30 minutes.
3. Centrifuge the samples at 4000 rpm for 20 minutes.

4. Transfer 400 ul supernatant to fresh plate. Add 400 ul cold isopropanol to plate (can be done while samples are centrifuging after lysis).
5. Invert several times and centrifuge 10 minutes
6. Pour out isopropanol and add 400 ul cold 70% ethanol
7. Invert or pipette gently several times and pour out ethanol
8. Aspirate under flowhood to dry
9. Add 100 ul TE and store at 4 C.

### **CTAB solutions**

#### **DNA Extraction Buffer**

working concentration:	stock:	amount for 1 liter
350 mM sorbitol	sorbitol	63.8 g
100 mM Tris-base	1 M Tris pH 7.5	100 ml
5 mM EDTA	0.5 M EDTA	10 ml

Add the above to 800 ml of ddH<sub>2</sub>O then pH to 7.5 using NaOH. Bring the volume up to 1 liter using ddH<sub>2</sub>O. Store the solution at 4°C until use. The day of use, add 3.8 g sodium bisulfite per liter of extraction buffer.

#### **Nuclear Lysis Buffer**

working concentration:	stock:	amount for 1 liter
200 mM Tris-base	1 M Tris pH 7.5	200 ml
50 mM EDTA	0.5 M EDTA	100 ml
2 M NaCl	5 M NaCl	400 ml
2% CTAB (mixed alkyltrimethylammonium bromide)	CTAB powder	20.0 g

Add the above to 200 ml ddH<sub>2</sub>O and then pH to 7.5 using NaOH. Bring up to 1 liter volume using ddH<sub>2</sub>O. Store the solution at room temperature until use.

#### **5% Sarkosyl**

for 1 liter: add 50 g N-lauroylsarcosine to ddH<sub>2</sub>O. Store at room temperature until use.

#### **TE**

Stock	Amount
1 M Tris pH 8.0	10 ml
0.5 M EDTA pH 8.0	2.0 ml

Combine the above and bring the volume to 1 liter with ddH<sub>2</sub>O.

#### **Making the Buffer Mix**

Mix 2.5 parts DNA Extraction Buffer, 2.5 parts Nuclear Lysis Buffer. Add 1 part 5% sarkosyl. Add sodium bisulfite to the extraction buffer the day of use.

Store at 4°C

**Modifications:** As for method #1-Sodium Bisulfite.

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**5 Omega EZ 96 Plant DNA kit** (manufacturer's protocol)**Part#:** D1086-02 for 5 x 96-well plates**Cost/sample:** ~\$1.05**Time for 192 samples:** 1 hour

