Axiom™ 2.0 Assay 96-Array Format Automated Workflow

Catalog Number 901758

Pub. No. MAN0017742 Rev. C.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the following product documentation: *Axiom™ 2.0 Assay 96-Array Format Automated Workflow User Guide—Applied Biosystems™ NIMBUS™ Target Preparation Instrument* (Pub. No. MAN0017740). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Introduction

The Axiom™ 2.0 Assay 96-Array Format Automated Workflow uses the Axiom™ 96 Sample Automated Target Preparation Solution method on the Applied Biosystems™ NIMBUS™ Instrument for target preparation and for GeneTitan™ reagent preparation to process 96 samples at a time.

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

1. Genomic DNA preparation, described in the *Axiom™ 2.0 Assay 96-Array Format Automated Workflow User Guide—Applied Biosystems™ NIMBUS™ Target Preparation Instrument* (Pub. No. MAN0017740).
2. Target preparation of the samples, performed using Axiom™ 2.0 target preparation for the NIMBUS™ Target Preparation Instrument, described in this document.
3. Array processing, described in *GeneTitan™ MC Protocol for Axiom™ Array Plate Processing Quick Reference* (Pub. No. MAN0017718).

IMPORTANT! This document contains an abbreviated set of instructions. Carefully read all the instructions in the target preparation chapter of the *Axiom™ 2.0 Assay 96-Array Format Automated Workflow User Guide—Applied Biosystems™ NIMBUS™ Target Preparation Instrument* before running the automated target preparation method.

The user guide covers the assay steps in more detail and provides information on running multiple plates per week through the automated target preparation process.

Stage 1: Amplify the genomic DNA

Time required

Note: A 22–24 hour incubation is required at the end of this stage.

Activity	Time
Hands-on time	~30 minutes
NIMBUS™ Instrument—DNA Amplification	~30 minutes
Incubation	23 ±1 hour
Total	**~24 hours**

Input required

The Amplification Sample Plate of genomic DNA samples in a round deepwell plate.

Equipment required

- Incubator/oven, temperature at 37°C
- Centrifuge, at room temperature

Reagent and sample plate handling

Module	Reagent and cap color	Place at room temperature	Deck loading instructions
From the Axiom™ 2.0 Reagent Kit.			
Module 1 –20°C	Axiom™ 2.0 Denat Soln 10X	✓ Thaw at room temperature.	Vortex and spin. Place in the cooling block.
	Axiom™ 2.0 Neutral Soln	✓ Thaw in room temperature water bath (~1 hour).	Vortex for 30 seconds. Pour in reservoir.
	Axiom™ 2.0 Amp Soln	✓ Thaw in room temperature water bath (~1 hour).	Vortex for 30 seconds. Pour in reservoir.
	Axiom™ Water	✓ Thaw in room temperature water bath (~1 hour).	Vortex for 30 seconds. Pour in reservoir.
	Axiom™ 2.0 Amp Enzyme	⊗ Do not thaw. Keep at –20°C until ready to use.	**Immediately before use:** Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
Sample Plate			
Thaw the gDNA Sample Plate at room temperature, then briefly centrifuge. **Note:** Do not place a frozen Sample Plate directly on the NIMBUS™ Instrument deck.			

Run the DNA amplification step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **DNA Amplification**, then click **Run**.
2. Set up the deck as indicated in the deck setup prompt and following screens, then click **Run**. See Figure 1.
3. When finished, remove the sample plate from deck position 3.
   a. Blot the top of the plate with a Kimwipes™ laboratory tissue to remove any droplets present.
   b. Tightly seal the plate.
   c. Vortex the plate for 30 seconds, then centrifuge briefly.
   d. Place in a preheated oven, then incubate at 37°C for 23 ±1 hour.
   e. After 22–24 hours of incubation, do one of the following:
      - Proceed directly to “Stage 2: Fragment and precipitate the DNA” on page 4.
      - Store the amplified DNA sample plate at –20°C.

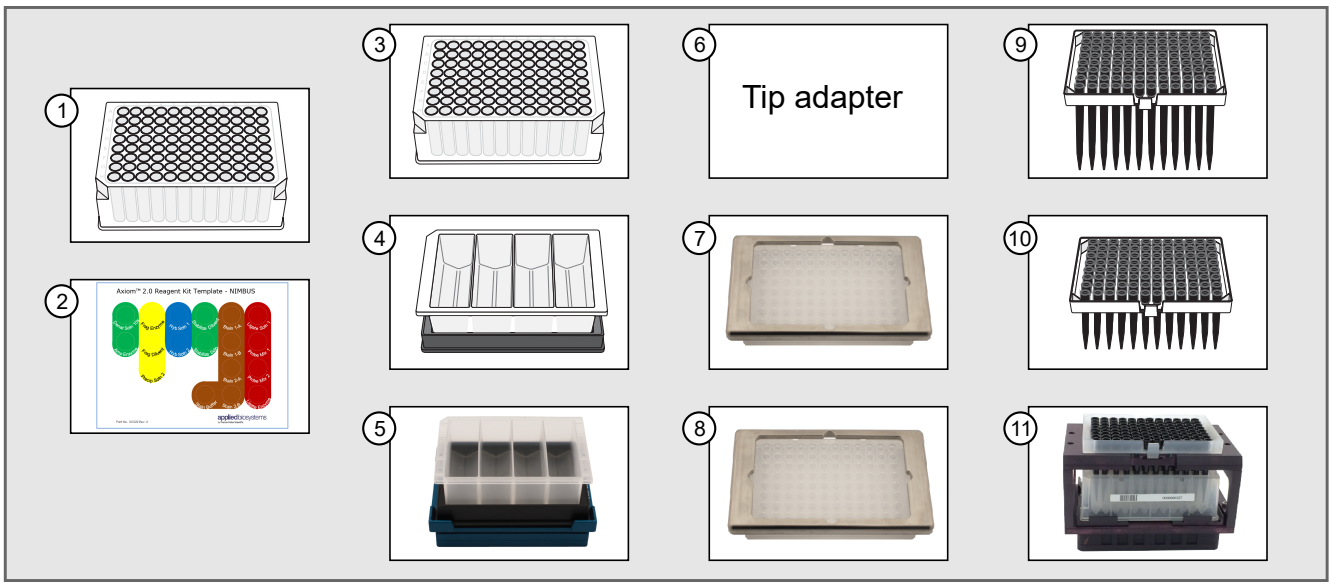
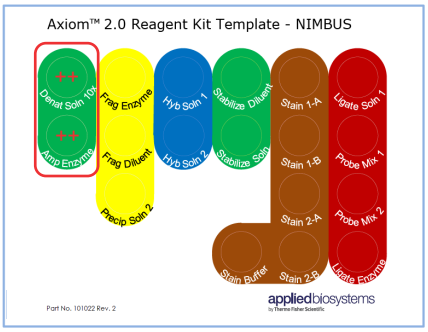


Figure 1 Stage 1B: Amplify the genomic DNA deck layout

① Round deepwell plate

② Cooling block and tube collar. Load reagent tubes only in the green column of the reagent block. Use the template as guidance for specific reagent placement.



