Sep 07, 2022

Integra Magbead DNA and RNA Extraction for isolated colonies

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dx.doi.org/10.17504/protocols.io.dm6gpjeqjgzp/v1

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ABSTRACT

This protocol is the process to extract DNA and RNA from isolated colonies. The extracted high-quality DNA or RNA are suitable for Next-Generation Sequencing (NGS).

DOI

dx.doi.org/10.17504/protocols.io.dm6gpjeqjgzp/v1

PROTOCOL CITATION

Sophana Chea, Sreyngim Lay, Mengheng Oum, Gechlang Tang, Cheata Hou, Manu Vanaerschot, Christina Yek, Cristina Tato, Jessica Manning, Vida Ahyong 2022. Integra Magbead DNA and RNA Extraction for isolated colonies . **protocols.io** https://protocols.io/view/integra-magbead-dna-and-rna-extraction-for-isolate-cf4mtqu6

KEYWORDS

Integra, DNA, RNA, Colony, isolated, Extraction

CREATED

Sep 02, 2022

LAST MODIFIED

Sep 07, 2022

PROTOCOL INTEGER ID

69485

GUIDELINES

Adapted from the ZymoBIOMICS MagBead DNA/RNA Kit Manual (Zymo Research, Cat#R2135).

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MATERIALS TEXT

1. RNase away spray for RNase decontaminants.
RNase AWAY™ Surface Decontaminant **Thermo Fisher Scientific Catalog #7002PK**

ZymoBIOMIC MagBead DNA/RNA **Zymo**
2. **Research Catalog #R2135**
3. 100% Molecular grade ethanol **Contributed by users**
4. Molecular Grade Isopropanol **Contributed by users**

Proteinase K w/ Storage buffer 20mg set **Zymo**
5. **Research Catalog #D3001-2-20**

DNase I Set **Zymo**
6. **Research Catalog #E1010**

Nuclease-free water
7. **Ambion Catalog #AM9932**

8. 1ml deep well sterile plate.

9. 2ml deep well sterile plate.

10. Hard-shell PCR Plates 96 V-well (Bio-Rad, Cat# HSP9601).

11. PCR Plate Seal, foil (Bio-Rad, Cat# MSF1001).

12. 96S Super Magnet. (ALPAQUA, Cat# A001322)

VIAFLO
96 channel pipette
Integra **VIAFLO 96**

SAFETY WARNINGS

All steps should be performed at **Room temperature** .

Perform the extraction in the extraction room separate from the PCR room.
Respect the Laboratory safety guideline for all steps of the protocol.
Wearing PPE is recommended.

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** When reusing tips, make sure to include a bit of extra air aspiration to avoid drops at the bottom of tips when aspirating volumes, and also a bit of extra air blows out at the end of dispensing steps in plates.

Buffer Preparation 30m

30m

1 1. Add **20 mL** isopropanol to the MagBead DNA/RNA Wash 1 concentrate.

2. Add **30 mL** isopropanol to the MagBead DNA/RNA Wash 2 concentrate.

3. Reconstitute lyophilized Proteinase K at **20 mg/mL** with Proteinase K Storage Buffer and mix by vortexing. Use immediately or store at **-20 °C** .

4. Reconstitute each vial of lyophilized DNase I with **2.25 mL** DNase/RNase-Free water in a conical tube.

For each sample to be treated, prepare DNase I Reaction Mix (scale up proportionally):
Add **45 µL** DNase I (reconstituted) and **5 µL** DNA Digestion Buffer in a nuclease-free tube.
mix by gentle inversion and place **On ice** until ready to use.

Make buffer plates prior to starting protocol 1h

1h

2 1. Pre-make Lysis Buffer plate with **520 µL** DNA/RNA Lysis buffer in 1ml deep well plate.

2. Pre-make Beads plate with **35 µL** ZymoBIOMIC MagBinding Beads into 96 V-well PCR plate.

For the Beads plate, make it immediately before starting, <1h prior to starting the protocol, to ensure the beads are kept in suspension.

3. Pre-make DNA/RNA Wash 1 plate with **520 µL** MagBead DNA/RNA Wash 1 into 1ml deep well plate. Make it two plates.

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4. Pre-make DNA/RNA Wash 2 plate with **520 µL** MagBead DNA/RNA Wash 2 into 1ml deep well plate. Make it two plates.
5. Pre-make 100% Ethanol plate with **1100 µL** of 100% Ethanol into a 2ml deep well plate. Make it three plates.
6. Pre-make Prep Buffer plate with **520 µL** DNA/RNA Prep Buffer into a 1ml deep well plate.
7. Pre-make water plate with **60 µL** Nuclease-free water in a 96 V-well PCR plate. Make it two plates.
8. Spin all plates down for **00:01:00** except for the bead plate. Perform a quick pulse spin down of the bead plate, just enough to get all the liquid down. Centrifuge the rest of the plate at 12 000 rpm for **00:01:00** .

Sample preparation and Proteinase K 31m

3 31m

1. Create a plate map so you know which sample you are adding to each well. Add **50 µL** of isolated colonies samples to plate 1 (leave column 12 for water control).
2. Top up the 1x DNA/RNA Shield to get **750 µL** .
3. Manually add **120 µL** of Proteinase K into the 0.2ml 8-strip well.
4. Use multichannel pipet to add **10 µL** of Proteinase K into each sample and mix (plate 1).
5. Load a set of Integra tips (tip set 1) onto the Integra.
6. **Program: Pipet/Mix 250ul, 15 cycles, speed 4.** Program the Integra to pipet **250 µL** of your samples up and down for **00:01:00** (15 cycles), then incubate at **Room temperature** for **00:30:00** . Keep tips.

