MAR 22, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.81wgby4m1vpk/v1

External link:
https://www.zymoresearch.com/

Protocol Citation: izabel.stohel@pnnl.gov 2023. DNA Extraction . **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgby4m1vpk/v1

Protocol status: Working

Created: Jan 18, 2023

Last Modified: Mar 22, 2023

PROTOCOL integer ID:
75433

DNA Extraction

In 2 collections

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ABSTRACT

A list of all protocols used, from Zymo Research with slight modification. Additionally, protocols for measurement of purity and concentration are listed as well.

D6010 is used when there are only a handful of samples and we use tubes, with the corresponding clean and concentrate protocol being D4013 . D4302, D4306, D4308 is the protocol used when there are a large number of samples and we are using a plate, and the corresponding clean and concentrate protocol is D4066. For bottoms of cores that have less DNA, protocol D6110 has been effective.

Keywords: DNA, Extraction, Zymo research, zymoresearch, D6010, D4013, D4066, D6110, clean, clean and concentrate, concentration, purity, nanodrop, qubit, D4302, D4306, D4308, plate protocol, epMotion, eppendorf, tubes

MATERIALS

Labels
1.5mL microcentrifuge tubes
Soil Sample
ZR Bashing Bead Lysis Tube
ZymoBIOMICS lysis solution
ZymoBIOMICS MagBinding Buffer
ZymoBIOMICS™ MagWash 1
ZymoBIOMICS™ MagWash 2
Silicon-A™ Plate
Collection Plate
DNA Pre-Wash Buffer
g-DNA Wash Buffer
Silicon-A™-HRC Plate
Elution Plate
Prep Solution
DNA Elution Buffer
DNase/RNase Free Water
Zymo-Spin III-F filter
Collection Tube
Genomic Lysis Buffer
Ethanol
Zymo-Spin IICR filter
Zymo-Spin III-HRC filter
ChIP DNA Binding Buffer
Zymo-Spin column
Zymo-Spin I-96-XL Plate
Qubit Buffer
Qubit Standards
bead/filter chamber of a ZR BashingBead Lysis/Filtration Tube
BashingBead buffer
50mL tube
Zymo-Spin V-E Column / Zymo-Midi Filter

DNA Extraction Protocol by Hand: Quick DNA Fecal/Soil Mic...

1 Print Labels for 1.5mL tubes (“Monet, Sample ID, Core Location, DNA")

2 Measure 200mg of soil in ZR Bashing Bead Lysis Tube, do not exceed 200mg as to not overwhelm the tube

3 Remove all the lids and add 750uL of ZymoBIOMICS lysis solution

4 Recap and put in a bead beater for 10 minutes

5 Centrifuge ZR Bashing Bead Lysis Tube in a microcentrifuge at 16,000 x g for 1 minute

6 Pipette 350uL of the solution in Zymo-Spin III-F filter, placed in a collection tube. Pipette the remaining solution into another Zymo-Spin III-F filter with collection tube. Now you have two sets of DNA from one original sample that can be combined later

7 Centrifuge at 8,000 x g for one minute (If you get a pellet, pipette supernatant to a fresh tube)

8 Discard the filter

9 Add 800uL of Genomic Lysis Buffer and 400uL of ethanol to the filtrate. Mix well

10 Transfer the solution to a Zymo-Spin IICR filter and collection tube. (Note: You cannot fit the entire solution in the filter, you will need to do the following steps at least twice:)

11 Centrifuge at 10,000 x g for 1 minute. Discard flow through

12 Add the rest of the solution from step 9 and repeat step 11

13 Put the filter in a new collection tube

14 Add 200uL of DNA pre-wash buffer to the Zymo-Spin IICR Column and centrifuge at 10,000 x g for 1 minute

15 Add 500uL of g-DNA wash buffer to the Zymo-Spin IICR Column

16 Centrifuge at 10,000 x g for 1 minute. Discard flow through

17 Repeat step 16

18 Get a new collection tube for the filter and change gloves

19 Add 100uL of elution buffer to the filter and let incubate at room temperature for 1 minute

20 Centrifuge at 10,000 x g for 1 minute

21 Add 600uL of prep solution to a new III-HRC filter with a collection tube and let incubate for 3 minutes. Then centrifuge at 8000 x g for 3 minutes. Discard flow through.

22 Put the III-HRC filter into a 1.5mL microcentrifuge tube with cap.

23 Discard the filter from step 19 and pipette the eluted DNA to the prepared III-HRC filter

24 The filter may be discarded and the eluted DNA is now in the 1.5mL microcentrifuge tube

25 Centrifuge at 16,000 x g for 3 minutes

Measuring Concentration and Purity with Nanodrop

26 Select dsDNA and follow the prompts from the Nanodrop.

26.1 Use 2uL of nuclease free water as the blank before you begin measuring samples

26.2 Once the blank has been calibrated use 2uL of sample to measure the concentration and purity

Measuring Concentration and Purity with Qubit

27 Set up two standards, (Note: keep buffer away from the light as much as possible)

27.1 Measure 190uL of buffer and 10uL of standard in Qubit measurement tube

28 Vortex the tubes briefly

29 Incubate in the dark for two minutes

30 Measure 198uL of buffer and 2uL of sample, vortex briefly and incubate in the dark for two minutes

31 Follow Qubit prompts and measure standards and then sample

DNA Clean and Concentrater-5 Protocol D4013

32 Add 200uL of DNA Binding Buffer to the eluted DNA

33 Transfer the mixture to a Zymo-Spin column in a collection tube. If you have multiple sets of DNA, combine them in this step

34 Centrifuge for 30 seconds at 16000 x g and discard flow through

35 Add 200uL of DNA Wash Buffer centrifuge for 30 seconds at 16000 x g then repeat this step again.

36 Put the filter in a 1.5mL microcentrifuge tube with cap

37 Add 100uL of DNA Elution Buffer to the filter, let incubate for 1 minute at room temperature

38 Centrifuge at 16000 x g for 1 minute. Eluted DNA is now ready for use

DNA Extraction 96 Plate (With Eppendorf epMotion) D4302,..