1. Add seeds and ¼ inch stainless steel beads/tumblers to each well of a 2 ml 96 deep-well plate. Make or buy bead dispenser. Seal plates with foil sealer or rubber plate covers and secure lid. Secure plate(s) on grinder/shaker and shake for recommended time (see Table 1) depending on grinder/shaker.
2. Add 300 µl Buffer P1 to each well of the 2 ml 96 deep-well plate.
3. Add 110 µl Buffer P2 to each well of the plate. Seal the plate with adhesive film and shake to mix. Add 10 µl RNase (20 mg/ml) and mix.
4. Incubate at 65°C for 10 min. Mix sample twice during incubation by shaking the plate.
5. Place the 2 ml 96-well collection plate into a centrifuge adapter and centrifuge at 5000 rpm for 15 min.
6. Place the E-Z 96™ HiBind DNA plate on top of a new 96-well plate (not supplied)
7. Carefully transfer 250 µl of the clear supernatant to each well of the E-Z 96™ DNA plate using a multiple channel transfer pipet. (Note: Mark the plate to identify the positions of samples).
8. Add 125 µl Buffer P3 into each well followed by addition of 250 µl of ethanol (96-100%). Seal the plate with tape. Mix by pipetting or shaking the plate.
9. Place the E-Z 96™ DNA plate on top of the 2 ml 96 deep well collection plate (supplied) and assemble them into a centrifuge adapter. Centrifuge at 5000 rpm for 5 min.
10. Remove the collection plate and discard the flow-through liquid. Reuse the collection plate.
11. Remove the tape pad and carefully add 500 µl of DNA Wash Buffer to each well.
12. Seal the E-Z 96™ DNA plate with new adhesive film.
13. Reassemble the E-Z 96™ DNA plate with the collection plate. Centrifuge at 5000 rpm for 5 min.
14. Wash the E-Z 96™ DNA plate with another 500 µl of DNA Wash Buffer by repeating steps 6-8. Discard the flow-through liquid in the collection plate, reuse the collection plate for next step.
15. Add 400 µl absolute ethanol (96-100%) to each well. Seal the E-Z 96™ DNA plate with adhesive film, and centrifuge at 5000 rpm for 5 min.
16. Remove the adhesive film, incubate the E-Z 96™ DNA plate at 70°C for 10-15 min in an incubator or oven to dry the membrane.  
*Note: Drying the membrane in this step is very important for DNA elution in the next step. The residue of the DNA Wash Buffer contains ethanol which will inhibit PCR and cause low yield of DNA.*
17. To elute the DNA, place the E-Z 96™ DNA plate on top of the 300 µl 96-well collection plate provided and add 100 µl of preheated (70°C) water or TE buffer to each well using a multichannel pipet. Seal the E-Z 96™ DNA plate with new adhesive film and incubate for 2 min at room temperature. Centrifuge at 5000 rpm for 5 min.  
*Tip: 100 µl water or TE buffer is sufficient to elute up to 85% of the DNA from each well of the E-Z 96™ DNA plate. A second elution step with the same 100 µl eluate containing DNA, reheated to 70°C, will increase yield by up to 10-15%.*

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## 6. MagAttract 96 DNA Plant (modified from manufacturer's protocol)

**Part#:** 67161 for 5 x 96-well plates

**Cost/sample:** ~\$0.85

**Time for 192 samples:** 1 hour

Things to do before starting

- *Add 125 ml isopropanol and 1 vial RNase A (1 x 220 µl) to each bottle of Buffer RPW (125 ml) before use.*
- *Shake the bottle containing MagAttract Suspension A and vortex for 5 minutes (before first use) or 1 minute (before subsequent uses) to ensure that the magnetic particles are fully resuspended before use.*

### Manual Procedure:

1. Add seeds and ¼ inch stainless steel beads/tumblers to each well. Make or buy bead dispenser. Seal plates with foil sealer or rubber plate covers and secure lid. Secure plate(s) on shaker/grinder and shake for recommended time (see Table 1) depending on grinder/shaker.
2. Carefully pierce foil and immediately pipet 400 µl Buffer RLT into each collection microtube.
3. Reseal the plate with aluminum adhesive and shake the entire rack in an upright position 20 times back and forth. Vortex the plate for 20 s.
4. Centrifuge the plates for 5 min at 4000 x g.
5. Add 65 µl of Buffer RB to each well of a flat-bottom microplate.
6. Add 20 µl of resuspended MagAttract Suspension A to each well of the 96-well flat-bottom microplate.  
*Note: Buffer RB and MagAttract Suspension A can be combined in appropriate proportions to make a master mix before starting the procedure. Add 85 µl of the master mix to each well of the 96-well flat-bottom microplate. Ensure that the MagAttract particles are fully resuspended.*
7. Transfer 200 µl plant lysate supernatant into each well of the microplate, and mix by pipetting up and down several times.
8. Incubate at room temperature (15–25°C) for 5 min. Mix once during incubation.  
*Note: Mixing can be done by pipetting using an 8-channel pipet. The pipet tips needed for this step can be reused if they are returned to the tip rack after use. To avoid cross-contamination, ensure that the same tip is always used for the same well.*
9. Place the plate or microcentrifuge tube on the magnet and remove the supernatant after magnetic separation
10. Wash the pelleted MagAttract particles by adding 200 µl Buffer RPW, resuspending the particles, placing the plate or tube on the magnet, and removing the supernatant.  
*Note: Resuspension of the magnetic particles should be done very carefully since the efficiency of washing is directly related to how well the particles are resuspended. Resuspension can be performed by pipetting (see previous note) or by vortexing. If 96-well plates are vortexed, the initial mixing should be done very carefully to avoid spilling the magnetic particle suspension out of the wells and contaminating others. If this is too difficult with an initial volume of 200 µl, add 100 µl of Buffer RPW, vortex, and then add an additional 100 µl.*
11. Wash the pelleted MagAttract particles by adding 200 µl ethanol (96–100%), resuspending the particles, placing the plate or tube on the magnet, and removing the supernatant.

