Mu-DNA: a modular universal DNA extraction method adaptable for a wide range of sample types V.2

Metabarcoding and Metagenomics

In 1 collection

Graham S Sellers¹, Cristina Di Muri¹, Africa Gómez¹, Bernd Hänfling¹

¹University of Hull

ABSTRACT

Mu-DNA is designed to be a simple and modular spin column-based DNA extraction ‘toolkit’ for the isolation of high purity DNA from multiple sample types. Here we present optimised Mu-DNA protocols for soil, tissue and water samples. Each protocol consists of five stages for DNA extraction: lysis, inhibitor removal, silica binding, wash and elution. Mu-DNA: Tissue has no inhibitor removal stage. All stages are designed to be modular and interchangeable between protocols. For example, an inhibitor removal stage can be added to a tissue protocol or a tissue protocol wash stage added to a soil protocol. The modular aspect allows for the creation of an optimised, bespoke DNA extraction for a specific sample type.

GUIDELINES

Materials required

Below are listed the materials required for all Mu-DNA extraction protocols. Companies used and product codes are provided.

Chemicals

Guanidine thiocyanate (Alfa Aesar: B21250.22)
Trisodium phosphate dodecahydrate (Sigma Aldrich: 04277-1KG)
Sodium chloride (Sigma Aldrich: S7653-250G)
Tris HCl (Alfa Aesar: J67233.22)
Disodium EDTA dihydrate (Sigma Aldrich: E5134-250G)
Sodium dodecyl sulphate (Alfa Aesar: J75819.22)
Proteinase K (Thermofisher: AM2542)
Ammonium acetate (Sigma Aldrich: A1542-500G)
Aluminium ammonium sulphate dodecahydrate (Alfa Aesar: 13802.22)
Calcium chloride dihydrate (Sigma Aldrich: 1.02382.0250)
Guadidine hydrochloride (Thermofisher: 10071503)

**Plastics**
- 2 mL screw cap tubes (Starlab: E1420-2341)
- 7 mL Bijou tubes (Sigma-Aldrich: 129A)*
- 1.5 mL tubes (Starlab: S1615-5510)
- 2 mL tubes (Starlab: E1420-2000)
- Spin Columns (NBS Biologicals: SD5005)

* these tubes are the ones used in our study and fit in a TissueLyser II but do not fit in many centrifuges. Alternatively you can use transport tubes (Axygen SCT-5ML-S, Fisher Scientific: 12559107) that fit in a Mobio Vortex Adapter and all centrifuges with 15 mL falcon tube fittings.

**Garnet beads**
Garnet grit was sourced from an abrasives company at the grades required (0.15 mm and 1 - 1.4 mm). Each grade was thoroughly washed with purified water through a suitable mesh sieve to remove any detritus and fine particles. After washing, the garnet grit was transferred to 250 mL conical flasks capped with aluminium foil and sterilised at 210°C for three hours. The garnet grit was left to cool before being transferred to sterile 50 mL falcon tubes. Alternatively sterile garnet bead tubes, or similar, can be purchased commercially (e.g. Qiagen).

**Stock solutions**
Stock solutions are given as compositions for 100 mL with the exception of PK.

1 M Tris HCl (pH 8):
Dissolve 15.7 g of Tris HCl in 75 mL ddH$_2$O. Adjust to pH 8 with 5 M NaOH. Bring to 100 mL with ddH$_2$O.

0.5 M EDTA (pH 8):
Dissolve 18.6 g of disodium EDTA dihydrate in 75 mL ddH$_2$O. Adjust to pH 8 with 5 M NaOH. Bring to 100 mL with ddH$_2$O.

20% SDS:
Dissolve 20 g sodium dodecyl sulphate in 75 mL ddH$_2$O, bring to 100 mL with ddH$_2$O.

PK:
To 7 mL ddH$_2$O add 0.5 mL 1 M Tris HCl (pH 8) and 100 mg Proteinase K, bring to 10 mL with ddH$_2$O.

