May 03, 2025

High Molecular Weight DNA Extraction from Actinomycetota

DOI
https://dx.doi.org/10.17504/protocols.io.q26g7mqr9gwz/v1

Ashley Paulsen[1], Larry Halverson[1]

[1]Iowa State University

Ashley Paulsen
Iowa State University

DOI: https://dx.doi.org/10.17504/protocols.io.q26g7mqr9gwz/v1

Protocol Citation: Ashley Paulsen, Larry Halverson 2025. High Molecular Weight DNA Extraction from Actinomycetota. **protocols.io** https://dx.doi.org/10.17504/protocols.io.q26g7mqr9gwz/v1

Protocol status: Working
We use this protocol and it's working

Created: March 05, 2025

Last Modified: May 03, 2025

Protocol Integer ID: 123879

Keywords: high molecular weight dna extraction from actinomycetota actinomycete, high molecular weight dna from actinomycete, high molecular weight dna extraction, extracting high molecular weight dna, actinomycetota actinomycete, weight dna extraction, actinomycete, chloroform dna extraction, extractions with an n50, extraction, dna, whole genome, read sequencing, genome

Funders Acknowledgements:
National Science Foundation
Grant ID: DGE-1545453
National Science Foundation
Grant ID: DRL-1759152
U.S. Department of Energy (DOE) Office of Biological and Environmental Research, Biological Systems Division
Grant ID: FWP No AL-18-380-055

Abstract

Actinomycetes are difficult to extract DNA from and excluding mechanical lysis makes it especially challenging. This protocol is a modified phenol-chloroform DNA extraction for extracting high molecular weight DNA from *Actinomycetes* for whole genome long-read sequencing and has been verified to produce extractions with an N50 of >30 kb. The main modifications include an *Actinomycetes* optimized lysis buffer and a PEG-based size selective precipitation step.

Materials

Growth Medium

- Tryptic Soy Broth **Fisher Scientific Catalog #**DF0370-07-5
- Glycine

Extraction

- 1X PBS (Phosphate-buffered saline)
- Sodium chloride **P212121**
- Sucrose **P212121**
- Tris HCl **P212121**
- EDTA
- Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**12671-19-1
- RNase A (10 mg/mL) **Thermo Fisher Scientific Catalog #**EN0531
- Sodium Dodecyl Sulfate **P212121**
- BioUltra TE-saturated phenol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**77607
- BioUltra Chloroform – isoamyl alcohol mixture 24:1 **Supelco Catalog #**25666
- ammonium acetate **P212121**
- Ethanol **P212121 Catalog #**BE-BDH1156
- 70% Ethanol
- 10 mM Tris-HCL pH 8.0

Size selective precipitation

- Poly(ethylene glycol) 8000 [PEG 8000] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**89510

Protocol materials

- 1X PBS (Phosphate-buffered saline)
- BioUltra TE-saturated phenol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**77607
- BioUltra Chloroform – isoamyl alcohol mixture 24:1 **Supelco Catalog #**25666
- Proteinase K **Life Technologies Catalog #**17916
- 10 mM Tris-HCL pH 8.0
- ammonium acetate **P212121**
- Tris HCl **P212121**
- EDTA
- RNase A (10 mg/mL) **Thermo Fisher Scientific Catalog #**EN0531
- Glycine
- 70% Ethanol
- Sodium chloride **P212121**
- Tryptic Soy Broth **Fisher Scientific Catalog #**DF0370-07-5
- Lysozyme **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**12671-19-1
- Sucrose **P212121**
- Sodium Dodecyl Sulfate **P212121**
- Ethanol **P212121 Catalog #**BE-BDH1156
- Poly(ethylene glycol) 8000 [PEG 8000] **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**89510

Before start

Reagents to prepare

half strength TSB plus glycine:
- 15 g/L Tryptic Soy Broth
- 0.5% glycine

Sucrose Lysis Buffer:
- 100 mM NaCl,
- 25% (w/v) sucrose
- 10 mM Tris-Cl pH 8
- 25 mM EDTA pH 8
- 1 mg/mL lysozyme
- 20 µg/ml RNase A - added immediately before use

10% (w/v) sodium dodecyl sulfate (SDS) solution
70% Ethanol
5M ammonium acetate - prepared fresh and chilled

Elution Buffer - 10 mM Tris-HCl pH 8

PEG precipitation buffer:
- 9% polyethylene glycol 8k (w/v)
- 1 M NaCl
- 10 mM Tris-HCl pH 8

Lysis

3h 10m

1 Grow cultures shaking in half strength TSB with 0.5% glycine for 36-48 hours.

2 Spin down at 4500 x g for 10 minutes, remove supernatant 10m

4500 x g, Room temperature, 00:10:00

3 Wash cells by re-suspending in 500 µL of PBS, centrifuging, and removing the supernatant

500 µL 1X PBS (Phosphate-buffered saline)

> Note
>
> At this point the cells can be frozen and stored at -80 °C if needed

4 Re-suspend in 200 µL 1X PBS (Phosphate-buffered saline)

5 Vortex with 5 mL of sucrose lysis buffer and incubate at 37 °C for 1h

01:00:00

5 mL Sucrose Lysis Buffer

6 Add 500 µL of 10% SDS (for a final concentration of 1%) and 50 µL Proteinase K **Life Technologies Catalog #**17916 (20 mg/mL stock solution). Mix by rotating the tube end over end 5 times.

