Quick Reference Card

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Axiom® 2.0 Automated Target Prep Protocol
Stage 1. DNA Amplification

Introduction

Running the Axiom 2.0 Assay requires the following sets of steps:

1. Genomic DNA Prep, described in either the *Axiom® 2.0 gDNA Sample Prep QRC* (P/N 702987) or the *Axiom® gDNA Sample Prep for Genome-Wide BOS 1 Array Plate QRC* (P/N 702975).
2. Target Prep of the samples, performed using either:
   - Automated Target Prep, described in this QRC.
   - Manual Target Prep, described in *Axiom® 2.0 Manual Target Prep Protocol QRC* (P/N 702989).
3. Array Processing, described in *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC* (P/N 702988).

This QRC describes the automated target prep, performed using the Biomek FX^P Target Prep Express.

IMPORTANT: This QRC contains an abbreviated set of instructions. You must carefully read all the instructions in *Chapter 3, Axiom 2.0 Assay: Target Prep with Biomek* of the *Axiom® 2.0 Assay Automated Workflow User Guide* (P/N 702963) before running the Automated Target Prep Method.

The *Axiom® 2.0 Assay Automated Workflow User Guide* covers the assay steps in more detail and provides information on running multiple plates per week through the automated target prep process.

WARNING: Make sure the thermal cycler lid is closed before homing the axes or starting a method.

NOTE: The Biomek FX^P should be homed before the first run of the day.

STAGE 1: DNA Amplification

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 Axiom 2.0 Assay is performed and should be free of DNA amplified in other procedures.

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 (P/N 901711) of the Axiom 2.0 Reagent Kit, as shown in Table 1.1:

Table 1.1. Reagents Preparation for Stage 1

Reagent	Temp Out of Module*	Treatment
Axiom 2.0 Amp Soln	Thaw at Room Temp (~1 hr)	Vortex twice
Axiom Water	Thaw at Room Temp	Vortex
Axiom 2.0 Denat Soln 10X	Thaw at Room Temp	Vortex and spin
Axiom 2.0 Neutral Soln	Thaw at Room Temp	Vortex and spin
Axiom 2.0 Amp Enzyme Soln	Keep at –20 °C	Just before use, flick tube 3X, spin, and place in the cold block
*Temp Out of Module: temperature reagent is held at immediately after removal from module		

4. Thaw Samples in gDNA Plate:

   NOTE: The gDNA samples must be at a volume of 20 µL for each sample. The DNA concentration is 5 ng/µL (for a total of 100 ng of gDNA in 20 µL) for gDNA that will be processed for the Axiom® Genome-Wide Pan-African Array. For gDNA that will be processed for all other Axiom genome-wide human arrays (CEU, ASI, EUR, and CHB), the concentration is 10 ng/µL (for a total of 200 ng gDNA in 20 µL).

   A. Bring your gDNA samples to room temperature on the bench top.

   B. Vortex and spin.

   C. 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 **OK**.

   NOTE: The deck setup is also shown in Figure 1.1 on the next page of this QRC.

6. When finished, remove the sample plate from the deck.
7. Blot the top of the plate with a Kimwipe. Tightly seal the plate.
8. Vortex and spin.
9. Place the sample plate in the preheated 37 °C oven and incubate for 22 to 24 hr.

2. What to do Next

After the incubation period, do one of the following:

- Proceed directly to *Stage 2. Fragmentation and Precipitation*.
- Store the sample plate at –20 °C