③ Round deepwell plate (gDNA Samples)

④ 4-column reservoir & Reservoir frame (Master Mix Reservoir)

⑤ 4-column reservoir, Reservoir frame, & Alpillo™ Plate Cushion
- Column 1: Axiom™ Water
- Column 2: Axiom™ 2.0 Neutral Soln
- Column 3: Axiom™ 2.0 Amp Soln
- Column 4: (Empty)

⑥ (Empty)

⑦ 96-well full-skirt plate & Plate collar

⑧ 96-well full-skirt plate & Plate collar

⑨ CO-RE™ II Filter Tips, 1,000 µL

⑩ CO-RE™ II Filter Tips, 300 µL

⑪ CO-RE™ II Filter Tips, 300 µL & Square deepwell plate

Stage 2: Fragment and precipitate the DNA

Time required

Activity	Time
Hands-on time	~25 minutes ~50 minutes if frozen amplified DNA from Stage 1
NIMBUS™ Instrument—Fragmentation • Deactivation incubation—20 minutes to deactivate the amplification reaction and 20 minutes to equilibrate to the fragmentation temperature • Fragmentation incubation—30 minutes	~1.5 hours
Off-line precipitation incubation at –20°C	3 hours (optional), or overnight (16–18 hours)
Total (does not include precipitation time)	**~2–2.5 hours**

Input required

The Sample Plate of amplified DNA from Stage 1 in a round deepwell plate.

Reagent and sample plate handling

Thaw and prepare reagents and sample plate according to the following table.

Module	Reagent and cap color	Thaw, then place on ice	Place on ice	Place at room temp	Deck loading instructions
Reagents from the Axiom™ 2.0 Reagent Kit					
Module 2-1 –20°C	Axiom™ Frag Enzyme	⊗ Do not thaw. Keep at –20°C until ready to use.			*Immediately before use:* Gently flick tube 3 times, centrifuge briefly. Place in the cooling block.
	Axiom™ 10X Frag Buffer	✓ Thaw in a small water bath[1]			Vortex. Pour in reservoir.
	Axiom™ Precip Soln 2	✓			Vortex, then centrifuge briefly. Place in the cooling block.
Module 2-2 2–8°C	Axiom™ Precip Soln 1		✓		Vortex. Pour in reservoir.
	Axiom™ Frag Diluent		✓		Vortex, then centrifuge briefly. Place in the cooling block.
	Axiom™ Frag Rxn Stop		✓		Vortex. Pour in reservoir.
User-supplied					
	Isopropanol 99.5%, 70 mL			✓	Pour in reservoir.
Note: Estimated reagent thawing time is 30 minutes.					
Amplified Sample Plate					
If the Amplified Sample Plate was *frozen* after the DNA amplification step.		Place the deep-well plate in a small water bath[1] for 50 minutes until all wells have thawed.	Centrifuge at 1,000 rpm for 30 seconds.		
If the Amplified Sample Plate was *not frozen* after the DNA amplification step.			Do not vortex. Remove the seal, then perform the mPCR spike-in step to DNA Amplification Plate before placing on deck.		

[1] For example, on the benchtop at room temperature, pour ultra-pure water into a small tray.

Note: If the Amplified Sample Plate or mPCR Reaction Plate was frozen at the end of Stage 1B, thaw the plates before beginning fragmentation.

Run the fragmentation step, then precipitate the samples

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Fragmentation**, then click **Run**.
2. Set up the deck as indicated in the deck setup prompt and following screens, then click **Run**.

   Note: The deck setup is also shown in Figure 2.
3. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start.

   The fragmentation method starts. The sample plate is incubated at 65°C to inactivate amplification. When complete the **Fragmentation—Cleanup** window appears.
4. Remove the Sample Plate from deck position 8.

   The Sample Plate is now known as the **Precipitation Plate**.
   a. Blot the top of the plate with a laboratory tissue, then seal tightly.
   b. Place the plate in a –20°C freezer overnight to precipitate the DNA.
5. Save or discard the labware as instructed.
6. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.
7. After the incubation period, proceed directly to “Stage 3: Centrifuge and dry pellets” on page 7.

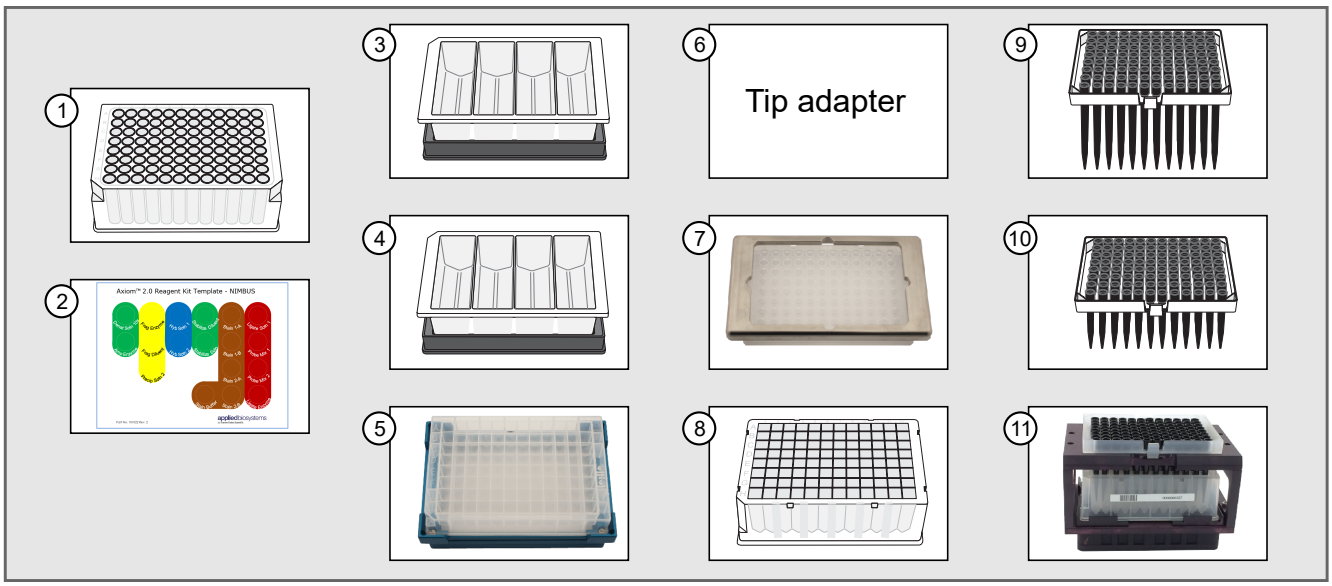
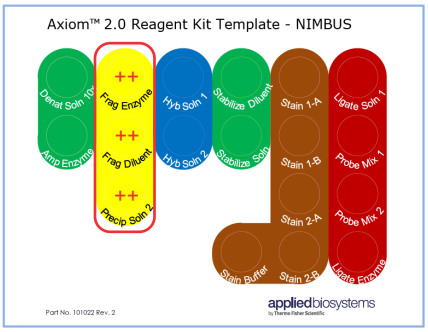


Figure 2 Stage 2: Fragment and precipitate the DNA deck layout

① Round deepwell plate (Amplified gDNA)

② Cooling block and tube collar. Load reagent tubes only in the yellow column of the reagent block. Use the template as guidance for specific reagent placement.



