CG000705 Rev A

USER GUIDE

Chromium Connect Automated Test Kit

FOR USE WITH

Chromium Automated Test Library and Gel Bead Kit, 24 rxns PN-1000578

Notices

Document Number

CG000705 • Rev A

Legal Notices

Instrument & Licensed Software Updates Warranties

Updates to existing Instruments and Licensed Software may be required to enable customers to use new or existing products. In the event of an Instrument failure resulting from an update, such failed Instrument will be replaced or repaired in accordance with the 10x Limited Warranty, Assurance Plan or service agreement, only if such Instrument is covered by any of the foregoing at the time of such failure. Instruments not covered under a current 10x Limited Warranty, Assurance Plan or service agreement will not be replaced or repaired.

Support

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Document Revision Summary

Document Number	CG000705
Title	Chromium Connect Automated Test Kit User Guide
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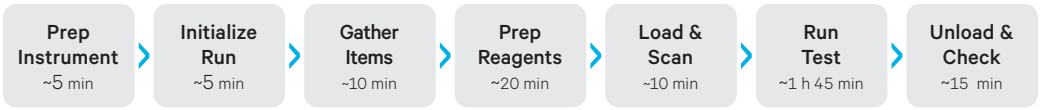
Objective

Chromium Connect is an automated platform for preparation of sequencing-ready libraries from input samples. Before executing an automated assay workflow on Chromium Connect using actual samples, an instrument functional test run can be performed with the Chromium Automated Test Kit. The automated functional test run uses test reagents, consumables, and a test sample to verify that the instrument hardware and software are operating as intended. After the functional test run, inspection of the final liquid volumes in plates and tubes on the instrument deck and a test run report are used as readouts to assess the run.

This document outlines the key steps for executing the Chromium Automated Test Kit workflow (see overview below).



Chromium Automated Test Kit

Chromium Automated Test Library and Gel Bead Kit, 24 rxns PN-1000578

Chromium Automated Test Library Kit Module, 24 rxns PN-1000574*
(store at ambient temperature)
**3 kits included per order*

Chromium Automated
Test Module, 24 rxns

	#
Test Module Strips	24 tube strips
Tube B	2
Tube P	2

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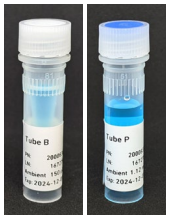

Chromium Automated Test Chip Kit, 48 rxns PN-1000575
(store at ambient temperature)

Chromium Automated
Test Chip Kit, 48 rxns

	#
Test Chip	6
50% Glycerol	6
Tube O	6
Test Sample	6

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Chromium Automated Test Kit

Chromium Automated Test Library and Gel Bead Kit, 24 rxns PN-1000578

Chromium Automated Test Gel Beads Kit, 24 rxns, PN-1000576
(store at ambient temperature)

Chromium Automated
Test Gel Beads Kit, 24 rxns

	#
Test Gel Beads	3 tube strips

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Test Index Kit, 96 rxns, PN-1000577
(store at ambient temperature)

Test Index Kit, 96 rxns

	#
Test Index Plate	1

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Additional Kits, Reagents & Equipment

The items in the table below have been tested by 10x Genomics and are required for this protocol. DO NOT substitute any of the listed materials.

Supplier	Description	Part Number (US)
Plastics		
Hamilton	CO-RE/CO-RE II Tips 50 µl Filtered Tips* CO-RE/CO-RE II Tips 300 µl Filtered Tips* 60 ml Reagent Reservoir Self-Standing Hamilton PCR ComfortLid	235948 235903 194051 814300
	**CO-RE pipette tips will be phased out and replaced by new CO-RE II pipette tips (same part number as CO-RE tips) in 2022/2023. CO-RE II tips include a new sealing surface to interface with the CO-RE II stop disk. Geometry that interfaces with the current CO-RE stop disk is identical between the two tip designs and performance remains unaffected.*	
Eppendorf	96-well Full-Skirted Plate 96-well Semi-Skirted Plate *(Blue color listed; other colors are acceptable)*	951020460 951020362
Thermo Fisher Scientific	MicroAmp 8-Tube Strip, 0.2 ml MicroAmp 8-Cap Strip, clear	N8010580 4323032
Kits & Reagents		
Thermo Fisher Scientific	Nuclease-free Water	AM9937
Millipore Sigma	Ethanol, Pure (200 Proof, anhydrous)	E7023-500ML
Equipment		
10x Genomics	10x Vortex Adapter Benchtop Vortex Benchtop Centrifuge Plate Centrifuge	330002 standard lab equipment standard lab equipment standard lab equipment
Additional materials for Chromium Connect maintenance Use only indicated cleaning agents. DO NOT use bleach or organic oxidizers		
Thor Labs	Lens tissues	MC-5
VWR	Microcide SQ Broad Spectrum Disinfectant	25099
Contec	70% Isopropanol (alternative to VWR disinfectant)	SB167030IR

