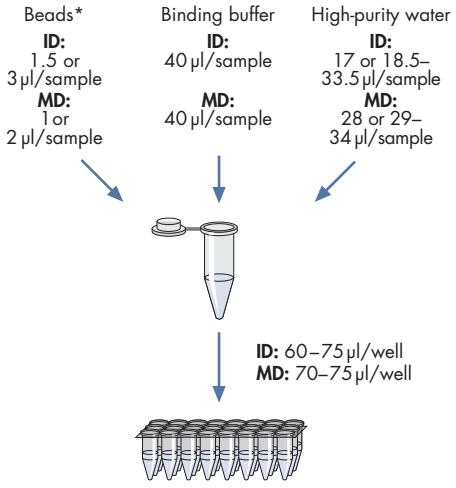
# PyroMark® Q96 Vacuum Workstation quick-start guide

This Technical Information summarizes the immobilization and preparation of PCR products for Pyrosequencing® using the PyroMark Q96 Vacuum Workstation. Before beginning, carefully read Section 5.3.3 of the *PyroMark Q96 ID User Manual* or Section 5.3.5 of the *PyroMark Q96 MD User Manual* and pay particular attention to the safety information.

## Immobilizing the PCR products

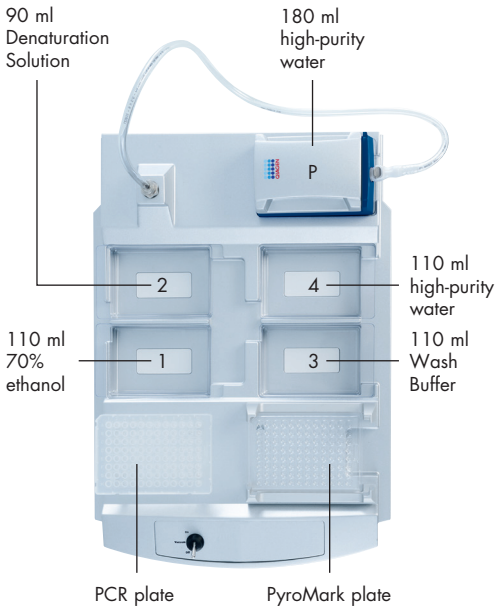
1. Make a master mix according to the flowchart to the right.
   **Note**: Before pipetting, gently shake the bottle of streptavidin-coated Sepharose® beads* to ensure a homogenous suspension.
2. Depending on sample volume and instrument type, pipet the correct amount of master mix into each necessary well of a PCR plate to give a total volume of 80 µl per well.
3. Add PCR product to each well, according to instrument type.
4. Seal the wells with strip caps and agitate the PCR plate at 1400 rpm for 5–10 min at room temperature (15–25°C) using an orbital shaker.




**Preparing the master mix to immobilize the PCR product.**

## Separation of DNA strands and release of samples into the PyroMark plate

1. Dilute sequencing primers with PyroMark Annealing Buffer (cat. no. 979009) and pipet into each necessary well of the instrument-specific PyroMark plate. Position the plate on the workstation.
   **ID**: 40 µl sequencing primer at 0.4 µM in the PyroMark Q96 Plate Low
   **MD**: 12 µl sequencing primers at 0.3 µM in the PyroMark Q96 HS Plate
2. Fill the workstation troughs according to the diagram to the right.
3. Start the pump and apply vacuum to the tool by opening the switch.
4. Flush the filter probes with high-purity water (Milli-Q® 18.2 MΩ x cm or equivalent) in the "Parking" trough (P). Refill the trough with fresh high-purity water for use in step 12.
5. Position the PCR plate on the workstation. Ensure that both plates are in the same orientation as when the samples were loaded.




* Streptavidin Sepharose High Performance (34 µm, 5 ml, GE Healthcare). Check the lot number of the Streptavidin Sepharose High Performance. For lot number 10057037 or higher, use 1.5 µl (ID) or 1 µl (MD) in the master mix. For lot numbers lower than 10057037, use 3 µl (ID) or 2 µl (MD). This is not a complete list of suppliers and does not include many important vendors of biological supplies.

6. With the vacuum switch ON, lower the vacuum tool into the wells of the PCR plate for 15 s to capture the beads with PCR product.
7. With vacuum ON, flush the tool with 70% ethanol (trough 1) for 5 s.
8. With vacuum ON, flush the tool with Denaturation Solution (trough 2) for 5 s.
8. With vacuum ON, flush the tool with Wash Buffer (trough 3) for 10 s.
10. With vacuum ON, raise the tool to beyond 90° vertical for 5 s.
11. Align the vacuum tool with the PyroMark plate and switch the vacuum OFF. Lower the vacuum tool into the wells and gently shake from side to side to release the beads.
12. With the vacuum OFF, agitate the vacuum tool in high-purity water (trough 4) for 10 s.
13. With vacuum ON, flush the filter probes with high-purity water (trough P) for 5 s.
14. Raise the vacuum tool to beyond 90° vertical for 5 s, then switch the vacuum OFF and store the tool in the "Parking" position.

**CAUTION:**

PyroMark Denaturation Solution contains sodium hydroxide, which is an eye and skin irritant.

Always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, see the *PyroMark Q96 ID User Manual* or the *PyroMark Q96 MD User Manual* (and the MSDS).

## Annealing sequencing primers to DNA strands

Place the PyroMark Q96 Plate Low in a prewarmed PyroMark Q96 Sample Prep Thermoplate Low. Alternatively, place the PyroMark Q96 HS Plate in a prewarmed PyroMark Q96 HS Sample Prep Thermoplate. Heat the Pyrosequencing samples on a heating block at 80°C for 2 minutes. Remove the plate from the thermoplate and allow the samples to cool to room temperature (15–25°C) for at least 5 minutes. The cooled plate can now be processed.

## Cleaning the vacuum workstation

Liquid waste and solutions remaining in the troughs of the vacuum workstation should be appropriately discarded at the end of the day. For details, see your instrument user manual.

For up-to-date licensing information and product-specific disclaimers, see the *PyroMark Q96 ID User Manual* or *PyroMark Q96 MD User Manual*. The *PyroMark Q96 ID User Manual* and *PyroMark Q96 MD User Manual* are available at www.qiagen.com or can be requested from QIAGEN Technical Services or your local distributor.

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