Mar 03, 2025 Version 1

Soil Metagenome PacBio V.1

Nature Communications

DOI
https://dx.doi.org/10.17504/protocols.io.rm7vzjqo8lx1/v3

Robert James[1,2], Gaetan Benoit[3], Sebastian Raguideau[2], Georgina Alabone[2], Christopher Quince[2,1]

[1]Quadram Institute Bioscience; [2]Earlham Institute; [3]Pasteur Institute

Quince_Group

Robert S James
Quadram Institute Bioscience

DOI: https://dx.doi.org/10.17504/protocols.io.rm7vzjqo8lx1/v3

External link: https://doi.org/10.1038/s41467-026-69760-y

Protocol Citation: Robert James, Gaetan Benoit, Sebastian Raguideau, Georgina Alabone, Christopher Quince 2025. Soil Metagenome PacBio. **protocols.io** https://dx.doi.org/10.17504/protocols.io.rm7vzjqo8lx1/v3

Manuscript citation:
Benoit G, James R, Raguideau S, Alabone G, Goodall T, Chikhi R, Quince C (2026) High-quality metagenome assembly from nanopore reads with nanoMDBG. Nature Communications 17(). doi: 10.1038/s41467-026-69760-y

Protocol status: In development
We are still developing and optimizing this protocol

Created: August 15, 2024

Last Modified: March 03, 2025

Protocol Integer ID: 105698

Keywords: Metagenomics, Soil, Long-reads, PacBio, Revio, soil metagenome pacbio this protocol, soil metagenome pacbio, acquisition workflow for pacbio revio, read sequencing, revio smrt cell, sequencing, complex soil sample, pacbio revio, using smrt bell library prep kit, smrt bell library prep kit, soil, kit, sequence

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

This protocol describes the sample collection to sequence acquisition workflow for PacBio Revio long-read sequencing of a complex soil sample using SMRT Bell library prep kit 2.0 and Revio SMRT cells.

Attachments

SPRI_man.pdf 565KB

FastDNA_SPIN_Kit_Pro... 221KB

Zymo_shield_man.pdf 628KB

Guidelines

- Fully equilibriate Ampure XP SPRI beads to Room temperature before use.
- Fully equilibriate Qubit solution to Room temperature before use.
- Fully equilibriate Tape station screen tape and reagents to Room temperature before use.
- Over drying DNA bound to Ampure XP SPRI beads can reduce Sample recovery.
- Additional time must be given for the Ampure XP beads to clear from the adapter ligation reaction due to the high viscosity of the solution. Failure to provide adequate time for the supernatant to clear can result in Sample loss.

Materials

Equipment

- Bench top centrifuge
- Thermal cycler
- Qubit 4 Fluorometer **Thermo Fisher Scientific Catalog #**Q33238
- Capillary electrophoresis instrument (e.g. Agilent Tapestation 4200)
- Rotational mixer
- Heat block
- Magnetic rack
- Fridge 4 °C
- Freezer -80 °C
- Ice bucket
- Pipette set (P10, P20, P200, P1000)
- Soil corer
- Soil Sieve
- Soil collection plate
- Plate sealer
- Top pan balance
- Weigh boat
- Spatula
- Measuring cylinder 100 mL
- Conical flask 250 mL
- P2 Solo device and compatible compute

Reagents

- Zymo DNA/RNA Shield **Fisher Scientific Catalog #**50-125-1706
- FastDNA™ SPIN Kit for Soil **MPBio Catalog #**116560200-CF
- Monarch RNase A **New England Biolabs Catalog #**T3018L
- Ethanol (100%, Molecular Biology Grade) **Fisher Scientific Catalog #**BP2818500
- Molecular Biology Grade Water **Fisher Scientific Catalog #**10154604
- Agencourt AMPure XP **Beckman Coulter Catalog #**A63880
- Qubit 1X dsDNA HS Assay Kit **Thermo Fisher Scientific Catalog #**Q33230
- Genomic DNA Reagents **Agilent Technologies Catalog #**5067-5366
- Genomic DNA ScreenTape **Agilent Technologies Catalog #**5067-5365

