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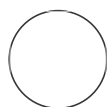
Protocol status: Working
 We use this protocol and it's working

Created: Jan 18, 2023

🌐 Annonaceae DNA extraction protocol from silicagel dried and herbarium preserved leaves

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Thomas LP Couvreur

DISCLAIMER

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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


Last Modified: Dec 05, 2023

PROTOCOL integer ID: 75464


Keywords: DNA extraction, NGS extraction, MATAB, CTAB, aDNA, Illumina sequencing, Angiosperm, magnoliales, herbarium

Funders
Acknowledgement:
ERC Consolidator
Grant ID: 865787


PROTOCOL MATERIALS

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


Step 4.2

 Gel Red Nucleic Acid Gel Stain Biotium Catalog ##41003


Step 7

 Na acetate 3M pH 4.8 or 5.2


Step 4

 Proteinase K Life Technologies Catalog #17916


In 2 steps

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


Step 3.3

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


Step 5

 Agarose Catalog #A5304

Step 7


 Isopropanol

Step 4

 DTT Merck MilliporeSigma (Sigma-Aldrich) Catalog #D0632



Step 2.1

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 1x1 cm and 3x3 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  4m minute pause in between each grind as not to over heat the samples.

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

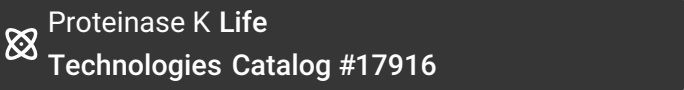
SPECIFICATIONS



file

Lysis buffer preparation and lysis

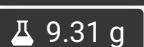
2

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

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

 milliQ water

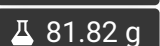
Final concentration

 EDTA



 Tris-HCL



 NaCl





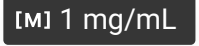
2.1 Preparation of **LB** for 48 samples:







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





Dissolve MATAB in  water bath and vortex (aprox 10 min).


Add  of  at  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 e **17h**




 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 **1d**


  24:00:00 at  -20 °C


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 **10m**

 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 **10m** 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 **30m**

Place the rack with the samples at 37°C for 00:30:00 .

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

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

2h

- 4 Add 360 μL of Isopropanol at -20°C 2h

- ! Add 60 μL of Na acetate 3M pH 4.8 or 5.2 pH=5 at 4°C

Slowly shake the tubes and then place the rack at -20°C for 02:00:00 to Overnight

- 4.1 Centrifuge 14000 rpm, -20°C , 00:10:00 10m

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 10m
each tube to wash pellet.


- 4.3 Centrifuge 14000 rpm, -20°C , 00:10:00 25m

- ! Remove liquid phase with P1000, be aware to not pipette DNA pellet.
Let dry for 00:15:00 at Room temperature .



DNA elution


5 Depending on DNA pellet size, add  60-100 μL of 20m



 1M TE buffer (1M Tris-HCl, 0.1M EDTA, pH 8.0), shake tubes.

 10 rpm, Room temperature

Elate  Overnight at  4 °C


5.1 Tubes can be stored for  48:00:00 at  4 °C before quality check and quantification. 2d

*


II

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

9

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.