

Materials List for:

Optimization and Comparative Analysis of Plant Organellar DNA Enrichment Methods Suitable for Next-generation Sequencing

Marisa E. Miller^{*1,2}, Katie L. Liberatore^{*1,3}, Shahryar F. Kianian^{1,3}

¹Cereal Disease Laboratory, United States Department of Agriculture-Agricultural Research Service

²Department of Horticultural Science, University of Minnesota

³Department of Plant Pathology, University of Minnesota

*These authors contributed equally

Correspondence to: Shahryar F. Kianian at Shahryar.Kianian@ars.usda.gov

URL: <https://www.jove.com/video/55528>

DOI: [doi:10.3791/55528](https://doi.org/10.3791/55528)

Materials

Name	Company	Catalog Number	Comments
2-mercaptoethanol (beta-mercaptoethanol; BME)	Sigma Aldrich	M3148-100ml	
2-propanol (Isopropyl alcohol/isopropanol), bioreagent	Sigma Aldrich	I9516	
agarose, Bio-Rad Certified Megabase agarose	Bio-Rad	1613108	
analytical balance	Mettler Toledo	AB54-S	
balance	Mettler Toledo	PB1502-S	
bovine serum albumin (BSA)	Sigma Aldrich	B4287-25G	
Ceramic grinding cylinders, 3/8in x 7/8in	SPEX SamplePrep	2183	
Cryogenic Blocks compatible with tissue homogenizer for holding 50 mL tubes	SPEX SamplePrep	2664	
DNaseI	Sigma	DN25	
ethanol, absolute	Decon Laboratories	2716	
Ethylenediamine Tetraacetic Acid (EDTA), 0.5 M Solution, pH 8.0	Fisher	BP2482-500	
gel imaging system			
gel stain			Such as GelRed or Ethidium Bromide
grinding pestle, wide tip for 2 mL conical tubes			
Guanidine-HCl, 8 M solution	ThermoFisher	24115	
LightCycler 480 SYBR Green I Master	Roche	4707516001	
liquid nitrogen			
Lysing enzymes from <i>Trichoderma harzianum</i>	Sigma	L1412	
Magnesium Chloride	G Bioscience	24115	
magnetic rack	ThermoFisher	A13346	
microcentrifuge tubes, LoBind 1.5 mL	Eppendorf	22431021	
microcentrifuge tubes, standard nuclease-free 1.5 mL	Eppendorf		

microcentrifuge, refrigerated	Sorvall	Legend X1R	Or equivalent product, must be capable of reaching at least 18,000 x g with rotors for 50 mL tubes, Oak Ridge tubes, and 1.5 mL tubes
microcentrifuge, room temperature	Eppendorf	5424	Or equivalent product, must be capable of reaching at least 18,000 x g with rotor for 1.5 mL and 2 mL microcentrifuge tubes
Microcon DNA Fast Flow Centrifugal Filter Units	EMD Millipore	MRCFOR100	
Miracloth, 1 square per sample cut to fit funnel	EMD Millipore	475855	
NEBNext Microbiome DNA Enrichment Kit	New England Biolabs	E2612L	
parafilm	Parafilm M	PM992	
plastic pots and trays			
polyvinylpyrrolidone (PVP)	Fisher	BP431-100	
Proteinase K	Qiagen	19131	
Pulsed-Field Gel Electrophoresis rig (e.g. CHEF DR III)	Bio-Rad	1703697	
purification beads, Agencourt AMPureXP beads	Beckman Coulter	A63881	
QIAamp DNA Mini Kit	Qiagen	51304	
Qiagen 20/g Genomic Tip DNA Extraction Kit	Qiagen	10223	
Qiagen Buffer EB (elution buffer)	Qiagen	19086	
Qiagen DNA Extraction Buffer Set	Qiagen	19060	
QiaRack	Qiagen	19015	
qPCR machine (e.g. Roche Light Cycler 480)	Roche		
qPCR plate sealing film	Roche	4729757001	
qPCR plate, 96 well plate	Roche	4729692001	
Qubit assay tubes	Life Technologies	Q32856	
Qubit Broad Spectrum assay kit	Life Technologies	Q32850	
Qubit High Sensitivity assay kit	Life Technologies	Q32851	
RNaseA	Qiagen	19101	
Serological pipettes (20 mL) and pipet-aid	Fisher	13-678-11	
Small funnels, 1 per sample			
Sodium Chloride	Ambion	AM9759	
Soft paintbrush, 2 per sample			
SPEX SamplePrep 2010 Geno/Grinder or another type of tissue homogenizer	SPEX SamplePrep		Or another comparable tissue homogenizer. If you do not have access to a tissue homogenizer, then grinding in a pre-chilled mortar and pestle will suffice (see protocol for details). However, a homogenizer will give more consistent results and total homogenization time is reduced.
Sucrose	Omnipure	8550	
TBE			

thermomixer			
Tris	Sigma	T2819-100ml	
Triton X-100	Promega	H5142	
tube rotater			
tubes, 50 mL conical polypropylene	Corning	352070	
tubes, 50 mL high-speed polypropylene	ThermoScientific/Nalgene	3119-0050	e.g. Nalgene Oakridge tubes or equivalent
vermiculite			
water bath			
water, sterile and certified Nuclease-free	Fisher	1481	
water, sterile milliQ			