High-Throughput RNA Extraction on Agilent Bravo

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We use this protocol and it's working

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ABSTRACT

Extracting bulk-RNA from biospecimens is a time-consuming process. Column-based extractions performed by hand must be done in batches and the number of samples that can be extracted per batch is dependent on a single person's abilities. Hence, extraction hundreds of samples can take weeks. With every round of extractions, reagents are susceptible to differing amounts of contamination and technical error, resulting in batch effects. Microbial RNA input for metagenomic next-generation sequencing is often found in very low quantities; therefore, contamination introduced during extraction may influence sequencing results and depict an inaccurate reflection of microbes present in the original sample.

Here, we outline a high-throughput, semi-automatic method to extract RNA using a Agilent Bravo liquid handler. This protocol utilizes a magnetic bead-based approach to extracting RNA and is performed in 96-well deep-well plates. All reagents are plated into 96-well deep-well plates before the start of the Bravo protocol. The liquid handler is programmed to aliquot, mix, and aspirate reagents from different decks of plates. The user is only responsible for putting these plates on the correct decks.

With this approach, we can extract a full plate of 96 samples within a day and reduce the susceptibility to contamination from user error.

Keywords: RNA, bead extraction, Bravo, RNA extraction, magbinding beads, magnetic bead extraction

GUIDELINES

Bravo protocols are optimized to operate specific depths that are dependent on plate type and manufacturer. For best practice, consistently use the same plates and tips from the same manufacturer to avoid pipetting errors. Even if the plates/tips are thought to be technically identical (based on dimensions), errors can occur.

This Bravo protocol was optimized for nasopharyngeal swabs processed with RNA Shield and bead bashed (before plating). To extract blood (PAXgene), plasma, BAL, etc., modifications such as centrifuging and/or Proteinase K treatment are required. Inquire with a member of Zinter team for specifics.

The plate shaker is a Bravo attachment that is controlled manually. The shaker in the Derisi Lab is attached to DECK 5.

The time required to pellet Magbinding beads may differ based on the type of magnet used. We use ring magnets to cluster the beads. Optimize before starting.

MATERIALS

1. Zymo Quick RNA Mag Bead Kit Cat. No. R2113
2. Bravo Lab Disposable Pipette Tips; Part Number: 19477-022; Tips, 250 µL, sterile, filtered, 96 in rack, case of 50, compatible with Bravo 96LT head
3. 96 Deep well, 2ml/well, Polypropylene, Round well, Round bottom, DNase/ RNase free Cat. No. awls-219021
4. Fisherbrand 96 Well 1 mL Deep Well Polypropylene Microplates Cat No. 12-566-611
5. BioRad Hard-Shell® 96-Well PCR Plates Cat. No. HSP9601

BEFORE START INSTRUCTIONS

For your sample type, ensure that it is processed/homogenized before plating into sample plate. Inquire with a member of Zinter team for specifics or refer to the Zymo kit manual.

Same Day Reagent Preparation

1

1. Plate and seal all reagents in the biosafety cabinet on the same day you plan to run the procedure with the following volumes:

	A	B	C	D
	Reagent	**Volume per well**	**Plate Quantity**	**Plate**
	Lysis Buffer	630 uL	1	1 mL deep-well

	A	B	C	D
	Magbeads	35 uL	1	Biorad 96-well PCR
	Ethanol	900 uL	1	1 mL deep-well
	Ethanol	600 uL	4	1 mL deep-well
	RNA Wash 1	530 uL	1	1 mL deep-well
	RNA Wash 2	530 uL	1	1 mL deep-well
	Prep Buffer	530 uL	1	1 mL deep-well
	Nuclease-free Water	50 uL	1	Biorad 96-well PCR
	DNase*	55 uL	1	Biorad 96-well PCR
	Initial Sample**	200 uL	1	2 mL deep-well
	Elution Plate	0 uL	1	Biorad 96-well PCR
	Waste	0 uL	3	2 mL deep-well
	Waste	0 uL	1	1 mL deep-well

* Prepare same day as experiment. When preparing DNAse, mix 54 uL DNAse and 6 uL of Digestion Buffer for n +1 samples to be extracted. Mix in a tube by inverting and proceed to plate the DNAse. Once plated, immediately place on ice.