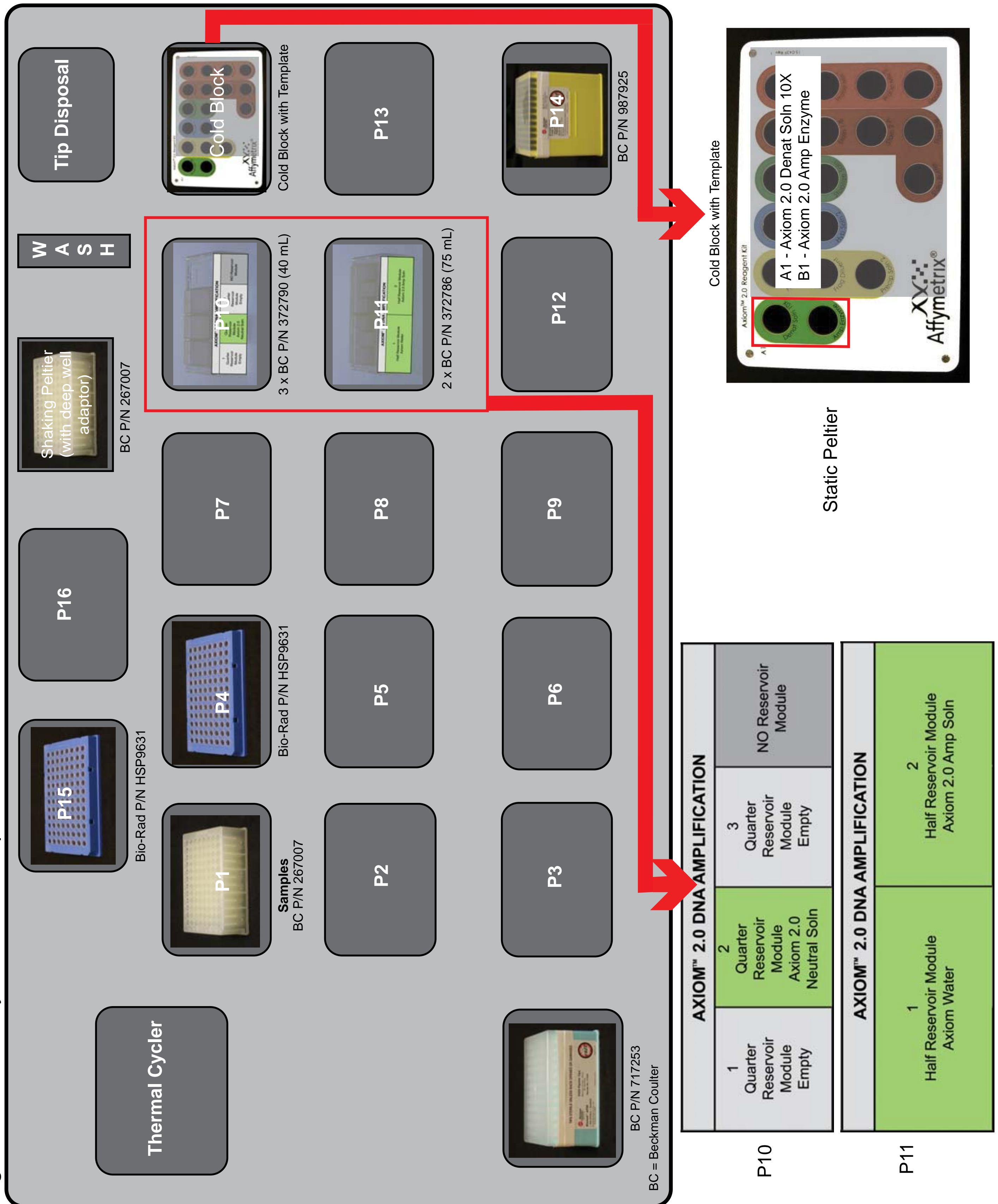

Figure 1.1. Deck Layout — DNA Amplification

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Axiom® 2.0 Automated Target Prep Protocol

Stage 2. Fragmentation and Precipitation

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Reagents and Samples Required

Reagents from Module 2-1 (P/N 901528) and Module 2-2 (P/N 901529) of the Axiom® 2.0 Reagent Kit; Isopropanol is user-supplied.

Prepare as shown in Table 2.1 and place in the appropriate place on the deck.

