An Efficient Field and Laboratory Workflow for Plant Phylotranscriptomic Projects

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Protocol Note

An efficient field and laboratory workflow for plant phylotranscriptomic projects[1]

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- *Premise of the study:* We describe a field and laboratory workflow developed for plant phylotranscriptomic projects that involves cryogenic tissue collection in the field, RNA extraction and quality control, and library preparation. We also make recommendations for sample curation.
- *Methods and Results:* A total of 216 frozen tissue samples of Caryophyllales and other angiosperm taxa were collected from the field or botanical gardens. RNA was extracted, stranded mRNA libraries were prepared, and libraries were sequenced on Illumina HiSeq platforms. These included difficult mucilaginous tissues such as those of Cactaceae and Droseraceae.
- *Conclusions:* Our workflow is not only cost effective (ca. $270 per sample, as of August 2016, from tissue to reads) and time efficient (less than 50 h for 10–12 samples including all laboratory work and sample curation), but also has proven robust for extraction of difficult samples such as tissues containing high levels of secondary compounds.

Key words: Caryophyllales; cryogenic field sampling; phylogenomics; phylotranscriptomics; RNA.

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Phylotranscriptomics, or using transcriptome sequences to investigate phylogenetic relationships and gene family evolution in nonmodel plants, has gained popularity in recent years due to decreases in cost and improvements in analysis pipelines (Wickett et al., 2014; Edger et al., 2015; Li et al., 2015; Yang et al., 2015; McKain et al., 2016). It is often possible to recover at least 15,000 genes from the target species using de novo–assembled transcriptome data (Yang and Smith, 2013). Among these, approximately 5000 are shared among most species within an order (Yang et al., 2015), with the rest being tissue- and/or taxon-specific. Together they provide enormously rich data both for phylogenetic reconstruction and for investigating gene family evolution that underlies lineage-specific adaptations.

Generating plant phylotranscriptomic data has become much easier over the past few years due to improvements in sequencing and extraction protocols but may still be challenging for a variety of reasons. Previous literature on phylotranscriptomic methods has focused on RNA extraction and fragment analyses of those extracted RNA samples (Johnson et al., 2012; Yockteng et al., 2013; Jordon-Thaden et al., 2015) and sequence data analyses (Yang and Smith, 2013, 2014). However, as phylotranscriptomic studies expand to nonmodel systems that often require field sampling, the logistics of obtaining fresh tissues becomes a limiting factor. Likewise, some taxa such as cacti pose special challenges due to high levels of mucilage (Jordon-Thaden et al., 2015). Moving forward, the issues of long-term preservation and curation of cryogenic genetic materials will also be of the utmost importance for laboratories seeking to pursue these studies.

From 2012 to 2015, we conducted field expeditions to remote localities in both the southwestern United States and northern Mexico to support National Science Foundation–funded projects on the evolution of Caryophyllales and gypsum-endemic plants. Together with samples from living collections, we generated a transcriptome data set of 200 species of plants (Appendix 1). During the process we have developed an optimized workflow, which is described below. In addition, we discuss alternative procedures that we tested, as well as considerations for project planning.

METHODS AND RESULTS

***Taxon sampling*—**The Caryophyllales phylotranscriptomics project emphasized a combination of broad taxon sampling across the order and in-depth sampling of lineages with key evolutionary transitions. These key transitions include the gain and loss of plant carnivory; the gain and loss of betalain pigmentation; transitions to saline, dry, or alpine habitats, and/or to specialized soil types; and transitions to C_4 and CAM photosynthesis. Of the transcriptomes we have generated for the Caryophyllales phylotranscriptomic project, half were collected from the field, with the remaining half from living collections (Appendix 1). Additional transcriptomes and genomes were obtained from publicly available databases such as Phytozome (Goodstein et al., 2012), the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA), and the 1000 Plants Initiative (1KP; Matasci et al., 2014).

***Field collection*—**We timed our field trips to coincide with the beginning of the flowering season as much as possible to optimize the chance of obtaining young flower and leaf buds. Our experience has been that mature vegetative tissue is more difficult to work with due to its low concentration of nuclear RNA (Johnson et al., 2012) and high level of chloroplast RNA and secondary compounds compared to developing tissues. It is also important to emphasize that field conditions are more difficult to control than greenhouse conditions. While this may impose limitations for researchers wishing to study differential gene expression, this is less problematic for phylotranscriptomic studies.

Compared to tissue preservation using an RNA stabilization solution (such as RNA*later*; Thermo Fisher Scientific, Waltham, Massachusetts, USA), tissue frozen in the field allows for biochemical analyses such as characterization of betalain and anthocyanin pigmentation, in addition to DNA and RNA sequencing, and hence this was our primary (and recommended) means of collection (Appendix 2). For all individuals frozen in liquid nitrogen, we also collected silica-preserved tissue from the same individual as a DNA backup, as well as herbarium specimens whenever possible. Because DNA may degrade relatively quickly for some groups in silica (e.g., Onagraceae), it is important to remove silica from the leaves once dried and place them in a −20°C freezer for long-term storage (Neubig et al., 2014).

***RNA extraction (less than 6 h for six samples)*—**We tested five alternative RNA extraction protocols. These include TRIzol option 1 from Jordon-Thaden et al. (2015), the Aurum Total RNA Mini Kit (Bio-Rad Laboratories, Hercules, California, USA) following the manufacturer's protocol, the QIAGEN RNeasy Mini Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol, the PureLink protocol (Appendix 3; Yockteng et al., 2013), and the hot acid phenol-LiCl-RNeasy Mini Kit protocol (Appendix 4, modified from Protocol 12 of Johnson et al. [2012]). We had approximately 10–30% success rate (see below for quality control) with Bio-Rad, QIAGEN, and TRIzol protocols, whereas the PureLink protocol had close to 100% success rate and only failed when the sample itself was degraded or highly mucilaginous. Although more time consuming, the hot acid phenol-LiCl-RNeasy Mini Kit protocol had great success with tissues that are highly mucilaginous like cacti (Appendix 4).

***Quality control and DNase digestion (less than 3 h for 12 samples)*—**For quality control of RNA, we used agarose gel for an initial assessment. If RNA was evident, removal of DNA was carried out following Jordon-Thaden et al. (2015) with minor modifications (Appendix 5). After that, we followed fig. 2 of Jordon-Thaden et al. (2015) for evaluating integrity of RNA on a 2100 Bioanalyzer (Agilent, Santa Clara, California, USA) or a Fragment Analyzer (Advanced Analytical Technologies, Ankeny, Iowa, USA). RNA concentration was measured with either a NanoDrop Spectrophotometer (Thermo Fisher Scientific) or a Qubit fluorometer (Thermo Fisher Scientific). We considered an RNA integrity number (RIN) of 6 or higher and concentration of 20 ng/μL or higher as successful. When RNA extraction failed, it was often due to either pellet loss (resulting in a completely empty gel with no DNA or RNA trace) or degradation (which shows up as smeared ribosomal RNA bands). RNA degradation can happen during collection, shipping, or in a suboptimal extraction, as for example with too much starting tissue. For difficult tissues that are mucilaginous, we reduced the amount of starting tissue by half.

RNA samples prepared at the Brockington Laboratory at the University of Cambridge, United Kingdom, were shipped on dry ice in cardboard freezer boxes to the University of Michigan for library preparation and sequencing. Dry ice shipments were sent on Monday or Tuesday to avoid delay over the weekend.

***Library preparation (less than 20 h for 12 samples)*—**We tested four different library preparation protocols. In 2012, we started with Illumina TruSeq version 2 (Illumina, San Diego, California, USA), with and without additional strand-specific steps (see Supplementary Methods in Yang et al. [2015]). In 2013, we began using the newly released TruSeq Stranded mRNA Library Prep Kit ("the Illumina kit"; Illumina), which was more streamlined and produced much higher strand specificity than the previous stranded protocol. In 2014, we switched to the KAPA Stranded mRNA-Seq kit ("the KAPA kit"; KAPA Biosystems, Wilmington, Massachusetts, USA; Appendix 6), which is considerably cheaper than the Illumina kit with indistinguishable results in terms of both success rate and strand specificity. The KAPA kit is also more streamlined with fewer bead washing steps and required roughly 15% less time. The cost is ca. US$30 per sample for the KAPA kit itself plus ca. US$20 per sample for consumables (magnetic beads, tips, tubes, and additional chemicals; we used leftover adapters from the Illumina kit, which lasted through more than 150 additional libraries from one 48-sample Illumina kit). We modified the manufacturer's protocol slightly to accommodate the increasing read length of newer Illumina platforms (125- or 150-bp paired-end; Appendix 6).

Quality control of the library was done at the University of Michigan DNA Sequencing Core using an Agilent 2100 Bioanalyzer followed by confirmation using qPCR. Although the minimal concentration of the library and percentage of adapter contamination allowed differ among sequencing platforms, we followed a few general rules. First, the peak of the library fragment size distribution should be approximately the read length plus adapter size. For example, for paired-end 125-bp sequencing on Illumina platforms, peak of library size distribution should be approximately 60 bp (adapter) + 125 bp (read) in each direction, making a total of 370 bp for the optimum library size (see Appendix 6 for modifications in library preparation to adjust library sizes). Second, although we do not quantify the library concentration in the laboratory, we visualized the library by loading 3 μL of library mixed with GelRed fluorescent stain (Biotium, Fremont, California, USA) onto a 1.5% agarose gel. As a rule of thumb, if the libraries were visible from the gel (even if only barely visible), they were sent to the DNA Sequencing Core for further quantification. Libraries were walked to the on-campus University of Michigan DNA Sequencing Core immediately in ambient temperature, or stored in −20°C for less than a month before walking to the sequencing core in ambient temperature.

***Sample curation (less than 1 h per sample)*—**We store all RNAs in a −80°C freezer on standard storage racks. Ideally, they would be stored long-term in liquid nitrogen vapor freezers. To prevent freeze/thaw of sensitive samples, we placed samples into labeled cardboard freezer boxes and recorded the sample locations in a database that is properly backed up (Appendix 7).

CONCLUSIONS

We have developed an effective phylotranscriptomics workflow involving cryogenic tissue collection in the field, RNA extraction of diverse taxa with close to 100% success rate, library preparation for Illumina platforms, and sample storage and curation. Future efforts should focus on streamlining the workflow given specific laboratory and field settings and as sequencing technologies continue to evolve. In addition, it would be ideal to collaborate with major tissue and seed banks such as the Millennium Seed Bank (Royal Botanic Gardens, Kew) and the Global Genome Initiative (Smithsonian Institution) (Gostel et al., 2016) when designing phylotranscriptomic projects.

