CarrierScan™ Assay 96-Array Format Automated Workflow

for Beckman Biomek FX^P (Windows® 7)

Pub. No. 703479 **Rev.** 3

CarrierScan Assay on the Biomek FX^P

The CarrierScan Assay 96-Array Format Automated Workflow leverages the Axiom 2.0 Assay method on the Biomek FX^P for target preparation and GeneTitan reagent preparation to process 96 samples at a time. Please note that the multiplex PCR (mPCR) and mPCR spike-in steps are not part of the automated method and are executed off-deck, manually.

Introduction

Running the CarrierScan Assay requires the following sets of steps:

1. Genomic DNA preparation, described in the *CarrierScan™ Assay 96-Array Format Automated Workflow for Biomek FX^P (Windows® 7) User Guide* (Pub. No. 703478)
2. Target preparation of the samples, performed using CarrierScan target preparation for Windows 7, described in this document.
3. Array processing, described in *GeneTitan™ MC Protocol for Axiom™ 2.0 Array Plate Processing Quick Reference* (Pub. No. 702988).

This document describes the manual mPCR preparation step and the automated target prep, performed using the Biomek FX^P Target Prep Express.

IMPORTANT! This document contains an abbreviated set of instructions. You must carefully read all the instructions in Chapter 4, *Multiplex PCR and Target preparation on the Biomek FX^P with Windows® 7* of the *CarrierScan™ Assay 96-Array Format Automated Workflow for Biomek FX^P User Guide* (Pub. No. 703478) before running the automated target preparation method.

The *CarrierScan™ Assay 96-Array Format Automated Workflow for Biomek FX^P User Guide* covers the assay steps in more detail and provides information on running multiple plates per week through the automated target preparation process.

Note: The Biomek FX^P should be homed before the first run of the day. If your Biomek FX^P has an on-deck thermal cycler, ensure that the lid is closed before homing the axes or starting a method.

New: An option for a three-hour DNA precipitation step is now available. See the *CarrierScan Assay 96-Array Format Automated Workflow for Biomek FX^P (Windows® 7) User Guide* (Pub. No. 703478) for details.

Additional notes:

- This CarrierScan assay format allows the user to run the CarrierScan Assay for 96 samples once using one CarrierScan™ Reagent Kit 96 Reactions (Cat. No. 931933).
- The mPCR preparation step requires the use of disposable reservoirs with a "trough within a trough" design which maximizes the amount of liquid accessible to pipette tips when using small amounts of reagent.
- Remove seals from plates carefully and discard used seals. Do not reuse seals.
- Unless otherwise specified, all reagent Modules are from the CarrierScan Reagent Kit 96 Reactions (Cat. No. 931933). Stage 1A requires the QIAGEN Multiplex PCR Plus Kit (Cat. No. 206152) and is sufficient to process 96 samples. See Chapter 3 of the *CarrierScan™ Assay 96-Array Format Automated Workflow for Biomek FX^P User Guide* (Pub. No. 703478) for a complete list of equipment and consumables required for each stage.

Note: CarrierScan arrays require a total of 150 ng of gDNA. mPCR requires 50 ng and DNA Amplification requires 100 ng.

Genomic DNA plate preparation

We recommend that you prepare your genomic DNA Sample Plate in a clean room. The clean room should be separate from the laboratory where the CarrierScan Assay is performed and should be free of DNA amplified in other procedures.

Stage 1A: Multiplex PCR

1. Preparation for Stage 1A: Multiplex PCR (mPCR)

Supplies required

- mPCR Sample Plate (10 µL volumes of gDNA at 5 ng/µL, with Control DNA)
- 10X Primer Mix from CarrierScan mPCR Module, –20°C, Part No. 931939
- Reagents from QIAGEN Multiplex PCR Plus Kit, –20°C, Cat. No. 206152

Instruments and setup

- Plate centrifuge at room temperature
- Approved thermal cycler
    - Must be programmed with **CarrierScan mPCR** protocol
        - 95°C for five minutes
        - 95°C for 30 seconds, 60°C for 90 seconds, and 72°C for 45 seconds—cycled 35 times
        - 68°C for ten minutes
        - 4°C hold
- Make sure heated lid option is used and thermal cycler is programmed to run in “9600 Mode” for Applied Biosystems 9700, Veriti™, and ProFlex™ and “Safe” mode for Eppendorf® Mastercycler® pro S.

Reagent preparation

1. Prepare reagents as shown in Table 1.

   Note: It is important that reagents are well mixed right before use.

   Table 1 Reagent preparation

Reagent	Quantity	Treatment
CarrierScan 10X Primer Mix	1 tube	Thaw, vortex, pulse-spin, keep on ice
QIAGEN Multiplex PCR Master Mix, 2X	3 tubes	Thaw, invert ten times to thoroughly mix, pulse-spin, keep on ice
QIAGEN Q-Solution, 5X	1 tube	Thaw, vortex, pulse-spin, keep on ice
QIAGEN RNase-free water	1 tube	Thaw, vortex, pulse-spin, keep on ice

2. Thaw mPCR Sample Plate

1. Bring mPCR Sample Plate to room temperature.
2. Vortex, pulse-spin, and place on cold aluminum block.

3. Prepare mPCR Master Mix

1. Prepare mPCR Master Mix as shown in Table 2.
2. Add water, Q-Solution, and primers to 15-mL conical tube. Vortex and pulse spin.
3. Add 2,400 µL of the QIAGEN 2X PCR Master Mix to the 15-mL tube.
    - All three QIAGEN 2X Multiplex PCR Master Mix supplied vials will be needed.
    - It is recommended to set a P1000 single channel pipette to 800 µL and remove this volume from the first vial.
    - Transfer this solution to the 15-mL tube. Change tips, and repeat this step for the remaining two vials.
4. Mix thoroughly, but gently, by inverting tube ten times.
5. Pulse spin and quickly proceed to next step.

