Ancient Plant aDNA Extraction protocol Vienna

Category	Experimental Procedures
Author	Miguel Vallebueno
Version	3

Labels:

In Brief

Protocol for extracting ancient DNA from ancient plant remains.

Based on the protocol used in Max Plank institute in Tuebingen by Kelly Swarts and Hernan Burbano.

15 - 30 mg of **tissue** is the recommended input amount.

UV tube material in advance

Irradiate for 30 min for each sample to process:

1 x 15ml tube

5 x DNA low binding 1.5 ml tube

1 x 2ml tube

1 x 50mL tube/column combo

Prepare Lysis PTB Buffer

Add extra 0.5 reaction

Lysis PTB Buffer

Reagent	Stock (M)	Final (mM)	Volume per sample ul	6.5	10.5
SDS	20%	4%	240	1560 ul	2520 ul
Tris	1	40	48	312 ul	504 ul
EDTA	0.5	40	96	624 ul	1008 ul
NaCl	5	20	4.8	31.2 ul	50.4 ul
H20			574	3731 ul	6027 ul
Add fresh on every use:					
Proteinase K	15 mg/ml	0.4 mg/ml	32	208 gr	336 gr
PTB	284.18 g/mol	2.5 mM	0.711 mg	4.62 mg	7.47 ul
DTT	3 M	50 mM	20	130 ul	210 ul

Reagent	Stock (M)	Final (mM)	Volume per sample ul	6.5	10.5
H20			to 1.2 ml	to 7.8 ml	12.6 ml
Final Volume			**1200**	**7800**	**ul**

Guanidine Binding Buffer

Add 0.5 reaction

Guanidine Binding Buffer (GBB)

Reagent	Stock (M)	Final (M)	Volume per sample	6.5	10.5
Guanidine GuHCL	95.53 g/mol	5	4.776 gr	31.044 gr (split into 2 tubes, 15.522 gr ea)	50.148 gr (split into 2 tubes, 25.074 gr ea)
Isopropanol	100%	40%	400 ul	2.6 ml	4.2 ml
Add fresh on every use just before adding sample:					
Sodium Acetate	3 M	120mM	400 ul	2.6 ml	4.2 ml
Water			fill up to 10 ml	fill up to 65 ml	fill up to 105 ml

Day 1 Prep

1. Collect UVd tubes into a zip locked bag
2. Aliquote in 1.2 ml of PTB Buffer for each sample
3. Aliquote the GBB Buffer in 15 ml tubes and UV for 30 min

Grinding and Incubation at 37°C

1. Weight 15 to 30 mg of tissue for each sample, record the weight
2. Add sample to tube filled with glass pellets
3. Add 1200 ul of freshly prepared PTB buffer
4. put in thermal mixer at 3700 at low rpm 37°C overnight

Day 2 Prep

1. Collect incubated tubes and centrifuge at 14,000 RPM for 10 min
2. Collect UV'ed tubes: 1.5 ml DNA low binding 1.5 tube, 2mL tube, 50mL column/tube
3. Collect P3 buffer (neutralization buffer from Quiagen), PE buffer, and TE solution (stock is 100X concentration)

Day 2 Purification

1. Add 325 ul of P3 Buffer to a new 1.5 low binding tube
2. Transfer supernatant to the tube containing P3 Buffer
3. Incubate 5 min on ice tube rack
4. Freeze at -20 leftover pellet
5. Centrifuge at 14,000 RPM for 5 min
6. Add sodium acetate to GBB buffer
7. Transfer 700 ul of supernatant to a QIAshredder spin column
8. Centrifuge 14,000 RPM for 2 min
9. Transfer 700 ul of supernatant to a QIAshredder spin column
10. Centrifuge 14,000 RPM for 2 min
11. Transfer the liquid from the collector into the 15ml tube with GBB buffer
12. Transfer the GBB mixed with the sample and the Acetate into 50 ml tube with column.
13. Centrifuge the 50 ml tube 4 min at 1500 RPM
14. Disassembly the column and transfer it to a 2ml tube
15. dry spin for 1 min
16. Add 700 ul of PE buffer and centrifuge 1 min at max speed
17. Discard flowthrough
18. Add 700 ul of PE buffer and centrifuge 1 min at max speed
19. Discard flowthrough
20. Dry spin at 1 min
21. Rotate the column 180 and dry spin for 1 min
22. Transfer column into a new 1.5 ml lowDNA binding tube
23. Add 50 ul of TE and incubate for 5 min
24. Centrifuge 12000 RPM for 1 min max speed
25. Add 50 ul of TE and incubate for 5 min
26. Centrifuge 12000 RPM for 1 min max speed
27. Transfer 2ul into new 1.5 tubes for concentration quantification using Quibit
28. Store DNA in fridge if using soon or in the freezer at -20°C

Attachments

FileID	Name
4737	C012HERB-Tehuacanextractions20180212-110218-1142-8.pdf

*This procedure was originally created by **Miguel Vallebueno***