Consumables

- Eppendorf lo-bind Falcon tubes 5 mL Catalog no. 0030122348
- Eppendorf lo-bind microfuge tubes 1.5 mL Catalog no. 0030108051
- Qubit™ Assay Tubes **Invitrogen - Thermo Fisher Catalog #**Q32856
- Thin-walled PCR tubes 0.2 mL Catalog no: AB-2000
- Cryovials 2 mL Cataloge no. 41121704
- Qubit tubes 0.2 mL Cataloge no. Q32856
- P1000 Wide bore pipette tips
- P1000 pipette tips
- P200 Pipette tips
- P20 pipette tips
- P10 pipette tips
- Crushed ice

Safety warnings

Safety information

- Binding Matrix contains components that, when in contact with human tissue, may cause irritation. Wear personal protective equipment to prevent contact with the skin or mucous membranes (gloves, lab coat, and eye protection).
- EtOH (100%) is a highly flammable liquid and vapour. It can cause serious eye irritation. Keep away from heat, hot surfaces, sparks, open flames and other sources of ignition.

Before start

- Dilute the concentrated SEWS-M solution with 100 mL of [M] 100 % (v/v) EtOH before use.
- Prepare 10 mL of fresh [M] 80 % (v/v) EtOH.

1. Soil sample collection and storage

1 Collect approximately 15 g of soil using a sterile soil corer or similar device and transfer to a sterile soil sieve and collection plate.

2 Homogenise the soil sample by passing it through the soil sieve and collecting the output on the collection plate below the sieve. The use of a sterile plate sealer can be used to facilitate sieve homogenisation.

3 Use a top pan balance, spatular and weigh boat to weigh 10-50 g of homogenised soil Sample and transfer to a 250 mL conical flask. Promptly suspend the soil in 100 mL of Zymo DNA/RNA shield for a final concentration of [M] 100-500 mg/mL.

4 Incubate for at least 04:00:00 at room temperature or 4 °C Overnight with occasional mixing. 16h

5 Gently mix the bulk sample by hand to form a homogenous solution then aspirate 1000 µL of the total sample using a P1000 pipette and wide bore tip. Transfer the Sample into a new and sterile 2 mL cryovial and close the lid securely.

6 Repeat step 5 until the total volume of sample has exhausted or the desired number of aliquots have been achieved.

7 Snap freeze and store Sample at -80 °C for future use.

2. DNA extraction and sample cleanup 1h 38m 15s

8 Defrost the Sample for 00:15:00 On ice prior to commencing DNA extraction. 15m

9 Transfer 1000 µL of the Sample to a new and clean 1.5 ml lo-bind Eppendorf tube using a P1000 pipette and wide bore pipette tip.

10 Centrifuge at 5000 x g, 00:04:00 to pellet the Sample . 4m

11 Aspirate and discard the supernatant without disturbing the pellet.

12 Resuspend the pellet in 978 µL of sodium phosphate buffer, then transfer the Sample to a new and clean lyzing matrix E tube.

13 Add 122 µL of MT buffer to the Sample , mix by inversion and incubate On ice for 00:05:00 . 5m

14 Place the matrix tube in a MPBio FastPrep instrument (or similar) and homogenise for 00:00:10 at 5.0 ms then return the Sample to ice. 10s

15 Incubate the Sample On ice for 00:05:00 . 5m

16 Repeat steps 14 and 15 then continue to step 17.

17 Add 250 µL of protein precipitation solution (PPS) to a new and clean 1.5 mL lo-bind Eppendorf tube and chill On ice .

18 Remove the lysing matrix-E tube from the ice and centrifuge at 14000 x g, 00:04:00 to pellet the debris. 4m

19 Decant the supernatant from the lysing matrix E tube into the pre chilled PPS without disturbing the pellet, then mix the Sample by inversion 10 times and place On ice .

20 Incubate the Sample On ice for 00:10:00 to facilitate protein precipitation. 10m

21 Remove the Sample from the ice and centrifuge at 14000 x g, 00:02:00 to pellet the protein precipitate. 2m

22 Homogenise the binding matrix immediately before use. Add 1000 µL of resuspended binding matrix to a clean 5 mL lo-bind tube.

Safety information

- Binding Matrix contains components that, when in contact with human tissue, may cause
irritation. Wear personal protective equipment to prevent contact with the skin or mucous
membranes (gloves, lab coat, and eye protection).