39 Print Labels for 1.5mL tubes (“Monet, Sample ID, Core Location, DNA) place on ZR BashingBead

Lysis Rack

40 Measure 200mg of soil in ZR Bashing Bead Lysis Tube, do not exceed 200mg as to not overwhelm the tube

41 Remove all the lids and add 750uL of ZymoBIOMICS lysis solution

42 Recap and put in a bead beater for 10 minutes

43 Centrifuge ZR Bashing Bead Lysis Tube in a microcentrifuge at 16000 x g for 1 minute

44 Add 600uL of ZymoBIOMICS MagBinding Buffer and 25uL of the beads to 3 96 well plates. Mix well

45 Add 200uL of supernatant to each 96 well plate so you have 3 sets of the sample

46 Load plate in the Eppendorf epMotion and begin protocol: (Note: since there are three plates, epMotion can only one run one a day, so this will take three days to complete)

46.1 a. Transfer the 96-well block to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand

46.2 b. Dispense 500 µl of ZymoBIOMICS™ MagBinding Buffer and mix well by pipette

46.3 c. Transfer the 96-well block to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.

46.4 d. Dispense 500 µl of ZymoBIOMICS™ MagWash 1 and mix well by pipette

46.5 e. Transfer the 96-well block to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.

46.6 f. Dispense 900 µl ZymoBIOMICS™ MagWash 2 and mix well by pipette or shaker plate for 1 minute.

46.7 g. Transfer the deep-well block to a magnetic stand until beads pellet, then aspirate and discard the supernatant. Remove the 96-Well Block from the magnetic stand.

46.8 h. Repeat the wash (Steps 46.6-46.7).

46.9 i. Transfer the 96-Well Block onto a heating element (55°C) until beads dry (approximately 30 minutes).

46.10 j. Heat again at 30°C for 30 minutes

46.11 k. Dispense 50 µl of ZymoBIOMICS™ DNase/RNase Free Water to each well and re-suspend beads. Mix the beads well for 10 minutes and then transfer the plate onto the magnetic stand for 2-3 minutes until the beads pellet.

46.12 l. Transfer the supernatant (containing the eluted DNA) to a clean elution plate or tube. The eluted DNA can be used immediately for molecular based applications or stored ≤ -20ºC for future use.

ZR-96 Genomic DNA Clean and Concentrator-5 Kit Protocol ...

47 Centrifuge plates at 3486 x g for 3 minutes before you start

48 Add 200uL of ChIP DNA Binding Buffer to each volume of DNA sample

49 Mix well and transfer mixtures to the provided Zymo-Spin I-96-XL Plate mounted on a collection plate and centrifuge for 5 minutes at 3486 x g

50 Repeat step 49 with duplicate samples

51 Add 200uL of DNA Wash Buffer and centrifuge for 5 minutes at 3486 x g

52 Centrifuge again to ensure all solution has filtered through to the collection plate

53 Put Zymo-Spin I-96-XL Plate on an Elution Plate and add 50uL of Elution Buffer

54 Centrifuge for 5 minutes at 3486 x g to elute the DNA.

50mL Tube DNA Extraction Protocol D6110

55 Print Labels for 50mL tubes (“Monet, Sample ID, Core Location, DNA). Add 2.5-5g of soil to the bead/filter chamber of a ZR BashingBead Lysis/Filtration Tube

56 Add 6mL of BashingBead buffer

57 Shake on bead beater for ten minutes

58 Centrifuge at 3486 x g for five minutes

59 Transfer the supernatant to a clean 50mL tube, there is likely a pellet

60 Add 18mL of Genomic Lysis Buffer to supernatant and mix well

61 Filter entire mixture using Zymo-Spin V-E Column / Zymo-Midi Filter with a new 50mL tube, Centrifuge at 2000 x g for five minutes

62 Repeat step 61, because the mixture won't all fit the first time

63 Centrifuge at 2000 x g for five minutes once more to ensure the solution has entirely filtered

64 Transfer the Zymo-Spin V-E Column to a collection tube and spin at 10000 x g for 1 minute in a microcentrifuge. (The filter disconnects from the column)

65 Add 300uL of DNA Pre-Wash Buffer to the filter and spin at 10000 x g for 1 minute and discard flow through

66 Add 400uL of g-DNA wash Buffer to the column and centrifuge at 10000 x g for 1 minute, discard flow through

67 Repeat step 66

68 Place Zymo-Spin III-HRC in a clean collection tube and add 600uL of prep solution. Let incubate for 3 minutes then centrifuge at 8000 x g for 3 minutes

69 Transfer the Zymo-Spin III-HRC filter to a 1.5mL microcentrifuge tube

70 Transfer the Zymo-Spin V-E Column to a clean collection tube and add 150uL of Elution Buffer directly to the column matrix, let incubate for a minute and then centrifuge at 10000 x g for 1 minute

71 Transfer the eluted DNA to the prepared III-HRC filter and centrifuge at 16000 x g for 3 minutes. Eluted DNA is ready for use.