

Dec 05, 2023

Annonaceae DNA extraction protocol from silicagel dried and herbarium preserved leaves

DOI

<https://dx.doi.org/10.17504/protocols.io.5qpvorqx9v4o/v1>



Vincent R C Soulé¹, Couvreur Thomas¹, Cedric Mariac¹

¹DIADÉ, Univ Montpellier, CIRAD, IRD, Montpellier, France



Couvreur LP Thomas

Institut de Recherche pour le Développement (IRD)

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.5qpvorqx9v4o/v1>

Protocol Citation: Vincent R C Soulé, Couvreur Thomas, Cedric Mariac 2023. Annonaceae DNA extraction protocol from silicagel dried and herbarium preserved leaves. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.5qpvorqx9v4o/v1>

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited



Protocol status: Working

We use this protocol and it's working

Created: January 18, 2023

Last Modified: December 05, 2023

Protocol Integer ID: 75464

Keywords: DNA extraction, NGS extraction, MATAB, CTAB, aDNA, Illumina sequencing, Angiosperm, magnoliales, herbarium, annonaceae dna extraction protocol from silicagel, annonaceae dna extraction protocol, dna extraction of sample, dna extraction, tropical plant family annonaceae for leaf, tropical plant family annonaceae, using silicagel, silicagel, extraction, dna, preserved leaf, herbarium sheet, herbarium, plant, quality of sample, sample

Funders Acknowledgements:

ERC Consolidator

Grant ID: 865787

Disclaimer

This project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation program (grant agreement No. 865787)

Abstract

This protocol is used for DNA extraction of samples from the tropical plant family Annonaceae for leaves dried using silicagel or sampled from herbarium sheets. This protocol is made for generation of small to long fragments (depending quality of sample) to prepare NGS libraries.

This protocol is designed to extract DNA in batches of 48 samples, but this can also be undertaken in 2 times 48 (96) samples.

Image Attribution

Art work by Vincent Soulé

Guidelines

Work in a clean environment to avoid contamination, use as much as possible filter tips, wear gloves and lab coat.

Wash work space and pipettes before and after use, with DNAaway and DNase away.

Manipulate with extreme caution rare or old samples.



Materials

2mL Screw tube

2mL secure lock ependorf tube

REF MATAB

Protocol materials

☒ RNase A Solution, 4mg/ml **Promega Catalog #A7973**

☒ 70% alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #793213**

☒ 1M TE buffer (1M Tris-HCl, 0.1M EDTA, pH 8.0)

☒ Agarose **Catalog #A5304**

☒ Gel Red Nucleic Acid Gel Stain **Biotium Catalog ##41003**

☒ Na acetate 3M pH 4.8 or 5.2

☒ Isopropanol

☒ Proteinase K **Life Technologies Catalog #17916**

☒ DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D0632**

Safety warnings

! Always work under an extractor hood when manipulating of Chloroform, DTT or Isopropanol

Leaf grinding

- 1 Prepare 48 2 mL Screw-Top tubes in rows of 8 in a 96 well rack. Add one 1/4" ceramic beads (MP Biomedical REF 116540422).
- 1.1 Add leaf sample inside the tube using clean tweezers. The leaf samples can be between 1×1 cm and 3×3 cm in size. Closes the tubes.
- 1.2 Grind samples using a MP FastPrep grinder, twice for  00:00:40 at 4m/second speed with a 2-minute pause in between each grind as not to over heat the samples.

4m

Equipment

FastPrep-24™ 5G

NAME

grinder

TYPE

MP Biomedicals™

BRAND

116005500

SKU

<https://www.fishersci.fr/shop/products/mp-biomedicals-fastprep-24-5g-instrument/15260488>

LINK

24 samples

SPECIFICATIONS



Lysis buffer preparation and lysis

- 2 Lysis buffer **LB** needs to be freshly made the day of the extraction using a previously made **LBmix** + MATAB,  Proteinase K **Life Technologies Catalog #17916** and DTT (DL-Dithiothreitol) (final concentration 1mM).



Preparation of **LBmix** ( 1000 mL) for 1000 samples:

 1000 mL miliQ water

Final

concentration

 9.31 g EDTA

[M] 25 millimolar (mM)

 15.76 g Tris-HCL

[M] 100 millimolar (mM)

 81.82 g NACl

[M] 1.4 Mass Percent

2.1 Preparation of **LB** for 48 samples:

In a 50mL tube add  2 g of MATAB + 20 ml of **LBmix** Final concentration

[M] 4 Mass / % volume

Disolve MATAB in  65 °C water bath and vortex (aprox 10 min).

Add  250 µL of  Proteinase K Life Technologies Catalog #17916 at

[M] 1 mg/mL then vortex.

Add  50 µL of  DTT Merck MilliporeSigma (Sigma-Aldrich) Catalog #D0632

then vortex.

Add **LBmix** to adjust volume to  50 mL and put solution at  65 °C

Final volume  50 mL

2.2 Using a p1000, add to each grinded sample 1 mL of still hot **LB**; close the tube then vortex each sample until the leaf powder is totally dissolved.

Place the rack at  65 °C for a minimum of  03:00:00 for silicagel preserved samples; for herbarium sampled leaves place at  65 °C for a recommended

 06:00:00 to  08:00:00 . Lysis can be done over night.

Shake the rack/tubes every 30 minutes during lysis.