③ 4-column reservoir & Reservoir frame (precipitation reagents)
- Column 1: (Empty)
- Column 2: Isopropanol
- Column 3: Isopropanol
- Column 4: Axiom™ Precip Soln 1

④ 4-column reservoir & Reservoir frame (fragmentation reagents)
- Column 1: Axiom™ 10X Frag Buffer
- Column 2: (Empty)
- Column 3: (Empty)
- Column 4: Axiom™ Frag Rxn Stop

⑤ Square 1.2-mL plate & Alpillo™ Plate Cushion

⑥ (Empty)

⑦ 96-well full-skirt plate & Plate collar

⑧ Square deepwell plate

⑨ CO-RE™ II Filter Tips, 1,000 µL

⑩ CO-RE™ II Filter Tips, 300 µL

⑪ CO-RE™ II Filter Tips, 300 µL & Square deepwell plate

Stage 3: Centrifuge and dry pellets

Time required

Activity	Time
Hands-on time	~10 minutes
Centrifugation	40 minutes
Drying	25 minutes
Total	**~75 minutes**

Input required

One plate of precipitated samples from Stage 2 in a square deepwell plate.

Equipment required

- Incubator/oven, temperature at 37°C
- Centrifuge, at 4°C

Centrifuge and dry the pellets

Note: Keep the centrifuge ready at 4°C.

1. Transfer the Precipitation Plate from the –20°C freezer to a pre-chilled centrifuge.
2. Centrifuge the plate for 40 minutes at 4°C at 3,200 x *g*.
3. Immediately after the 40-minute centrifugation time, empty the liquid from the plate using the following steps:
   a. Remove the seal.
   b. Invert the plate over a waste container, then allow the liquid to drain.

      Note: It is normal for the intensity of the blue color between pellets to vary and the color variation does not indicate any significant differences in the yield of precipitated DNA.
   c. While still inverted, gently press the plates on a pile of laboratory tissues on a bench, then allow them to drain for 5 minutes. Transfer the plates to a new pile of tissues twice during the 5-minute time frame.
4. Turn the plate right side up and place in an oven for 20 minutes at 37°C to dry.
5. Do one of the following:
   - Proceed directly to “Stage 4A and 4B: Resuspension and hybridization preparation” on page 8, even if some droplets of liquid remain. Leave the sample plate at room temperature. It is helpful to start preparing reagents for Stage 4A and 4B while centrifuging and drying pellets.
   - Store the plates for resuspension later in the same day. Tightly seal the plates.
     – If resuspension is carried out within 4 hours, keep the plates at room temperature.
     – If resuspension is carried out in more than 4 hours, store the plates in a refrigerator (2–8°C).
   - Store the plates for resuspension on another day. Tightly seal the plate and store at –20°C.

Stage 4A and 4B: Resuspension and hybridization preparation

We recommend thawing the Module 2-1 and Module 2-2 reagents before starting Stage 4A to minimize any time lapses between stages. The resuspension step must be immediately followed by hybridization preparation (see "Stage 4B: Prepare Hybridization Master Mix" on page 10).

Time required

Activity	Time
Off-deck shaking	10 minutes
Hands-on time	~15 minutes
Frozen pellet equilibration to room temperature	1.5 hours
NIMBUS™ Instrument—Resuspension	4 minutes
NIMBUS™ Instrument—Hybridization preparation	15 minutes
Total	**~2.25 hours**

Input required

- **Stage 4A: Resuspension**—Pelleted DNA from Stage 3 in a square deepwell plate.
- **Stage 4B: Hybridization preparation**—Resuspended DNA from Stage 4A in a square deepwell plate.

Reagent and plate handling

Thaw and prepare reagents according to the following table.

Module	Reagent and cap color	Place on ice	Place at room temperature	Deck loading instructions
Reagents from the Axiom™ 2.0 Reagent Kit				
Module 2-1 –20°C	Axiom™ Hyb Buffer	✓		Vortex. Pour in reservoir.
	Axiom™ Hyb Soln 1		✓	Vortex, then centrifuge briefly. Place in the cooling block.
Module 2-2 2–8°C	Axiom™ Resusp Buffer		✓	Vortex. Pour in reservoir.
	Axiom™ Hyb Soln 2	✓		Vortex, then centrifuge briefly. Place in the cooling block.
Note: Estimated reagent thawing time is 1 hour.				
Precipitation Plates				
• Plates with fresh DNA pellets that were stored at 2–8°C after stage 3 should be allowed to warm to room temperature for 30 minutes. • Plates with frozen DNA pellets at –20°C after stage 3 should be allowed to equilibrate at room temperature for 1.5 hours.				

IMPORTANT! The resuspension reagent must be at room temperature for *1 hour* before proceeding with this step. Failure to equilibrate to room temperature causes incomplete resuspension of pellets and compromises results.

Stage 4A: Prepare the resuspension buffer

The resuspension stage must be immediately followed by "Stage 4B: Prepare Hybridization Master Mix" on page 10.

Run the resuspension step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Resuspension**, then click **Run**.
2. Set up the deck as indicated in the deck setup prompt and following screens, then click **Run**. See Figure 3.
3. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start the resuspension step.

   The resuspension method starts. When complete the **Resuspension—Cleanup** window appears.

4. Remove the Sample Plate from deck position 5.

   This plate contains the DNA pellets in Axiom™ Resusp Buffer.

   a. Blot the top of the plate with a laboratory tissue to remove droplets present.

   b. Seal the plate tightly.

5. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.