23 Gently decant the Sample directly into the binding matrix without disturbing the protein pellet, secure the lid and mix the Sample by inversion until a homogeneous solution has formed.

24 Incubate the Sample on a rotational mixer at 30 rpm, Room temperature , 00:10:00 . 10m

25 Add 1000 µL of DES to a new and clean 1.5 mL lo-bind tube and place the tube into an active heat block set to 56 °C .

26 Remove the Sample from the rotational mixer and transfer 750 µL of sample to an MPbio spin filter using a wide bore P 1000 pipette tip. Return the Sample to the rotational mixer.

27 Centrifuge the spin filter at 14000 x g, 00:02:00 and discard flow through. 2m

28 Repeat steps 26 and 27 until all the binding matrix has been processed through the spin filter.

29 Add 500 µL of prepared SEWS-M buffer to the Sample bound binding matrix, close the lid securely and suspend the beads in the SEWS-M solution by flicking the tube.

Safety information

EtOH (100%) is a highly flammable liquid and vapour. It can cause serious eye irritation. Keep away from heat, hot surfaces, sparks, open flames and other sources of ignition.

30 Centrifuge the Sample at 14000 x g, 00:02:00 then discard the flow through. 2m

31 Repeat steps 29 and 30 then continue to step 32.

32 Centrifuge the Sample at 14000 x g, 00:02:00 to collect excess EtOH. 2m

33 Remove the spin filter from the catch tube and place it into a new and clean 1.5 mL lo-bind Eppendorf tube.

34 Allow the binding matrix to air dry for 00:02:00 . 2m

35 Add 100 µL of 56 °C DES elution buffer to the binding matrix and agitate the spin filter until a slurry has formed.

36 Incubate the Sample at 56 °C for 00:10:00 with intermittent agitation. 10m

37 Centrifuge the Sample at 14000 x g, 00:02:00 . Discard the spin filter and retain the Sample eluate. 2m

38 Allow the Sample to equilibrate to Room temperature and add 1 µL RNase A at [M] 20 mg/mL. Mix tube by flicking and then incubate at Room temperature for 00:02:00. 2m

39 Add 60 µL of Room temperature Ampure XP beads to the Sample and mix by flicking the tube until a homogeneous solution has formed.

40 Incubate the Sample on a rotational mixer for 00:10:00 at Room temperature. 10m

41 Briefly spin down the Sample and place on a magnetic rack.

42 Once the Sample has cleared, aspirate and discard the supernatant without disturbing the beads.

43 Gently add 200 µL of [M] 80 % (v/v) EtOH across the beads and incubate on the magnetic rack for at least 00:00:30. 30s

44 Aspirate and discard the supernatant without disturbing the beads.

45 Repeat steps 43 and 44 then continue to step 46.

46 Remove the Sample from the magnetic rack and briefly centrifuge at < 1000 x g to collect any residual EtOH then return the Sample to the magnetic rack. 5s

47 Promptly remove the excess EtOH with a P10 pipette and tip without disturbing the beads.

48 Allow the beads to air dry for 00:00:30 or until the beads are satin in appearance. Do not over dry the beads, avoid excess EtOH carry over. 30s

49 Remove the Sample from the magnetic rack and add 46 µL of molecular grade water to the beads.

50 Resuspend the beads in the water by flicking the tube until a homogenous solution has formed.

51 Incubate the Sample at 37 °C for 00:10:00 then return the Sample to the magnetic rack. 10m

52 Once the solution has cleared, Quantify 1 µL of Sample using a Qubit 4 fluorometer or similar device.

PacBio SPK 2.0 library preparation 5h 6m

53 DNA blunting

Prepare the following reaction on ice. Scale the reaction volume by sample number.

	A	B
	Reagent	**Volume (µl)**
	Enzyme Dilution Buffer	4.0
	DNA Prep Additive	1.0

54 Prepare the following reaction in a 0.2 ml thin walled PCR tube for each sample and return to ice. Use DNA Prep Additive from step 53.

	A	B
	Reagent	**Volume (µl)**
	DNA	45.0
	DNA Prep Buffer	7.0

	A	B
	NAD	1.0
	DNA Prep Additive	1.0
	DNA Prep Enzyme	1.0
	Total	**55**

55 Mix the sample by flicking and spin down to collect.

56 Incubate the Sample in a thermal cycler at 37 °C for 00:15:00 then place the sample On ice . 15m

57 DNA damage repair (FFPE).
Add 2 µL of DNA damage repair mix v2 to each Sample and mix by flicking then spin down to collect.