5 M Ammonium acetate:
Dissolve 38.6 g ammonium acetate in 75 mL ddH$_2$O, bring to 100 mL with ddH$_2$O.

180 mM Aluminium etc.:
Dissolve 8.2 g aluminium ammonium sulphate dodecahydrate in 75 mL ddH$_2$O, bring to 100 mL with ddH$_2$O.

3% Calcium chloride:
Dissolve 3 g calcium chloride dihydrate in 75 mL ddH$_2$O, bring to 100 mL with ddH$_2$O.

5.5 M Guanidine HCl:
Dissolve 52.6 g guanidine hydrochloride in 75 mL ddH$_2$O, bring to 100 mL with ddH$_2$O.

**Working solutions**

All working solutions are composites of stock solutions. All working solution compositions are given for a 100 mL final volume. However, working solutions may not be required in this amount. Some working solutions are designed to be easily created from combinations of stock solution volumes. For these solutions the number of volumes required per stock solution is given in brackets after the amount for 100 mL. Also note that some working solutions consist of a single stock solution.

**Lysis Solution.**
To 75 mL ddH$_2$O add 6.7 mL 1 M Tris HCl (pH 8), 5.3 mL 0.5 M EDTA (pH 8), 1.7 g guanidine thiocyanate, 8.7 g trisodium phosphate dodecahydrate and 0.2 g sodium chloride. Stir mixture until all solids dissolve. Adjust to pH 9.0 with 5 M HCl. Bring to final 100 mL volume with ddH$_2$O.

**Soil Lysis Additive.**
To 50 mL (8 volumes) 180 mM Aluminium etc. add 43.75 mL (7 volumes) ddH$_2$O and 6.25 mL (1 volume) 20% SDS. Vortex briefly to mix.

**Tissue Lysis Additive.**
20% SDS

**Water Lysis Additive.**
To 93.75 mL (15 volumes) ddH$_2$O add 6.25 mL (1 volume) 20% SDS. Vortex briefly to mix.

**Flocculant Solution.**
To 50 mL (2 volumes) 5 M Ammonium acetate add 25 mL (1 volume) 180 mM Aluminium etc. Vortex briefly before adding 25 mL (1 volume) 3% Calcium chloride. Vortex briefly to mix.

**Binding Solution.**
5.5 M Guanidine HCl

**Tissue Binding Solution.**
To 50 mL (1 volume) 5.5 M Guanidine HCl add 50 mL (1 volume) 100% ethanol. Vortex briefly to mix.

**Wash Solution.**
To 20 mL (2 volumes) ddH$_2$O add 80 mL (8 volumes) 100% ethanol.

**Elution Buffer.**
To 75 mL ddH$_2$O add 1 mL 1 M Tris HCl (pH 8) and 0.2 mL 0.5 M EDTA (pH 8). Bring to 100 mL with ddH$_2$O.
SAFETY WARNINGS

- Reagents and extraction waste chemicals are damaging to the environment. Dispose of extraction waste responsibly.
- Do not add bleach to any reagents or extraction waste containing guanidine thiocyanate or guanidine hydrochloride as toxic gases and chemicals are released.

BEFORE START INSTRUCTIONS

Incubator
Preheat incubating apparatus to 55°C.

Samples
Defrost samples (if necessary) at room temperature. Samples stored in ethanol and other storage buffers (e.g. RNAlater) are best air dried on sterile blotting paper (or similar) to remove as much storage buffer as possible prior to lysis.

Working solutions
Create the relevant working solutions in sufficient volumes required for the extraction method.

**Important:** Incubate Soil Lysis Additive, Tissue Lysis additive, Water Lysis Additive, Binding Solution and Tissue Binding Solution at 55°C until required. If any precipitate is present mix gently until redissolved. Use solutions while still warm.

Lysis master mix
To reduce processing time when extracting from a large number of samples it is possible to create a master mix of reagents used in the lysis stage of each protocol (Soil: Lysis Solution and Soil Lysis Additive, Tissue: Lysis Solution, Tissue Lysis Additive and PK, Water: Lysis Solution and Water Lysis Additive). Heat the master mix at 55°C until required, mix gently occasionally. This prevents the formation of precipitates that interfere with lysis. Use the master mix while still warm.