6. Immediately proceed to “Resuspend the samples by off-deck shaking” on page 9.

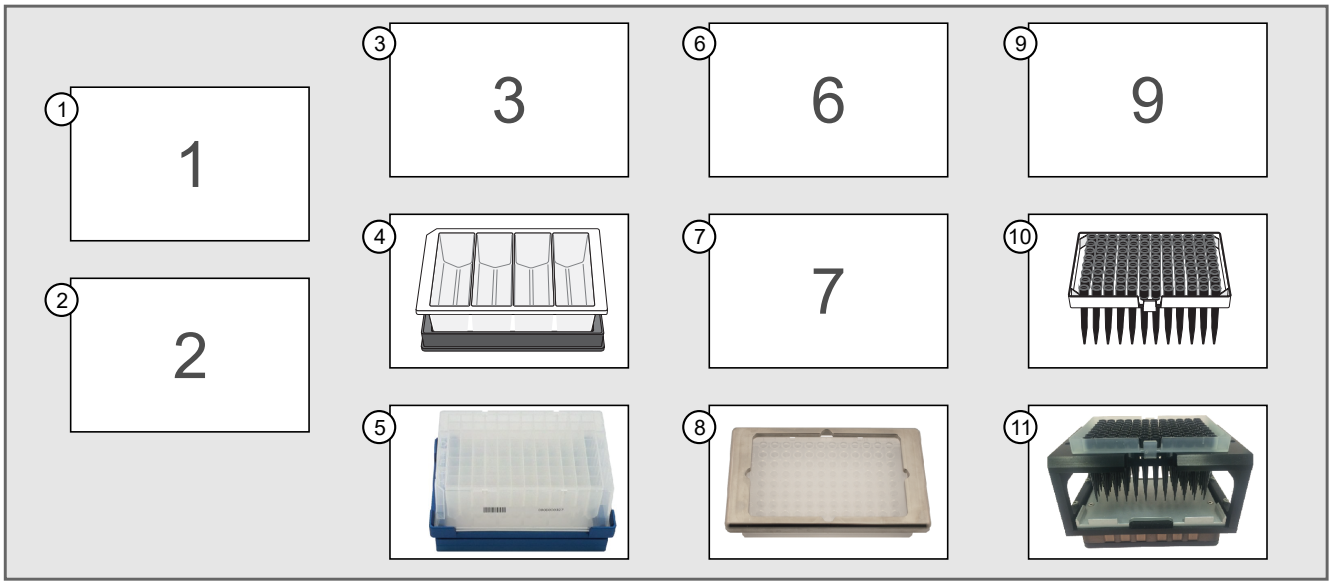


Figure 3 Stage 4A: Prepare the resuspension buffer deck layout.

① (Empty)
② (Empty)
③ (Empty)
④ 4-column reservoir & Reservoir frame (fragmentation reagents)
- Column 1: Axiom™ Resusp Buffer
- Column 2: (Empty)
- Column 3: (Empty)
- Column 4: (Empty)

⑤ Square deepwell plate & Alpillo™ Plate Cushion (Precipitation Plate with DNA pellets)
⑥ (Empty)
⑦ (Empty)
⑧ 96-well full-skirt plate & Plate collar
⑨ (Empty)
⑩ CO-RE™ II Filter Tips, 300 µL
⑪ CO-RE™ II Filter Tips, 300 µL

Resuspend the samples by off-deck shaking

After completion of the on-deck method to aliquot the Axiom™ Resusp Buffer to the square deepwell plate containing the DNA pellets, resuspension is carried out by shaking off-deck.

1. Seal the plate tightly.

   Blue pellets should be visible at the bottom of the wells.

2. Place the sample plate onto one of the following shakers for 10 minutes:
   - Thermo Scientific™ Digital Microplate Shaker, set at 900 rpm.
   - Thermo Scientific™ Compact Digital Microplate Shaker, set at 900 rpm.
   - Boekel Scientific™ Jitterbug™, set at speed of 7.

3. Inspect the plate from the bottom. If the pellets are not dissolved, repeat the shaking step.

4. Centrifuge the plate in a room temperature centrifuge at 1,000 rpm for 30 seconds.

5. Proceed to “Stage 4B: Prepare Hybridization Master Mix” on page 10.

Stage 4B: Prepare Hybridization Master Mix

Perform the off-deck shaking to resuspend the samples before starting this stage.

Run the hybridization preparation step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Hybridization Preparation**, then click **Run**.
2. Set up the deck as indicated in the deck setup prompt and following screens, then click **Run**. See Figure 4.
3. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start the fragmentation step.

   The hybridization method starts. When complete the **Hybridization Preparation—Cleanup** window appears.
4. Remove the Sample Plate from deck position 3.
   a. Blot the top of the plate with a laboratory tissue to remove any droplets.
   b. Seal the plate tightly.
5. When deck cleanup is complete, click **Finish**, then click **Yes** in the confirmation window.
6. Do one of the following:
   - Proceed directly to “Stage 4C: Perform sample QC” on page 11.
   - Tightly seal the Sample Plate and store at –20°C.

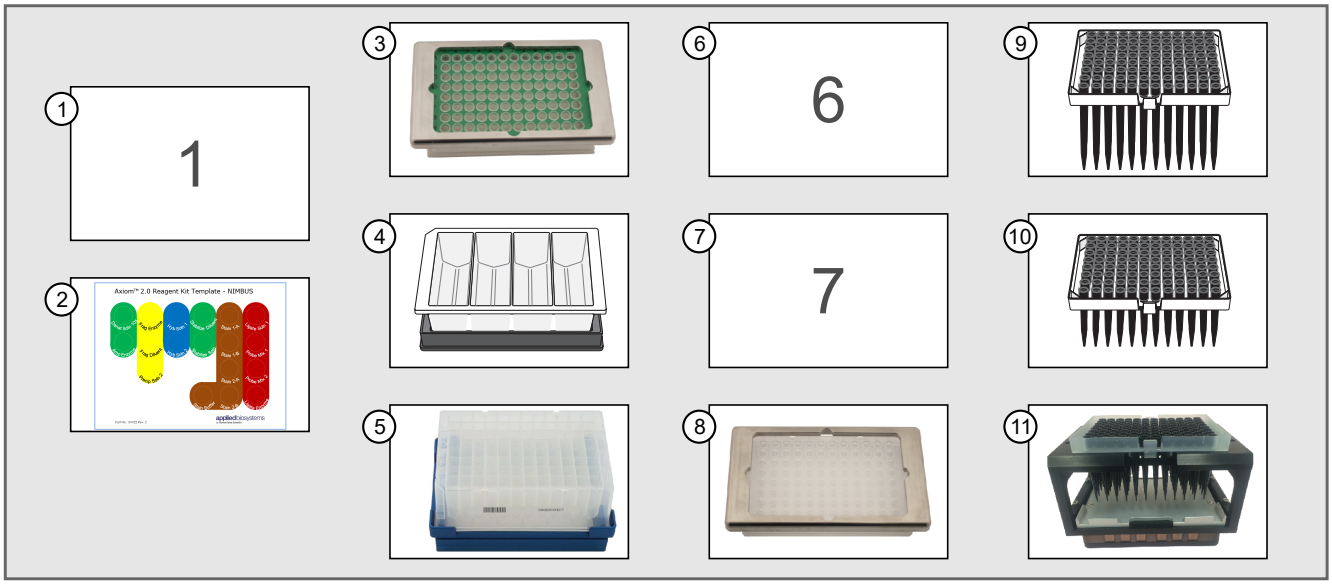
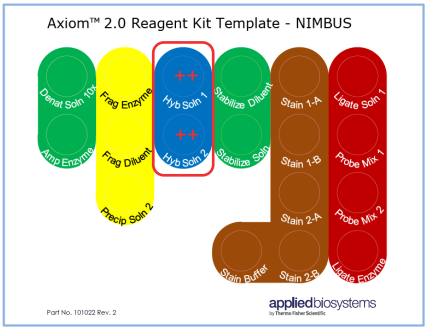


Figure 4 Stage 4B: Prepare Hybridization Master Mix deck layout.

① (Empty)

② Cooling block and tube collar. Load reagent tubes only in the blue column of the reagent block. Use the template as guidance for specific reagent placement.