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Appendix 1. Voucher information for the accessions used in this study.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Achatocarpaceae	*Achatocarpus gracilis* H. Walter	Michael J. Moore et al. 2704 (OC)	Jalisco, Mexico: La Huerta, Estacion Biologica Chamela, along Sendero Perico	Field	PureLink	Unpublished
Achatocarpaceae	*Phaulothamnus spinescens* A. Gray	Michael J. Moore 1677 (OC)	Texas, USA: Kleberg, along Kleberg County Rd. 1155 S, approx. 0.1 mi. N of jct. w/ FM 771.	Field	Bio-Rad	SRX998856 (Brockington et al., 2015)
Agdestidaceae	*Agdestis clematidea* Moc. & Sessé ex DC.	Michael J. Moore et al. 2669 (OC)	Veracruz, Mexico: San Andres Tuxtla, in thicket immediately adjacent to rd. in Montepio	Field	PureLink	Unpublished
Amaranthaceae	*Froelichia latifolia* R. A. McCauley	Michael J. Moore 1665 (OC)	Texas, USA: Caldwell, along FM 713 betw. McMahan and Delhi, just W of jct. w/ Taylorville Rd.	Field	Bio-Rad	SRX998855 (Brockington et al., 2015)
Amaranthaceae	*Gomphrena decumbens* Jacq.	Michael J. Moore et al. 2734 (OC)	Distrito Federal, Mexico: Coyoacan, at Instituto de Biologia at UNAM	Cultivated	PureLink	Unpublished
Amaranthaceae	*Gossypianthus lanuginosus* (Poir.) Moq.	Michael J. Moore 1807 (OC)	Texas, USA: Llano, along TX 71 at roadside historical marker, where Honey Creek passes under the hwy.	Field	Bio-Rad	Unpublished
Amaranthaceae	*Guilleminea densa* (Humb. & Bonpl. ex Schult.) Moq.	Michael J. Moore et al. 2445 (OC)	Chihuahua, Mexico: N end of Sierra de Fernando, on Rancho Puerto de Lobos	Field	Bio-Rad	Unpublished
Amaranthaceae	*Iresine arbuscula* Uline & W. L. Bray	Michael J. Moore et al. 2678 (OC)	Veracruz, Mexico: San Andres Tuxtla, Estacion Biologica Los Tuxtlas, along stream bed approx. 100 m upstream of path crossing	Field	PureLink	Unpublished
Amaranthaceae	*Iresine rhizomatosa* Standl.	Michael J. Moore & J. Lee 2943 (OC)	Cultivated at Missouri Botanical Garden	Cultivated	PureLink	Unpublished
Amaranthaceae	*Nelsia quadrangula* (Engl.) Schinz	Millennium Seed Bank accession 0468510	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Amaranthaceae	*Nitrophila occidentalis* (Moq.) S. Watson	Michael J. Moore et al. 3242 (OC)	California, USA: Inyo, along CA 190 approx. 1.3 mi. NE of jct. w/ US 395 in Olancha, just S of Owens Lake bed	Field	PureLink	Unpublished
Amaranthaceae	*Polycnemum majus* A. Braun	Gudrun Kadereit s.n.	Cultivated at Universitat Mainz	Cultivated	PureLink	Unpublished
Amaranthaceae	*Tidestromia lanuginosa* (Nutt.) Standl.	Michael J. Moore et al. 2259 (OC)	New Mexico, USA: Sierra, E side of Caballo Mountains, ca. 0.3 mi. N of jct. of Slater Rd. and Apache Gap Ranch entrance	Field	Bio-Rad	Unpublished
Anacampserotaceae	*Grahamia kurtzii* (Bacigalupo) G. D. Rowley	Sukkulenten-Sammlung Zürich accession 100046/0	Cultivated at Sukkulenten-Sammlung Zürich	Cultivated	Bio-Rad	Unpublished
Anacampserotaceae	*Talinopsis frutescens* A. Gray	Michael J. Moore et al. 2441 (OC)	Chihuahua, Mexico: N end of Sierra de Fernando, on Rancho Puerto de Lobos	Field	Trizol	Unpublished
Ancistrocladaceae	*Ancistrocladus robertsoniorum* J. Léonard	Michael J. Moore & J. Lee 2940 (OC)	Missouri Botanical Garden, cultivated in Climatron	Cultivated	PureLink	Unpublished
Apiaceae	*Heracleum mantegazzianum* Sommier & Levier	Hannah E. Marx 2014-016 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished
Apiaceae	*Heracleum sphondylium* L.	Hannah E. Marx 2014-010 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Apiaceae	*Meum athamanticum* Jacq.	Hannah E. Marx 2014-027 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Asteraceae	*Centaurea uniflora* Turra	Hannah E. Marx 2014-028 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Asteraceae	*Gaillardia multiceps* Greene	Michael J. Moore 1737 (OC)	Texas, USA: Winkler, along Winkler County Rd. 101, ca. 5 mi. N of jct. w/ TX 302	Field	Bio-Rad	Unpublished
Basellaceae	*Anredera cordifolia* (Ten.) Steenis	Cultivated at Cambridge University Botanic Garden 19770198	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Boraginaceae	*Tiquilia hispidissima* (Torr. & A. Gray) A. T. Richardson	Michael J. Moore 1736 (OC)	Texas, USA: Winkler, along Winkler County Rd. 101, ca. 5 mi. N of jct. w/ TX 302	Field	Bio-Rad	Unpublished
Brassicaceae	*Nerisyrenia linearifolia* (S. Watson) Greene	Michael J. Moore 1755 (OC)	Texas, USA: Culberson, along FM 652 ca. 25 mi. W of Orla	Field	Bio-Rad	Unpublished
Cactaceae	*Opuntia arenaria* Engelm.	Michael J. Moore et al. 2911 (OC)	New Mexico, USA: Dona Ana, cultivated at UTEP Botanical Garden; originally collected from Anapra, NM	Cultivated	PureLink	Unpublished
Cactaceae	*Rhipsalis baccifera* (Sol.) Stearn subsp. *baccifera*	Michael J. Moore 2938 (OC)	Cultivated at Oberlin College greenhouse	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Arenaria serpyllifolia* L.	Michael J. Moore 1164 (OC)	Ohio, USA: Erie, in lawn behind my house (4910 State Route 113 E)	Field	Bio-Rad	Unpublished
Caryophyllaceae	*Cerastium alpinum* L. var. *lanatum* (Lam.) Hegetschw.	Alplains accession 07471.06	Cultivated at Matthaei Botanical Gardens, University of Michigan	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Cerastium arvense* L.	Michael J. Moore 1767 (OC)	New Mexico, USA: Otero, along NM 130 a few mi. E of jct. w/ rd. to Sunspot	Field	Bio-Rad	SRX998858 (Brockington et al., 2015)
Caryophyllaceae	*Cerastium fontanum* Baumg. subsp. *vulgare* (Hartm.) Greuter & Burdet	Michael J. Moore 1163 (OC)	Ohio, USA: Erie, in lawn behind my house (4910 State Route 113 E)	Field	Bio-Rad	Unpublished
Caryophyllaceae	*Corrigiola litoralis* L.	Cultivated at Cambridge University Botanic Garden SFB_221	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Drymaria cordata* (L.) Willd. ex Schult.	Lucas C. Majure 3403 (FLAS)	Florida, USA: Alachua, Gainesville, University of Florida campus	Field	Bio-Rad	SRX998854 (Brockington et al., 2015)
Caryophyllaceae	*Drymaria* sp.	Michael J. Moore et al. 2679 (OC)	Veracruz, Mexico: Coscomatepec, along paved rd. from Fortin to Huatusco, a few km N of Coscomatepec	Field	PureLink	Unpublished
Caryophyllaceae	*Drymaria subumbellata* I. M. Johnst.	Michael J. Moore et al. 2503 (OC)	Durango, Mexico: on the W side of the Sierra de Tlahualilo	Field	Bio-Rad	Unpublished
Caryophyllaceae	*Eremogone hookeri* (Nutt. ex Torr. & A. Gray) W. A. Weber subsp. *desertorum* (Maguire) W. A. Weber	Alplains accession 35665.38	Cultivated at Matthaei Botanical Gardens, University of Michigan	Cultivated	Bio-Rad	Unpublished
Caryophyllaceae	*Eremogone procera* (Spreng.) Rchb.	Gudrun Kadereit s.n.	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Gypsophila repens* L.	Cambridge University Botanic Garden 10007025, 19610163, 19860039	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Herniaria latifolia* Lapeyr.	Cambridge University Botanic Garden 10005718	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Honckenya peploides* (L.) Ehrh.	Millennium Seed Bank accession 0286981	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Caryophyllaceae	*Illecebrum verticillatum* L.	N/A	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Lepyrodiclis stellarioides* Fisch. & C. A. Mey.	Millennium Seed Bank accession 0653842	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Paronychia drummondii* Torr. & A. Gray	Michael J. Moore 1670 (OC)	Texas, USA: Caldwell, along FM 713 between McMahan and Delhi, just W of jct. w/ Taylorville Rd.	Field	PureLink	Unpublished
Caryophyllaceae	*Paronychia jamesii* Torr. & A. Gray	Michael J. Moore et al. 2931 (OC)	New Mexico, USA: Eddy, along gravel rd. leading W from US 285 along N edge of Seven Rivers Hills	Field	PureLink	Unpublished
Caryophyllaceae	*Schiedea globosa* H. Mann	Cambridge University Botanic Garden SFB_256	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Scleranthus polycarpos* L.	Gudrun Kadereit s.n.	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Silene acaulis* (L.) Jacq. subsp. *acaulescens* Hitchc. & Maquire	Alplains accession 01224.31	Cultivated at Matthaei Botanical Gardens, University of Michigan	Cultivated	Bio-Rad	Unpublished
Caryophyllaceae	*Spergularia marina* (L.) Besser	Michael J. Moore et al. 3185 (OC)	California, USA: Kern, Tejon Ranch: Amargo Springs	Field	PureLink	Unpublished
Caryophyllaceae	*Telephium imperati* L.	Cambridge University Botanic Garden 19910346	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Caryophyllaceae	*Velezia rigida* L.	Gudrun Kadereit s.n.	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Chenopodiaceae	*Anabasis articulata* (Forssk.) Moq.	LS168	Cultivated at Universitat Mainz	Cultivated	PureLink	Unpublished
Chenopodiaceae	*Arthrocnemum macrostachyum* (Moric.) K. Koch	LS33	Cultivated at Universitat Mainz	Cultivated	PureLink	Unpublished
Chenopodiaceae	*Atriplex* sp.	Michael J. Moore et al. 3295 (OC)	Utah, USA: Wayne, a few hundred yards W of Coal Mine Rd., approx. 3.2 mi. N of jct. w/ UT 24	Field	PureLink	Unpublished
Chenopodiaceae	*Caroxylon vermiculatum* (L.) Akhani & Roalson	LS178	Cultivated at Universitat Mainz	Cultivated	PureLink	Unpublished
Chenopodiaceae	*Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch	Michael J. Moore et al. 2991 (OC)	New Mexico, USA: Chaves, along gravel rd. just N of US 380 opposite rest area	Field	PureLink	Unpublished
Chenopodiaceae	*Corispermum hyssopifolium* L.	Millennium Seed Bank accession 0000170	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Chenopodiaceae	*Eokochia saxicola* (Guss.) Freitag & G. Kadereit	LS70	Cultivated at Universitat Mainz	Cultivated	PureLink	Unpublished
Chenopodiaceae	*Extriplex californica* Moq.	Michael J. Moore et al. 3214 (OC)	California, USA: San Luis Obispo, Morro Bay State Park/Morro Estuary Natural Preserve: just E of State Park Rd./Main St., approx. 0.7 mi. SW of jct. w/ S Bay Blvd.	Field	Bio-Rad	Unpublished
Chenopodiaceae	*Grayia spinosa* (Hook.) Moq.	Michael J. Moore et al. 3268 (OC)	Nevada, USA: Douglas, near two-track rd. that runs S from Mel Drive	Field	Bio-Rad	Unpublished
Chenopodiaceae	*Kali collina* (Pall.) Akhani & Roalson	Millennium Seed Bank accession 0496298	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Chenopodiaceae	*Kalidium cuspidatum* (Ung.-Sternb.) Grubov	LS97	Cultivated at Universitat Mainz	Cultivated	PureLink	Unpublished