   Table 2 mPCR Master Mix

Reagent	120 reactions
RNase-free Water	240 µL
Q-solution	480 µL
10X mPCR Primer Mix	480 µL
2X QIAGEN Multiplex PCR Master Mix	2,400 µL
Total	**3,600 µL**

4. Add mPCR Master Mix to samples

1. Carefully pour the prepared mPCR Master Mix into a 25-mL reservoir.
2. Use a P200 multichannel pipette to transfer 30 µL of mPCR Master Mix to each well of the mPCR Sample Plate.
3. Seal plate. Gently vortex. Pulse spin.
4. Load plate onto thermal cycler within five minutes.

5. Discard any leftover reagents

6. Run the CarrierScan mPCR thermal cycler protocol

Load the plate on the thermal cycler and run the **CarrierScan mPCR** protocol.

7. Freeze the mPCR Reaction Plate or proceed

After the mPCR protocol finishes, you can either:

- Store the mPCR Reaction Plate at –20°C.
- Proceed to *Stage 2: Fragmentation and precipitation* if *Stage 1B: DNA amplification* is complete.

Stage 1B: DNA amplification

1. Performing DNA amplification

1. Set the incubator/oven temperature at 37°C.
2. Set the centrifuge temp at room temperature.
3. Prepare reagents from Module 1 (Part No. 906011) of the CarrierScan Reagent Kit, as shown in the following table:

Reagent	Temp out of module[1]	Treatment
Amp Solution	Thaw at room temperature (~one hour)	Vortex for 30 seconds to thoroughly mix
Water	Thaw at room temperature	Vortex
10X Denat Solution	Thaw at room temperature	Vortex and spin
Neutral Solution	Thaw at room temperature	Vortex for 30 seconds to thoroughly mix
Amp Enzyme	Keep at –20°C	Just before use, flick tube three times, spin, and place in the cold block

[1] Temp out of module: temperature reagent is held at immediately after removal from module.

4. Thaw samples in gDNA Plate:
   a. Bring your gDNA samples to room temperature on the benchtop.
   b. Vortex, pulse-spin, and leave at room temperature.
5. Run Biomek method:
   a. Select the **DNA Amplification** step, then click **OK**.
   b. Set up the deck as indicated in the deck setup prompt, then click **Run**.
   Note: The deck setup is also shown in Figure 1.
6. When finished, remove the Sample Plate from the deck.
7. Blot the top of the plate with a laboratory tissue. Tightly seal the plate.
8. Vortex plate for 30 seconds and pulse-spin to 1,000 rpm.
9. Place the Sample Plate in the preheated 37°C oven and incubate for 22 to 24 hours.

2. What to do next

1. After the incubation period, do one of the following:
2. Proceed directly to *Stage 2: Fragmentation and purification*.
3. Store the Sample Plate at –20°C.

