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

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Graham S Sellers[1], Cristina Di Muri[1], Africa Gómez[1], Bernd Hänfling[1]

[1]University of Hull

EvoHull

Graham S Sellers
University of Hull

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We use this protocol and it's working

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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)
Guanidine hydrochloride (Thermofisher: 10071503)

Plastics

2 mL screw cap tubes (Starlab: E1420-2341)
7mL 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_2O. Adjust to pH 8 with 5 M NaOH. Bring to 100 mL with ddH_2O.

***0.5 M EDTA (pH 8)*:**

Dissolve 18.6 g of disodium EDTA dihydrate in 75 mL ddH_2O. Adjust to pH 8 with 5 M NaOH. Bring to 100 mL with ddH_2O.

***20% SDS*:**

Dissolve 20 g sodium dodecyl sulphate in 75 mL ddH_2O, bring to 100 mL with ddH_2O.

PK:
To 7 mL ddH_2O add 0.5 mL 1 M Tris HCl (pH 8) and 100 mg Proteinase K, bring to 10 mL with ddH_2O.

5 M Ammonium acetate:
Dissolve 38.6 g ammonium acetate in 75 mL ddH_2O, bring to 100 mL with ddH_2O.

180 mM Aluminium etc.:
Dissolve 8.2 g aluminium ammonium sulphate dodecahydrate in 75 mL ddH_2O, bring to 100 mL with ddH_2O.

3% Calcium chloride:
Dissolve 3 g calcium chloride dihydrate in 75 mL ddH_2O, bring to 100 mL with ddH_2O.

5.5 M Guanidine HCl:
Dissolve 52.6 g guanidine hydrochloride in 75 mL ddH_2O, bring to 100 mL with ddH_2O.

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_2O 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_2O.

Soil Lysis Additive:
To 50 mL (8 volumes) ***180 mM Aluminium etc.*** add 43.75 mL (7 volumes) ddH_2O 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_2O 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_2O add 80 mL (8 volumes) 100% ethanol.

Elution Buffer:
To 75 mL ddH_2O 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_2O.

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

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 breifly
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. Witout 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 transterred 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 transterred 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 transterred 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 transterred 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 transterred 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.