12. Repeat step 7, aspirating as much ethanol as possible.
13. Dry the MagAttract particles for 5–10 min at room temperature (15–25°C).
14. Resuspend the MagAttract particles in 100 µl Buffer AE.
15. Incubate at room temperature for 5 min.
16. Place the plate on a magnet and transfer the DNA eluates to a clean 96-well round-bottom microplate.

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**7 Macherey Nagel Nucleospin 96 plant** (modified from manufacturer's protocol)

**Part#** 740661.4 (4 96-well plates) or 740661.24 (24 96-well plates)

**Cost/sample:** ~\$1.05

**Time for 192 samples:** 1.5 hours

*Before starting with the preparation, set incubator or oven to 56°C. Equilibrate buffer CE to 70°C. Prepare buffer C5 and RNase A solution.*

1. **Homogenize and lyse sample material.** Add seeds and ¼ inch stainless steel beads/tumblers to each well. Make or buy bead dispenser. Seal plates with foil sealer or rubber plate covers and secure lid. Secure plate(s) on shaker/grinder and shake for recommended time (see Table 1) depending on grinder/shaker. Add 600 µl C1 and close wells again using Adhesive Aluminum Foil. Mix by vigorous shaking for 15-30 sec. Spin briefly for 30 sec at 1,500 x g to collect any sample from the cap strips. Incubate the sealed Round-well Block at 56°C for 30 min. Mix by vortexing briefly twice during incubation period.

*Depending on plant sample and available methods, buffer C1 may be added to the plant material in a Round-well Block before homogenization by the appropriate mechanical method. Add 10 µl RNase A to the C1 lysis solution.*

2. **Clear lysate**

Centrifuge the samples for 20 min at full speed (4,000 x g). Remove cap strips.

3. **Adjust binding conditions**

Transfer 300 µl clear supernatant to a Round-well Block. Add 300 µl buffer C4 and 200 µl ethanol. Close the individual wells with Cap Strips. Mix by vigorous shaking for 15-30 sec (or pipette up and down). Spin briefly for 30 sec at 1,500 x g to collect any sample from cap strips.

*Buffer C4 and ethanol can be premixed.*

4. **Transfer lysate to NucleoSpin® Plant Binding Plate**

Place the NucleoSpin® Plant Binding Plate on an MN Square-well Block. Remove the first Cap Strip and transfer samples from the Round-well Block into the wells of the NucleoSpin® Plant Binding Plate. Do not moisten the rims of the individual wells while dispensing samples. After transfer seal the openings of the NucleoSpin® Plant Binding Plate with Gas-permeable Foil.

5. **Bind DNA to silica membrane**

Place the NucleoSpin® Plant Binding Plate on a MN Square-well Block and place both into the rotor buckets. Centrifuge at 5,600 –6,000 x g for 5 min.

*Typically, lysates will pass through the columns within 1 min. The centrifugation process can be extended to 20 min, if the lysates have not passed completely.*

6. **Wash silica membrane**

*1st wash*

add 500  $\mu$ l CW to each well of the NucleoSpin® Plant Binding Plate. Centrifuge again at 5,600 – 6,000 x g for 2 min.

*2nd wash*

add 700  $\mu$ l C5 to each well of the NucleoSpin® Plant Binding Plate. Centrifuge again at 5,600 – 6,000 x g for 2 min. Replace MN Square-well Block.