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Chenopodiaceae	*Kraschenninnikovia lanata* (Pursh) A. Meeuse & A. Smit	Michael J. Moore et al. 2311 (OC)	New Mexico, USA: Sierra, along WSMR Rte. 6 just S of Big Gyp Mountain	Field	Trizol	Unpublished
Chenopodiaceae	*Salsola* sp.	N/A	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Chenopodiaceae	*Sarcocornia pacifica* (Standl.) A. J. Scott	Michael J. Moore et al. 3216 (OC)	California, USA: San Luis Obispo, Morro Bay State Park/Morro Estuary Natural Preserve: just E of State Park Rd./Main St., approx. 0.7 mi. SW of jct. w/ S Bay Blvd.	Field	PureLink	Unpublished
Chenopodiaceae	*Stutzia covillei* (Torr. ex S. Watson) S. Watson	Michael J. Moore et al. 3228 (OC)	California, USA: Inyo, along Searles Dry Lake Rd., approx. 0.5 mi. E of jct. w/ Trona-Wildrose Rd.	Field	PureLink	Unpublished
Chenopodiaceae	*Suaeda linearis* (Elliott) Moq.	Michael J. Moore 1679 (OC)	Texas, USA: Kleberg, Riviera Beach, along beach just N of parking area at end of FM 771. GPS coordinates refer to parking area.	Field	Bio-Rad	Unpublished
Chenopodiaceae	*Tecticornia pergranulata* (J. M. Black) K. A. Sheph. & Paul G. Wilson	LS28	Cultivated at Universitat Mainz	Cultivated	PureLink	Unpublished
Cyperaceae	*Carex capillaris* L.	Hannah E. Marx 2014-030 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Cyperaceae	*Carex nigra* (L.) Reichard	Hannah E. Marx 2014-031 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Didiereaceae	*Alluaudia dumosa* (Drake) Drake	Desert Botanical Garden accession 1987-0301-0201	Cultivated at Desert Botanical Garden	Cultivated	PureLink	Unpublished
Didiereaceae	*Alluaudia humbertii* Choux	Desert Botanical Garden accession 1974-0229-01-1	Cultivated at Desert Botanical Garden	Cultivated	PureLink	Unpublished
Didiereaceae	*Alluaudia procera* (Drake) Drake	Desert Botanical Garden accession 1956-5744-02-3 G	Cultivated at Desert Botanical Garden	Cultivated	PureLink	Unpublished
Didiereaceae	*Alluaudiopsis marnieriana* Rauh	Sukkulenten-Sammlung Zürich accession 81 2213/0	Cultivated at Sukkulenten-Sammlung Zürich	Cultivated	Hot acid Phenol	Unpublished
Didiereaceae	*Ceraria pygmaea* (Pillans) G. D. Rowley	Sukkulenten-Sammlung Zürich accession 90 1893/b	Cultivated at Sukkulenten-Sammlung Zürich	Cultivated	Hot acid Phenol	Unpublished
Didiereaceae	*Decarya madagascariensis* Choux	Michael J. Moore & J. Lee 2944 (OC)	Missouri Botanical Garden, cultivated in nonpublic arid plant greenhouse	Cultivated	PureLink	Unpublished
Didiereaceae	*Didierea madagascariensis* Baill.	Desert Botanical Garden accession 1996-0312-01-1	Cultivated at Desert Botanical Garden	Cultivated	PureLink	Unpublished
Didiereaceae	*Didierea trollii* Capuron & Rauh	Desert Botanical Garden accession 1984-0024-0202 G	Cultivated at Desert Botanical Garden	Cultivated	PureLink	Unpublished
Didiereaceae	*Portulacaria afra* Jacq.	Desert Botanical Garden accession 1988-0583-02-1 G	Cultivated at Desert Botanical Garden	Cultivated	PureLink	Unpublished
Droseraceae	*Aldrovanda vesiculosa* L.	Michael J. Moore 1652 (OC)	Virginia, USA: Caroline, ponds at Meadowview Biological Station	Cultivated	Bio-Rad	SRX998847 (Brockington et al., 2015)
Droseraceae	*Drosera binata* Labill.	Cambridge University Botanic Garden	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Droseraceae	*Drosera burmannii* Vahl	Matthaei Botanical Gardens	Cultivated at Matthaei Botanical Gardens, University of Michigan	Cultivated	PureLink	Unpublished

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Drosophyllaceae	*Drosophyllum lusitanicum* (L.) Link	Cambridge University Botanic Garden	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Fabaceae	*Lotus corniculatus* L.	Hannah E. Marx 2014-026 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Frankeniaceae	*Frankenia salina* (Molina) I. M. Johnst.	Michael J. Moore et al. 3209 (OC)	California, USA: San Luis Obispo, Morro Bay State Park/Morro Estuary Natural Preserve: just E of State Park Rd./Main St., approx. 0.7 mi. SW of jct. w/ S Bay Blvd.	Field	PureLink	Unpublished
Geraniaceae	*Geranium sylvaticum* L.	Hannah E. Marx 2014-014 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Gisekiaceae	*Gisekia pharnaceoides* L.	Millennium Seed Bank accession 0586315	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Hydrophyllaceae	*Nama carnosa* (Woot.) C. L. Hitchc.	Michael J. Moore 1750 (OC)	Texas, USA: Culberson, along FM 652 ca. 25 mi. W of Orla	Field	Bio-Rad	Unpublished
Kewaceae	*Kewa bowkeriana* (Sond.) Christenh.	Botanischen Gartens, Technische Universität Dresden accession 99 Bonn 931 (B:09964)	Cultivated at Cambridge University Botanic Garden	Cultivated	Hot acid Phenol	Unpublished
Lentibulariaceae	*Pinguicula vulgaris* L.	Hannah E. Marx 2014-021 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Limeaceae	*Limeum aethiopicum* Burm. f.	Cultivated at Cambridge University Botanic Garden s.n.	Cultivated at Cambridge University Botanic Garden	Cultivated	Hot acid Phenol	Unpublished
Loasaceae	*Mentzelia humilis* (Urb. & Gilg) J. Darl.	Michael J. Moore 1749 (OC)	Texas, USA: Culberson, along FM 652 ca. 25 mi. W of Orla	Field	Bio-Rad	Unpublished
Macarthuriaceae	*Macarthuria australis* Hügel ex Endl.	Kevin Thiele 5141 (UWA)	Western Australia, Australia: Perth. Cultivated at Cambridge University Botanic Garden.	Cultivated	PureLink	Unpublished
Melanthiaceae	*Veratrum album* L.	Hannah E. Marx 2014-025 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Molluginaceae	*Glinus lotoides* L. var. *virens* Fenzl	Millennium Seed Bank accession 0197698	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Molluginaceae	*Pharnaceum exiguum* Adamson	Millennium Seed Bank accession 0467649	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Molluginaceae	*Suessenguthiella caespitosa* Friedrich	Millennium Seed Bank accession 0467650	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Montiaceae	*Calandrinia grandiflora*	Cambridge University Botanic Garden 20070025	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Montiaceae	*Calyptridium pygmaeum* Parish ex Rydb.	Michael J. Moore et al. 3165 (OC)	California, USA: San Bernardino, approx. 0.2 mi. N of Forest Rd. 2N86, approx. 0.4 mi. NE of Bluff Lake	Field	PureLink	Unpublished
Montiaceae	*Calyptridium umbellatum* (Torr.) Hershkovitz	Michael J. Moore et al. 3142 (OC)	California, USA: San Bernardino, Mt. Baldy ski area	Field	Bio-Rad	Unpublished
Montiaceae	*Cistanthe grandiflora* (Lindl.) Schltdl.	Chileflora s.n.	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Montiaceae	*Claytonia nevadensis* S. Watson	Thomas R. Stoughton et al. 2027 (RSA)	California, USA: Alpine, about 250 yards W of Round Top Lake, near trail	Field	Bio-Rad	Unpublished
Montiaceae	*Claytonia virginica* L.	Michael J. Moore 1156 (OC)	Ohio, USA: Erie, in woods behind my house (4910 State Route 113 E)	Field	Bio-Rad	Unpublished
Montiaceae	*Lewisia nevadensis* (A. Gray) B. L. Rob.	Michael J. Moore et al. 3168 (OC)	California, USA: San Bernardino, near edge of meadow, along small creek that feeds into Bluff Lake	Field	PureLink	Unpublished
Montiaceae	*Montia chamissoi* (Ledeb. ex Spreng.) Greene	Michael J. Moore et al. 3167 (OC)	California, USA: San Bernardino, near edge of meadow, along small creek that feeds into Bluff Lake	Field	PureLink	Unpublished
Montiaceae	*Phemeranthus parviflorus* (Nutt.) Kiger	Michael J. Moore et al. 2214 (OC)	New Mexico, USA: Socorro, just E of Quebradas Backcountry Byway	Field	Trizol	Unpublished
Nepenthaceae	*Nepenthes alata* Blanco	Cambridge University Botanic Garden 20160588, 20160946	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Nepenthaceae	*Nepenthes ventricosa* Blanco	Cambridge University Botanic Garden 20050134	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Nyctaginaceae	*Abronia bigelovii* Heimerl	Michael J. Moore et al. 2189 (OC)	New Mexico, USA: Sandoval, just N of Cabezon Rd., N of White Mesa Bike Trails	Field	Bio-Rad	Unpublished
Nyctaginaceae	*Abronia fragrans* Nutt. ex Hook.	Michael J. Moore et al. 2992 (OC)	New Mexico, USA: Chaves, along gravel rd. just N of US 380 opposite rest area	Field	PureLink	Unpublished
Nyctaginaceae	*Abronia glabrifolia* Standl.	Michael J. Moore et al. 3318 (OC)	Colorado, USA: Mesa, along Mitchell Rd. approx. 1.0 mi. W of jct. w/ CO 139	Field	PureLink	Unpublished
Nyctaginaceae	*Abronia latifolia* Eschsch.	Michael J. Moore et al. 3204 (OC)	California, USA: San Luis Obispo, Montana de Oro State Park: along path from parking lot at end of Sand Spit Rd. to beach, just up the dunes from the beach	Field	PureLink	Unpublished
Nyctaginaceae	*Abronia maritima* Nutt. ex S. Watson	Michael J. Moore et al. 3217 (OC)	California, USA: San Luis Obispo, Morro Strand State Beach: just W of Hwy. 1 in Cayucos	Field	Trizol	Unpublished
Nyctaginaceae	*Abronia nealleyi* Standl.	Michael J. Moore 1751 (OC)	Texas, USA: Culberson, along FM 652 ca. 25 mi. W of Orla	Field	Bio-Rad	SRX998850 (Brockington et al., 2015)
Nyctaginaceae	*Abronia umbellata* Lam.	Michael J. Moore et al. 3203 (OC)	California, USA: San Luis Obispo, Montana de Oro State Park: along path from parking lot at end of Sand Spit Rd. to beach, approx. 100 yards from parking lot	Field	PureLink	Unpublished
Nyctaginaceae	*Acleisanthes acatitensis* M. J. Moore & E. Loke (ined.)	Michael J. Moore et al. 2505 (OC)	Durango, Mexico: on the W side of the Sierra de Tlahualilo	Field	PureLink	Unpublished
Nyctaginaceae	*Acleisanthes acutifolia* Standl.	Michael J. Moore et al. 2447 (OC)	Chihuahua, Mexico: N end of Sierra de Fernando, on Rancho Puerto de Lobos	Field	Bio-Rad	Unpublished
Nyctaginaceae	*Acleisanthes chenopodioides* (A. Gray) R. A. Levin	Michael J. Moore et al. 2246 (OC)	New Mexico, USA: Sierra, Armendaris Ranch, along rd. leading to highest point in Fra Cristobal Mountains	Field	Bio-Rad	Unpublished
Nyctaginaceae	*Acleisanthes lanceolata* (Wooton) R. A. Levin var. *lanceolata*	Michael J. Moore 1741 (OC)	Texas, USA: Winkler, along Winkler County Rd. 101, ca. 5 mi. N of jct. w/ TX 302	Field	Bio-Rad	SRX998849 (Brockington et al., 2015)