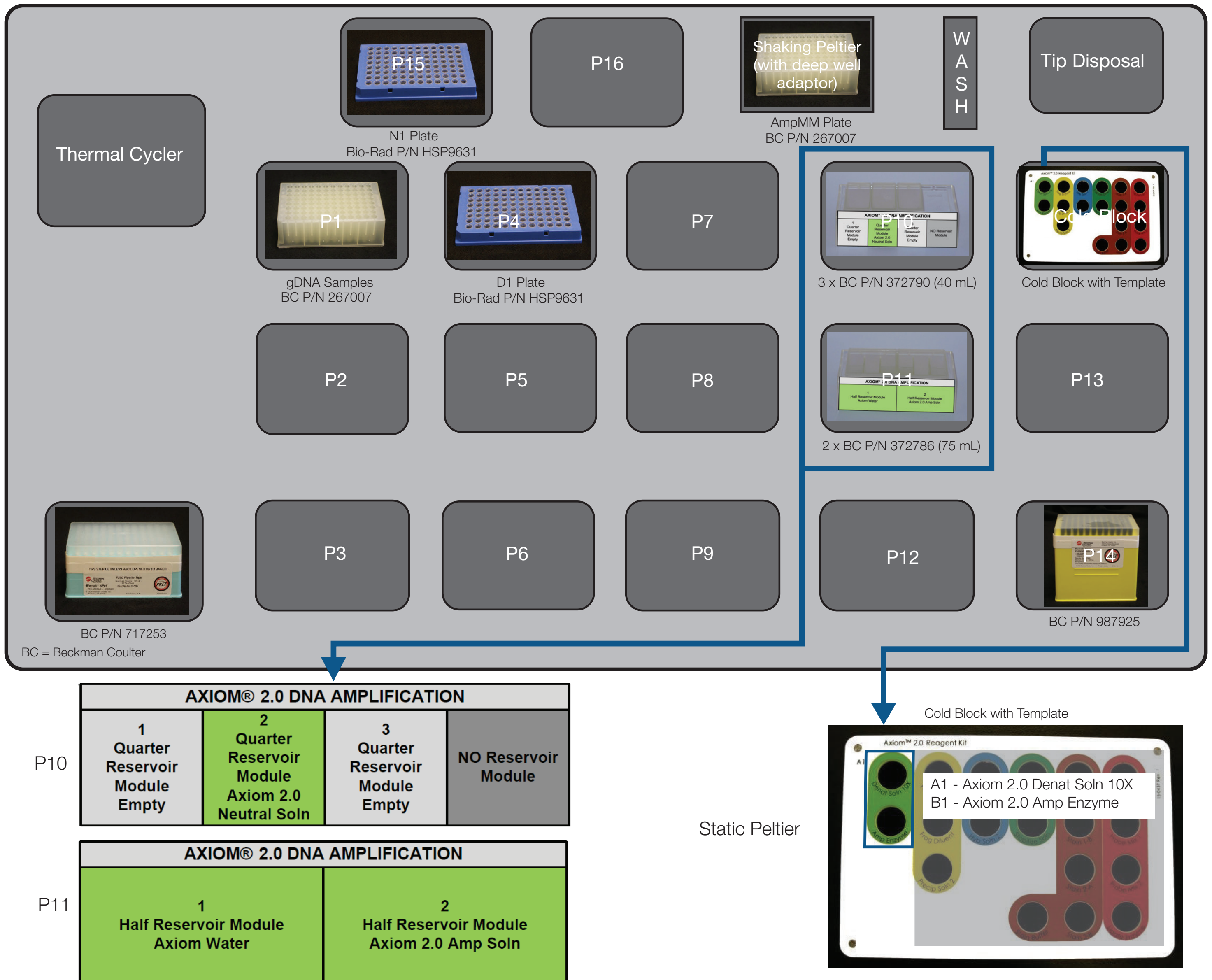


Figure 1. Deck Layout—DNA Amplification

Note: Deck images in this document display the blue Bio-Rad® Hard-Shell® 96-well plate (Cat. No. HSP-9631). However, the white version of this plate (Cat. No. HSP-9601) is an acceptable alternative.

Stage 2: Fragmentation and purification

Reagents and samples required

Reagents from HT Target Prep Module 2-1 (Part No. 906012) and HT Target Prep Module 2-2 (Part No. 906013) of the CarrierScan Reagent Kit; Isopropanol is user-supplied. Prepare as shown in the table below and place in the appropriate place on the deck.

Reagent	Temp out of module[1]	Treatment
10X Frag Buffer	Thaw at room temperature	Vortex
Frag Diluent	Place on ice	Vortex and spin
Frag Enzyme	Keep at –20°C	Just before use, flick tube three times, spin, and place in the cold block
Frag Reaction Stop	Room temperature	Vortex
Precip Solution 1	Place on ice	Vortex
Precip Solution 2	Thaw at room temperature	Vortex and spin
Isopropanol	Not applicable	Room temperature

[1] Temp out of module: temperature reagent is held at immediately after removal from module.

Note: If the plate of amplifed DNA samples or mPCR Reaction Plate was frozen at the end of Stage 1, thaw the plates before beginning Stage 2. See instructions in Chapter 2 of the *CarrierScan™ Assay 96-Array Format Automated Workflow for Biomek FXP User Guide* (Windows® 7) (Pub. No. 703478) for notes on thawing and spinning down prior to changing the seal to avoid cross-contamination.

1. If frozen, thaw the Sample Plate

1. Place the deepwell plate in a small bath of room temperature Millipore water for ~50 minutes (until all wells have thawed).
2. Pulse-spin plate to 1,000 rpm.
3. Remove the seal and blot the top of the plate with a laboratory tissue.
4. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 seconds to thoroughly mix.
6. Pulse-spin plate to 1,000 rpm.

2. If Sample Plate is not frozen

1. Do not vortex.
2. Remove the seal and continue to *3: Spike mPCR reaction into Amplification Plate* (below) before placing on deck.

3. Spike mPCR reaction into Amplification Plate

1. Vortex mPCR Reaction Plate and pulse spin.
2. Carefully transfer 10 µL of the mPCR reaction into the corresponding well of the Amplification Plate. Ensure complete transfer of liquid from pipette tip.
3. Securely seal Amplification Plate to minimize evaporation during next steps. Vortex, and pulse spin.
4. Immediately proceed to next step: *4. Fragment samples*.

4. Fragment samples

1. Run Biomek method:
   a. Select the **Fragmentation** step, then click **OK**.
   b. Setup the deck as indicated the deck setup prompt, then click **Run**.
      Note: The deck layout is also shown in Figure 2.