Table 2.1. Reagent Preparation for Stage 2

Reagent	Temp Out of Module*	Treatment
Axiom 10X Frag Buffer	Thaw at Room Temp	Vortex
Axiom Frag Diluent	Place on ice	Vortex and spin
Axiom Frag Enzyme	Keep at –20 °C	Just before use, flick tube 3X, spin, and place in the cold block
Axiom Frag Rxn Stop	Room Temp	Vortex
Axiom Precip Soln 1	Place on ice	Vortex
Axiom Precip Soln 2	Thaw at Room Temp	Vortex and spin
Isopropanol	Not Applicable	Room Temp
Notes: *Temp Out of Module: temperature reagent is held at immediately after removal from module		

1. If frozen, thaw the Sample Plate

1. Place the deep well plate in a small bath of room temperature Millipore water for ~ 50 min (until all wells have thawed).
2. Spin at 1000 rpm for 30 sec.
3. Remove the seal and blot the top of the plate with a Kimwipe.
4. Tightly reseal the plate with a fresh seal.
5. Vortex the plate for 30 sec to thoroughly mix.
6. Spin at 1000 rpm for 30 sec.

2. 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 **OK**.

     NOTE: The deck layout is also shown in Figure 2.1 on the next page of this QRC.

3. 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 Kimwipe.
3. Tightly seal the plate.
4. Place the plate in a –20 °C freezer overnight to precipitate.

4. Centrifuge and Dry Pellets

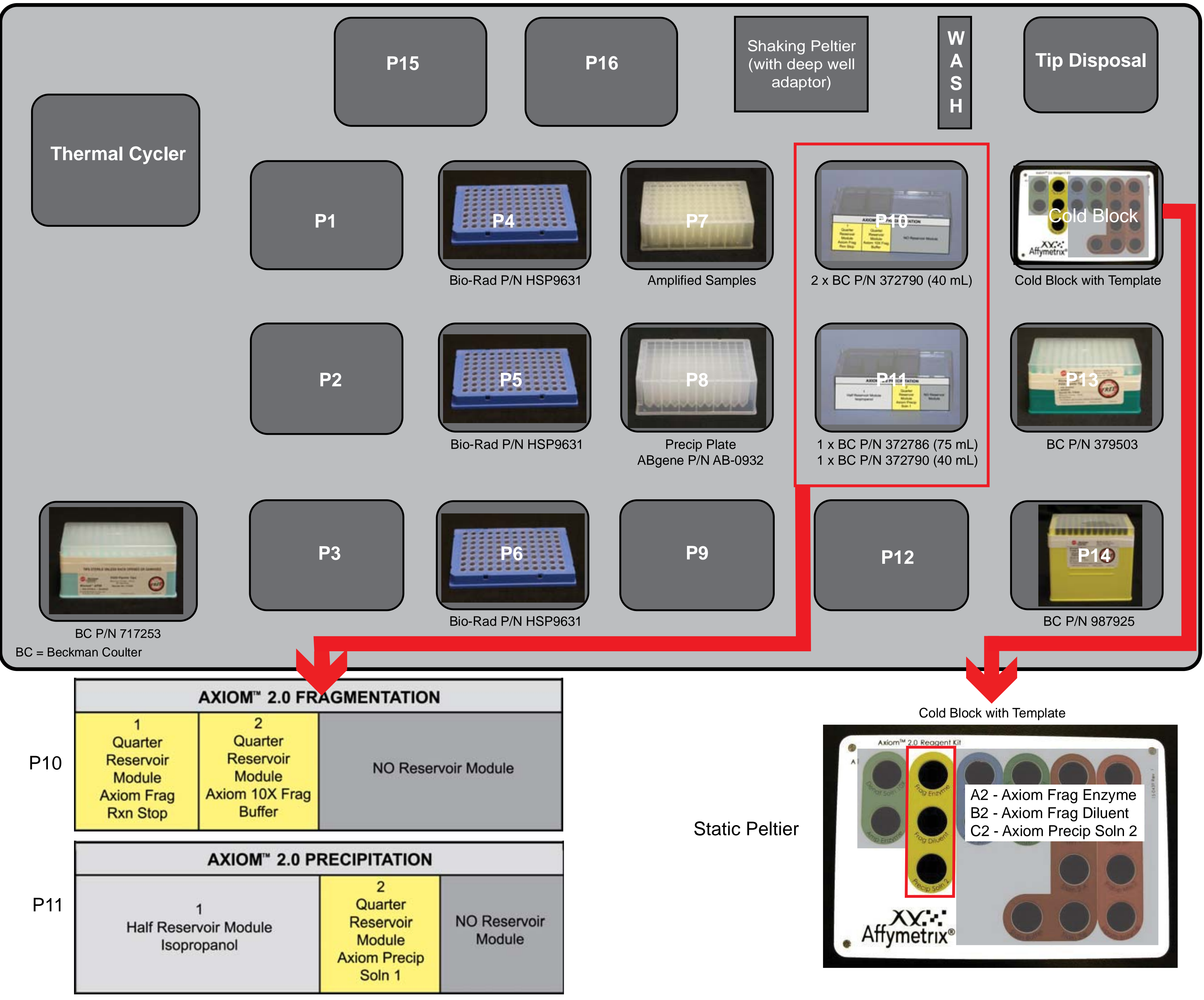
1. Preheat the oven to 37 °C; cool centrifuge to 4 °C
2. Centrifuge the plate at 3200 xg (or rcf) at 4 °C for 40 min.
3. Remove the seal, then invert the plate over a waste container to allow the liquid to drain.
4. While still inverted, gently press the top of the plate on a stack of Kimwipes.
5. Leave plate inverted on Kimwipes for 5 min.
6. Turn the plate right side up and place in the preheated oven for 20 min.