③ 96 half-skirt plate on holder and plate collar on top

④ 4-column reservoir & Reservoir frame (fragmentation reagents)
- Column 1: (Empty)
- Column 2: Axiom™ Hyb Buffer
- Column 3: (Empty)
- Column 4: (Empty)

⑤ Square deepwell plate on Alpillo™ Plate Cushion (Precipitation Plate with resuspended DNA samples)

⑥ (Empty)

⑦ (Empty)

⑧ 96-well full-skirt plate & Plate collar

⑨ CO-RE™ II Filter Tips, 1,000 µL

⑩ CO-RE™ II Filter Tips, 300 µL

⑪ CO-RE™ II Filter Tips, 300 µL

Stage 4C: Perform sample QC

Note: We strongly recommend that you run 2 quality process controls during this step:

- A gel to verify successful fragmentation
- An OD quantification of each resuspended sample

Time required

Activity	Time
Hands-on time	~5 minutes ~15 minutes if frozen from Stage 4B
NIMBUS™ Instrument—Sample QC	~12 minutes
Total	**~25 minutes**

Input required

One plate of hybridization ready samples from Stage 4B.

Equipment required

- Microplate reader
- E-Gel™ base device

Reagents required

Quantity	Reagent
User-supplied	
20 mL	Gel diluent: TrackIt™ Cyan/Orange Loading Buffer, diluted 100-fold
20 mL	Nuclease-free water, ultrapure MB grade (Thermo Fisher Scientific, Cat. No. 71786; for OD and gel plate preparation)
60 µL	25 bp DNA Ladder

Run the sample QC step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Sample QC**, then click **Run**.
2. Set up the deck as indicated in the deck setup prompt and following screens, then click **Run**. See Figure 6.
3. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start the sample QC step.
   The sample QC method starts. When complete the **Sample QC—Cleanup** window appears.
4. Remove the Sample Plate from position 3. Tightly seal the Hyb Ready Plate.
5. Save or discard the labware as instructed.
6. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.
7. Run fragmentation QC gels.
   a. Tightly seal the Gel QC Plate, vortex, and briefly centrifuge.
   b. Onto a 4% E-Gel™ Agarose 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.
   c. Run for 22 minutes.

d. Review gel image.

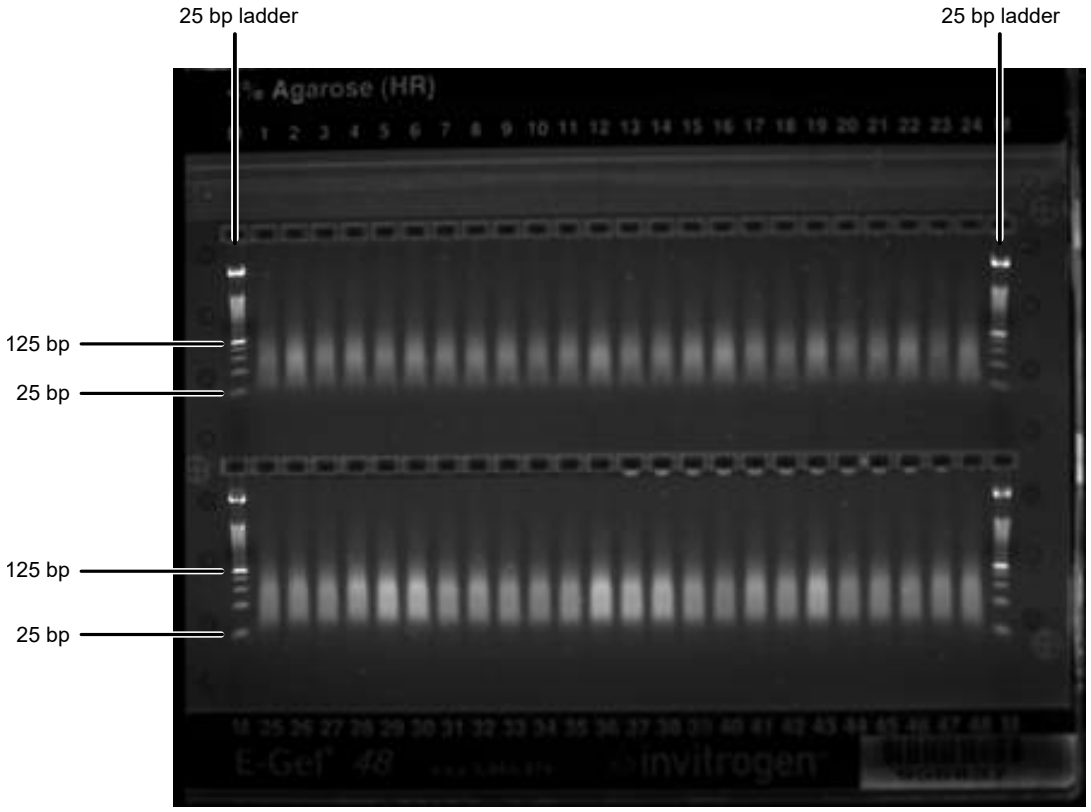


Figure 5 Fragments fall between 125 bp and 25 bp on a successful gel image.

8. Quantify the resuspended samples.
   a. Quantify the samples prepared in the OD Plate.
   b. Evaluate the OD reading for each sample.

      Median yield = 1,200 µg/well.

9. Do one of the following:
   - If the GeneTitan™ MC Instrument is free, and if the gel and OD quantification results are good, proceed directly to “Stage 5: Prepare the hybridization tray” on page 14.
   - If the GeneTitan™ MC Instrument is not free, then store the sealed Hyb Ready Plate at –20°C.

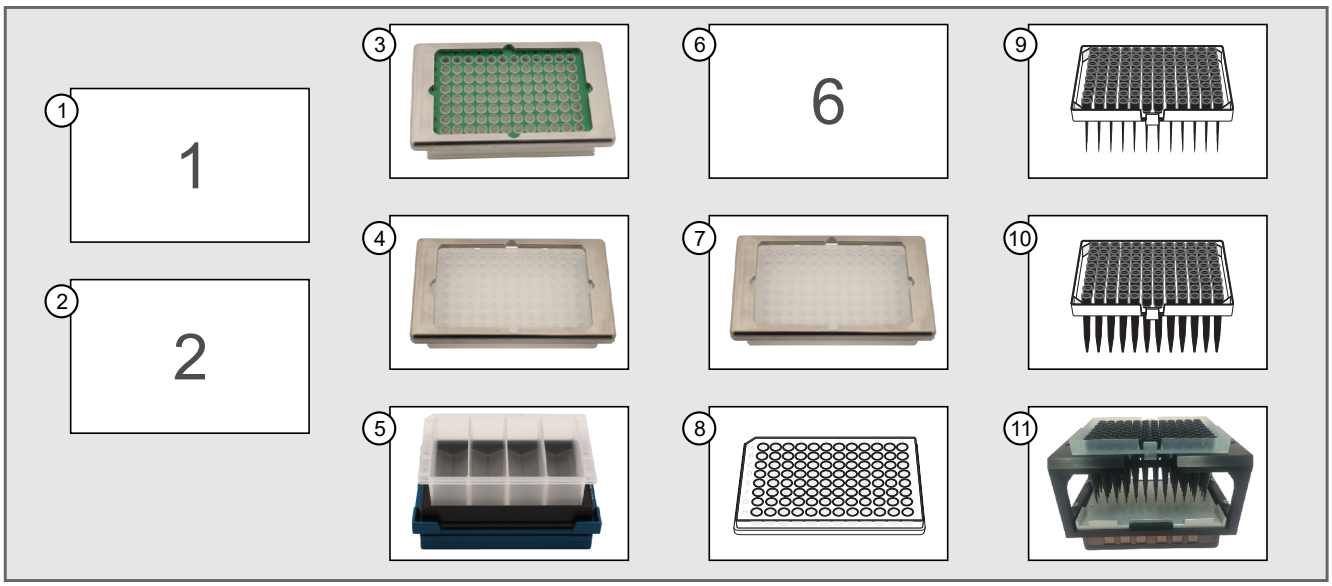


Figure 6 Stage 4C: Perform sample QC deck layout.

