

I. Introduction

This protocol is used in conjunction with PrepX kits to accommodate rapid, walkaway automation of PCR cleanup of sequencing libraries on the Apollo™ Library Prep System. When run with the PCR Cleanup 48 script, this protocol can be used to cleanup 48 samples with a run time of 1 hour and 35 minutes (2 hours and 5 minutes total preparation time). **Read this Protocol-At-A-Glance in its entirety before you begin, with particular attention paid to the Apollo System Best Practices.**

II. Materials Required

The following reagents and consumables were used to validate this protocol and script. **Do not make any substitutions.**

Reagents and Consumables	Source	Cat. No.	Quantity	Usage/48-rxn run
AMPure XP Beads	Agencourt	A63882	450 ml	3,760 µl
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	48 tips
Apollo Reservoirs	Takara Bio	640087	Box of 100 reservoirs	4 reservoirs
Apollo Microtiter Plates	Takara Bio	640083	Box of 25 plates	1 plate
Apollo 1.1 ml MiniTubes	Takara Bio	640088	Box of 960 minitubes	8 tubes
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	16 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	16 strips

Additional Materials Required

- Single-channel pipettes: 10 µl, 20 µl, 200 µl, and 1,000 µl
- Eight-channel pipettes (recommended): 20 µl and 200 µl
- Filter pipette tips: 2 µl, 20 µl, 200 µl, and 1,000 µl
- PCR thermal cycler
- 100% ethanol (EtOH; molecular biology grade)

III. Apollo System Best Practices

- **Read this Protocol-At-A-Glance in its entirety before you begin.**
- Clean the work surfaces, including the retention plates, with 70% ethanol at least once a week.
NOTE: While the standard cleaning procedure is sufficient to clean Apollo system surfaces for RNA work, the user may wish to use additional decontamination solutions to remove nucleases. As these are known to be corrosive and may damage the system, ensure any nuclease decontamination is immediately followed by a cleanup with 70% EtOH
- **Restart the instrument before every run. Also, between each subprotocol, perform a power cycle by turning the instrument off, waiting 1 min, and then turning it back on.**
- Discard any deformed plastics.
- Separate partial tube strips with scissors and remove resulting plastic overhangs.
- Spin down reagents before placing them on the deck to avoid air bubbles. **Bubbles at the bottoms of tubes must be removed to ensure accurate volume delivery.**
- Ensure plastics are properly seated on the deck surface **with caps/lids removed**. Be sure to push any tubes down completely and evenly prior to installing the metal retention plates.
- Empty the waste box before every run. **An accumulation of tips in the waste box may cause the run to fail.**

IV. Protocols

A. Protocol: Sample and Reagent Prep

Materials Required

Reagents	Storage conditions	Source
PCR product	–20°C	User
AMPure XP Beads	4°C	Agencourt
Nuclease-free water	Room temperature	User
100% ethanol	Room temperature	User

Reagents and Consumables	Source	Cat. No.	Quantity	Usage/48-rxn run
Apollo Reservoirs	Takara Bio	640087	Box of 100 reservoirs	4 reservoirs
Apollo 1.1 ml MiniTubes	Takara Bio	640088	Box of 960 minitubes	8 tubes
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	16 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	16 strips

NOTE: AMPure XP Beads need to come to room temperature before the container is opened. Therefore, **we strongly recommend preparing ~1-ml aliquots upon receipt** and then refrigerating the aliquots. Individual tubes can be removed for each experiment, allowing them to come to room temperature more quickly (≥ 30 minutes). This will also decrease the chance of bead contamination. Mix well to disperse the beads before adding them to your reactions. The beads are viscous, so pipette slowly.

1. If your PCR product is less than 50 μ l, add water to reach 50 μ l. If your PCR product is more than 50 μ l, split each product into 50 μ l aliquots.
2. Prepare up to 16 strips of 6-well strip tubes by cutting two tubes from 16 8-tube strips and trimming the resulting plastic overhangs. The samples and reagents must be distributed in sets of six to be in the correct orientation for processing up to 48 samples (Figure 1). **Do not use 8-tube strips instead of 6-tube strips.**
3. On the benchtop, aliquot the reagents into the consumables as described in the table below:

Component	Consumable	Volume per tube or reservoir
PCR product	Apollo 0.2 ml PCR 8-Tube Strips, Clear	50 μ l
AMPure XP Beads	Apollo 1.1 ml MiniTubes	470 μ l
Nuclease-free water	1 Apollo Reservoir	45 ml
100% EtOH	1 Apollo Reservoir	45 ml

*The AMPure XP Beads should be warmed to room temperature and mixed well prior to use.

B. Protocol: PCR Cleanup

Materials Required

Reagents	Current temperature	Source
PCR product	On ice	User
AMPure XP Beads	Room temperature	Agencourt
Nuclease-free water	Room temperature	User
100% EtOH	Room temperature	User

Apollo consumables	Source	Cat. No.	Quantity	Usage/48-rxn run
Apollo Filter Tips	Takara Bio	640084	Box of 960 tips	48 tips
Apollo Reservoirs	Takara Bio	640087	Box of 100 reservoirs	4 reservoirs
Apollo Microtiter Plates	Takara Bio	640083	Box of 25 plates	1 plate
Apollo 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640082	Box of 125 strips	16 strips
Apollo Caps for 0.2 ml PCR 8-Tube Strips, Clear	Takara Bio	640086	Box of 125 strips	16 strips

1. Turn on the instrument or, if the instrument is already on, perform a power cycle by turning the instrument off, waiting 1 min, and then turning it back on.
2. To access the PCR Cleanup 48 script, press **Utility Apps > PCR Cleanup 48**. The **Cooling** indicator will appear.
3. Load consumables onto the Apollo system work surface according to the layout in Figure 1. First, load the consumables that do not initially hold reagents (table above). Just before the run, load the consumables containing reagents, but not samples, onto the system.
4. When the **Cooling** indicator has disappeared, and the **Run** button has appeared, load the samples according to the touch screen and the layout shown in Figure 1.