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Nyctaginaceae	*Acleisanthes obtusa* (Choisy) Standl.	Michael J. Moore 1697 (OC)	Texas, USA: Zapata, immediately behind the Holiday Inn Express on the S side of Zapata, along US 83	Field	Bio-Rad	SRX998848 (Brockington et al., 2015)
Nyctaginaceae	*Anulocaulis annulatus* (Coville) Standl.	Michael J. Moore et al. 3237a (OC)	California, USA: Inyo, mouth of Surprise Canyon	Field	PureLink	Unpublished
Nyctaginaceae	*Anulocaulis eriosolenus* (A. Gray) Standl.	Michael J. Moore et al. 2362 (OC)	Chihuahua, Mexico: along MEX 16 in Sierra Peguis	Field	Bio-Rad	Unpublished
Nyctaginaceae	*Anulocaulis leiosolenus* (Torr.) Standl. var. *gypsogenus* (Waterf.) Spellenb. & T. Wootten	Michael J. Moore 1070 (OC)	Texas, USA: Culberson, along FM 652 25 mi. W of jct. w/ US 285 in Orla	Field	Bio-Rad	SRX717838 (Yang et al., 2015)
Nyctaginaceae	*Boerhavia ciliata* Brandegee	Michael J. Moore et al. 2760 (OC)	Nuevo Leon, Mexico: Mier y Noriega, large gypsum exposure about 35 km SSW of Doctor Arroyo	Field	PureLink	Unpublished
Nyctaginaceae	*Boerhavia purpurascens* A. Gray	Michael J. Moore et al. 2201 (OC)	New Mexico, USA: Socorro, along wash E of County Rd. 12	Field	Bio-Rad	Unpublished
Nyctaginaceae	*Boerhavia torreyana* (S. Watson) Standl.	Michael J. Moore et al. 2202 (OC)	New Mexico, USA: Socorro, along wash E of County Road 12	Field	Bio-Rad	Unpublished
Nyctaginaceae	*Bougainvillea stipitata* Griseb. var. *grisebachiana* Heimerl	Kew Living Collection #1986-4920	Cultivated at Royal Botanic Gardens, Kew	Cultivated	PureLink	SRX718672 (Yang et al., 2015)
Nyctaginaceae	*Colignonia ovalifolia* Heimerl	Crug Farms accession BSWJ10644	Cultivated at Cambridge University Botanic Garden	Cultivated	Hot acid Phenol	Unpublished
Nyctaginaceae	*Commicarpus scandens* (L.) Standl.	Michael J. Moore et al. 2726B (OC)	Jalisco, Mexico: Autlan de Navarro, along gravel rd. leading from the outskirts of El Grullo to El Chacalito	Field	PureLink	Unpublished
Nyctaginaceae	*Cyphomeris gypsophiloides* (M. Martens & Galeotti) Standl.	Michael J. Moore 1714 (OC)	Texas, USA: Val Verde, along US 90 W of Langtry	Field	Bio-Rad	SRX998857 (Brockington et al., 2015)
Nyctaginaceae	*Guapira obtusata* (Jacq.) Little	Kew Living Collection #2011-994	Cultivated at Royal Botanic Gardens, Kew	Cultivated	PureLink	SRX718384 (Yang et al., 2015)
Nyctaginaceae	*Mirabilis multiflora* (Torr.) A. Gray	Michael J. Moore 1771 (OC)	New Mexico, USA: Lincoln, along NM 55 approx. 0.5 mi. W of jct. w/ US 54	Field	Bio-Rad	SRX998851 (Brockington et al., 2015)
Nyctaginaceae	*Mirabilis pringlei* Weath.	Michael J. Moore et al. 2725 (OC)	Jalisco, Mexico: Autlan de Navarro, along gravel rd. leading from the outskirts of El Grullo to El Chacalito	Field	PureLink	Unpublished
Nyctaginaceae	*Neea psychotrioides* Donn. Sm.	Michael J. Moore et al. 2675 (OC)	Veracruz, Mexico: San Andres Tuxtla, Estacion Biologica Los Tuxtlas, near the collections building	Field	PureLink	Unpublished
Nyctaginaceae	*Nyctaginia capitata* Choisy	Michael J. Moore et al. 2585 (OC)	Coahuila, Mexico: in Valle Padilla along rd. leading W away from ranch house	Field	Bio-Rad	Unpublished
Nyctaginaceae	*Okenia hypogaea* Schltdl. & Cham.	Michael J. Moore et al. 2673 (OC)	Veracruz, Mexico: San Andres Tuxtla, along the face and near the base of large foredunes near the beach	Field	PureLink	Unpublished
Nyctaginaceae	*Pisonia aculeata* L.	Kew Living Collection #2011-448	Cultivated at Royal Botanic Gardens, Kew	Cultivated	PureLink	SRX718389 (Yang et al., 2015)
Nyctaginaceae	*Pisonia umbellifera* (J. R. Forst. & G. Forst.) Seem.	Kew Living Collection #1986-3623	Cultivated at Royal Botanic Gardens, Kew, United Kingdom	Cultivated	PureLink	SRX998852 (Brockington et al., 2015)

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Nyctaginaceae	*Salpianthus purpurascens* (Cav. ex Lag.) Hook. & Arn.	Michael J. Moore et al. 2724 (OC)	Jalisco, Mexico: Autlan de Navarro, along arroyo bed across the street from the campus of CUCSUR in Autlan de Navarro	Field	PureLink	Unpublished
Nyctaginaceae	*Tripterocalyx carneus* (Greene) L. A. Galloway	Michael J. Moore et al. 3027 (OC)	New Mexico, USA: Socorro, along US 380 at picnic area a few mi. W of Bingham	Field	PureLink	Unpublished
Nyctaginaceae	*Tripterocalyx crux-maltae* (Kellogg) Standl.	Michael J. Moore et al. 3267 (OC)	Nevada, USA: Douglas, approx. 0.6 mi. S along two-track road that runs S from Mel Drive	Field	Bio-Rad	Unpublished
Orchidaceae	*Dactylorhiza alpestris* (Pugsley) Aver.	Hannah E. Marx 2014-019 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Orobanchaceae	*Bartsia alpina* L.	Hannah E. Marx 2014-020 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Petiveriaceae	*Monococcus echinophorus* F. Muell.	I. R. Telford & G. Butler 9043 (CANB)	Queensland, Australia: Moreton, Mount French, 6 km SW of Boonah. [Cultivated at Booderee National Park, Jervis Bay Territory.]	Cultivated	PureLink	Unpublished
Petiveriaceae	*Rivina humilis* L.	Michael J. Moore 1651 (OC)	Cultivated	Cultivated	Bio-Rad	SRX718277 (Yang et al., 2015)
Petiveriaceae	*Seguieria aculeata* Jacq.	Kew Living Collection #1991-169	Cultivated at Royal Botanic Gardens, Kew	Cultivated	PureLink	SRX718486 (Yang et al., 2015)
Petiveriaceae	*Trichostigma octandrum* (L.) H. Walter	Michael J. Moore et al. 3358 (OC)	Florida, USA: Miami-Dade, The Kampong: S fenceline immediately adjacent to Biscayne Bay	Cultivated	PureLink	Unpublished
Phytolaccaceae	*Anisomeria littoralis* (Poepp. & Endl.) Moq.	Chileflora s.n.	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Phytolaccaceae	*Ercilla volubilis* (Bertero) Moq.	Michael J. Moore 1649 (OC)	Oberlin greenhouse	Cultivated	Bio-Rad	SRX998846 (Brockington et al., 2015)
Phytolaccaceae	*Phytolacca dioica* L.	Kew Living Collection #1963-34101	Cultivated at Royal Botanic Gardens, Kew, United Kingdom	Cultivated	PureLink	SRX998853 (Brockington et al., 2015)
Plumbaginaceae	*Acantholimon lycopodioides* (Girard) Boiss.	Euroseeds s.n.; Cultivated at Cambridge University Botanic Garden 20150471	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Plumbaginaceae	*Aegialitis annulata* R. Br.	David Warmington s.n.	Queensland, Australia: near Cairns. Cultivated at Cambridge University Botanic Garden.	Cultivated	PureLink	Unpublished
Plumbaginaceae	*Limonium californicum* (Boiss.) A. Heller	Michael J. Moore et al. 3210 (OC)	California, USA: San Luis Obispo, Morro Bay State Park/Morro Estuary Natural Preserve: just E of State Park Rd./Main St., approx. 0.7 mi. SW of jct. w/ S Bay Blvd.	Field	PureLink	Unpublished
Plumbaginaceae	*Plumbago auriculata* Lam.	Michael J. Moore et al. 3360 (OC)	Florida, USA: Miami-Dade, The Kampong: betw. tennis court and main house	Cultivated	PureLink	Unpublished
Poaceae	*Dactylis glomerata* L.	Hannah E. Marx 2014-011 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Poaceae	*Deschampsia cespitosa* (L.) P. Beauv.	Hannah E. Marx 2014-012 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished
Polemoniaceae	*Polemonium caeruleum* L.	Hannah E. Marx 2014-004 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished
Polygonaceae	*Antigonon leptopus* Hook. & Arn.	Michael J. Moore 1811 (OC)	Texas, USA: Travis, in overgrown lot on N side of house at 3104 Tom Green St., Austin	Field	Bio-Rad	SRX998859 (Brockington et al., 2015)
Polygonaceae	*Bistorta bistortoides* (Pursh) Small	Michael J. Moore et al. 3333 (OC)	Colorado, USA: Larimer, approx. 0.25 mi. E of Forest Rd. 69, S of Red Feather Lakes	Field	PureLink	Unpublished
Polygonaceae	*Chorizanthe angustifolia* Nutt.	Michael J. Moore et al. 3201 (OC)	California, USA: San Luis Obispo, Montana de Oro State Park: along path from parking lot at end of Sand Spit Rd. to beach, approx. 100 yards from parking lot	Field	PureLink	Unpublished
Polygonaceae	*Coccoloba pubescens* L.	Michael J. Moore & J. Lee 2942 (OC)	Missouri Botanical Garden, cultivated in Climatron	Cultivated	PureLink	Unpublished
Polygonaceae	*Coccoloba uvifera* (L.) L.	Michael J. Moore 2665 (OC)	Oberlin greenhouse	Cultivated	PureLink	Unpublished
Polygonaceae	*Dedeckera eurekensis* Reveal & J. T. Howell	Michael J. Moore et al. 3138 (OC)	California, USA: Los Angeles. Cultivated at Rancho Santa Ana Botanic Garden.	Cultivated	PureLink	Unpublished
Polygonaceae	*Emex spinosa* (L.) Campd.	Millennium Seed Bank accession 0210373	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Polygonaceae	*Eriogonum arcuatum* Greene var. *arcuatum*	Michael J. Moore et al. 2215 (OC)	New Mexico, USA: Socorro, just E of Quebradas Backcountry Byway	Field	PureLink	Unpublished
Polygonaceae	*Eriogonum callistum* Reveal	Michael J. Moore et al. 3179 (OC)	California, USA: Kern, Tejon Ranch: Tehachapi Mtns., on calcareous outcrop near crest	Field	PureLink	Unpublished
Polygonaceae	*Eriogonum deflexum* Torr.	Lucas C. Majure and Michael J. Moore 5367 (DES)	Arizona, USA: Maricopa, Desert Botanical Garden	Field	PureLink	Unpublished
Polygonaceae	*Eriogonum inflatum* Torr. subsp. *inflatum*	Michael J. Moore et al. 3227 (OC)	California, USA: Kern, along Short Canyon Rd., approx. 2.6 mi. W of Bradys	Field	PureLink	Unpublished
Polygonaceae	*Eriogonum longifolium* Nutt. var. *longifolium*	Michael J. Moore et al. 2918 (OC)	New Mexico, USA: Eddy, Yeso Hills, on long WNW/ENE trending ridge of gypsum, approx 1.0 mi. along gravel rd. leading E from US 62/180	Field	PureLink	Unpublished
Polygonaceae	*Eriogonum rotundifolium* Benth.	Michael J. Moore 1769 (OC)	New Mexico, USA: Otero, next to parking lot at Holiday Inn Express, near US 54/70 jct. in southern end of Alamogordo	Field	PureLink	Unpublished
Polygonaceae	*Muehlenbeckia platyclada* (F. J. Müll.) Meisn.	Michael J. Moore 1170 (OC)	Cultivated at Oberlin greenhouse	Cultivated	Bio-Rad	Unpublished
Polygonaceae	*Oxytheca perfoliata* Torr. & A. Gray	Millennium Seed Bank accession 0266864	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Polygonaceae	*Persicaria virginiana* (L.) Gaertn.	Michael J. Moore 1162 (OC)	Ohio, USA: Erie, along the bank of the creek behind my house	Field	Bio-Rad	Unpublished
Polygonaceae	*Podopterus cordifolius* Rose & Standl.	Michael J. Moore et al. 2714 (OC)	Jalisco, Mexico: La Huerta, behind farmhouse approx. 0.5 km landward from main coastal hwy.	Field	PureLink	Unpublished