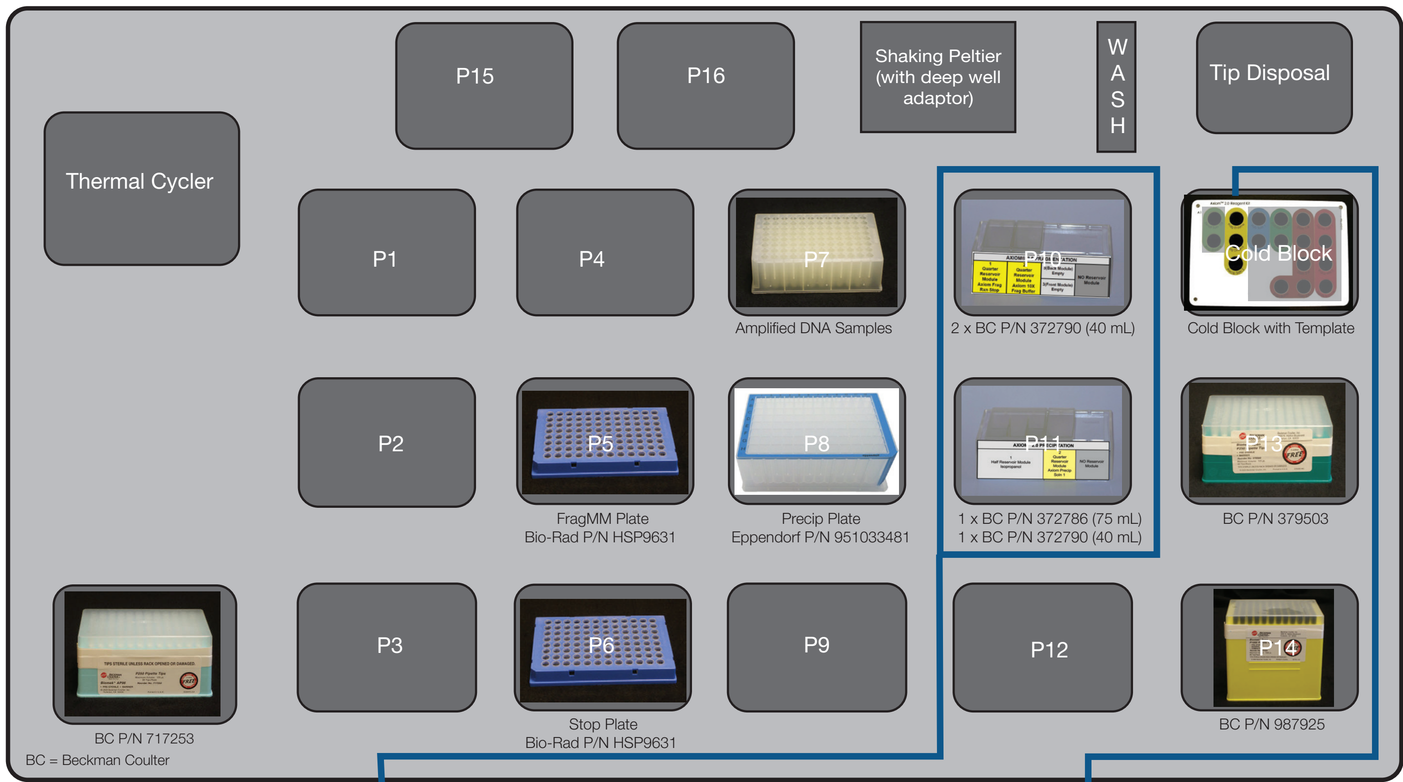
5. Precipitate samples

1. When the Biomek method is finished remove the Sample Plate (Precipitation Plate; position P8) from the deck.
2. Blot the top of the plate with a laboratory tissue.
3. Tightly seal the plate.
4. Place the plate in a –20°C freezer overnight to precipitate. A three-hour precipitation workflow option is also available. See the *CarrierScan Assay 96-Array Format Automated Workflow for Biomek FXP (Windows® 7) User Guide* (Pub. No. 703478) for details.
5. Discard used labware, reagents and tips from the deck.

6. What to do next

After the incubation period, do the following:

- Proceed directly to *Stage 3: Centrifugation and drying pellets*.



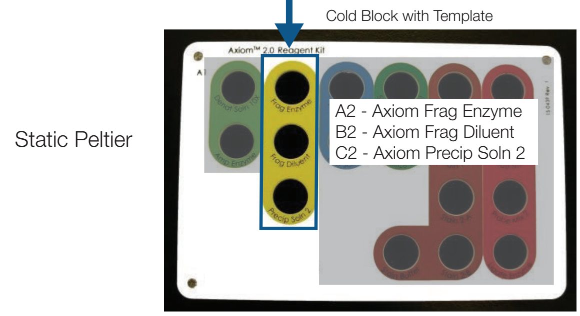
P10

AXIOM® 2.0 FRAGMENTATION			
1 Quarter Reservoir Module Axiom Frag Rxn Stop	2 Quarter Reservoir Module Axiom 10X Frag Buffer	3(Back Module) Empty 3(Front Module) Empty	NO Reservoir Module

P11

AXIOM® 2.0 PRECIPITATION		
1 Half Reservoir Module Isopropanol	2 Quarter Reservoir Module Axiom Precip Soln 1	NO Reservoir Module

Static Peltier



See the note on page 4 regarding Bio-Rad HSP-9631.

Figure 2. Deck Layout—Fragmentation

Stage 3: Centrifugation and drying pellets

1. Centrifuge and dry pellets

1. Preheat the oven to 37°C; cool centrifuge to 4°C.
2. Centrifuge the plate at 3,200 rcf at 4°C for 40 minutes.
3. Remove the seal, then invert the plate over a waste container to allow the liquid to drain.
4. While still inverted, gently press the top of the plate on a stack of laboratory tissues.
5. Leave plate inverted on the tissues for five minutes.
6. Turn the plate right side up and place in the preheated oven for 20 minutes.

2. What to do next

After the pellets have been dried, do one of the following:

- Proceed directly to *Stage 4: Resuspension, hybridization preparation, and QC* (even if some droplets of liquid remain).
- Store plates for resuspension later in the same day:
  - If resuspension will be carried within four hours, tightly seal and keep the plates at room temperature.
  - If resuspension will be carried out in more than four hours, tightly seal and store the plates in a refrigerator (2–8°C).
- Store plates for resuspension on another day:
  - Tightly seal the Sample Plate and store at –20°C.

Stage 4: Resuspension, hybridization preparation, and QC

Reagents and samples required

Reagents from HT Target Prep Module 2-1 (Part No. 906012) and HT Target Prep Module 2-2 (Part No. 906013) of the CarrierScan Reagent Kit; Invitrogen TrackIt™ reagents are user-supplied.

Reagent	Temp out of module[1]	Treatment
Hyb Buffer	Place on ice	Vortex
Hyb Solution 1	Thaw at room temperature	Vortex and spin
Resuspension Buffer	Warm to room temperature (one hour minimum)	Vortex
Hyb Solution 2	Place on ice	Vortex and spin
Nuclease-free water	Not applicable	Not applicable
TrackIt Cyan/Orange Gel Loading Buffer (diluted 1:1,000)	Not applicable	See gel QC instructions
TrackIt 25 bp DNA Ladder (diluted 1:15) or similar product	Not applicable	See gel QC instructions

[1] Temp out of module: temperature reagent is held at immediately after removal from module.

1. Prepare the Precipitation Plate

Before proceeding with resuspension:

1. Plates stored at 2-8°C after Stage 3 must be allowed to warm to room temperature for 30 minutes.
2. Plates frozen at –20°C after Stage 3 must be allowed to equilibrate at room temperature for 1.5 hours.

