

May 29, 2023 Version 2

## 🌐 Low-cost museum DNA extraction using magnetic beads V.2

DOI

[dx.doi.org/10.17504/protocols.io.4r3l27ebxg1y/v2](https://dx.doi.org/10.17504/protocols.io.4r3l27ebxg1y/v2)



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**Protocol Citation:** Andie C Hall, Owain N Powell, Piotr Cuber, Ben Price 2023. Low-cost museum DNA extraction using magnetic beads. [protocols.io https://dx.doi.org/10.17504/protocols.io.4r3l27ebxg1y/v2](https://dx.doi.org/10.17504/protocols.io.4r3l27ebxg1y/v2) Version created by **Ben Price**

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**Protocol status:** Working

**We use this protocol and it's working**

**Created:** May 29, 2023

**Last Modified:** October 27, 2023

**Protocol Integer ID:** 82583

**Keywords:** DNA extraction, historical, aDNA, museum specimen, silica, magnetic bead

## 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).

v2 uses 5x lysate volume of binding buffer as tests have shown improved recovery over 10x volume.

v2 replaces SeraSil beads with Qiagen MagAttract beads which provide a much cheaper option to G-Biosciences beads.

## 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 (GEN0786-915)

Option 2: MagAttract Suspension G magnetic beads (Qiagen: 1026901)

## buffer preparation

### 1 Lysis buffer C

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

To make  mix together:  molecular grade water,  1M Tris pH8,  0.5M EDTA (pH 8),  Proteinase K (20mg/ml), and  Tween 20.

This is sufficient for 1 extraction plate of 95 samples and 1 negative control (  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  of guanidine hydrochloride and fill up with molecular water to . Heat briefly in a microwave until the buffer is warm to the touch and shake until the salt is fully dissolved. Add  of 2-propanol,  of 3M sodium acetate buffer solution (pH 5.2) and  of Tween 20.

This is sufficient for 6 extraction plates of 95 samples and 1 negative control (  each). Store in a fridge for up to 4 weeks. Seal the bottle with Parafilm to avoid evaporation.

### 3 Wash buffer

Option 1: To  of Qiagen buffer PE concentrate, add  of 96-100% Ethanol.

Option 2: To  1M Tris-HCl, add  97% Ethanol and  molecular grade water.

*Note: Kevin Beentjes (Naturalis) has compared the Qiagen PE and "DIY" wash buffers with no apparent difference in fragment recovery.*

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

### 4 Elution buffer

To make  of elution buffer, combine  of molecular water,  of 1 M Tris-HCl (pH 8.0),  of 0.5 M EDTA (pH 8.0) and  of Tween 20.



This is sufficient for 10 96-well extraction plates (50  $\mu\text{L}$  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** (GEN078 6-915)

Option 2: **MagAttract Suspension G magnetic beads** (Qiagen: 1026901)

*Note: MagAttract beads are much cheaper and have very similar recovery for fragments 50bp and above, with minor loss of 35bp fragments. SeraSil beads only recover from 75bp (SeraSil 700) or 100bp (SeraSil 400).*

The original Rohland protocol washes the beads before use, but we have found unwashed beads work just as effectively. If washing follow these steps:






1. Fully resuspend the stock suspension of silica beads by vortexing.
2. Transfer 10  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  of lysis buffer C to each sample tube / well. Volume can be adjusted to ensure coverage of tissue, however be mindful that 5x volume of binding buffer is required further down the protocol.
2. Incubate overnight at 56  $^{\circ}\text{C}$  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  450  $\mu\text{L}$  of binding buffer D (= 5x volume of lysate) to lysate in tube / deep-well plate.
5. Add  10  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  -  100  $\mu\text{L}$  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 tested on the Kingfisher Flex and Apex platforms. The protocol starts after step 5 above, i.e. prepare the deep-well plate with  90  $\mu\text{L}$  lysate +  450  $\mu\text{L}$  binding buffer D and  10  $\mu\text{L}$  silica beads.

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

## Protocol references

Petra Korlević, Erica McAlister, Matthew Mayho, Alex Makunin, Paul Flicek, Mara K N Lawniczak, A Minimally Morphologically Destructive Approach for DNA Retrieval and Whole-Genome Shotgun Sequencing of Pinned Historic Dipteran Vector Species, *Genome Biology and Evolution*, Volume 13, Issue 10, October 2021, evab226, <https://doi.org/10.1093/gbe/evab226>

Rohland, N., Glocke, I., Aximu-Petri, A. *et al.* Extraction of highly degraded DNA from ancient bones, teeth and sediments for high-throughput sequencing. *Nat Protoc* **13**, 2447–2461 (2018). <https://doi.org/10.1038/s41596-018-0050-5>