5. What to do Next

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

- Proceed directly to *Stage 3. Resuspension and Hybridization Preparation* (even if some droplets of liquid remain).
- Tightly seal the sample plate and store at –20 °C.

Figure 2.1. Deck Layout — Fragmentation and Precipitation



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Axiom® 2.0 Automated Target Prep Protocol
Stage 3. Resuspension and Hyb Preparation

Reagents and Samples Required

Reagents from Module 2-1 (P/N 901528) and Module 2-2 (P/N 901529) of the Axiom® 2.0 Reagent Kit; TrackIt reagents are user-supplied.

Table 3.1. Reagent Preparation for Stage 3

Reagent	Temp Out of Module*	Treatment
Axiom Hyb Buffer	Place on ice	Vortex
Axiom Hyb Soln 1	Thaw at Room Temp	Vortex and spin
Axiom Resusp Buffer	Warm to Room Temp (~ 1 hr)	Vortex
Axiom Hyb Soln 2	Place on ice	Vortex and spin
Nuclease-free water	Not Applicable	Not Applicable
TrackIt Cyan/Orange Gel Loading Buffer (diluted 1:1000)	Not Applicable	See Gel QC Instructions
TrackIt 25 bp DNA Ladder (diluted 1:15)	Not Applicable	See Gel QC Instructions
*Temp Out of Module: temperature reagent is held at immediately after removal from module		

1. Prepare the Precipitation Plate (only if frozen)

1. Place the plate of precipitated samples on the bench top and equilibrate to room temperature 1 to 1.5 hr.

2. Resuspend the Samples

1. Label two of the Bio-Rad plates as follows:
   - Hyb Ready <sample identifier>
   - Gel QC
2. Run Biomek method:
   - **A.** Select the ***Resuspension and Hybridization Preparation*** step, then click **OK**.
   - **B.** Setup the deck as indicated in the Biomek deck setup prompt, then click **OK**.

     NOTE: The deck setup is also shown in Figure 3.2 on the next page. Refer to the *Axiom® 2.0 Assay Automated Workflow User Guide* (P/N 702963) for special instructions if an ABI thermal cycler will be used in Stage 4.

3. Run Fragmentation QC Gels

1. Tightly seal the Gel QC plate, vortex and spin.
2. Onto a 4% agarose e-gel load:
   - 20 µL from each well of the Gel QC plate.
   - 15 µL diluted TrackIt 25 bp ladder to marker wells.
   - 20 µL water to any unused wells.
3. Run for 22 min.
4. Review gel image (see Figure 3.1).