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Polygonaceae	*Podopterus mexicanus* Humb. & Bonpl.	Michael J. Moore et al. 2721 (OC)	Jalisco, Mexico: La Huerta, Estacion Biologica Chamela, along Arroyo Colorado near the end of the Eje Central	Field	PureLink	Unpublished
Polygonaceae	*Polygonum* sp.	Michael J. Moore et al. 3219 (OC)	California, USA: San Luis Obispo, Morro Strand State Beach: just W of Hwy. 1 in Cayucos	Field	PureLink	Unpublished
Polygonaceae	*Polygonum* sp.	Michael J. Moore et al. 3263 (OC)	California, USA: alpine, along Round Top Lake Trail	Field	PureLink	Unpublished
Polygonaceae	*Polygonum aviculare* L.	Michael J. Moore 2667 (OC)	Ohio, USA: Lorain, adjacent to Woodland St. parking lot on campus of Oberlin College	Field	Bio-Rad	Unpublished
Polygonaceae	*Polygonum dentoceras* T. M. Schust. & Reveal	Michael J. Moore et al. 3353 (OC)	Florida, USA: Highlands, just S of the Denny's parking lot on the E side of US 27, approx. 0.6 mi. N of jct. w/ Schumacher Rd./Sebring Pkwy.	Field	PureLink	Unpublished
Polygonaceae	*Pterostegia drymarioides* Fisch. & C. A. Mey.	Millennium Seed Bank accession 0496863	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Polygonaceae	*Rumex alpinus* L.	Hannah E. Marx 2014-013 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Polygonaceae	*Rumex arifolius* Aiton	Hannah E. Marx 2014-015 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Polygonaceae	*Ruprechtia coriacea* (H. Karst.) S. F. Blake	Michael J. Moore et al. 3364 (OC)	Florida, USA: Miami-Dade, The Kampong: N side of Bay Breeze Ave. near main entrance to property. Area B1. Original collecting locality: Venezuela, Sangre de Toro, Biscochito	Cultivated	PureLink	Unpublished
Polygonaceae	*Ruprechtia salicifolia* (Cham. & Schltdl.) C. A. Mey.	Sunshine Seeds s.n.	Cultivated at Cambridge University Botanic Garden	Cultivated	PureLink	Unpublished
Polygonaceae	*Sidotheca caryophylloides* (Parry) Reveal	Michael J. Moore et al. 3164 (OC)	California, USA: San Bernardino, just S of CA 18 approx. 80 yards E of jct. w/ Rim of the World Dr.	Field	PureLink	Unpublished
Polygonaceae	*Stenogonum salsuginosum* Nutt.	Michael J. Moore et al. 3060 (OC)	New Mexico, USA: San Juan, along Rd. 6893 approx. 2.4 mi. N of jct. w/ US 64	Field	PureLink	Unpublished
Polygonaceae	*Triplaris weigeltiana* (Rchb.) Kuntze	Michael J. Moore & J. Lee 2941 (OC)	Missouri Botanical Garden, cultivated in Climatron	Cultivated	PureLink	Unpublished
Primulaceae	*Primula auriculata* Lam.	Hannah E. Marx 2014-006 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished
Primulaceae	*Primula farinosa* L.	Hannah E. Marx 2014-001 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished

Appendix 1. Continued.

Family	Taxon name	Collection or accession no. (Herbarium)[a,b]	Collection locality	Field or cultivated?	RNA extraction	SRA accession no. (Publication)
Primulaceae	*Primula grandis* Trautv.	Hannah E. Marx 2014-003 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished
Ranunculaceae	*Caltha fistulosa* Schipcz.	Hannah E. Marx 2014-009 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished
Ranunculaceae	*Caltha palustris* L.	Hannah E. Marx 2014-002 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Ranunculaceae	*Ranunculus aconitifolius* L.	Hannah E. Marx 2014-022 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Ranunculaceae	*Ranunculus acris* L.	Hannah E. Marx 2014-017 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Ranunculaceae	*Ranunculus caucasicus* M. Bieb.	Hannah E. Marx 2014-005 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished
Ranunculaceae	*Trollius europaeus* L.	Hannah E. Marx 2014-018 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Rosaceae	*Alchemilla mollis* (Buser) Rothm.	Hannah E. Marx 2014-007 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: invaded plot	Field	PureLink	Unpublished
Rosaceae	*Alchemilla xanthochlora* Rothm.	Hannah E. Marx 2014-008 (UI)	Provence-Alpes-Côte d'Azur, France: Hautes-Alpes, Villar-d'Arêne: Jardin Botanique Alpin du Lautaret: native plot	Field	PureLink	Unpublished
Sarcobataceae	*Sarcobatus vermiculatus* (Hook.) Torr.	Michael J. Moore 1773 (OC)	New Mexico, USA: Torrance, along US 60 ca 5 mi. E of jct. w/ NM 42, near S end of Laguna del Perro	Field	Bio-Rad	Unpublished
Scrophulariaceae	*Leucophyllum frutescens* (Berl.) I. M. Johnst.	Michael J. Moore 1810 (OC)	Texas, USA: Travis, on the campus of the University of Texas, behind the Littlefield House	Cultivated	Bio-Rad	Unpublished
Stegnospermataceae	*Stegnosperma halimifolium* Benth.	Desert Botanical Garden accession 1973-0120-01-1 W	Cultivated at Desert Botanical Garden	Cultivated	Bio-Rad	Unpublished
Talinaceae	*Talinum paniculatum* (Jacq.) Gaertn.	Michael J. Moore 1789 (OC)	Cultivated; purchased from Oasis Nursery in Stillwater, OK	Cultivated	Bio-Rad	Unpublished

[a] Herbaria codes are from Index Herbariorum (http://sweetgum.nybg.org/science/ih/).

[b] For samples without herbaria listed, we have provided the accession number from the living collection (e.g., Cambridge University Botanic Garden, Sukkulenten-Sammlung Zürich), seed bank (e.g., Millennium Seed Bank), or seed supplier.

Appendix 2. Two alternative setups for field collection with liquid nitrogen.

I. Setup 1 (prepared by Michael Moore and Ya Yang): Driving to field sites and collecting within a short hike.

The field collection setup uses the trunk of the field vehicle as storage and as a wind-blocking, sample processing workbench (Fig. A2-1A).

Fig. A2-1. (A) Field collection setup, with the trunk of the field vehicle doubling as a wind-blocking, sample processing workbench. (B) Placing sample bottles directly into the liquid nitrogen tank for the duration of the trip.

A. Field supplies:

Field supplies do not need to be RNase-free, given that the tissue sample itself contains RNase. RNase will be deactivated at the first step of RNA extraction.

1. Plant press, straps, cardboard, blotting paper, and newspaper; (optional) field press
2. Coin envelopes for seeds
3. 2 × 3-in, 2-mm thick, clear reclosable bags, one per sample
4. GPS unit and maps
5. Black Sharpies (blue rub off more easily) (Sanford L.P., Downers Grove, Illinois, USA), pens
6. Field notebook
7. Silica gel in bulk
8. Coffee filters to place leaf samples in, to be secured using a large paper clip and dried in silica gel. Alternatively, tea bags can be used, with a small stapler to close tea bags.
9. Field guide and keys
10. Hand lens
11. Voucher shipping supplies: shipping tape, strings for tying up specimen into 2-in bundles with a cardboard on both ends, and cardboard shipping boxes
12. Tools: clippers, Hori-Hori, hammer, scissors
13. Liquid nitrogen tank (shown in Fig. A2-1A; MVE Doble 47, Princeton Cryo, Pipersville, Pennsylvania, USA)
14. Cryogenic gloves, at least mid-arm length
15. Single-edge razor blades
16. Long metal tongs (e.g., VWR 82027-366; VWR, Radnor, Pennsylvania, USA)
17. 8-mL Nalgene Boston Round Bottles, high-density polyethylene, narrow mouth (VWR 16056-988), two bottles per sample

Notes:

***Choice of liquid nitrogen containers*—**There are many options for appropriate liquid nitrogen containers to bring in the field, including nitrogen Dewars of varying sizes and dry shippers that possess an absorbent material that leaves a dry interior. There are pros and cons to both styles of containers: Dewars often contain larger interiors but care must be taken with the presence of liquid nitrogen, including proper personal protective equipment such as cold gloves and eye protection. Dry shippers often have very small interiors and are not appropriate for large numbers of samples. We recommend the MVE Doble series containers, which are combination Dewars/dry shippers that are designed for medium-term sample storage (up to two months) as well as shipment. The Doble series containers can be filled to the top, and the exterior of the tank will absorb some of the nitrogen but the interior will maintain liquid. We used the Doble 47 container, which has an interior capacity of 47 L. Filled to the top, the tank has stayed reliably cold for over four weeks on multiple trips throughout southwestern North America during the summer months, despite repeated jostling on rough unimproved roads. However, these tanks do occupy space, which must be considered when planning a trip.

***Methods of freezing plant tissue in the field*—**We have attempted multiple methods of freezing plant tissue in nitrogen in the field, ranging from placing tissue directly into nitrogen-filled containers to placing tissue into bottles and then placing the bottles into nitrogen. Likewise, we have also experimented with leaving tissue-filled bottles in nitrogen for the remaining duration of a field expedition vs. freezing them in nitrogen and then removing them and placing them in dry ice containers for the remaining duration of a field expedition. The former strategy ensures that samples stay appropriately cold with minimal risk of thawing during

Appendix 2. Continued.

travel, but not all bottles/containers can withstand being at the temperature of liquid nitrogen for several weeks. The latter strategy obviates this problem, but comes at the cost of having to obtain dry ice at regular intervals, often every day of the trip, due to the relatively rapid sublimation of dry ice even within a cooler. Because of this, we recommend the former strategy of placing tissue first into bottles and then placing the bottles into liquid nitrogen and leaving them there until returning to the laboratory.

