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We use this protocol and it's working

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Museum DNA extraction

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ABSTRACT

Modified from Korlevic et al 2021& Rohland et al 2018 for high throughput DNA extraction from historical specimens in natural history collections. **Please cite these papers when using this method.**

An in depth SOP by Korlevic can be found here: https://dx.doi.org/10.17504/protocols.io.ewov1o3molr2/v1

Post lysis the protocol can be run by hand or on a magnetic bead based robot (e.g. Kingfisher Flex / Apex).

MATERIALS

Lysis buffer C:
200mM Tris pH8, 25mM EDTA pH8, 0.05% (vol/vol) Tween 20, 0.4mg/ml PK

Binding buffer D:
5M guanidine hydrochloride, 40% (vol/vol) 2-propanol, 0.12M sodium acetate, 0.05% (vol/vol) Tween 20.

Wash buffer:
Option 1: Qiagen PE wash buffer
Option 2: DIY wash buffer:
10mM Tris pH8, 80% (vol/vol) ethanol

Elution buffer:
10mM Tris pH8, 1mM EDTA pH8, 0.05% (vol/vol) Tween 20

Silica magnetic beads:
Option 1: G-Biosciences Silica magnetic bead suspension (GENO786-915)
Option 2: SeraSil-Mag™ 400 magnetic beads (VWR: 29-3573-69)

buffer preparation

1 **Lysis buffer C**
200mM Tris pH8, 25mM EDTA pH8, 0.05% Tween 20, 0.4mg/ml PK

To make 10 mL mix together: 7295 µL molecular grade water, 2000 µL 1M Tris pH8,
500 µL 0.5M EDTA (pH 8), 200 µL Proteinase K (20mg/ml), and 5 µL Tween 20.

This is sufficient for 1 extraction plate of 95 samples and 1 negative control (90 µL each).

2 **Binding buffer D**

5 M guanidine hydrochloride, 40% (vol/vol) 2-propanol, 0.12 M sodium acetate and 0.05% (vol/vol) Tween 20.

In a glass or plastic bottle, weigh 124.2 g of guanidine hydrochloride and fill up with molecular water to 150 mL. Heat briefly in a microwave until the buffer is warm to the touch and shake until the salt is fully dissolved. Add 104 mL of 2-propanol, 10.4 mL of 3M sodium acetate buffer solution (pH 5.2) and 130 µL of Tween 20.

This is sufficient for 3 extraction plates of 95 samples and 1 negative control (900 µL each). Store in a fridge for up to 4 weeks. Seal the bottle with Parafilm to avoid evaporation.

3 **Wash buffer**

Option 1: To 100 mL of Qiagen buffer PE concentrate, add 400 mL of 96-100% Ethanol.
Option 2: To 5 mL 1M Tris-HCl, add 412 mL 97% Ethanol and 83 mL molecular grade water.
Note: Kevin Beentjes (Naturalis) has compared the Qiagen PE and "DIY" wash buffers with no apparent difference in recovery.

This makes 500 mL of wash buffer, sufficient for 6 extraction plates of 96 wells (750 µL each). This buffer can be stored at room temperature for at least 1 year.

4 **Elution buffer**

To make 50 mL of elution buffer, combine 49.4 mL of molecular water, 500 µL of 1 M Tris-HCl (pH 8.0), 100 µL of 0.5 M EDTA (pH 8.0) and 25 µL of Tween 20.

This is sufficient for 10 96-well extraction plates 50 µL each). This buffer can be stored at room temperature for at least 1 year.

silica bead preparation

8s

5 Option 1: **G-Biosciences Silica magnetic beads** (GENO78 6-915)
Option 2: **SeraSil-Mag™ 400 magnetic beads** (VWR: 29-3573-69)
Note: Kevin Beentjes (Naturalis) has used the SeraSil beads without washing them with similar recovery to the G-Biosciences beads.

The original Rohland protocol washes the G-Biosciences beads before use:

1. Fully resuspend the stock suspension of silica beads by vortexing.
2. Transfer 10 µL of silica bead suspension per reaction to a 2.0-ml lobind tube, including an excess of 5% (e.g., for a plate take 1 mL).
3. Place the tube on a magnet to collect the beads.
4. Pipette off and discard the supernatant.
5. Remove the tube from the magnet, add 500 µL of elution buffer and resuspend the beads by vortexing for 8 seconds.
6. Spin the tubes briefly in a microcentrifuge to collect the suspension at the bottom.
7. Place the tube back on the magnet to collect the beads.
8. Pipette off and discard the supernatant.
9. Repeat steps 5 to 8 for a total of two washes.
10. Resuspend the beads in a volume of elution buffer equivalent to the initial volume used (e.g., for a plate add 1 mL).

Manual protocol

6 Tissue can be added to the plate/tubes either before or after the addition of lysis buffer, however tissue from dried specimens (e.g. pinned insects) should be added to wells/tubes with lysis buffer to prevent static displacement. If tissue was previously stored in ethanol this should be dried off before lysis.

1. Add 90 µL of lysis buffer C to each sample tube / well. Volume can be adjusted to ensure coverage of tissue, however be mindful that 10x volume of binding buffer is required further down the protocol.
2. Incubate overnight at 56 °C on a shaking incubator. The longer the incubation the more the tissue / specimen is digested. Shorter incubations are recommended for better voucher recovery with whole body lysis.
3. Pipette lysate into a new tube / deep-well plate. If tissue / voucher is to be recovered then add appropriate volume of 80% ethanol to cover sample in original tube / well.
4. Add 900 µL of binding buffer D (= 10x volume of lysate) to lysate in tube / deep-well plate.
5. Add 10 µL of silica magnetic bead suspension to lysate and binding buffer.
6. Vortex for 5sec.
7. Rotate (mix) the tubes / plates for 15min at room temperature.
8. Spin briefly in a microcentrifuge to collect the suspension at the bottom and place them on an appropriate magnet.
9. Once solution clears pipette off the supernatant and discard.
10. Remove from the magnet, add 250 µL of wash buffer and vortex for 8sec.
11. Spin briefly to collect the suspension at the bottom and place back on the magnet.
12. Repeat wash (steps 9 - 11) twice for a total of three washes.
13. Aspirate any remaining drops of liquid and dry the beads at room temperature by leaving them on the magnet with open lids. Usually a few minutes is sufficient.
14. Remove from the magnet, add 50 µL - 100 µL of elution buffer depending on original sample size and anticipated DNA recovery.
15. Vortex until all beads have been resuspended and then briefly spin down.

16. Incubate for 2min at room temperature.
17. Place back on the magnet, wait until the solution clears and transfer the supernatant to a fresh tube / plate.

Proceed to library preparation or freeze extracted DNA.

Automated protocol

7 The manual protocol has been modified and tested on the Kingfisher Flex and Apex platforms. The protocol starts after step 5 above, i.e. prepare the deep-well plate with 90 µL lysate + 900 µL binding buffer D and 10 µL silica beads.

The kingfisher protocol uses onboard "tip mixing" rather than vortexing / mixing to resuspend beads and skips all centrifugation steps.