** When preparing initial sample, plate and immediately place on ice.

Bravo Set-Up

2 To begin Bravo set up, switch the instrument on BEFORE opening bravo software (wait about about a minute). NP Swab protocol in DeRisi Lab : **GAR_magbead_v6.pro**

3 Retrieve 11 tip boxes, spray down Bravo decks and biosafety cabinet first with 70% ethanol, then with RNAse-zap/Windex, and again with ethanol.

Bravo Protocol (Pre-Programmed)

4 Place RNA Lysis Buffer on DECK 1, MagBinding beads on DECK 2, Sample plate on DECK 5, Waste (2 mL) on DECK 8, and Tip Boxes on DECKS 3 and 9.

5 Transfer 600 µl RNA Lysis Buffer to Sample plate.
a. This is done in 4 x 150 µl transfers
b. Mix and shake Sample plate on DECK 5 for 5 minutes (1/min)

6 Place RNA Lysis Buffer on DECK 1, MagBinding beads on DECK 2, Sample plate on DECK 5, Waste (2 mL) on DECK 8, and Tip Boxes on DECKS 3 and 9.

7 Transfer 600 µl RNA Lysis Buffer to Sample plate.
a. This is done in 4 x 150 µl transfers
b. Mix and shake Sample plate on DECK 5 for 5 minutes

8 Replace RNA Lysis Buffer on DECK 1 with Ethanol (900 ul).

9 Transfer 800 µl Ethanol to the sample and mix well.
a. This is done in 6 x 133.4 µl transfers.
b. Mix and shake Sample plate gently on DECK 5 for 5 minutes (half speed).

10 Remove seal from MagBinding Beads plate. Replace tip boxes on DECK 3 and DECK 9 with new boxes.

11 Transfer 30 µl MagBinding Beads to Sample plate.
a. Important: MagBinding Beads settle quickly, ensure that beads are kept in suspension while dispensing. This can be done by adding a mixing step to the MagBinding Beads plate before transferring to the Sample plate.
b. Mix and shake Sample plate gently on DECK 5 for 20 minutes (half speed).

12 Move the Sample plate/tube to the magnetic stand at DECK 7. Incubate for 4 minutes until beads

have pelleted.

13 Aspirate and discard the cleared supernatant.
a. This is done in 11 x 145.5 µl transfers.

14 Move Sample plate back to DECK 5. Replace MagBinding Beads with RNA Wash 1 on DECK 2. Replace Waste (2 ml) plate on DECK 8 with an empty Waste (2 ml) plate.

15 Transfer 500 µl RNA Wash 1 to the Sample plate.
a. This is done in 4 x 125 µl transfers.
b. Mix and shake Sample plate on DECK 5 for 5 minutes

16 Move the Sample plate/tube to the magnetic stand at DECK 7. Incubate for 4 minutes until beads have pelleted.

17 Aspirate and discard the cleared supernatant.
a. This is done in 4 x 125 µl transfers.

18 Move Sample plate back to DECK 5. Replace RNA Wash 1 with RNA Wash 2 on DECK 2. Replace tip boxes on DECK 3 and DECK 9 with new boxes.