We recommend placing samples in small, thick-walled, high-density polyethylene bottles of 30 mL size or less depending on tissue size; Nalgene manufactures a wide range of such bottles. In practice, 8-mL bottles have been most useful to us given the number of tissues collected; we have successfully accumulated nearly 500 8-mL bottles within a Doble 47 by the end of a four-week expedition. It is important to note that the caps will come unscrewed for a small proportion of bottles if placed in nitrogen for an extended period; however, we were able to minimize the loss to <1% of bottles if the caps are screwed on as tightly as possible before being placed in nitrogen. For important samples, we take the precaution of freezing at least two bottles of tissue to ensure that at least one will survive its time in the tank. In earlier iterations of this sampling protocol, we drilled a small hole into the caps of the bottles to allow nitrogen to contact the tissue immediately, but this resulted in no improvement in transcriptome quality and allowed small fragments of tissue to escape the bottle. Finally, it is important to write the sample number on a sheet of paper that is small enough to be easily placed and retrieved (e.g., 1 × 1 cm) within the bottle; writing on the outside of a plastic bottle cannot be counted on to survive several weeks in nitrogen.

Tissue sampling itself should proceed quickly, although there is leeway in how much time can elapse between removing a living plant from the soil in the field and freezing the tissue, depending on the goals of sampling. For our project, where transcript expression levels themselves were not a primary consideration, we generally place samples in nitrogen within 60 min of removing the plant from the soil or clipping a branch from a large individual, although even longer times have yielded successful, high-quality RNA isolations. If longer than 30 min is unavoidable, as might be the case if hiking several kilometers away from the field vehicle to a collecting site, it is important to keep the plant in a bag to keep it moist but not let the bag heat up too much by leaving it in the sun. Prior to placing tissue in sample bottles, it is important to break up tissues into pieces small enough that they can be easily retrieved for RNA isolation, especially for succulent or aquatic tissue as they will turn into a block of ice.

B. Field procedure:

1. Remove plant material sufficient for RNA, DNA, and voucher material and take it back to the vehicle for processing. Choose at least one plant with many flower buds and young leaves, and the rest with mature flowers and fruits for voucher specimens.
2. Label two Nalgene bottles for each sample. Write collection numbers on the bottle in two places each with a Sharpie so that if one number is rubbed off the other one remains. Put young leaves and flower buds from one single plant in both bottles. Choose young and vigorously growing tissue and avoid mature tissue if possible. Also avoid fruits and open flowers to avoid additional alleles once pollinated. For succulent tissue or large flower buds, cut the tissue into small pieces using a razor blade into paper punch size. Switch blades in between individuals.
3. Write the collection number on a small piece of paper and place it in the bottle after placing tissue in the bottle. This helps ensure that it is easy to remove the paper to check the sample ID without removing plant material. Cut the paper instead of tearing it so that it has smooth edges that will not entangle sample tissue fragments.
4. Close the lid of the bottle as tight as possible and place it into the liquid nitrogen tank for the duration of the trip (Fig. A2-1B). Bottles will float in the tank and will bounce against each other on rough roads, which may cause the numbers written with a Sharpie to rub off but the collection number on the piece of paper inside will be the backup. Although the nitrogen never comes in contact with the tissue directly, the tissue becomes frozen very quickly.
5. To prepare the silica-dried tissue for DNA extraction, cut a piece of coffee filter in half. Put 1–2 g of healthy leaf material from the same plant as the frozen tissue into the coffee filter, fold it, and secure it with large paper clip so that the material will not come in contact with silica gel directly. This will make replacing and reusing silica gel much easier. Write the collection number on the outside of the coffee filter. Place the coffee filter pack into a small, resealable bag (e.g., Ziploc bag [SC Johnson, Racine, Wisconsin, USA]), write the collection number on the bag, and fill the bag with silica gel.
6. Press 3–5 voucher specimens for each collection. Record collection date, location, habitat, plant habit, color, and other specimen information. See Gostel et al. (2016) for additional information on vouchers.
7. Check silica gel bags and vouchers each evening. Change newspaper and silica gel if they are saturated with water.
8. Once back in the laboratory, with cryogenic gloves on, use a pair of long metal tongs to retrieve bottles from the liquid nitrogen and place them into labeled freezer boxes for storage (see sample curation protocol) or shipping. Do this in the same room as the −80°C freezer, so that the bottles go directly into the −80°C freezer as soon as possible. Use styrofoam coolers with dry ice to place bottles in after retrieving them from nitrogen, to aid in sorting the samples without allowing them to thaw, prior to placing them in the freezer. Write box numbers on the cardboard storage box before placing them on dry ice to pre-cool.

II. Setup 2 (prepared by Hannah Marx): Collecting based on a field station or a local research laboratory by flying to the field site.

A. Field supplies:

In addition to the supplies listed for Setup 1, also bring:

1. A field dryer as described in Blanco et al. (2006)
2. Metal-lined 2-L coffee thermos for transporting liquid nitrogen in the field
3. Instead of 8-mL bottles, use six 2-mL Safe-Lock tubes (Eppendorf, Hamburg, Germany) per sample. RNA can be directly extracted from this tube. Carbide beads can be placed in the tube prior to collection (and frozen with the samples) or just prior to extraction.
4. Instead of a 47-L liquid nitrogen tank, use a 10-L cryogenic liquid nitrogen container with straps and carry bag and a normal holding time of 88 days (SKU YDS-10; Hardware Factory Store, Los Angeles, California, USA).

Notes:

All field supplies except the 10-L Dewar and liquid nitrogen can fit into one duffel bag (15 × 15 × 30 in) and checked for air travel (Fig. A2-2). The liquid nitrogen container was shipped empty to a field station near the collecting site. Liquid nitrogen was ordered from Airgas Inc. (Radnor Township, Pennsylvania, USA) and delivered to fill the Dewar at the field site. Refer to Federal Aviation Administration (FAA) regulation 49 CFR 175.10(a)(23) for specifics on taking liquid nitrogen or dry ice on an airplane.

Appendix 2. Continued.

Fig. A2-2. Field supplies laid out before packing for air travel. All field supplies except the 10-L Dewar and liquid nitrogen can fit into one duffel bag and checked for air travel.

B. Field procedure:

1. For field sampling, fill the 2-L thermos three-quarters full with liquid nitrogen and bring this into the field with the cap screwed on halfway. **Do not seal 2-L thermos lid completely!** The liquid nitrogen needs to vent to prevent pressure buildup. Use winter gloves to hold it while hiking (Fig. A2-3).

Fig. A2-3. Field collection setup with a 2-L thermos.

Appendix 2. Continued.

2. For each sample, place about 0.1 g (roughly equal to two hole punches) of tissue directly into a 2-mL Safe-Lock tube, label with the collection number using the black Sharpie, and drop the tube into the 2-L thermos. Because the tubes are in the thermos for less than a day, there is not a problem with labels rubbing off as long as black Sharpies are used.
3. Collect six replicates for each individual and place in individual 2-mL Safe-Lock tubes. After finishing, **do not screw the thermos lids completely**. Collect silica-preserved samples and vouchers as detailed in Gostel et al. (2016).
4. At the end of the day, transfer and organize sample tubes into freezer boxes. Store freezer boxes temporarily on dry ice if still in the field or in a −80°C freezer if near a laboratory.
5. At the end of the field trip, ship three replicates for each individual back on dry ice for extraction, and save the remaining three as backup, usually stored in a laboratory at a research station near the location where they were sampled. Ship Eppendorf tubes in cardboard freezer boxes to prevent the dry ice from breaking the tubes.

Appendix 3. RNA extraction using the PureLink reagent (ca. 4–6 h). Prepared by Ya Yang and Michael Moore.

A. Planning/Overview:

It is recommended to process six samples per day and two tubes per sample. For each sample, try different tissue types (flower buds vs. leaf) or different amounts of tissue (more vs. less if only vegetative tissue is available) for the two tubes. Twelve tubes at a time is optimum with a 24-place standard room temperature centrifuge. RNA extraction involves significant handling time and little wait, and there is little benefit to extracting more at a time. Because the entire procedure is carried out in a fume hood, make sure that it will be available for the entire day. Typical workflow consists of:

- Day 1: RNA extraction of six samples in 12 tubes.
- Day 2: RNA extraction for another six samples in 12 tubes. Proceed to DNase digestion and Bioanalyzer for all 12 samples. Normally at least one of the two tubes per sample will be successful and Bioanalyzer takes 12 mRNA samples per run.
- Days 3 and 4 (or once having 12 samples passing quality control): Library preparation. Currently we multiplex 10–11 libraries per lane on the Illumina HiSeq 2500 V4 platform (San Diego, California, USA).

B. Tools and equipment:

1. Access to a fume hood during the entire duration of extraction.
2. Tweezers with insulated handle and smooth tips (for easy cleaning).
3. Tissue homogenizer. We currently use the FastPrep-24 benchtop homogenizer with CoolPrep 24 × 2-mL adapter (MP Biomedicals, Santa Ana, California, USA).
4. Tube rack for holding lysing matrix tubes in liquid nitrogen. We recommend CoolRack Thermoconductive Tube Racks (BioCision, San Rafael, California, USA) to prepare frozen tissue before homogenization. Certain plastic racks work as well, but some will crack. If using a plastic rack, drill a hole at the bottom of each well to allow liquid nitrogen to go through. You may also need to cut a plastic rack short so that it fits into a styrofoam shipping container.
5. Two styrofoam shipping boxes with lids. The first box is to hold the tube rack in liquid nitrogen (a shallow one is preferred for easy maneuverability); the second box is to hold dry ice.
6. Waste beaker
7. Liquid nitrogen
8. Benchtop liquid nitrogen container (e.g., Nalgene Dewar Flasks, high-density polyethylene; Thermo Fisher Scientific, Waltham, Massachusetts, USA)
9. A room temperature centrifuge
10. A refrigerated centrifuge
11. A set of designated pipettes for RNA work: P1000, P200, and P20
12. Vortexer
13. To pick up tubes sitting on the rack in liquid nitrogen, use a winter glove underneath a nitrile glove on one hand (Jordon-Thaden et al., 2015; latex will crack in liquid nitrogen, while cryogenic gloves are too bulky to handle small bottles) and a nitrile glove only on the other hand for holding tweezers with insulated handle.

C. Reagents:

1. Squirt bottle with 70% ethanol
2. Ambion PureLink Plant RNA Reagent (Thermo Fisher Scientific). Store at 4°C.
3. RNase-free water (store at 4°C and aliquot in 50-mL tubes on bench)
4. 75% ethanol. Store at 4°C. Make 48 mL at a time with 36-mL 200 proof ethanol and 12-mL RNase-free water in a 50-mL centrifuge tube. It is good for 48 extractions.
5. 5 M NaCl solution in 50-mL tubes on bench. Dissolve 2.922 g NaCl powder in RNase-free water for each 10 mL of final volume.
6. Chloroform in non-inflammable cabinet. It is light sensitive and dissolves plastic, so only aliquot at use.
7. Isopropyl alcohol stored in a fireproof cabinet. Aliquot in 50-mL tubes on bench.
8. 3 M KOAc, pH 5.2 (optional, for mucilaginous tissue)

D. Consumables:

1. Kimwipe (Kimberly-Clark Professional, Roswell, Georgia, USA)
2. Paper towel
3. RNase-free 1.5-mL, 5-mL, and 50-mL tubes
4. RNase-free barrier tips (1000 μL, 200 μL, and 20 μL)
5. Lysing Matrix A in 2-mL tube (MP Biomedicals)
6. Ambion RNase*Zap* RNase Decontamination Solution (Thermo Fisher Scientific)
7. Disposable nitrile and latex gloves

E. General considerations for working with RNA:

1. RNA is a less stable molecule than DNA and is prone to degradation. RNases are also abundant within plant tissue and readily degrade RNA. All reagents, containers, and tips used for RNA-related work should be RNase-free. Clean the work surface and pipettes with RNase*Zap* before use. However, unless one is particularly unclean, RNase contamination is not the cause of most failed RNA extractions.

Appendix 3. Continued.