① (Empty)
② (Empty)
③ 96 half-skirt plate on holder with plate collar on top (hybridization ready samples)
④ 96-well full-skirt plate & Plate collar (GelQC)
⑤ 4-column reservoir, Reservoir frame, & Alpillo™ Plate Cushion
- Column 1: Water
- Column 2: Gel diluent
- Column 3: (Empty)
- Column 4: (Empty)

⑥ (Empty)
⑦ 96-well full-skirt plate & Plate collar (DilQC)
⑧ 96-well UV plate
⑨ CO-RE™ II Filter Tips, 50 µL
⑩ CO-RE™ II Filter Tips, 300 µL
⑪ CO-RE™ II Filter Tips, 50 µL

Stage 5: Prepare the hybridization tray

Time required

Table 1 Sample denaturation and hybridization tray transfer.

Activity	Time
Hands-on time	~10 minutes
Off-deck step: Denaturation in a thermal cycler	15 minutes
NIMBUS™ Instrument—Prepare Hybridization Tray	1 minute
Total	**~30 minutes**

Table 2 Hybridization.

Activity	Time
Hands-on time	~45 minutes, including denaturation time
Hybridization in the GeneTitan™ MC Instrument	23.5 hours to 24 hours

Input required

One plate of hybridization-ready samples from Stage 4B.

Reagents, equipment, and labware required

Quantity	Item	Instruction
Reagents from the Axiom™ 2.0 Reagent Kit		
2 bottles/1 L	Axiom™ Wash Buffer A	Room temperature. Invert 2-3X for mixing before filling GeneTitan™ bottle.
1 bottle	Axiom™ Wash Buffer B	Room temperature. Invert 2-3X for mixing before filling GeneTitan™ bottle.
1 bottle	Axiom™ Water	Room temperature.
Equipment		
1	GeneTitan™ MC Instrument	Available for hybridization.
1	Thermal cycler programmed with the **Axiom 2.0 Denature** protocol	**Axiom 2.0 Denature** protocol Use the heated lid option when setting up or running the protocol. • 95°C for 10 minutes • 48°C for 3 minutes • 48°C hold
1	96-well block warmed in a 48°C oven[1]	Keep in a 48°C oven.
Labware		
1	Axiom™ Array Plate (96-array format)	Warm the array plate in the pouch at room temperature for at least 25 minutes.
1	Hybridization tray[2]	Room temperature.

[1] The block coming out the 48°C oven is warm to the touch. Gloves and mitts can be used if it feels too hot.

[2] From the Axiom™ GeneTitan™ Consumables Kit (Cat. No. 901606).

Prepare samples that have been stored at –20°C

1. Warm up the Hyb-Ready Plate at room temperature for 5 minutes.
2. Check to ensure that the Hyb-Ready Plate is well sealed. If the plate is not well sealed:
   a. Briefly centrifuge the plate and carefully remove the old seal.
   b. If there is condensation on the top of the plate, blot dry with a laboratory tissue.
   c. Use a fresh seal and tightly reseal the plate.
3. Vortex the Hyb-Ready Plate briefly, then centrifuge at 1,000 rpm for 30 seconds.
4. Place the Hyb-Ready Plate at room temperature.

Prepare the array plate

1. Warm the array plate on the benchtop before setting up hybridization on the GeneTitan™ MC Instrument.
2. Leave the unopened array plate in the pouch at room temperature for at least 25 minutes.
3. At the end of the array warm-up time, open the pouch, then scan the array plate barcode into the GeneTitan™ Array Plate Registration file.

Prepare the GeneTitan™ MC Instrument

1. Launch the GeneChip™ Command Console™ software, then select **GCC GeneTitan Control**.
2. Upload the GeneTitan™ Array Plate Registration file.
3. Select the **System Setup** tab.
4. For **Setup Option**, select **Hyb-Wash-Scan**.
5. Click **Next**.
6. Complete the following in the **Plate information** section:
   a. **Barcode**: Scan or manually enter the Axiom™ array plate barcode, then click **Next**.
   b. **Protocol Name**: Select the protocol name, then click **Next**.
7. Fill the Wash A, Wash B, and Rinse bottles with Axiom™ Wash Buffer A, Axiom™ Wash Buffer B, and Axiom™ Water from Module 3, respectively.
8. Empty the Waste bottle.

Denature the samples

1. Ensure that the thermal cycler is powered on and the **Axiom 2.0 Denature** protocol with the heated lid option is selected.
2. Open the lid of the thermal cycler, then place the sealed Hyb-Ready Plate on the thermal cycler. Check the integrity of the seal as evaporation during denaturation can negatively affect assay performance.
3. Close the lid.
4. Start the **Axiom 2.0 Denature** protocol.
5. After the **Axiom 2.0 Denature** protocol is complete, remove the plate from the thermal cycler, then place the denatured samples on deck position 5.

IMPORTANT! Avoid leaving denatured samples at room temperature for any length of time. When you are ready to transfer the plate from the thermal cycler to the NIMBUS™ Instrument deck at the end of the Axiom 2.0 Denature protocol, place it on a heat block pre-heated at 48°C. Placing the plate on a heat block minimizes sample cooling as you return to the instrument deck.

Run the prepare hybridization tray step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Prepare Hybridization Tray**, then click **Run**.
2. Set up the deck as indicated in the deck setup prompt and following screens, then click **Run**. See Figure 7.
3. After ensuring that the deck layout is correct, click **Run**, then click **Yes** in the confirmation window to start the Prepare Hybridization Tray step.

   The prepare hybridization tray step starts. When complete, the **Prepare Hybridization Tray—Cleanup** window appears.
4. Immediately remove the hybridization tray from deck position 8 and examine to ensure that there are no air bubbles present. Puncture any air bubbles that you may see using a clean pipette tip for each sample.
5. Load the array plate and hybridization tray into the GeneTitan™ MC Instrument. Hybridization continues on the GeneTitan™ MC Instrument for 23.5 to 24 hours.
6. Click **Finish** when deck cleanup is complete. Click **Yes** in the confirmation window.
7. When hybridization is approximately 1.5 hours from completion (22 hours after the start of hybridization), proceed to “Stage 6: Prepare GeneTitan™ reagent trays” on page 17.