NOTE: Ensure plastics are properly seated on the deck surface with caps carefully removed. Be sure to push any tubes down completely and evenly prior to installing the metal retention plates.

5. Install the metal retention plates on Blocks 3 and 4.
6. Empty the waste box and remove any used consumables from the system.

NOTE: An accumulation of tips in the waste box may cause the run to fail.

7. Close the instrument door and press **Run**.

NOTE: The run time is 1 hour and 45 minutes.

8. When the run is complete, remove the cleaned PCR products from Block 3, Rows 7–12 (Products), cap the tubes, and place them on ice. The final cleaned PCR volume should be ~10 µl per tube.
9. Turn off the instrument.

NOTE: Be sure to remove any used consumables from the system.

10. **Recommended:** proceed to nucleic acid quantification with the Agilent High Sensitivity DNA Kit (Agilent, Cat. No. 5067-4626).

PCR Cleanup (48 samples)

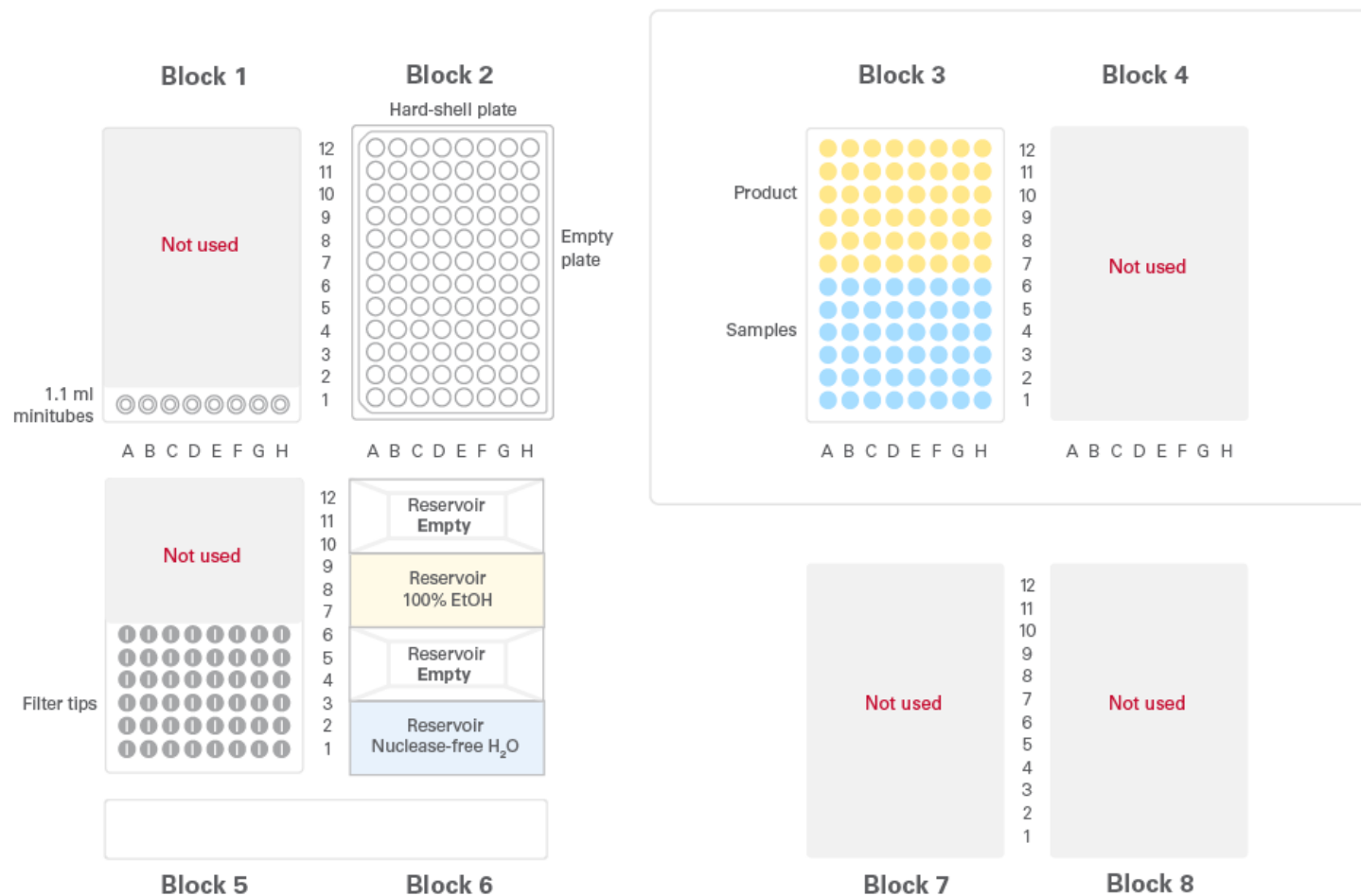


Figure 1. Deck layout for post-PCR cleanup of 48 samples. For this protocol, use script PrepX Cleanup 48. Run time is 1 hour and 45 minutes.

- Sample
- Product
- AMPure XP beads
- Filter tip
- Empty well in microtiter plate

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