2. Run the Biomek method

1. Select the **Resuspension, Hybridization Preparation, and QC** step, then click **OK**.
2. Setup the deck as indicated in the Biomek deck setup prompt, then click **Run**.

   Note: The deck setup is also shown in Figure 4. Refer to the *CarrierScan™ 2.0 Assay 96-Array Format Automated Workflow for Beckman Biomek FX^P User Guide* (Pub. No. 703478) for special instructions if an Applied Biosystems thermal cycler is used in Stage 5.

3. Resuspend by off-deck shaking

1. When prompted by the method, remove the Sample Plate (Resuspension Plate) from deck position 3 of the deck.
   - Click **OK** to allow the method to continue.
2. Blot the top of the plate with a laboratory tissue.
3. Seal the plate tightly; blue pellets should be visible at the bottom of the wells.
4. Place the Sample Plate onto one of the following shakers for ten minutes:
   - Thermo Scientific™ Compact Digital Microplate Shaker: set at 900 rpm
   - Jitterbug™: set at speed of 7
5. Inspect the plate from the bottom. If the pellets are not dissolved, repeat the shaking step (Step 4).
6. Pulse-spin the plate to 1,000 rpm in a room temperature centrifuge.
7. When prompted by the method, remove the seal and return the Sample Plate (Resuspension Plate) on deck position 3. Click **OK** to allow the method to continue.

4. Run fragmentation QC gels

1. Tightly seal the Gel QC Plate, vortex, and pulse- spin.
2. Onto a 4% agarose e-gel load:
   - 20 µL from each well of the Gel QC Plate.
   - 15 µL of diluted TrackIt 25 bp ladder to marker wells.
   - 20 µL of water to any unused wells.
3. Run for 22 minutes.
4. Review gel image (see Figure 3).

5. Quantitate the resuspended samples

1. Quantitate the samples prepared in the OD Plate.
2. Assess the OD reading for each sample.
   - Median Yield = 1,200 µg/well

What to do next

Do one of the following:

- If the GeneTitan MultiChannel Instrument is available, and if the gel QC and quantitation results were acceptable, proceed to *Stage 5: Preparation for the GeneTitan™ Instrument*.
- Tightly seal the Hyb-Ready Plate and store at –20°C. (This plate is referenced as Hyb Rxn in the Biomek software.)

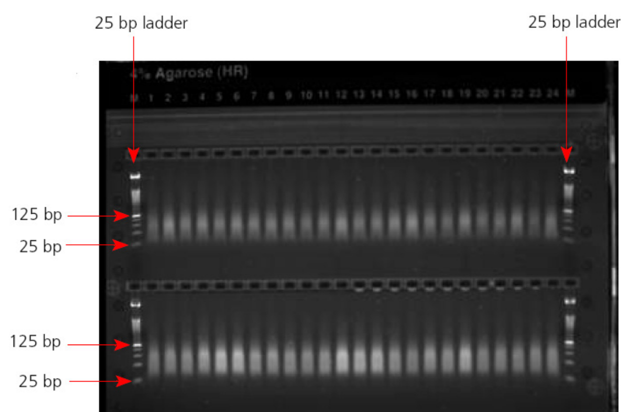
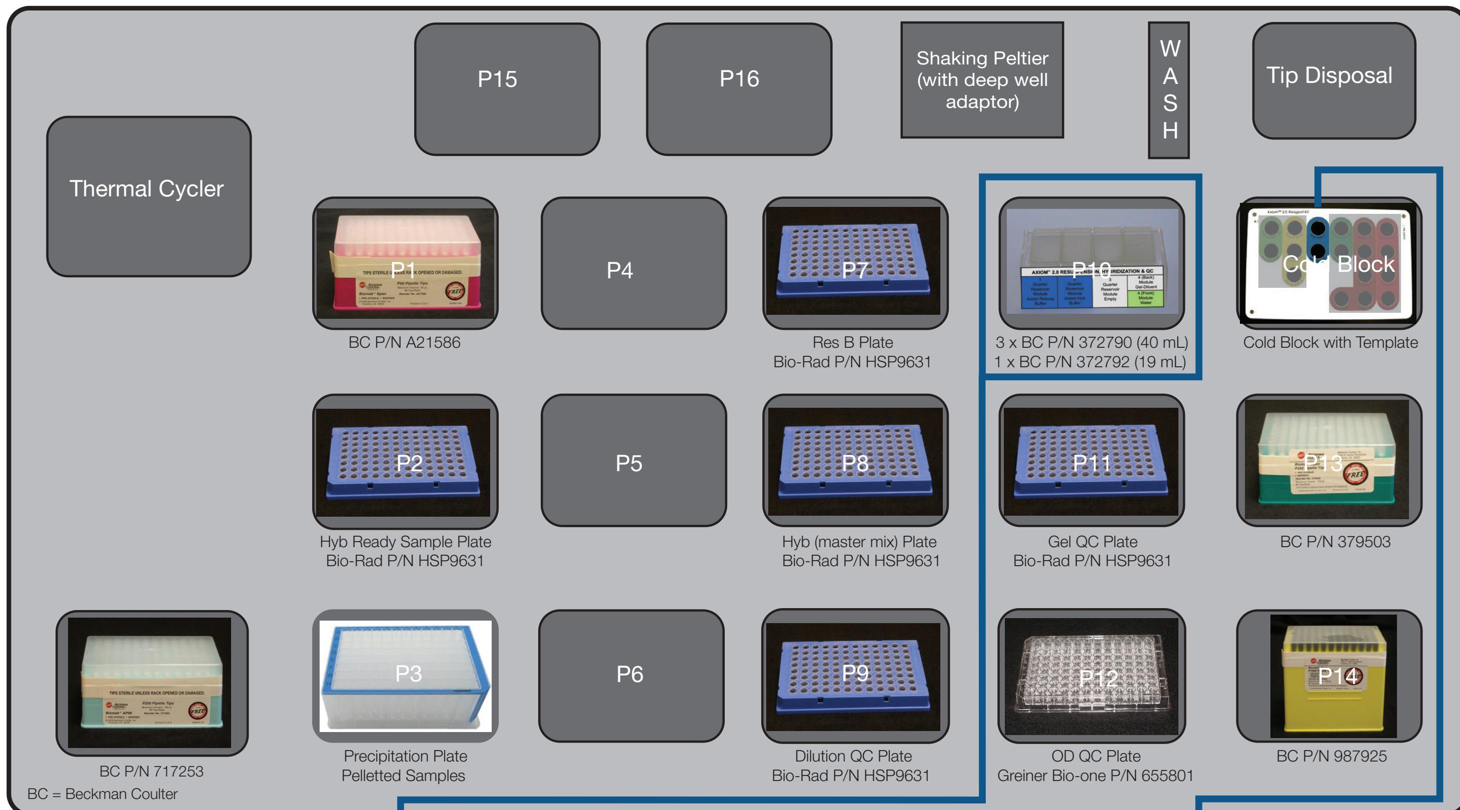