7 Incubate at 50 °C for 02:00:00, mix every 30 minutes by slowly rotating end-over-end 3 times. 2h

Extraction

45m

8 Pour the viscous lysate into fresh 15 mL conical centrifuge tubes

9 Add 5 mL recently opened BioUltra Phenol to each Falcon tube containing lysate.

BioUltra TE-saturated phenol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**77607

Note

buffer saturated phenol is preferable as the pH is around 8.0
Phenol should be used with 3 months of opening for optimum results

10 Place on a mixer at ~20 rpm end-over-end rotation for 10 minutes, if a fine emulsion has not formed after a minute gradually increase the rotation speed. 10m

Note

A HulaMixer is recommended, but a hematology mixer can be used instead.

11 Spin in a centrifuge at 6000 rpm for 10 minutes. 10m

6000 rpm, Room temperature, 00:10:00

12 Due to the sucrose, the aqueous phase will migrate below the organic. Pipette off the organic phase and discard it, removing as much as possible without removing too much of the aqueous phase. Protein may show as a white precipitate and should be removed as much as possible.

13 Pour the aqueous phases into new 15 ml Falcon tubes and add 2.5 mL of phenol and 2.5 mL of 24:1 chloroform-isoamyl alcohol to each.

BioUltra TE-saturated phenol **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**77607

BioUltra Chloroform – isoamyl alcohol mixture 24:1 **Supelco Catalog #**25666

14 Place on a mixer at ~20 rpm end-over-end rotation for 10 minutes, if a fine emulsion has not formed after a minute gradually increase the rotation speed. 10m

15 Centrifuge at 6000 rpm for 10 minutes. 10m
6000 rpm, Room temperature, 00:10:00

16 Pipette off the organic phase and discard it, removing as much as possible without removing too much of the aqueous phase.

17 Centrifuge at 6000 rpm for 5 minutes and ensure complete removal of the organic phase. 5m
6000 rpm, Room temperature, 00:10:00

18 Pour aqueous phase into a new 50 mL conical centrifuge tube.

Precipitation and elution 1d 0h 19m

19 Add 2 mL of cold 5M ammonium acetate. Gently rock the tube back and forth 3 times to mix.
ammonium acetate **P212121**

> Note
>
> Ammonium acetate solution should be used within a week of preparing, the fresher the better.

20 Add 15 mL ice-cold 100% ethanol and incubate tubes in 4 °C Overnight to maximize precipitation.

> Note
>
> Chill the ethanol before hand

21 Melt the tip of a glass pipet in a bunsen burner to make a small hook

22 Hook out the mass of precipitated DNA and dip it into a container 70% ethanol. Try to get it in one piece, but if it breaks then you can just go back for the rest. Gently work the mass off of the hook and into a labeled microcentrifuge tube

23 Add 1 mL 70% ethanol to the tube

24 Spin down at 10,000 x g for 2 min then remove as much of the 70% ethanol as possible 2m
10000 x g, 00:02:00

25 Wash again with 1 mL 70% ethanol

26 Spin down at 10,000 x g for 2 min then remove as much of the 70% ethanol as possible 2m
10000 x g, 00:02:00

27 Let the remaining ethanol evaporate by leaving at room temperature for 15 minutes. Leaving the tubes on their side in a laminar flow hood speeds this up significantly. 15m

28 Add 500 µL 10 mM Tris elution buffer and incubate without mixing at 4 °C for 24:00:00 to allow the pellet to fully re-suspend into a translucent viscous gel. 1d
10 mM Tris-HCL pH 8.0

Note

This can be decreased if the pellet is small.
It's important that freshly eluted HMW DNA is given time to relax before being handled and 4°C facilitates this relaxing.

Homogenization 1h

29 1) Slowly pipette mix DNA 5-10 times with wide bore pipette (or a cut p200)

30 2) Incubate on 37 °C heating block 30-60 minutes 1h

31 3) Slowly pipette mix 5-10 times with wide bore pipette

32 4) Perform nanodrop in triplicate; bottom, middle, top (I always use this order just for consistency) If the readings are too different then repeat the homogenization.

33 5) Once nanodrop readings are consistent the DNA is ready for Qubit and intended uses

Optional Size selection step 17m

34 Take an aliquot of DNA and add to a clean 1.5 mL micro centrifuge tube. Add an equal volume of precipitation buffer and gently flick to mix.

35 Incubate sample at Room temperature for 6-18 hours. Longer incubation times increases recovery.

36 Centrifuge for 15 minutes at 10,000 g 15m
10000 rpm, 00:15:00

37 Pipette off supernatant, being VERY careful not to disturb the pellet

Note

Pipette slowly, keeping tip at the liquid-air interface
Leave 10-20 µL behind

38 Add 1 mL of 70% ethanol and gently tap to mix

39 Centrifuge for 2 minutes at 10,000 g 2m
10000 x g, 00:02:00

40 Pipette off and discard supernatant. The pellet should be slightly more secure at this point, but care should still be taken not to disturb it.

41 Repeat the ethanol wash and let pellet dry for ~15 minutes.

42 Add desired volume of 10 mM Tris elution buffer and incubate at 4 °C

10 mM Tris-HCL pH 8.0