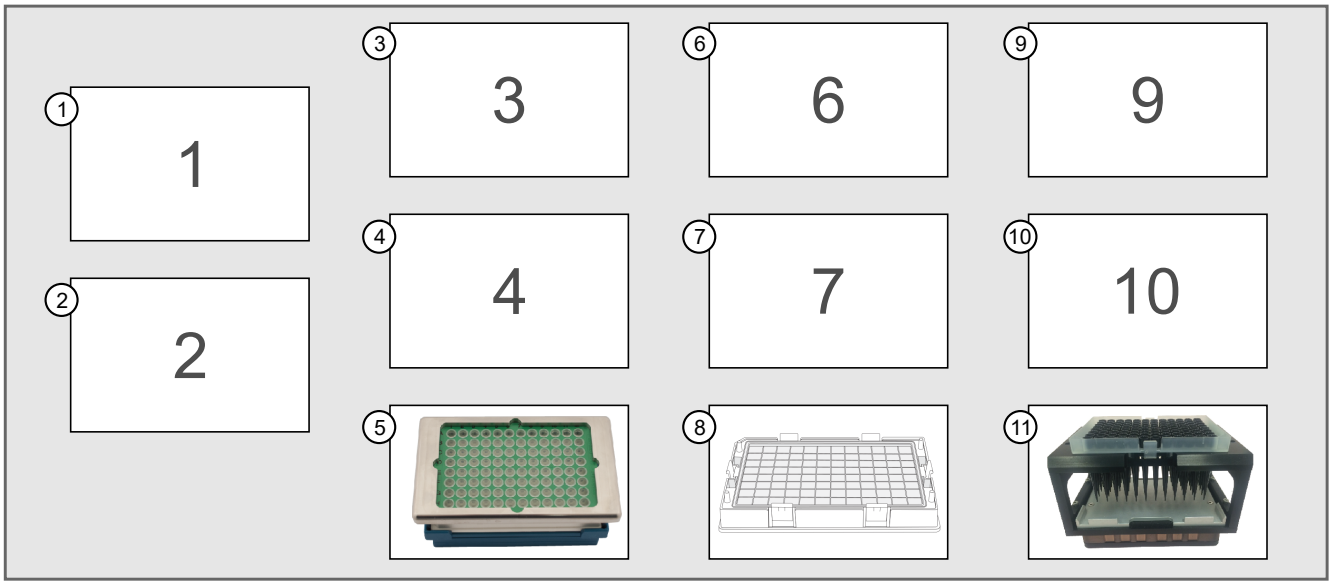


Figure 7 Stage 5: Prepare the hybridization tray deck layout.

① (Empty)
② (Empty)
③ (Empty)
④ (Empty)
⑤ 96 half-skirt plate on holder with plate collar & Alpillo™ Plate Cushion (denatured hybridization ready samples)
⑥ (Empty)
⑦ (Empty)
⑧ Hybridization tray
⑨ (Empty)
⑩ (Empty)
⑪ CO-RE™ II Filter Tips, 300 µL

Stage 6: Prepare GeneTitan™ reagent trays

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

The method for Stage 6 consists of 2 parts:

- Part 1: Preparation of the scan tray, stain 2 tray, and the stabilize tray.
- Part 2: Preparation of the stain 1-1 and stain 1-2 trays and the ligation tray.

After part 1 of the method is completed, a dialog box for labware change appears prompting you to remove labware from specific deck positions, then replace them with new labware. After the labware change, the run proceeds with part 2 of the method.

Time required

Activity	Time
Prepare reagents (thaw and organize reagents)	~30 minutes
Hands-on time	~15 minutes
NIMBUS™ Instrument—Prepare GeneTitan™ reagent trays (with labware change) • Runtime for part 1 • Runtime for part 2	~45 minutes • 21 minutes • 16 minutes
Total	**~90 minutes**

Equipment and labware required

Quantity	Item
Equipment	
1	GeneTitan™ MC Instrument
1	Mini microcentrifuge (microfuge with microtube rotor)
1	Vortexer
1	GeneTitan™ ZeroStat AntiStatic Gun
GeneTitan™ labware	
1	Scan tray with cover and protective base[1]
5	Stain tray[1]
5	Cover for stain tray[1]

[1] From the Axiom™ GeneTitan™ Consumables Kit (Cat. No. 901606).

Reagents required and reagent handling

Prepare reagents according to the following table.

Module	Reagent and cap color	Thaw, then place on ice	Place on ice	Place at room temp	Deck loading instructions
Module 4-1 –20°C	Axiom™ Ligate Buffer			✓	Vortex for 30 seconds. [1] Pour in reservoir.
	Axiom™ Ligate Enzyme	⊗ Do not thaw. Keep at –20°C until ready to use.			**Immediately before use:** Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Ligate Soln 1	✓			Vortex, then centrifuge briefly. Place in the cooling block.
	Axiom™ Probe Mix 1	✓			Vortex, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stain Buffer	✓			Vortex, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stabilize Soln	✓			Vortex, then centrifuge briefly. Place in the cooling block.
Module 4-2 2°C to 8°C	Axiom™ Ligate Soln 2			✓	Vortex, then centrifuge briefly. Place in 24-Position Tube Rack.
	Axiom™ Probe Mix 2[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Wash A			✓	Vortex for 30 seconds.[1] Pour in reservoir.
	Axiom™ Stain 1-A[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stain 1-B[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stain 2-A[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stain 2-B[2]		✓		Gently flick tube 3 times, then centrifuge briefly. Place in the cooling block.
	Axiom™ Stabilize Diluent		✓		Vortex, then centrifuge briefly.[1] Place in the cooling block.
	Axiom™ Water			✓	Pour in reservoir.
	Axiom™ Hold Buffer[2]			✓	Vortex for 30 seconds. Pour in reservoir.
Estimated reagent thawing time is ~1 hour.					

[1] Check for precipitate. If precipitate is present, repeat the vortex and centrifuge step.

[2] These solutions are light sensitive. Keep tubes out of direct light for a prolonged length of time.

Run the prepare GeneTitan™ reagent plates step

1. In the **Axiom NIMBUS Target Preparation Setup** window, select **Prepare GeneTitan Reagent Plates**, then click **Run**.
2. Set up the deck as indicated in the deck setup prompt and following screens, then click **Run**. See Figure 8.

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

   When part 1 is complete, the **Labware Change** window appears.
3. Complete the labware change on the NIMBUS™ deck. See Figure 9.
4. When ready, click **Continue**.
5. Prepare the GeneTitan™ MC Instrument. See *GeneTitan™ MC Protocol for Axiom™ Array Plate Processing Quick Reference* (Cat. No. MAN0017718).
6. Treat the stain and scan tray lids with the antistatic gun.
7. When the method is complete, examine each tray to:
   - Ensure all the wells have been filled. If any wells do not contain reagents, then manually add reagents to these wells.
   - Ensure that there are no air bubbles present. Puncture any air bubbles using a pipette tip.
8. Cover the reagent trays and scan tray with lids.
9. Transfer the reagent trays, scan tray to the GeneTitan™ MC Instrument and load. See *GeneTitan™ MC Protocol for Axiom™ Array Plate Processing Quick Reference* (Cat. No. MAN0017718).