*Optional 3rd wash*

add 700  $\mu$ l C5 to each well of the NucleoSpin® Plant Binding Plate. Centrifuge again at 5,600 – 6,000 x g for 10 min.

*During this step, as much of ethanolic buffer C5 as possible is removed by centrifugation. Allowing DNA binding plate to sit open in a 37°C incubator for 30 min can help with removal of ethanol buffer.*

**7. Elute highly pure DNA**

Place NucleoSpin® Plant Binding Plate on a Round-well Block (low). Dispense 100  $\mu$ l pre-warmed buffer CE (70°C) to each well of the NucleoSpin® Plant Binding Plate. Dispense the buffer directly onto the membrane. Incubate at room temperature for 1 min. Centrifuge at 5,600 – 6,000 x g for 2 min. Remove the NucleoSpin® Plant Binding Plate from the Round-well Block (low).

*Elution buffer volume may be increased to 150  $\mu$ l or elution step may be repeated. Yields will be 10 to 20% higher when eluting in 200  $\mu$ l buffer CE depending on the total amount of DNA. The concentration of DNA, however, will be much lower than with 100  $\mu$ l. Elution can also be done in TE buffer (at least pH 8.0) as well. Elution efficiency will decrease when using elution buffers with pH 8.0.*

*If DNA does not amplify for PCR, a standard ethanol precipitation can be done to remove any impurities or additional ethanol.*

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**8 Sigma Extract-N-Amp™ Plant** for 1000 samples including 1000 PCRs (not sold separately)

**Part #:** XNAR

**Cost/Sample:** ~\$1.20

**Time for 192 samples:** 30 minutes

1. Add seeds and ¼ inch stainless steel beads/tumblers to each well. Make or buy bead dispenser. Seal plates with foil sealer or rubber plate covers and secure lid. Secure plate(s) on shaker/grinder and shake for recommended time (see Table 1) depending on grinder/shaker.
2. Add 600  $\mu$ l dH<sub>2</sub>O to ground seed tissue and mix vigorously.
3. Add 45  $\mu$ l Extraction Solution and 5  $\mu$ l of Seed Preparation Solution to new plate, add 5  $\mu$ l of seed suspension and incubate at 55°C for 10 min.
4. Heat 95°C for 3 min.
5. Add 50  $\mu$ l of Neutralization Solution B and vortex.
6. Use 4  $\mu$ l aliquot directly for PCR.

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**9 Whatman binding plate** (modified from manufacturer's protocol)

**Whatman DNA Binding Plate****Part#7700-2810****Whatman Lysate Clarification Plate****Part#7720-2830****Cost/sample: \$0.35**

Buffer	Qty needed (per Plate)	Formulation
P1 Buffer	20mL	100 mM Tris, 20 mM EDTA, pH 7.8. Prior to use add RNase A to a final concentration of 0.1 mg/mL.
P2 Buffer	20mL	0.2 N NaOH, 1% (w/v) SDS
P3 Buffer	20mL	4 M Guanidine-HCl, 0.75 M Potassium acetate, pH 4.5
Wash Buffer 1	40mL	4 M Guanidine-HCl, 0.75 M Potassium acetate, pH 4.6
Wash Buffer 2	40mL	40% (v/v) 100 mM Tris, 20 mM EDTA, 0.4M NaCl, pH 7.5. Requires addition of ethanol prior to use. Final concentration 60% (v/v) ethanol.
Elution Buffer	10mL	10 mM Tris, 0.1 mM EDTA, pH 7.5

\*Buffers were also tested with Guanidine thiocyanate in place of Guanidine-HCl.

