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Nucleic Acid Purification: Bead Clean

Madeline Y Mayday[1], Lillian M Khan[2], Eric D. Chow[2], Matt S. Zinter[3], Joseph L. DeRisi[2]

[1]Yale University; [2]UCSF School of Medicine, Department of Biochemistry & Biophysics;

[3]UCSF School of Medicine, Benioff Children's Hospital, Department of Pediatrics, Division of Critical Care

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Madeline Mayday
Yale University, University of California, San Francisco

ABSTRACT

Preparation of high-quality sequencing libraries is a costly and time-consuming component of metagenomic next generation sequencing (mNGS). While the overall cost of sequencing has dropped significantly over recent years, the reagents needed to prepare sequencing samples are likely to become the dominant expense in the process. Furthermore, libraries prepared by hand are subject to human variability and needless waste due to limitations of manual pipetting volumes. Reduction of reaction volumes, combined with sub-microliter automated dispensing of reagents without consumable pipette tips, has the potential to provide significant advantages. Here, we describe the integration of several instruments, including the Labcyte Echo 525 acoustic liquid handler and the iSeq and NovaSeq Illumina sequencing platforms, to miniaturize and automate mNGS library preparation, significantly reducing the cost and the time required to prepare samples. Through the use of External RNA Controls Consortium (ERCC) spike-in RNAs, we demonstrated the fidelity of the miniaturized preparation to be equivalent to full volume reactions. Furthermore, detection of viral and microbial species from cell culture and patient samples was also maintained in the miniaturized libraries. For 384-well mNGS library preparations, we achieved a savings of over 80% in materials and reagents alone, and reduced preparation time by 90% compared to manual approaches, without compromising quality or representation within the library.

This automated, miniaturized protocol for bead cleaning can be used with the High-Throughput Echo Library Prep Protocol for processing up to 384 samples simultaneously. To facilitate the miniaturized columes required by this protocol, we have included the .STL file for our 3D-printed adaptor, which, when placed on the magnet, raises the height of the PCR plate, thereby lowering the beads inside of the well and allowing for elutions in as low as 6uL.

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PROTOCOL CITATION

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automated, miniaturized, protocol, NGS, nMGS, library prep, library preparation, Labcyte Echo, sequencing, Illumina, bead clean, size selection, Ampure, Biomek

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15421

PARENT PROTOCOLS

In steps of

High-Throughput Echo Library Prep Protocol
High-Throughput Echo Library Prep Protocol
High-Throughput Echo Library Prep Protocol
High-Throughput Echo Library Prep Protocol

GUIDELINES

Different SPRI beads for DNA could be used, but the ratios should be tested to obtain desired fragment size and recovery before use in protocol.

Robot guidelines:
For this protocol, we used the Beckman Coulter Biomek NXP with a 384-multichannel head and 384 barrier pipet tips. It would be possible to use a 96-multichannel head if samples are arranged in each quadrant of a 384-well plate, but a single 384-well bead clean expedites the process.

Any liquid handler used should be calibrated appropriately for each piece of labware, including plates, magnets, and reservoirs. Prior to beginning a library preparation, extensive testing should be performed using water to confirm each portion of the protocol runs smoothly prior to loading actual experimental samples.

In this protocol we use the Alpaqua's 384-Post Magnet designed for PCR plates, but the elution volume needed for the miniaturized library prep protocol is 6uL-- which is less than the advertised volume of 15uL. To compensate for this, be sure to obtain an adaptor to raise the PCR plate from the stand and therefore decrease elution volume.
Adaptor design: **alpaquamag384platform.stl**

Robotic calibrations:
Be sure to set the aspiration speed on the liquid handler used for bead cleans to have a slow aspiration speed for all steps to reduce bead loss. (**Recommended aspiration speed: 0.1uL/sec if possible**)

All mixing steps should be programed to be total volume to be mixed minus one microliter, and to pipette 20 times slowly. (i.e. Total volume is 20uL in each well; mix 19uL of the sample 20uL)

MATERIALS TEXT

MATERIALS

Ampure XP beads **Beckman Coulter Catalog #A63881**

384 Post Magnet Plate **Contributed by users Catalog #SKU A001222**

BEFORE STARTING

Calibrate and test protocol to ensure it runs smoothly for all calibrations before using it to clean up real samples.

Decide which ratio of AMPure XP beads to use with sample and ensure all volumes for beads and elution are correct in the robot's methods. Refer to High-Throughput Echo Library Prep Protocol for the ratios recommend at each step.

Bring AMPure XP beads to room temperature before using in the protocol.

Prepare fresh 80% Ethanol for the washes.

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Prep Reagents

1 Prepare all necessary reagents and bring to room temperature:

Reagent	Catalog Number
AMPure XP beads	A63881
80% Ethanol	
Desired Elution Buffer (H2O, elution buffer, etc)	
Alpaqua 384 well Post Magnetic Plate	SKU A001222
Low elution adaptor	

Add Beads

2 Aspirate desired volume of Ampure beads from reservoir and mix well into sample plate by mixing total volume minus one microliter less -- 20 times.

Bead volumes for each bead clean are available in the High-Throughput Echo Library Prep Protocol.

Incubate

3 Incubate samples and beads at room temperature for 5 minutes.

4 Transfer plate onto Alpaqua 384 Post Magnet Plate with 3D-printed adaptor and incubate at room temperature for another 5 minutes to allow for the formation of a magnetic pellet.

alpaquamag384platform.stl

It is imperative that the magnet used can elute, at the very minimum, 6uL of your chosen elution buffer. It is important to elute cDNA in as little volume as possible for a miniaturized protocol.

Ethanol washes

5 Aspirate 25uL freshly prepared 80% ethanol from reservoir and add to samples while on the magnetic rack. Incubate at room temperature for 30 seconds.

6 Slowly aspirate supernatant and discard. Repeat the wash step for a total of 2 washes.

Air dry pellet

7 Remove and discard the supernatent for the second wash step. Allow the magnetic pellet to air dry while on the magnetic rack for a maximum of 90 seconds.

Do not overdry the beads. This could cause undesired loss of cDNA.

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Resuspend

8 Remove plate from magnetic stand, and resuspend beads in desired elution buffer and volume. Mix well.

Volume and type of eluent are specified in the High-Throughput Echo Library Prep Protocol.

Reminder: Regular elution buffer is used for Post-Second Strand Synthesis, Post- End Prep, and Post- USER/PCR bead cleans. **Barcodes suspended in nuclease-free water will be used to elute in Post-Adaptor Ligation bead clean.**

Incubate

9 Incubate at room temperature for 5 minutes. Transfer plate onto Alpaqua 384 Post Magnet Plate with adaptor with 3D-printed adaptor and incubate at room temperature for another 5 minutes.

Recovery of Purified cDNA

10 Slowly aspirate supernatant, now containing cDNA, and transfer to final PCR plate.

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