Figure 3. Gel image. Example of good fragmentation QC gel. Fragments are between 125 and 25 bp.



P10

AXIOM® 2.0 RESUSPENSION, HYBRIDIZATION, & QC			
1 Quarter Reservoir Module Axiom Resusp Buffer	2 Quarter Reservoir Module Axiom Hyb Buffer	3 Quarter Reservoir Module Empty	4(Back Module) Gel-Diluent
			4(Front Module) Water

13 mL

To fill line

Static Peltier

Cold Block with Template

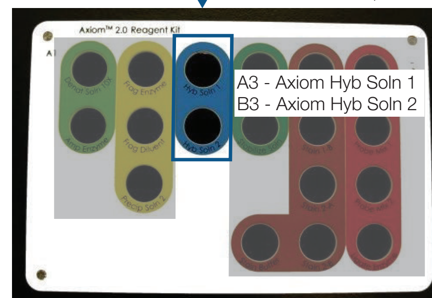


Figure 4. Deck Layout—Resuspension, Hybridization Preparation, and QC

See the note on page 4 regarding Bio-Rad HSP-9631.

Stage 5: Preparation for the GeneTitan™ Instrument

Important guidelines for this stage

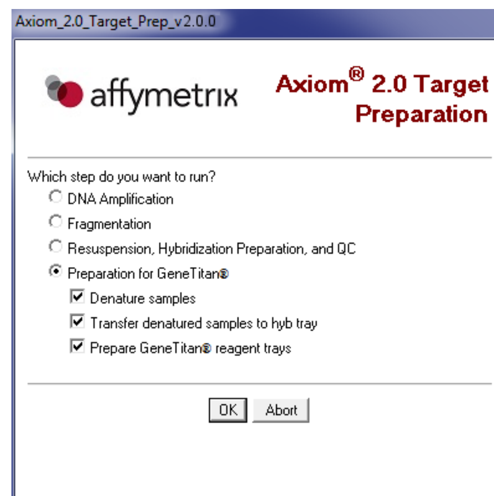
Begin this stage 45 minutes prior to when the array plate currently in the GeneTitan Instrument will finish hybridization.
This stage takes approximately 40 minutes to run.

The Preparation for GeneTitan stage has two different sets of steps:

- Denature samples and transfer denatured samples to hybridization tray
- Prepare GeneTitan reagent trays

The sets of steps are selected in the Axiom 2.0 Target Prep dialog box.

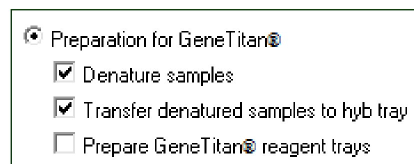
You can perform each part of the stage separately, or you can run both parts at the same time for the high-throughput workflow. For the high-throughput workflow, you are preparing reagent trays for the array plate that is currently finishing the hybridization step in the GeneTitan™ Multi-Channel (MC) Instrument, while preparing another hybridization tray that will be loaded into the GeneTitan MC Instrument with a new array plate to begin the Hybridization step.



Denaturation and hyb sample transfer

1. Prepare the hyb-ready samples and array plate:
   a. Warm the array plate to room temperature for at least 25 minutes before setting up hybridization on the GeneTitan MC Instrument. Open the pouch and scan the barcode into the GeneTitan Array Plate Registration file at the end of the array warm up time.
   b. Ensure that the Hyb-Ready Plate is sealed well. If not, centrifuge the plate, then change the seal. Evaporation can harm the assay performance.
      - If the Hyb-Ready Plate was stored at –20°C, warm at room temperature for 5 minutes before checking the seal.
   c. Vortex the Hyb-Ready Plate briefly, then pulse-spin to 1,000 rpm.
   d. Leave the Hyb-Ready Plate at room temperature.
2. Prepare the GeneTitan MC Instrument.

   Note: If using an off-deck thermal cycler, refer to the *CarrierScan™ Assay 96-Array Format Automated Workflow for Biomek FXP User Guide* (Windows® 7) (Pub. No. 703478) for special instructions.
3. Run the Biomek method:
   a. Select the **Preparation for GeneTitan** step, and the following sub-steps:
      - **Denature samples**
      - **Transfer denatured samples to hybridization tray**
   b. Click **OK**.
   c. Setup the deck as indicated in the deck setup prompt, then click **OK**.
      The deck setup is also shown in Figure 5.



IMPORTANT! Clean the metal lid and pad by wiping with 70% ethanol.