19 Transfer 500 µl RNA Wash 2 to the Sample plate.
a. This is done in 4 x 125 µl transfers.
b. Mix and shake Sample plate on DECK 5 for 5 minutes

20 Move the Sample plate/tube to the magnetic stand at DECK 7. Incubate for 4 minutes until beads have pelleted.

21 Aspirate and discard the cleared supernatant.
a. This is done in 4 x 125 µl transfers.

22 Move Sample plate back to DECK 5. Replace Ethanol (900 ul) with Ethanol 1/4 on DECK 1.

23 Transfer 500 µl Ethanol 1/4 to the Sample plate.
a. This is done in 4 x 125 µl transfers.
b. Mix and shake Sample plate on DECK 5 for 5 minutes

24 Move the Sample plate/tube to the magnetic stand at DECK 7. Incubate for 4 minutes until beads have pelleted.

25 Aspirate and discard the cleared supernatant.
a. This is done in 4 x 125 µl transfers.

26 Move Sample plate back to DECK 5. Replace Waste (2 ml) plate on DECK 8 with an empty Waste (2 ml) plate. Replace Ethanol 1/4 with Ethanol 2/4 on DECK 1. Replace tip boxes on DECK 3 and DECK 9 with new boxes.

27 Transfer 500 µl Ethanol 2/4 to the Sample plate.
a. This is done in 4 x 125 µl transfers.
b. Mix and shake Sample plate on DECK 5 for 5 minutes

28 Move the Sample plate/tube to the magnetic stand at DECK 7. Incubate for 4 minutes until beads have pelleted.

29 Aspirate and discard the cleared supernatant.
a. This is done in 4 x 125 µl transfers.

30 Move Sample plate back to DECK 5. Replace RNA Wash 2 with DNase plate on DECK 2 and confirm it is unsealed. Replace tip boxes on DECK 3 and DECK 9 with new boxes.

31 Transfer 50 µl DNase treatment to the Sample platea. Mix and shake Sample plate gently on DECK 5 for 10 minutes (half speed).

32 Replace DNase plate on DECK 2 with Prep Buffer plate.

33 28. Transfer 500 µl Prep Buffer to Sample plate.
a. This is done in 4 x 125 µl transfers.
b. Mix and shake Sample plate on DECK 5 for 10 minutes.

34 Move the Sample plate/tube to the magnetic stand at DECK 7. Incubate for 4 minutes until beads have pelleted.

35 Aspirate and discard the cleared supernatant.
a. This is done in 4 x 137.5 µl transfers.

36 Move Sample plate back to DECK 5. Replace tip boxes on DECK 3 and DECK 9 with new boxes. Replace Ethanol 2/4 with Ethanol 3/4 on DECK 1

37 Transfer 500 µl Ethanol 3/4 to the Sample plate.
a. This is done in 4 x 125 µl transfers.

b. Mix and shake Sample plate on DECK 5 for 5 minutes

38 Move the Sample plate/tube to the magnetic stand at DECK 7. Incubate for 4 minutes until beads have pelleted.

39 Aspirate and discard the cleared supernatant.
a. This is done in 4 x 125 µl transfers.

40 Move Sample plate back to DECK 5. Replace Ethanol 3/4 with Ethanol 4/4 on DECK 1. Replace Waste (2 ml) plate on DECK 8 with an empty Waste (2 ml) plate.

41 Transfer 500 µl Ethanol 4/4 to the Sample plate.
a. This is done in 4 x 125 µl transfers.
b. Mix and shake Sample plate on DECK 5 for 5 minutes

42 Move the Sample plate/tube to the magnetic stand at DECK 7. Incubate for 4 minutes until beads have pelleted.

43 Aspirate and discard the cleared supernatant.
a. This is done in 4 x 125 µl transfers.

44 Replace Prep Buffer with Water plate on DECK 2. Dry the beads for 30 minutes or until dry.

45 Confirm Water plate is unsealed. Place Elution PCR plate on DECK 4. Move Sample plate back to DECK 5. Replace tip boxes on DECK 3 and DECK 9 with new boxes.

46 Transfer 45 µl of Water to the Sample plate on DECK 5.
a. Mix and shake Sample plate on DECK 5 for 10 minutes

47 Move sample plate to DECK 7. Incubate for 4 minutes.

48 Transfer 40 µl from the Sample plate to Elution plate on DECK 4. Freeze Elution plate and Sample plate in -80°C and discard all plates and tip boxes.