2.3 If needed, the process can be stopped after lysis, and the tubes can be conserved up to

 24:00:00 at  -20 °C



17h



1d



Chloroform DNA Isolation

3 For herbarium samples, limit to one round of chloroform isolation.

12m



After the lysis step, let the samples get to room temperature.

Under an extractor hood, add  700 μL of 24:1 Chloroform : Isoamyl alcohol. Close the tube and shake vigorously by inversion the rack for  00:02:00 to  00:10:00

- 3.1 Centrifuge  4000 rpm, Room temperature, 00:10:00

Prepare in a new rack of 48 2mL tubes.

- 3.2 Under an extractor hood, transfer 8 by 8, using a multichannel pipette,  800 μL of the aqueous supernatant phase into the new 2mL tubes. Avoid pipetting the interphase pellet.

- 3.3 Add  10 μL of  RNase A Solution, 4mg/ml **Promega Catalog #A7973** at [M] 0.5 mg/mL

Place the rack with the samples at  37 $^{\circ}\text{C}$ for  00:30:00 .

- 3.4 Under an extractor hood, proceed with the second chloroform cleaning step. Add  700 μL of 24:1 Chloroform Isoamyl alcohol; close tubes and shake the rack of tubes.

- 3.5 Centrifuge  4000 rpm, Room temperature, 00:10:00

During centrifugation, prepare and name new 2ml eppendorf safe lock tubes.

- 3.6 Under an extractor hood, transfer 8 by 8 using a multichannel pipette,  600-800 μL of the aqueous supernatant phase into the new 2mL tubes. Avoid pipetting the interphase pellet.

DNA precipitation

- 4 Add  360 μL of  Isopropanol at  -20 $^{\circ}\text{C}$

Add  60 μL of  Na acetate 3M pH 4.8 or 5.2 pH=5 at  4 $^{\circ}\text{C}$

Slowly shake the tubes and then place the rack at  -20 $^{\circ}\text{C}$ for  02:00:00 to  Overnight

- 4.1 Centrifuge  14000 rpm, -20 $^{\circ}\text{C}$, 00:10:00

Slowly remove liquid phase using a pipette with P1000, be aware to not pipette DNA pellet.



Washing DNA

25m

4.2 Add  700 μL of  70% alcohol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #793213** in each tube to wash pellet.

10m

4.3 Centrifuge  14000 rpm, -20°C , 00:10:00
Remove liquid phase with P1000, be aware to not pipette DNA pellet.
Let dry for  00:15:00 at  Room temperature .

25m



DNA elution

5 Depending on DNA pellet size, add  60-100 μL of  1M TE buffer (1M Tris-HCl, 0.1M EDTA, pH 8.0) , shake tubes.
 10 rpm, Room temperature
Eluate  Overnight at  4°C

20m



5.1 Tubes can be stored for  48:00:00 at  4°C before quality check and quantification.

2d



DNA quantification

55m

6 Transfer  6 μL of each samples to a 96 well PCR plate.
Use  2 μL for DNA quantification using TECAN spark or nanoquant with nanoquant 16 holes plate; or quantify one by one using Nanodrop.





Equipment

SPARK

NAME

Microwell plate reader

TYPE

TECAN

BRAND

SPARK

SKU

<https://www.tecan.com/blog/spark-multimode-microplate-reader-for-high-performance-cell-based-fluorescence-assays>

LINK

DNA quality check on gel

55m

7 To the  4 μL left add  6 μL 1.5X red blue yellow loading buffer.

55m

Prepare 1% of  Agarose **Catalog #A5304** gel with TAE 1X.

Add your samples and:

+ promega 100pb dna ladder

+ promega 2.5kb lambda eco R1 hind3 dna ladder on gel well

Then proceed with gel electrophoresis at 135V  00:40:00 on TAE 0.5X.

Put gel  00:15:00 in 1X

 Gel Red Nucleic Acid Gel Stain **Biotium Catalog ##41003**

Place gel in imaging machine. Turn on UV light, take a picture.

DNA conservation

55m

8 Extracted DNA needs to be stored at  -20 °C .

For long term conservation, transfert to barcoded screw-top tube on 96 rack.

For the GLOBAL projet we used **Thermo Scientific™ Matrix™ 0.5mL 2d barcoded.**

Expected result



Expected result

Within the GLOBAL project, around 3600 DNA extractions were undertaken, some on silicagel leaves others on herbarium preserved leaves.

For silicagel dried samples we extracted 1050 specimens, with a max concentration of 979 ng/ul and a minimum of 0.9 ng/ul for a total elution volume of 100 ul. On average we had 213 ng/ul (Standard error: 205.8).

For herbarium preserved leaves 2600 samples were extracted: the highest concentration was 1088ng/ul and the lowest was 0,1 ng/ul for a total elution volume of 60 ul. On average we had 230 ng/ul per extraction (Standard error: 221.8).

Concentrations below 10 ng/ul were rarely used to sequenced or frequently failed.

DNA size ranged between 100pb-2.5kb, but longer fragments were also possible.

Protocol references

Part of this protol follows:

Couvreur TLP, Helmstetter AJ, Koenen EJM, Bethune K, Brandão RD, Little SA, Sauquet H, Erkens RHJ (2019) Phylogenomics of the Major Tropical Plant Family Annonaceae Using Targeted Enrichment of Nuclear Genes. *Frontiers in Plant Science* 9. <https://doi.org/10.3389/fpls.2018.01941>