58 Incubate at 37 °C for 00:30:00 then place the Sample On ice . 30m

59 End-repair and A-tailing.
Add 3 µL of End Prep Mix to each Sample mix by flicking and spin down to collect.

60 Incubate the Sample at 20 °C for 00:30:00 , then at 65 °C for 00:30:00 then place the sample On ice 1h

61 SMRT bell adapter ligation
Defrost one barcoded overhang SMRT bell adapter per sample, mix by flicking, spin down and place On ice

62 Add 5 µL of a single barcoded overhang SMRT bell adapter to each Sample , mix by flicking, spin down and place On ice .

63 Add 30 µL of Ligation mix, 1 µL of Ligation aditive and 1 µL of Ligation Enhancer to each sample, mix by flicking, spin down and return to ice.

64 Incubate the sample at 20 °C for 01:00:00 then at 4 °C for up to 16 h. 1h

65 Incubate the Sample at 65 °C for 00:10:00 then place On ice . 10m

66 Nuclease treatment

Prepare the following reaction and place on ice. Scale the reaction volume by sample number.

	A	B
	Reagent	Volume (µl)
	Enzyme A	4.0
	Enzyme B	1.0
	Enzyme C	1.0
	Enzyme D	2.0

67 Add 8 µL of Enzyme mix to eacg Sample and mix by flicking then spin down to collect.

68 Incubate at 37 °C for 01:00:00 then place the sample On ice . 1h

69 Spri cleanup.
Add 47.25 µL (0.45x) of SPRI beads at Room temperature and mix by flicking.

70 Incubate on a rotational mixer for 00:10:00 . 10m

71 Briefly spin to collect the Sample .

72 Place the sample on a magnetic rack and allow the solution to fully clear.

73 Aspirate and discard the supernatant without disturbing the beads.

74 Add 200 µL of [M] 80 % (v/v) EtOH across the beads and allow to sit for 00:00:30 . 30s

75 Aspirate and discard the EtOH.

76 Repeat steps 74 and 75, then continue.

77 Spin down to collect the tube contents and return to the magentic rack.

78 Promptly remove residual EtOH with a pippette.

79 Add 31 µL of PB 1X Elution buffer to the beads and mix by flicking.

80 Incubate the Sample at 37 °C for 00:30:00 with occasional mixing. 30m

81 Place sample on a magnetic rack and allow the sample to clear.

82 Aspirate 31 µL of Sample and transfer to a new 1.5 ml lo-bind tube.

83 Add 1 µL of Sample to 9 µL of water and mix by flicking.

84 Quantify 2 µL of diluted sample using a Qubit fluorometer and HS assay kit.
Quantify 1 µL of diluted library on a tape station or similar fragment analyser.

85 Dilute SPRI bead clean up

Add 350 µL of SPRI beads to 650 µL of 1 x PB elution buffer and mix by flicking.

86 Dilute the Sample to < 10 ng/µL with PB elution buffer.

87 Add 3.7 x volume of Sample of dilute SPRI beads to the Sample and mix by flicking.

88 Place on a rotational mixer for 00:30:00 at Room temperature

89 Briefly spin the sample to collect and place on a magnetic rack, then allow the sample to clear.

90 Aspirate and retain the supernatant in a new 1.5 lo-bind tube.

91 Add 250 µL of [M] 80 % volume EtOH across the beads and allow to sit for 00:00:30 . 30s

92 Remove and discard the EtOH without disturbing the beads.

93 Repeat the wash step and briefly spin to collect residual EtOH.

94 Remove residual EtOH then add 21 µL of 1x PB elution buffer to the beads and mix by flicking.

95 Incubate the Sample at 37 °C for 00:30:00 with occasional mixing then return to the magnetic rack. 30m

96 Dilute 1 µL of final library in 9 µL of molecular grade water and retain for QC.

97 Aspirate 20 µL of final library to a new 1.5 ml lo-bind tube and place on ice.

98 Store final library at 4 °C for imminent SMRT cell loading or a -20 °C for longterm storage.