4. Quantitate the Resuspended Samples

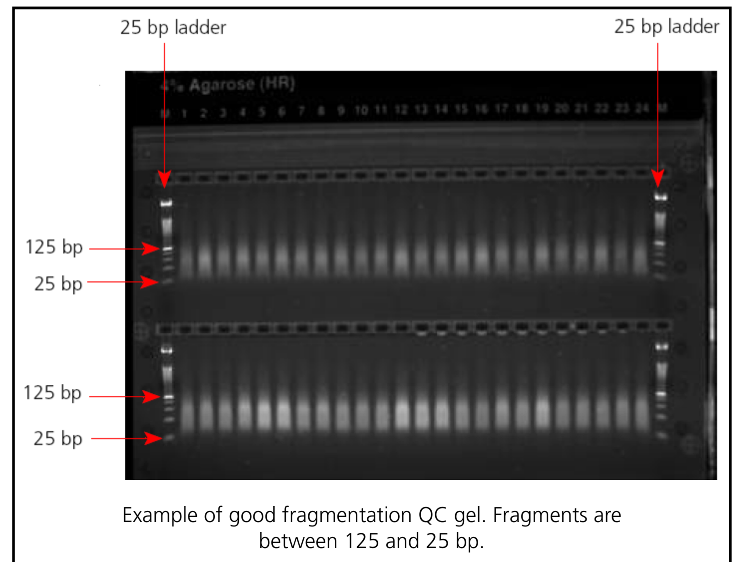
1. Quantitate the samples prepared in the OD plate.
2. Assess the OD reading for each sample.

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 4. Preparation for GeneTitan.*
- Tightly seal the Hyb Ready plate and store at –20 °C. (This plate is referenced as Hyb Rxn in the Biomek software.)

Figure 3.1. Gel Image



Example of good fragmentation QC gel. Fragments are between 125 and 25 bp.