1. Add 200  $\mu$ l P1 Buffer to homogenized tissue. Resuspend completely with a pipette or brief vortexing.
2. Add 200  $\mu$ l P2 Buffer and vortex.
3. Add 200  $\mu$ l P3 Buffer. Mix the plate by shaking gently and incubate at 60°C for 30 min.
4. Spin plate for 15 min at 5,600 – 6,000 x g.
5. Place Lysate Clarification Plate on new 96 well plate and transfer 400  $\mu$ l lysate to Lysate Clarification Plate and spin at 1800 x g.
6. Place DNA Binding Plate on 2 mL waste collection plate and transfer clarified lysate to DNA binding plate.
7. Centrifuge the DNA Binding Plate for 2.5 min at 1800 x g

8. Add 400  $\mu$ l Wash Buffer 1 to each well of the DNA Binding Plate and centrifuge for 2.5 min at 1800 x g.
9. Pour out flow-through. Add 400  $\mu$ l Wash Buffer 2 to each well of the DNA Binding Plate, place back on waste collection plate and spin for 4.5 min at 1800 x g.
10. Remove the stacked plates from the centrifuge and blot the bottom of the binding plate with paper towel.
11. Place the DNA Binding Plate on top of a 250  $\mu$ l collection plate and add 50  $\mu$ l of Elution Buffer to the center of each well. Incubate at room temperature for one minute. Spin for 2.5 min at 1800 x g.
12. Repeat step 11.

## #10 Promega SV Genomic DNA Purification System

Part#A2360

Cost/sample:1.60

### CTAB solutions

#### **DNA Extraction Buffer**

working concentration:	stock:	amount for 1 liter
350 mM sorbitol	sorbitol	63.8 g
100 mM Tris-base	1 M Tris pH 7.5	100 ml
5 mM EDTA	0.5 M EDTA	10 ml

Add the above to 800 ml of ddH<sub>2</sub>O then pH to 7.5 using NaOH. Bring the volume up to 1 liter using ddH<sub>2</sub>O. Store the solution at 4°C until use. The day of use, add 3.8 g sodium bisulfite per liter of extraction buffer.

#### **Nuclei Lysis Buffer**

working concentration:	stock:	amount for 1 liter
200 mM Tris-base	1 M Tris pH 7.5	200 ml
50 mM EDTA	0.5 M EDTA	100 ml
2 M Guanidine thiocyanate	5 M NaCl	400 ml
2% CTAB (mixed alkyltrimethylammonium bromide)	CTAB powder	20.0 g

Add the above to 200 ml ddH<sub>2</sub>O and then pH to 7.5 using NaOH. Bring up to 1 liter volume using ddH<sub>2</sub>O. Store the solution at room temperature until use.

#### **5% Sarkosyl**

for 1 liter: add 50g N-lauroylsarcosine to ddH<sub>2</sub>O. Store at room temperature until use.

#### **TE**

Stock	Amount
1 M Tris pH 8.0	10 ml
0.5 M EDT A pH 8.0	2.0 ml

Combine the above and bring the volume to 1 liter with ddH<sub>2</sub>O

### **Making the Buffer Mix**

Mix 2.5 parts DNA Extraction Buffer, 2.5 parts Nuclei Lysis Buffer. Add 1 part 5% Sarkosyl. Add sodium bisulfite to the extraction buffer the day of use.  
Store at 4°C

1. Grind seed using liquid nitrogen in 1.5 microfuge tube with plastic pestle.
2. Add 400µl CTAB buffer mix and incubate at 60°C for 30 min. mixing occasionally.
3. Spin tubes for 15 min at 14000 rpm.
4. Transfer 250µl lysate to binding membrane and spin at 13000 x g for 3 min
5. Remove minicolumn from the Assembly and discard the liquid in the Collection Tube. Replace the minicolumn into the Collection Tube.
6. Add 650 µl Wizard® SV Wash Solution (with 95% ethanol added) to each assembly. Centrifuge at 13,000 x g for 1 minute. Discard flow-through. Repeat for a total of 4 washes.
7. Discard flow-through and replace minicolumn in tube. Spin at 13,000 x g for 3 minutes to remove any remaining ethanol.
8. Transfer the Wizard® SV Minicolumn to a new 1.5 m. tube. Add 100µl of room temperature Nuclease-Free Water. Incubate for 2 minutes at room temperature.
9. Centrifuge tube at 13,000 x g for 1 min and repeat step 8.