4. After the the denaturation is complete, press **OK** at the prompt to transfer samples from thermal cycler to hybridization tray.
5. Ensure that there are no air bubbles present in the hybridization tray. Puncture any air bubbles that you see using a pipette tip.
6. Load the hybridization tray and array plate in the GeneTitan MC Instrument.

   See *GeneTitan™ MC Protocol for Axiom™ 2.0 Array Plate Processing QRC* (Pub. No. 702988).
7. What to do next
   - Near the end of the 23.5 to 24 hour hybridization period in the GeneTitan MC Instrument, select **Preparation for GeneTitan™** and the sub-step **Prepare GeneTitan™ reagent trays** on the Biomek FXP software.

Figure 5. Deck Layout—Denaturation and Transfer to Hyb Plate

See the note on page 4 regarding Bio-Rad HSP-9631.

Prepare reagent trays for the GeneTitan™ Instrument

IMPORTANT! The reagent trays prepared are for use with a CarrierScan array plate that is:

- already on the GeneTitan™ Multi Channel (MC) Instrument
- has completed the hybridization stage
- is ready for transfer to the fluidics area

Start deck setup for this stage 45 minutes before the array plate currently in the GeneTitan MC Instrument finishes hybridization. This step of the method takes approximately 30 minutes to run. The reagent trays for the fluidics stage on the GeneTitan MC Instrument CANNOT be prepared in advance. Do not prepare these plates if there is not an array plate ready for the fluidics stage. After being prepared, these plates must be loaded onto the instrument as soon as possible and cannot be stored.

1. Prepare the reagents from Modules 3-1 and 3-2 of the CarrierScan Reagent Kit, as shown in the table below:

Reagent	Temp out of module [1]	Treatment
HT Target Prep Module 3-1 (Part No. 906014)		
Ligate Buffer	Thaw at room temperature	1. Place on bench top at room temp for 30 minutes 2. Vortex for 30 seconds 3. Examine for precipitate. If any, warm bottle with your hands and vortex again for 30 seconds
Ligate Enzyme	Keep at –20°C until ready to use	Just before use: 1. Flick 2 to 3 times to mix 2. Spin 3. Place in the cold block
Ligate Solution 1	Thaw at room temperature	Vortex and spin
Probe Mix 1	Thaw at room temperature	Vortex and spin
Stain Buffer	Thaw at room temperature	Vortex and spin
Stabilize Solution	Thaw at room temperature	Vortex and spin
HT Target Prep Module 3-2 (Part No. 906015)		
Ligate Solution 2	Thaw at room temp (do not place on ice!)	Vortex and spin
Probe Mix 2[2]	Place on ice	Flick 2 to 3 times to mix, then spin
Wash A	Leave on bench	1. Vortex twice 2. Place on bench for 30 minutes 3. Look for precipitate 4. Vortex again if necessary
Stain 1-A[2]	Place on ice	Flick 2 to 3 times to mix, then spin
Stain 1-B[2]	Place on ice	Flick 2 to 3 times to mix, then spin
Stain 2-A[2]	Place on ice	Flick 2 to 3 times to mix, then spin
Stain 2-B[2]	Place on ice	Flick 2 to 3 times to mix, then spin
Stabilize Diluent	Place on ice	1. Vortex and spin 2. Look for precipitate. If any, warm tube to room temperature and vortex again
Water	Place on ice	Not applicable
Hold Buffer[2]	Room temperature	Vortex

[1] Temp out of module: temperature the reagent is held at immediately after removal from module.
[2] These solutions are light sensitive. Keep tubes out of direct light for a prolonged period of time.

2. Prepare the GeneTitan Multi-Channel Instrument.

   See *GeneTitan™ MC Protocol for Axiom™ 2.0 Array Plate Processing QRC* (Pub. No. 702988).

3. Run the Biomek method:

   a. Select the **Preparation for GeneTitan™** step, and the following substep:

      • **Prepare GeneTitan™ reagent trays**.

   b. Click **OK**.

   c. Set up the deck as indicated in the deck setup prompt, then click **OK**.
      The deck setup is also shown in Figure 6, below.

IMPORTANT! Label the stain trays and deionize them using an antistatic gun.

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4. Deionize the stain and scan tray lids with the antistatic gun.
5. Cover the reagent trays and scan tray with lids.
6. Examine each tray to ensure that all appropriate wells contain reagents (manually add if not present) and puncture any bubbles with a clean pipette tip.
7. Immediately load the reagent and scan trays into the GeneTitan MC Instrument.

   See *GeneTitan™ MC Protocol for Axiom™ 2.0 Array Plate Processing QR* (Pub. No. 702988).