---

Mu-DNA: Soil

1. The entire protocol is scalable depending on initial sample weight or transferred lysate amount. Where relevant, scalable volumes are shown in brackets next to reagent volumes used, e.g. (2 X volume). Large sample weights increase volumes at each step and will require extended centrifuge times under lower xg to obtain optimal results.

**Important:** Incubate Soil Lysis Additive and Binding Solution at 55°C until required. If any precipitate is present mix gently until redissolved. Use solutions while still warm.

LYSIS
The following steps are based on a 0.25 g sample.

1. Add 0.5 g (2 X sample weight) of 1 - 1.4 mm diameter sterile garnet beads to a 2 mL screw cap tube
2. Add up to 0.25 g of sample to tube
3. Add 550 μL of Lysis Solution and 200 μL of Soil Lysis Additive, vortex briefly
4. Place in TissueLyser II (or similar apparatus) at 30 hz for 10 mins
5. Centrifuge at 10,000 xg for 1 min at room temperature
6. Without disturbing the pellet excessively transfer all available supernatant to a 1.5 mL tube
7. Centrifuge at 10,000 xg for 1 min at room temperature
8. Without disturbing the pellet, transfer the supernatant to a fresh 1.5 mL tube

**INHIBITOR REMOVAL**
The following steps are based on 500 - 650 μL of transferred lysate.

1. Add 300 μL (0.6 X volume) of Flocculant Solution, vortex briefly and incubate at 4°C or on ice for a minimum of 10 mins
2. Centrifuge at 10,000 xg for 1 min at room temperature
3. Without disturbing the pellet, transfer the supernatant to a 2 mL tube

**SILICA BINDING**
The following steps are based on 600 - 700 μL of transferred supernatant.

1. Add 1200 μL (2 X volume) of Binding Solution, vortex briefly to mix
2. Transfer 650 μL of the mixture to a spin column
3. Centrifuge at ≥ 10,000 xg for 1 min at room temperature, discard the flow-through
4. Repeat steps 2 and 3 until all the mixture has passed through the spin column

**WASH**

1. Add 500 μL of Wash Solution to the spin column
2. Centrifuge at 10,000 xg for 1 min at room temperature, discard the flow-through
3. Centrifuge at 10,000 xg for 2 min at room temperature, replace collection tube with a fresh 1.5 mL tube

**ELUTION**

1. Add 100 μL of Elution Buffer directly to the spin column membrane and incubate for 1 min at room temperature
2. Centrifuge at 10,000 xg for 1 min at room temperature
3. DNA is now in the 1.5 mL tube
   Optional: For increased DNA yield repeat steps 1 and 2 a further time.

**Troubleshooting**
Spin column clogged: Centrifuge at higher xg for 2 mins. Alternatively, heat spin column (including collection tube) and binding mixture tube contents (previously transferred supernatant and Binding Solution mixture) at 55°C for 5 min then centrifuge at higher xg for 2 mins. Continue with protocol.

Mu-DNA: Tissue

2. The entire protocol is scalable depending on initial sample weight or transferred lysate amount. Where relevant, scalable volumes are shown in brackets next to reagent volumes used, e.g. (2 X volume).

Important: Incubate Tissue Lysis Additive and Tissue Binding Solution at 55°C until required. If any precipitate is present mix gently until redissolved. Use solutions while still warm.

LYSIS

1. Place up to 40 mg of tissue into a 1.5 mL tube
2. Add 260 μL of Lysis Solution, 20 μL Tissue Lysis Additive and 20 μL PK
3. Grind tissue with a tube pestle, vortex briefly
4. Incubate at 55°C with occasional vortexing until all tissue is dissolved (>3 hours or overnight).
5. Centrifuge at 10,000 xg for 1 min at room temperature
6. Without disturbing the pellet, transfer the supernatant to a fresh 1.5 mL tube

SILICA BINDING
The following steps are based on 300 μL of transferred supernatant.