2. RNases are not removed by autoclaving so avoid using glassware. Use RNase-free tubes to aliquot reagents.
3. Keep tissue frozen until PureLink is added, which deactivates RNase.
4. Regularly replace the electrophoresis buffer, ideally each time before running new samples.
5. Always wear disposable gloves and change them frequently.
6. Avoid freezing and thawing of RNA. Place RNA samples in 4°C if processing within the next day or two; store in −80°C if processing later.

F. Safety:

1. The PureLink reagent contains 2-mercaptoethanol and sodium azide. Sodium azide may react with lead and copper plumbing to form explosives. Do not pour down the drain. The reagent has a very strong odor and causes headache and dizziness when inhaled. Work in the hood and temporarily dispose of tips and tubes in the hood in a resealable bag. At the end of the day, seal the waste air tight with double layers of resealable bags and put in hazardous waste disposal.
2. Use proper protection when handling liquid nitrogen, including proper gloves, closed-toe shoes, long pants that are not tucked into shoes (to prevent trapping liquid nitrogen), and proper eye protection. Keep only a thin layer of liquid nitrogen at the bottom of the styrofoam box. Avoid tilting the styrofoam box to prevent sudden movement of the rack that may cause the liquid nitrogen to splash.

G. Sample preparation:

1. Fill the benchtop liquid nitrogen container with approximately 3 L of liquid nitrogen. Obtain enough crushed dry ice to fill the styrofoam box one third full. Place the CoolRack (or other rack you choose to use) in the shallow styrofoam box and pour approximately 1 L of liquid nitrogen into the styrofoam box. Let the rack chill for a few minutes. Pre-cool the refrigerated centrifuge to 4°C and check to make sure that it has the microtube adapter instead of the plate adapter in it.
2. Wipe down the workspace with 70% alcohol followed by RNase*Zap*.
3. Write numbers 1–12 on 12 lysing matrices. Tap down the beads. Slightly loosen the caps so that they are easy to unscrew in liquid nitrogen. Place the lysing matrices on the rack in liquid nitrogen to allow them to chill.
4. Gather the following: tweezers with smooth tips, 70% ethanol, RNase*Zap*, Kimwipes, waste jar, pen, winter gloves and nitrile gloves, styrofoam box with dry ice, box with chilled lysing matrices in liquid nitrogen, laboratory notebook, and laptop. Take the tissue storage box out of the −80°C freezer and immediately place it on dry ice.
5. Spray the tweezers using 70% ethanol, wipe with a Kimwipe, apply RNase*Zap*, and wipe again with another Kimwipe. Twist open the bottle and put the lid on the side, check the sample number on the bottle and on the paper slip inside, and put the slip in the bottle lid on the side. Dip the tweezers in liquid nitrogen to chill. Remove <0.1 g of tissue from the bottle (approximately the size of a punch hole; can skip weighing to avoid thawing). Record tissue types in laboratory notebook.
6. Clean and prepare the tweezers by spraying with 70% ethanol and RNase*Zap* as in step 5, and dip in liquid nitrogen to chill before proceeding to the next sample. Add liquid nitrogen to the styrofoam box when it is low.
7. Tape the openings of the cryogenic adapter, leaving 12 (six on each side) open, to prevent dry ice from flying out when shaking. Transfer <0.5 g of crushed dry ice to the FastPrep adapter. Use small pieces so that it is easier to balance.
8. Grind frozen tissue in the FastPrep-24 using the "cryogenic" cycle at 4 m/s for 40 s. After finishing, immediately move lysing matrices back to the rack in liquid nitrogen to avoid thawing. Tap down the bead gently on the bench while waiting for 5 min as required by the FastPrep. Do not tap too hard because the tubes are now brittle and may crack. Add liquid nitrogen to the styrofoam box if needed. Check for leftover dry ice in the adapter. There should be a small amount of dry ice powder left. If the tissue thaws at any point before adding the extraction buffer, you will get degraded RNA.
9. Add more dry ice to the adapter and grind for another 40 s. Put lysing matrix back onto the rack in liquid nitrogen. The tissue should be in very fine powder. If not, repeat for a third round of grinding.

H. RNA extraction:

1. Move the styrofoam container containing the samples in liquid nitrogen and a vortexer to the fume hood and complete all of the following steps in the hood. Line the waste beaker with a resealable bag. The samples need to be kept frozen until the PureLink reagent is added.
2. Take the PureLink reagent out of the 4°C refrigerator and aliquot 6.3 mL. Tap the frozen tube gently on the counter before opening it so that the beads and most of the powder are at the bottom of the tube instead of stuck to the lid. Add 0.5 mL of PureLink reagent to the frozen, ground plant tissue. Tighten the lid before vortexing the tube until the sample is thoroughly resuspended with no clumps at the bottom of the tube. Put the tube in a clean rack at room temperature. Return the PureLink reagent bottle back to 4°C refrigerator.
3. (Optional) Add one-third volume KOAc (3 M, pH 5.2) to the lysate. Vortex to mix. This step is used for mucilaginous tissue.
4. Incubate the tube horizontally for 5 min at room temperature. While waiting, label 12 1.5-mL tubes with numbers 1–12. Add 0.1 mL of 5 M NaCl to each empty new tube.
5. Centrifuge the sample tubes at 12,000 × *g* for 2 min at room temperature.
6. Use 200-µL tips to transfer the supernatant to the new tubes with 5 M NaCl. Do not use 1000-µL tips because liquids within them are more difficult to control. Pipette up and down gently to mix the supernatant with NaCl after transfer.
7. Aliquot 4 mL of chloroform. Add 0.3 mL of chloroform to each sample. Move quickly so that chloroform does not drip from the pipette tip. Close the lid tight and mix thoroughly by vortex.
8. Centrifuge the sample at 12,000 × *g* for 10 min at 4°C to separate the phases.
9. While waiting, label a new set of 12 tubes with the sample ID on top and the date and tube number on the side of the tube. Add an equal volume of isopropyl alcohol equal to that of the aqueous phase (usually 350–400 µL) to each empty tube.
10. Transfer the upper, aqueous phase using 200-µL tips to the new tubes with isopropyl alcohol. Make sure not to disturb the middle layer. Mix and let stand at room temperature for 10 min. Set aside the tube containing the waste and discard later so that gloves do not get dirty.

Appendix 3. Continued.

11. While waiting, make a 1.5% agarose gel.
12. Centrifuge the sample tubes at 12,000 × *g* for 10 min at 4°C.
13. Decant the supernatant, **taking care not to lose the pellet**. Touch the lip of the tube on a paper towel to clean up (make sure use a new spot for each tube). Add 1 mL of 75% ethanol to the pellet.
14. Centrifuge at 12,000 × *g* for 2 min at room temperature. Decant the supernatant carefully, **taking care not to lose the pellet.** The pellet is even looser than in the previous step. Touch the lip of the tube on a paper towel before closing the lid.
15. Briefly centrifuge to collect the residual liquid and remove it with a 20-µL pipette. Leave the tube open to dry for 15–30 min.
16. Add 30 µL of RNase-free water to the RNA pellet. Pipette the liquid up and down over the pellet to resuspend the RNA. It is OK if the solution is still cloudy after mixing. It will be cleaned up at the DNase step.
17. Visualize 3 µL of RNA on the 1.5% agarose gel. It is OK to use a DNA ladder. Purified RNA can be kept at 4°C for a day or two, or at −80°C for long-term storage. Alternatively, proceed immediately to the DNase step.
18. Pour waste into waste container. Wash room temperature racks with tap water. Pour waste liquid into the extraction waste collection bottle in the fume hood. Discard tips and tubes in the sealed bag to the hazardous waste bucket. Allow leftover dry ice and liquid nitrogen to evaporate on the laboratory bench and wash the containers and rack sitting in liquid nitrogen the next day.

Appendix 4. RNA extraction for mucilage tissue using hot acid phenol-LiCl-RNeasy Mini Kit (ca. 2 days). Notes and modifications from Protocol 12 in appendix S1, Johnson et al. (2012). Prepared by Alfonso Timoneda and Tao Feng.

A. Equipment:

Only equipment that is not required by the default PureLink protocol (Thermo Fisher Scientific, Waltham, Massachusetts, USA) is listed.

1. 15-mL RNase-free Falcon tubes (instead of snap cap tube as Johnson et al. [2012])
2. Adapter for 15-mL Falcon tubes in refrigerated centrifuge
3. Water bath or dry heating block that holds 15-mL tubes
4. Mortar and pestle. Rinse mortar and pestle with water immediately after use and then autoclave at 120°C for 2 h wrapped in aluminum foil. Autoclaving will not destroy all RNases, but it is OK to have some RNase before the extraction buffer is added because plant tissue contains RNases itself.

B. Reagents:

1. Saturated acid phenol (pH 4.3)
2. Chloroform : isopropyl alcohol (24 : 1), RNase free
3. Isopropyl alcohol
4. 4 M LiCl solution
5. 70% ethanol made with RNase-free H_2O, store at 4°C
6. Prepare RNA extraction buffer as follows. We did not filter purify them.

Final concentration:

100 mM Tris (pH 9.0)

1% sodium dodecyl sulfate (SDS)

100 mM LiCl

10 mM ethylenediaminetetraacetic acid (EDTA)

For 100 mL:

10 mL 1 M Tris (pH 9.0)

10 mL 10% SDS

2.5 mL 4 M LiCl

2.0 mL 0.5M EDTA (pH 8.0)

Bring the volume up to 100 mL using RNase-free water and keep at 4°C

C. Safety:

1. Avoid inhaling or skin contact with phenol or chloroform : isoamyl alcohol. Handle solution with these chemicals in a fume hood and minimize the time tubes are outside the fume hood. Refer to the Material Safety Data Sheet of both chemicals for details. Use protective goggles during the whole process and change gloves immediately after any chemical spillage. Phenol and RNA extraction buffer liquid waste should be stored in a separate waste bottle in the fume hood and disposed of separately.
2. HCl produces toxic vapor that can damage mucous membranes. Work in a fume hood and do not inhale while adjusting the pH of the Tris solution. Some institutions separate chlorinated and non-chlorinated chemicals for disposal. In this case, the saturated acid phenol and all wastes from step 1 to 11 should be disposed of with chlorinated waste.

D. Modification to Protocol 12 in appendix S1, Johnson et al. (2012):

1. Starting material: instead of 1 g, use 0.2 to 0.4 g or even less for Cactaceae.
2. The spatulas were cleaned between samples using ethanol and chilled before touching the powder, otherwise the tissue powder will melt in contact with the metal and stick to it.
3. For some samples, especially Cactaceae, the pellet is very small and looks clean, and would be lost with the LiCl precipitation. In these cases, skip steps 17–18.
4. For step 24, elute RNA twice from the column using 65°C RNase-free water instead of 95°C.

Appendix 5. DNase digestion (~1 h). Modified from the manufacturer's protocol and from Jordon-Thaden et al. (2015). Prepared by Ya Yang.

A. Equipment:

In addition to the equipment required for the PureLink RNA extraction protocol (Thermo Fisher Scientific, Waltham, Massachusetts, USA), you will need:

1. Dry heating block that holds 1.5-μL tubes (preferred) or an incubator
2. Invitrogen TURBO DNA-*free* Kit (Thermo Fisher Scientific), stored in −20°C freezer
3. Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano Kit (Agilent, Santa Clara, California, USA). Sequencing cores also usually provide Bioanalyzer service.