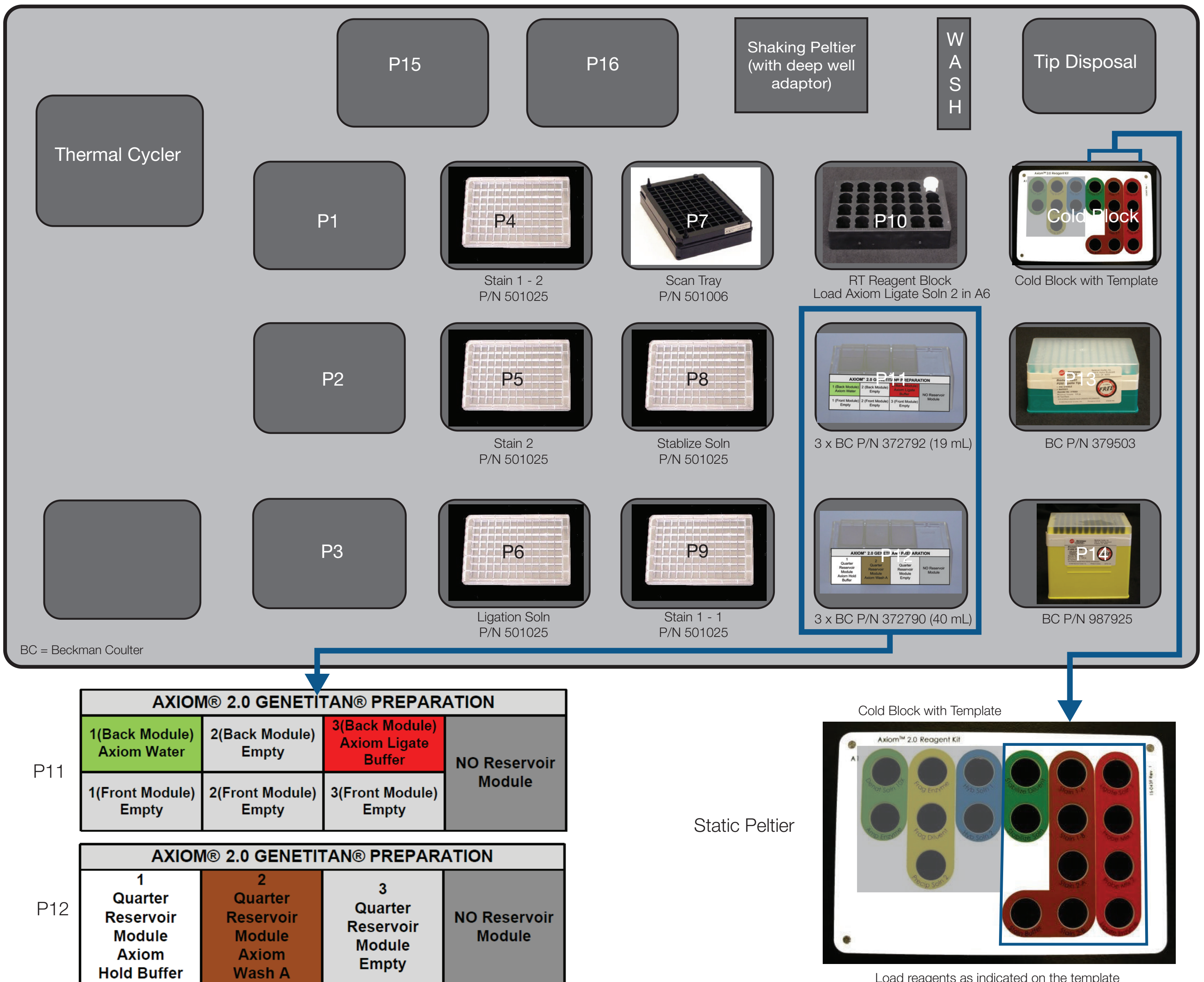


Figure 6. Deck Layout—Reagent Tray Preparation

Stage 5 for high-throughput workflow

In the high-throughput workflow you:

1. Denature the hybridization ready samples and transfer them to a hybridization tray for loading into the GeneTitan™ MC Instrument for the Hybridization stage.
2. Prepare reagent trays for another hybridization tray and array plate that is finishing the Hybridization stage.

IMPORTANT! The reagent trays that are prepared in the high-throughput workflow are not for use with the hybridization tray currently being prepared on the Biomek workstation. Rather, these reagent trays are for a CarrierScan array plate that is already in the GeneTitan MC Instrument and completing the Hybridization stage.

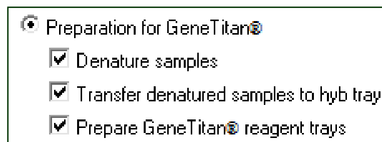
To perform Stage 5 for high-throughput:

1. Prepare the reagents from Modules 3-1 and 3-2 of the CarrierScan Reagent Kit , as shown in the table on page 9.
2. Prepare the Hyb-Ready Plate (Sample Plate):

   Vortex briefly; pulse-spin to 1,000 rpm. Leave the Hyb-Ready Plate at room temperature.
3. Prepare the GeneTitan MC Instrument

   See *GeneTitan™ MC Protocol for Axiom™ 2.0 Array Plate Processing QRC* (Pub. No. 702988).

   Note: If using an off-deck thermal cycler, refer to the *CarrierScan™ Assay 96-Array Format Automated Workflow for Biomek FX^P User Guide* (Windows® 7) (Pub. No. 703478) for special instructions.
4. Run the Biomek method:
   a. Select the **Preparation for GeneTitan™** step, and the following sub-steps:
      - **Denature samples**
      - **Transfer denatured samples to hyb tray**
      - **Prepare GeneTitan™ reagent trays**



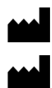
   b. Click **OK**.
   c. Setup the deck as indicated in the deck setup prompt, then click **OK**. The deck setup is also shown in Figures 5 and 6 of this QR.

IMPORTANT! Clean the metal lid and pad by wiping with 70% ethanol.

IMPORTANT! Label the stain trays and treat them with the antistatic gun.

5. Treat the stain and scan tray lids with the antistatic gun.
6. Cover the reagent trays and scan tray with covers.
7. Examine each tray to ensure that all appropriate wells contain reagents (manually add if not present) and puncture any bubbles with a clean pipette tip.
8. Immediately load the reagent trays and scan tray into the GeneTitan Instrument—do NOT click **OK** on the Biomek workstation prompt until all reagents trays have been loaded into the GeneTitan Instrument.
9. Return to the Biomek workstation and click **OK** when prompted to resume the method. Denatured samples are transferred to the hybridization tray.
10. Load the hybridization tray and array plate into the GeneTitan Instrument.

    See the *GeneTitan™ MC Protocol for Axiom™ 2.0 Array Plate Processing Quick Reference* (Pub. No. 702988).

 Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania — Products: CarrierScan™ Reagent Kit

Affymetrix Pte Ltd | 7 Gul Circle #2M-01 | Keppel Logistics Building | Singapore 629563 — Products: CarrierScan™ array plates

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