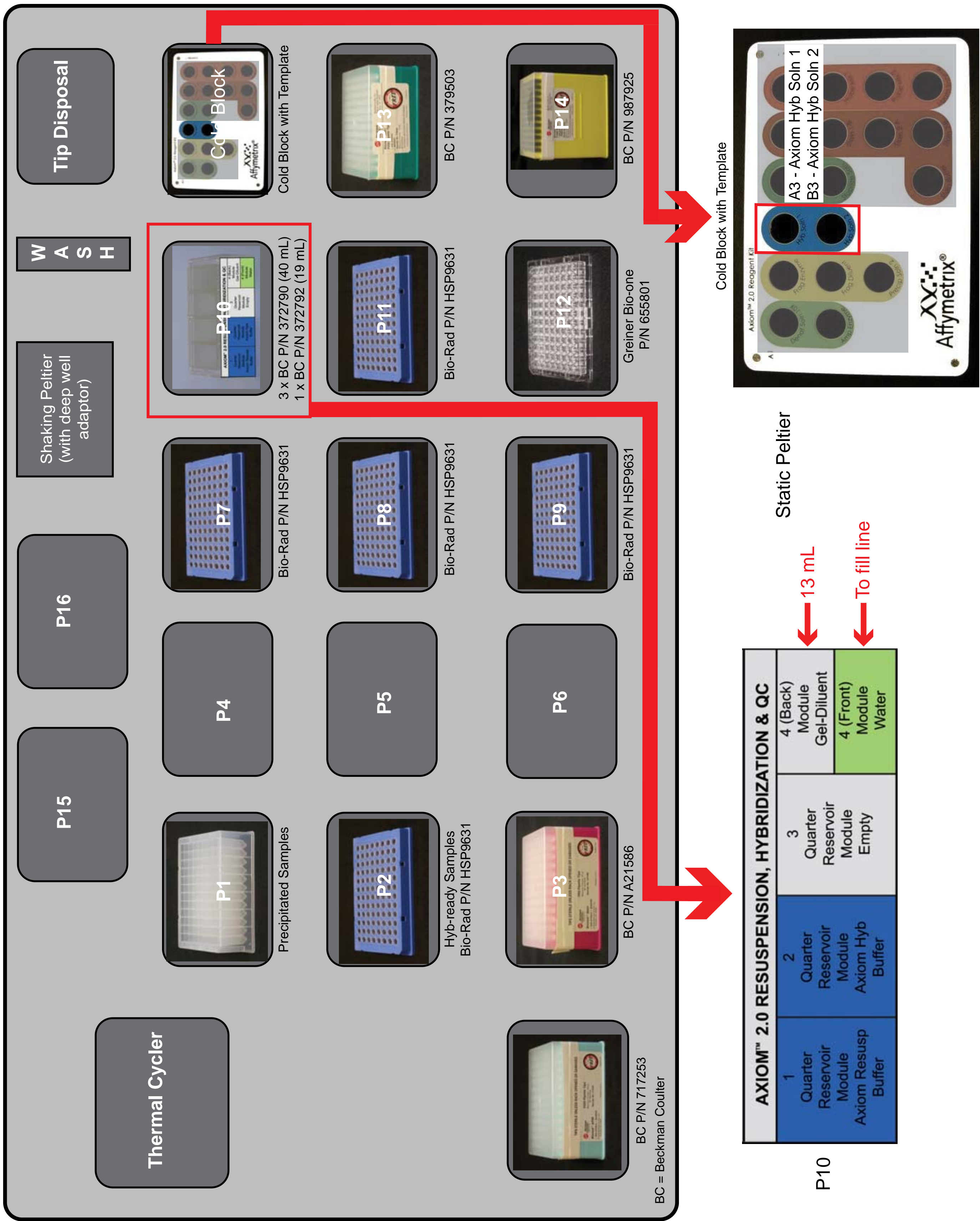


Figure 3.2. Deck Layout — Resuspension and Hybridization Preparation

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Axiom® 2.0 Automated Target Prep Protocol

Stage 4. Preparation for the GeneTitan® Instrument

Important Guidelines for this Stage

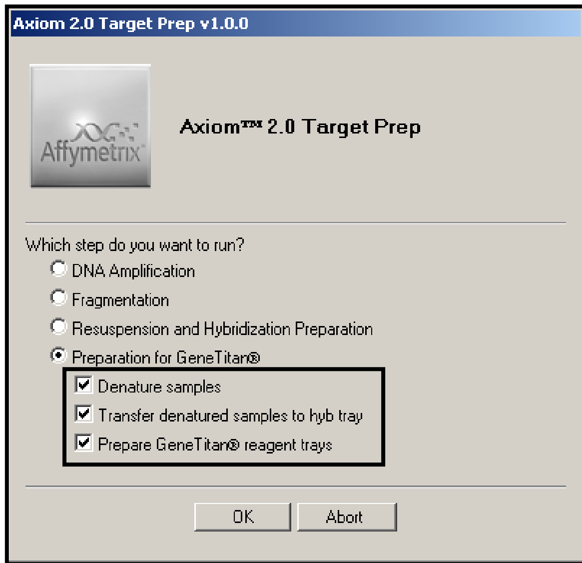
Begin this stage 45 min prior to when the array plate currently in the GeneTitan® Instrument will finish hybridization.
This stage takes approximately 40 min to run.

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

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

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

Figure 4.1. Axiom 2.0 Target Prep Step Selection



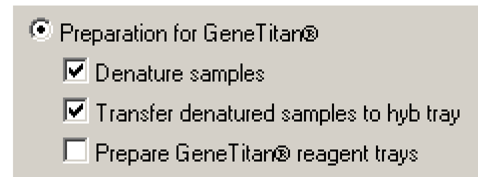
You can perform each part of the stage separately, or you can run both parts at the same time for the high-throughput workflow. For the high-throughput workflow, you are preparing reagent trays for the array plate that is currently finishing the Hybridization step in the GeneTitan® Multi-Channel (MC) Instrument, while preparing another hyb 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 Plate (sample plate):
   Vortex briefly; spin at 1000 rpm for 30 sec; then place on ice.
2. Run Biomek method:
   A. Select the ***Preparation for GeneTitan*** step, and the following sub-steps:
      - ***Denature samples***
      - ***Transfer denatured samples to hyb 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 4.2 on the next page of this QRC.