1. Add 600 μL (2 X volume) Tissue Binding Solution, vortex briefly to mix
2. Transfer 650 μL of the mixture to a spin column
3. Centrifuge at ≥ 10,000 xg for 1 min at room temperature, discard the flow-through
4. Repeat steps 2 to 3 until all the mixture has passed through the spin column

WASH

1. Add 500 μL of Wash Solution to the spin column
2. Centrifuge at 10,000 xg for 1 min at room temperature, discard the flow-through
3. Repeat steps 1 and 2 a second time
4. Centrifuge at 10,000 xg for 2 min at room temperature, replace collection tube with a fresh 1.5 mL tube

ELUTION

1. Add 200 μL of Elution Buffer directly to the spin column membrane and incubate for 1 min at
room temperature
2. Centrifuge at 10,000 xg for 1 min at room temperature
3. DNA is now in the 1.5 mL tube
Optional: For increased DNA yield repeat steps 1 and 2 a further time.

Troubleshooting

Lysate thickens and/or becomes white: Heat lysate at 55°C for 5 min, vortex briefly and proceed with protocol.
Lysate and Tissue Binding Solution mixture becomes cloudy: Heat mixture at 55°C for 5 min and proceed with protocol.
Spin column clogged: Centrifuge at higher xg for 2 mins. Alternatively, heat spin column (including collection tube) and binding mixture tube contents (previously transferred supernatant and Tissue Binding Solution mixture) at 55°C for 5 min then centrifuge at higher xg for 2 mins. Continue with protocol.

Mu-DNA: Water

3. For water samples vacuum filtered through 47 mm diameter cellulose nitrate membrane filters or similar. The entire protocol is scalable based upon transferred supernatant volumes. Where relevant, scalable volumes are shown in brackets next to reagent volumes used, e.g. (2 X volume).

Important: Incubate Water Lysis Additive and Binding Solution at 55°C until required. If any precipitate is present mix gently until redissolved. Use solutions while still warm.

LYSIS

1. Add 1 g each of 0.15 mm and 1 - 1.4 mm diameter sterile garnet beads to 7 mL Bijou tube (or similar tube able to hold the filter).
2. Roll filter and place in tube
3. Add 750 μL of Lysis Solution and 250 μL of Water Lysis Additive
4. Place in TissueLyser II (or similar apparatus) at 30 hz for 5 mins
5. Centrifuge at 4,000 xg for 1 min at room temperature
6. Transfer all available supernatant from within the garnet beads to a 1.5 mL tube
7. Centrifuge at 10,000 xg for 1 min at room temperature
8. Without disturbing the pellet, transfer the supernatant to a fresh 1.5 mL tube

**INHIBITOR REMOVAL**
The following steps are based on 500 - 650 μL of transferred lysate.

1. Add 200 μL (0.3 X volume) of Flocculant Solution, vortex briefly and incubate at 4°C or on ice for a minimum of 10 mins
2. Centrifuge at 10,000 xg for 1 min at room temperature
3. Without disturbing the pellet, transfer the supernatant to a 2 mL tube

**SILICA BINDING**
The following steps are based on 600 - 700 μL of transferred supernatant.

1. Add 1200 μL (2 X volume) of Binding Solution, vortex briefly to mix
2. Transfer 650 μL of the mixture to a spin column
3. Centrifuge at ≥ 10,000 xg for 1 min at room temperature, discard the flow-through
4. Repeat steps 2 to 3 until all the mixture has passed through the spin column

**WASH**

1. Add 500 μL of Wash Solution to the spin column
2. Centrifuge at 10,000 xg for 1 min at room temperature, discard the flow-through
3. Centrifuge at 10,000 xg for 2 min at room temperature, replace collection tube with a fresh 1.5 mL tube

**ELUTION**

1. Add 100 μL of Elution Buffer directly to the spin column membrane and incubate for 1 min at room temperature
2. Centrifuge at 10,000 xg for 1 min at room temperature
3. DNA is now in the 1.5 mL tube
Optional: For increased DNA yield repeat steps 1 and 2 a further time.

**Troubleshooting**

**Spin column clogged:** Centrifuge at higher xg for 2 mins. Alternatively, heat spin column (including collection tube) and binding mixture tube contents (previously transferred supernatant and Binding Solution mixture) at 55°C for 5 min then centrifuge at higher xg for 2 mins. Continue with protocol.