Sample binding and washing 35m

4 35m

7. **Program: Pipet 250ul.** Add **500 µL** total of Lysis Buffer to the sample plate (plate 1).
8. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix samples and buffer for **00:02:00** . Keep tips.
9. Aliquot **35 µL** of MagBinding Beads into 96 V-well PCR plate.
10. **Program: Pipet/Mix 20ul, 10 cycles, 2 times, speed 4.** Program the Integra to mix the MagBinding Beads plate, so the beads are fully resuspended.
11. **Program: Pipet 30ul.** Add **30 µL** of MagBinding Beads into the sample plate (plate 1).
12. **Program: Pipet/Mix 250ul, 30 cycles, speed 3.** Program the Integra to mix the sample and MagBinding Beads plate, so the beads are fully resuspended. Continue this Integra Program to mix the sample and MagBinding Beads for **00:20:00** .
13. Transfer the plate/tube to the magnetic stand for **00:05:00** until beads (DNA) have pelleted, transfer the cleared supernatant (RNA) into a new 96 V-well plate.

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DNA Purification (Beads) 45m

5 45m

14. Change new Integra tips.
15. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of **500 µL** MagBead DNA/RNA Wash 1 into sample plate and mix well.
16. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the Wash 1 buffer with the beads. Keep tips.
17. Place the 96-well magnetic stand underneath the sample plate for **00:02:00** until a bead ring forms.
18. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
19. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of **500 µL** MagBead DNA/RNA Wash 2 into sample plate and mix well.
20. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the Wash 2 buffer with the beads. Keep tips.
21. Place the 96-well magnetic stand underneath the sample plate for **00:02:00** until a bead ring forms.
22. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
23. Change new Integra tips.
24. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of **500 µL** 100% Ethanol into sample plate and mix well.
25. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix 100% Ethanol with the beads. Keep tips.
26. Place the 96-well magnetic stand underneath the sample plate for **00:02:00** until a bead ring forms.
27. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
28. Repeat step 24.
29. Dry the beads for **00:10:00** on the magnetic stand.
30. Change new Integra tips.
31. **Program: Pipet 30ul, speed 5.** Dispense a total of **30 µL** nuclease-free water into the sample plate.
32. **Program: Pipet/Mix 20ul, 30 cycles, speed 7.** Program the Integra to mix nuclease-free water with the beads. Keep tips.
33. **Program: Manual Pipet 30ul, speed 3.** Transfer the plate to the magnetic stand and pellet the beads for **00:05:00** , then aspirate and dispense the eluted DNA to a new 96 V-well plate.
34. Store DNA sample immediately at **-80 °C** .

RNA Purification (Supernatant) 45m

45m

35. Change the new Integra tip.

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36. **Program: Pipet 230ul, 3 times, speed 7.** Dispense a total of **690 µL** 100% Ethanol to the supernatant.
37. **Program: Pipet/Mix 250ul, 30 cycles, speed 7.** Program the Integra to mix 100% Ethanol with the supernatant. Keep tips.
38. Aliquot **35 µL** of MagBinding Beads into 96 V-well PCR plate.
39. **Program: Pipet/Mix 20ul, 10 cycles, 2 times, speed 4.** Program the Integra to mix the MagBinding Beads plate, so the beads are fully resuspended.
40. **Program: Pipet 30ul.** Add **30 µL** of MagBinding beads into the sample plate.
41. **Program: Pipet/Mix 250ul, 10 cycles, speed 3.** Program the Integra to mix the sample and MagBinding beads
plate, so the beads are fully resuspended. Continue this Integra Program to mix the sample and MagBinding Beads for **00:10:00** .
42. Transfer the plate to the magnetic stand for **00:05:00** until beads have pelleted, then discard the cleared supernatant.
43. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of **500 µL** MagBead DNA/RNA Wash 1 into sample plate.
44. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the Wash 1 buffer with the beads. Keep tips.
45. Place the 96-well magnetic stand underneath the sample plate for **00:02:00** until a bead ring forms.
46. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
47. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of **500 µL** MagBead DNA/RNA Wash 2 into sample plate.
48. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the Wash 2 buffer with the beads. Keep tips.
49. Place the 96-well magnetic stand underneath the sample plate for **00:02:00** until a bead ring forms.
50. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
51. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of **500 µL** 100% Ethanol into the sample plate.
52. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix 100% Ethanol with the beads. Keep tips.
53. Place the 96-well magnetic stand underneath the sample plate for **00:02:00** until a bead ring forms.
54. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
55. Repeat step 51.
56. **DNase I** treatment, use multiple channel pipet to transfer **50 µL** of DNase I Reaction Mix and mix gently for **00:10:00** .

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57. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of **500 µL** DNA/RNA Prep Buffer into sample plate.
58. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the DNA/RNA Prep Buffer with the beads. Keep tips.
59. Place the 96-well magnetic stand underneath the sample plate for **00:02:00** until a bead ring forms.
60. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
61. Repeat step 57 to 60.
62. **Program: Pipet 30ul, speed 5.** Dispense a total of **30 µL** nuclease-free water into the sample plate.
63. **Program: Pipet/Mix 20ul, 30 cycles, speed 7.** Program the Integra to mix nuclease-free water with the beads. Keep tips.
64. **Program: Manual Pipet 30ul, speed 3.** Transfer the plate to the magnetic stand and pellet the beads for **00:05:00** , then aspirate and dispense the eluted RNA to a new 96 V-well plate.
65. Store RNA sample immediately at **-80 °C** .

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