   IMPORTANT: Clean the metal lid and pad by wiping with 70% ethanol.
3. Prepare the GeneTitan MC Instrument.
   NOTE: If using an off-deck thermal cycler, refer to the *Axiom® 2.0 Assay Automated Workflow User Guide* (P/N 702963) for special instructions.
4. Once the GeneTitan Instrument is ready, return to Biomek and press OK at prompt to transfer samples from thermal cycler to hyb tray.
5. Load the hyb tray and array plate in the GeneTitan MC Instrument.
   See *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC* (P/N 702988).

Figure 4.2. Deck Layout — Denaturation and Transfer to Hyb Plate

Prepare Reagent Trays for the GeneTitan Instrument

IMPORTANT: The reagent trays prepared are for use with an Axiom array plate that is already in the GeneTitan MC instrument and is completing the hybridization stage.

1. Prepare the reagents from Module 4 of the Axiom 2.0 Reagent Kit , as shown in Table 4.1 below:

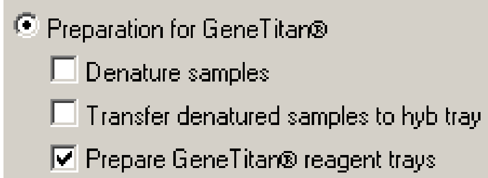
Table 4 1. Reagent Preparation

Reagent	Temp Out of Module*	Treatment
Module 4-1 (P/N 901278)		
Axiom Ligate Buffer	Thaw at Room Temp	1. Place on bench top at room temp for 30 min 2. Vortex twice 3. Examine for precipitate If any: ▪ Warm bottle with your hands and vortex again for thirty seconds
Axiom 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
Axiom Ligate Soln 1	Thaw at Room Temp	Vortex and spin
Axiom Probe Mix 1	Thaw at Room Temp	Vortex and spin
Axiom Stain Buffer	Thaw at Room Temp	Vortex and spin
Axiom Stabilize Soln	Thaw at Room Temp	Vortex and spin
Module 4- 2 (P/N 901276)		
Axiom Ligate Soln 2	Thaw at Room Temp (do not place on ice!)	Vortex and spin
Axiom Probe Mix 2#	Place on ice	Flick 2 to 3 times to mix, then spin
Axiom Wash A	Leave on bench	1. Vortex twice 2. Place on Bench for 30 min 3. Look for precipitate 4. Vortex again if necessary
Axiom Stain 1-A#	Place on ice	Flick 2 to 3 times to mix, then spin
Axiom Stain 1-B#	Place on ice	Flick 2 to 3 times to mix, then spin
Axiom Stain 2-A#	Place on ice	Flick 2 to 3 times to mix, then spin
Axiom Stain 2-B#	Place on ice	Flick 2 to 3 times to mix, then spin
Axiom Stabilize Diluent	Place on ice	1. Vortex and spin 2. Look for precipitate If any: ▪ Warm tube to room temperature and vortex again
Axiom Water	Place on ice	N/A
Axiom Hold Buffer#	Room Temp	Vortex

Notes:
* Temp Out of Module: temperature the reagent is held at immediately after removal from module.
# These solutions are light sensitive. Keep tubes out of direct light for a prolonged period of time.
N/A: not applicable in this case

2. Run Biomek method:
   - **A.** Select the ***Preparation for GeneTitan*** step, and the following sub-step:
     - ***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 Figure 4.3 of this QRC.