B. Procedure:

1. Take the DNase buffer out of the −20°C freezer to thaw at room temperature. Turn on the dry heater or incubator to preheat to 37°C.
2. The two tubes per sample can be combined to increase yield and diversity of genes (total of ca. 50 μL). Vortex the DNase buffer and spin it down briefly. Add 0.1 volume of 10× Turbo DNase buffer to each tube. For 50 μL of RNA add 5 μL of buffer.
3. Add 1 μL of DNase from the TURBO DNA-*free* Kit to the RNA. Watch closely to make sure the 1 μL of DNase is indeed transferred into the RNA solution. Vortex briefly to mix.
4. Incubate at 37°C for 30 min. While waiting, label new 1.5-mL storage tubes with the collection number on top and the tube number at the date of extraction on the side.
5. Add vortexed DNase Inactivation Reagent in the TURBO DNA-*free* Kit (typically 0.1 volume; 5 μL for 50 μL of starting RNA) and mix by vortexing briefly. Incubate at room temperature for 5 min, vortex occasionally.
6. Centrifuge at 10,000 × *g* for 2 min, transfer supernatant to the new, pre-labeled storage tubes, and aliquot 3 μL for Bioanalyzer. Place cleaned RNA in 4°C if the library prep will be performed in the following day or two. Otherwise store at −80°C.
7. Run the cleaned RNA on a Bioanalyzer using the Agilent RNA 6000 Nano Kit chips. Mucilaginous tissue can give distorted Bioanalyzer traces, but in most cases will yield successful RNA-seq libraries in subsequent steps. Repeat the DNase digestion a second time if a high-molecular-weight DNA band shows up. Chloroplast rRNA gives additional bands and can appear as a smear on an agarose gel but will be distinguishable on Bioanalyzer trace.

Appendix 6. Stranded mRNA library preparation (ca. 2 d for 12 libraries and 2.5 d for 20 libraries). Prepared by Ya Yang and Michael Moore.

A. Equipment and consumables:

Items required in addition to the PureLink RNA extraction (Thermo Fisher Scientific, Waltham, Massachusetts, USA) protocol:

1. A thermocycler with a dedicated PCR block to store all the programs; access to the machine should be ensured at all times throughout the duration of the protocol.
2. Minicentrifuge for quick spins of 1.5-mL tubes, 2-mL tubes, and PCR strips
3. Magnetic-ring stand (96 well). We used one from Ambion (AM10050; Thermo Fisher Scientific), but it often resulted in bead loss. We recommend Agencourt SPRIPlate 96R Ring Magnet Plate (Beckman Coulter, Brea, California, USA) and DynaMag-96 side magnet (12331D; Thermo Fisher Scientific); both have a stronger magnet.
4. Agencourt AMPure XP beads, 5 mL (A63880, Beckman Coulter). Larger volumes are available but beads only have a shelf life of one year.
5. Indexed adapters. We used the leftover adapters from the Illumina TruSeq Stranded mRNA library preparation kit (Illumina, San Diego, California, USA). Indexed adapters can also be purchased separately. See the Illumina website (http://support.illumina.com/) for adapter sequences.
6. 0.2-mL PCR strips, RNase free
7. 80% ethanol, 1.6 mL per sample, made fresh for each library prep with RNase-free water
8. 0.01 M Tris-HCl (pH 8). Dilute with RNase-free water from 1 M stock solution.
9. KAPA Stranded mRNA-Seq Kits (KAPA Biosystems, Wilmington, Massachusetts, USA). There are other mRNA library kits that may require shorter handling time. Illumina NeoPrep is not working reliably yet as of July 2016, but it looks like a promising future alternative to hand prep.

B. Notes and modifications to the manufacturer's instruction (2015 version):

1. Library preparation is carried out in PCR strips. To avoid contamination, do not use multi-channel pipettes and only open one tube at a time.
2. Briefly vortex and spin down all stock reagent tubes before opening them.
3. Use P10 or P2 to pull up the leftover ethanol (usually 1–2 µL) while air-drying the beads.
4. Because most RNA-Seq library preparation kits are optimized for differential gene expression studies that use relatively short read lengths, we modified the protocol to produce larger insertion sizes to accommodate paired-end 125-bp or 150-bp reads.
   a. Lower fragmentation temperature and/or shorten fragmentation time: 85°C for 6 min.
   b. Use 0.7× (35 µL) instead of 1× (50 µL) AMPure beads for the final cleanup step after PCR enrichment. Doing so is also more effective in removing leftover adapter.
5. Use 1–2-µL Illumina TruSeq adapter per sample.
6. Use 12 cycles for PCR enrichment.

Appendix 7. Sample curation. Prepared by Ya Yang and Stephen Smith.

This protocol is for curating tissues and RNA samples at a moderate scale (several hundred to a few thousand samples).

A. Considerations on facilities:

Freezers fail periodically. Ultra-low-temperature freezers should be equipped with a temperature monitor and an alarm system, and should be connected to a backup power generator. Ideally, transfer samples to a liquid nitrogen vapor system for long-term storage.

B. Organizing samples:

1. To fit the 8-mL bottles into standard freezer racks, we use standard storage boxes (2-in Cardboard Cryovial Storage Box only, 5 1/4 × 5 1/4 × 1 7/8 in; Dot Scientific, Burton, Michigan, USA) with 16 cell dividers (16-cell cardboard divider, cell opening 30.23 mm/1.19 in, outside dimensions 4 7/8 × 4 7/8 in; Dot Scientific). Plastic storage boxes should not be used in ultra-low-temperature freezers because they get brittle. Tape should not be used because it tends to fall off of bottles and boxes in the freezer.
2. When organizing bottles into storage boxes upon returning from collection trips, verify the collection number by reading the paper slip inside of the bottle if the number written on the outside of the bottle is rubbed off, and record the precise location of each sample in a database. Always sort samples in insulated containers with ample fresh dry ice to avoid thawing.
3. Fig. A7-1 shows how we organize sample tubes in cardboard freezer boxes for long-term storage. The collection number can be written on the box cover if needed. Identifying information for each box should be clearly indicated on both the cover and the body of the storage box. Cell location ID is recorded as A1, A2, … to D4. All information should be recorded in a database or a spreadsheet that is write-protected and properly backed up. The database schema is shown in Fig. A7-2.

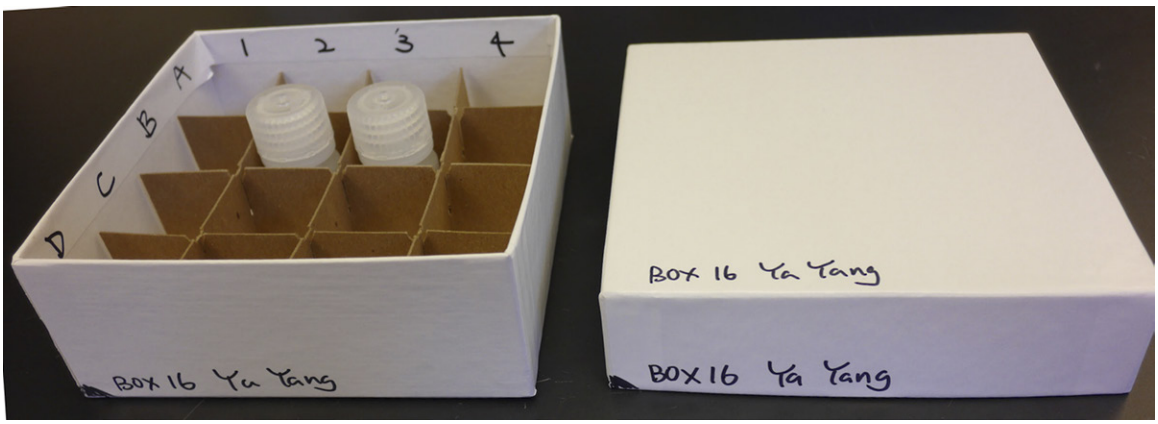

Fig. A7-1. Sample tubes organized in cardboard freezer boxes for long-term storage.

Appendix 7. Continued.

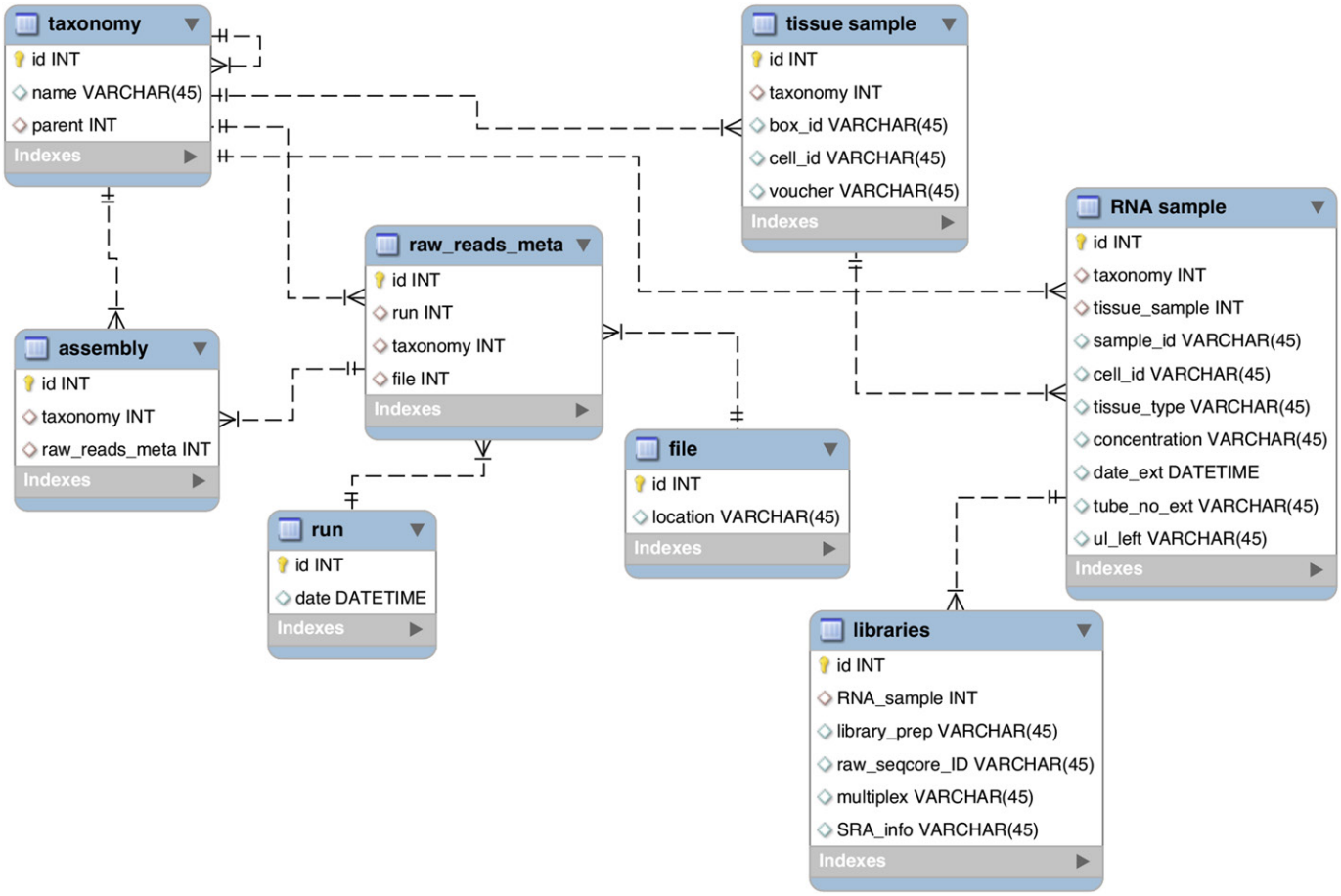

Fig. A7-2. Database schema used to organize sample, extraction, and library information, as well as metadata on sequencing reads and assembly files. These were started as spreadsheets on Google Drive, but were developed as an SQL database as the number of samples grew.