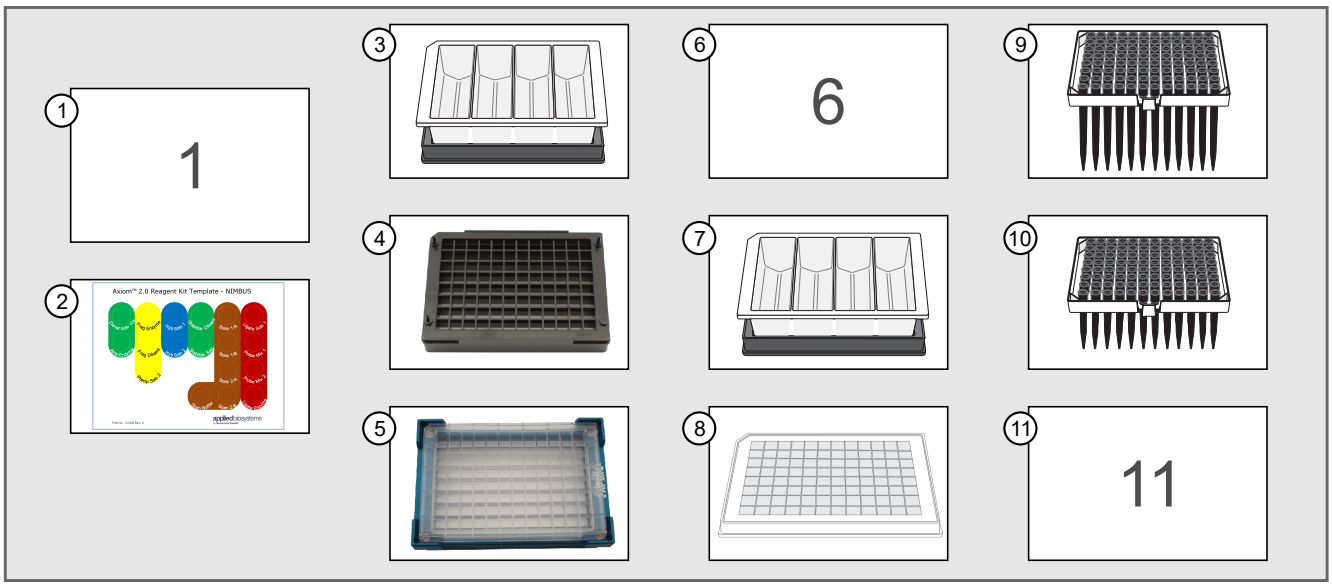
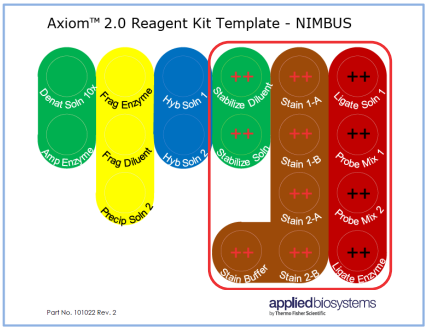


Figure 8 Stage 6: Prepare GeneTitan™ reagent plates, part 1 deck layout

① (Empty)

② Cooling block and tube collar. Load reagent tubes in the last 3 columns (green, brown, and red) of the reagent block. Use the template as guidance for specific reagent placement.



③ 4-column reservoir & Reservoir frame (master mix reservoir)
- Column 1: (Empty)
- Column 2: (Empty)
- Column 3: (Empty)
- Column 4: (Empty)

④ Scan tray

⑤ Stain 2 tray and Alpillo™ Plate Cushion

⑥ (Empty)

⑦ 4-column reservoir & Reservoir frame (fragmentation reagents)
- Column 1: Axiom™ Water
- Column 2: Axiom™ Hold Buffer
- Column 3: Axiom™ Wash A
- Column 4: Axiom™ Ligate Buffer

⑧ Stabilize tray

⑨ CO-RE™ II Filter Tips, 1,000 µL

⑩ CO-RE™ II Filter Tips, 300 µL

⑪ (Empty)

Stage 6: Prepare GeneTitan™ reagent plates, part 2

1. Remove the following labware:
   - Deck position 4: Scan tray
   - Deck position 5: Stain 2 tray
   - Deck position 8: Stabillize Tray
2. Discard the 4-column reservoir and frame from deck position 7
3. Add the labware indicated in the following deck layout image.

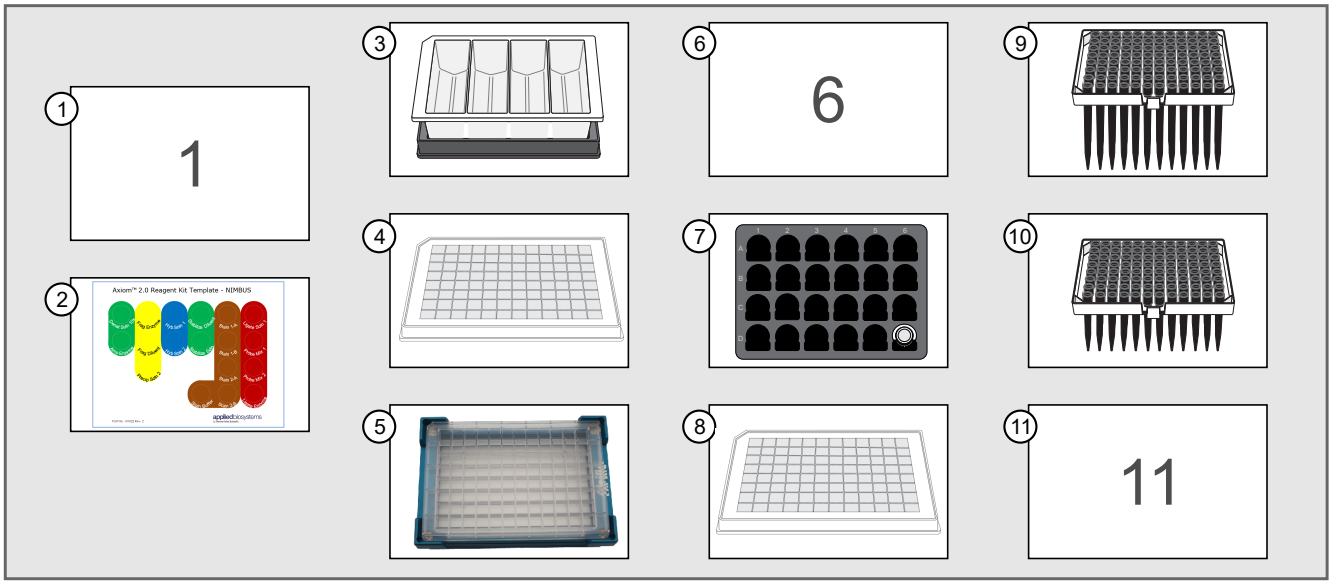


Figure 9 Labware change deck layout

① (Empty)
② Keep: Cooing block and tube collar.
③ Keep: 4-column reservoir & Reservoir frame
④ **Add**: Ligation tray
⑤ **Add**: Stain 1-1 Tray on Alpillo™ Plate Cushion
⑥ (Empty)
⑦ **Add**: 24-Position Tube Rack with insert in D6
• Axiom™ Ligate Soln 2 in D6 tube insert
⑧ **Add**: Stain 1-2 Tray
⑨ Keep: CO-RE™ II Filter Tips, 1,000 µL
⑩ Keep: CO-RE™ II Filter Tips, 300 µL
⑪ (Empty)

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