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



3. Prepare the GeneTitan® Multi-Channel Instrument.
   See *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC* (P/N 702988).
4. Treat 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 the GeneTitan MC Instrument.
   See *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC* (P/N 702988).

Figure 4.3. Deck Layout — Reagent Tray Preparation

Thermal Cycler

P15

P16

Shaking Peltier (with deep well adaptor)

WASH

Tip Disposal

P1

P4 — Stain 1 - 2, P/N 501025

P7 — Scan Tray, P/N 501006

P10 — RT Reagent Block, Load Axiom Ligate Soln 2 in A6

Cold Block — Cold Block with Template

P2

P5 — Stain 2, P/N 501025

P8 — Stablize Soln, P/N 501025

P11 — 3 x BC P/N 372792 (19 mL)

P13 — BC P/N 379503

P3

P6 — Ligation Soln, P/N 501025

P9 — Stain 1 - 1, P/N 501025

P12 — 3 x BC P/N 372790 (40 mL)

P14 — BC P/N 987925

BC = Beckman Coulter

P11

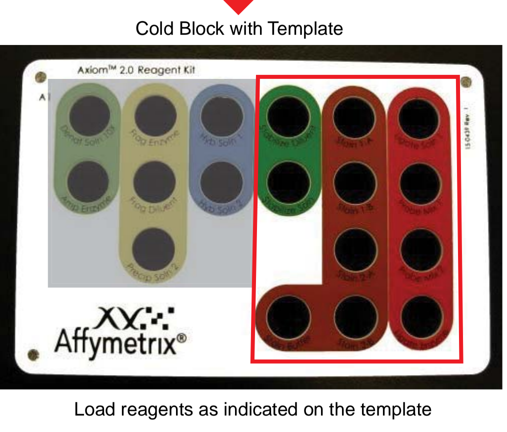
AXIOM™ 2.0 GENETITAN® PREPARATION			
1 (Back Module) Axiom Water	2 (Back Module) Empty	3 (Back Module) Axiom Ligate Buffer	NO Reservoir Module
1 (Front Module) Empty	2 (Front Module) Empty	3 (Front Module) Empty	

P12

AXIOM™ 2.0 GENETITAN® PREPARATION			
1 Quarter Reservoir Module Axiom Hold Buffer	2 Quarter Reservoir Module Axiom Wash A	3 Quarter Reservoir Module Empty	NO Reservoir Module

Static Peltier

Cold Block with Template



Load reagents as indicated on the template

Stage 4 for High-Throughput Workflow

In the high-throughput workflow you:

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

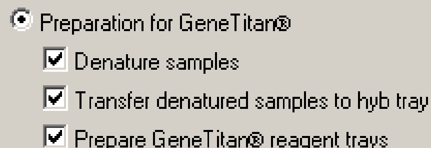
IMPORTANT: The reagent trays prepared in the high-throughput workflow are not for use with the hyb tray currently being prepared on the Biomek workstation, but for an Axiom array plate that is already in the GeneTitan MC Instrument and is completing the hybridization stage.

To perform Stage 4 for high-throughput:

1. Prepare the reagents from Module 4 of the Axiom® 2.0 Reagent Kit , as shown in Table 4.1 on page 9.
2. Prepare the Hyb Ready Plate (sample plate):

   Vortex briefly; spin at 1000 rpm for 30 sec; then place on ice.
3. Run 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 4.2 and 4.3 of this QRC.

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

   IMPORTANT: Label the stain trays and treat them with the antistatic gun.
4. Prepare the GeneTitan MC Instrument

   See *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC* (P/N 702988).

   NOTE: If using an off-deck thermal cycler, refer to the *Axiom® 2.0 Assay Automated Workflow User Guide* (P/N 702963) for special instructions.
5. Treat the stain and scan tray lids with the antistatic gun.
6. Cover the reagent trays and scan tray with lids.
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 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 hyb tray.
10. Load the hyb tray and array plate into the GeneTitan Instrument.

    See *GeneTitan® MC Protocol for Axiom® 2.0 Array Plate Processing QRC* (P/N 702988).

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