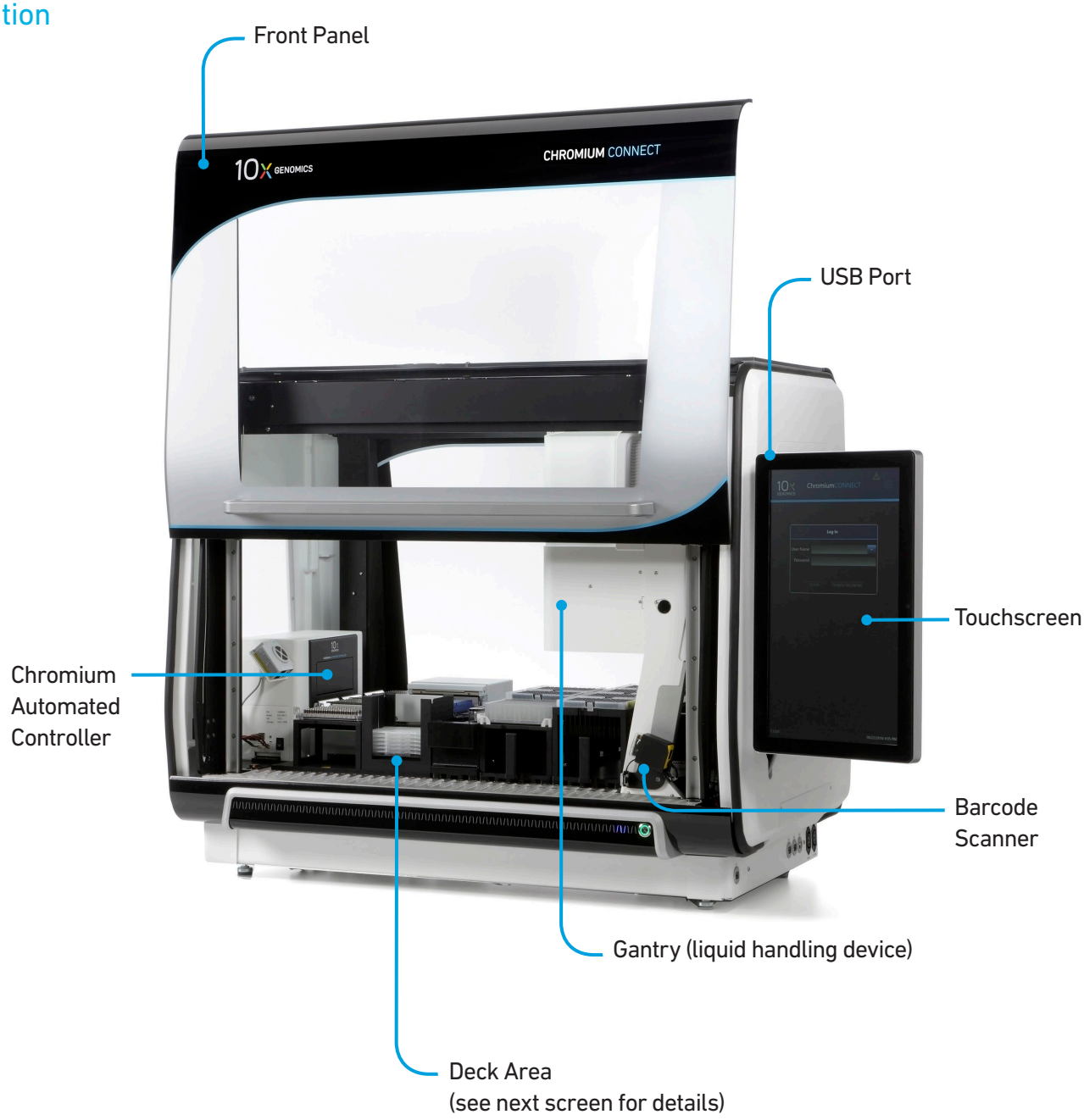
Automated Test Kit Workflow

Instrument Orientation

Deck Orientation

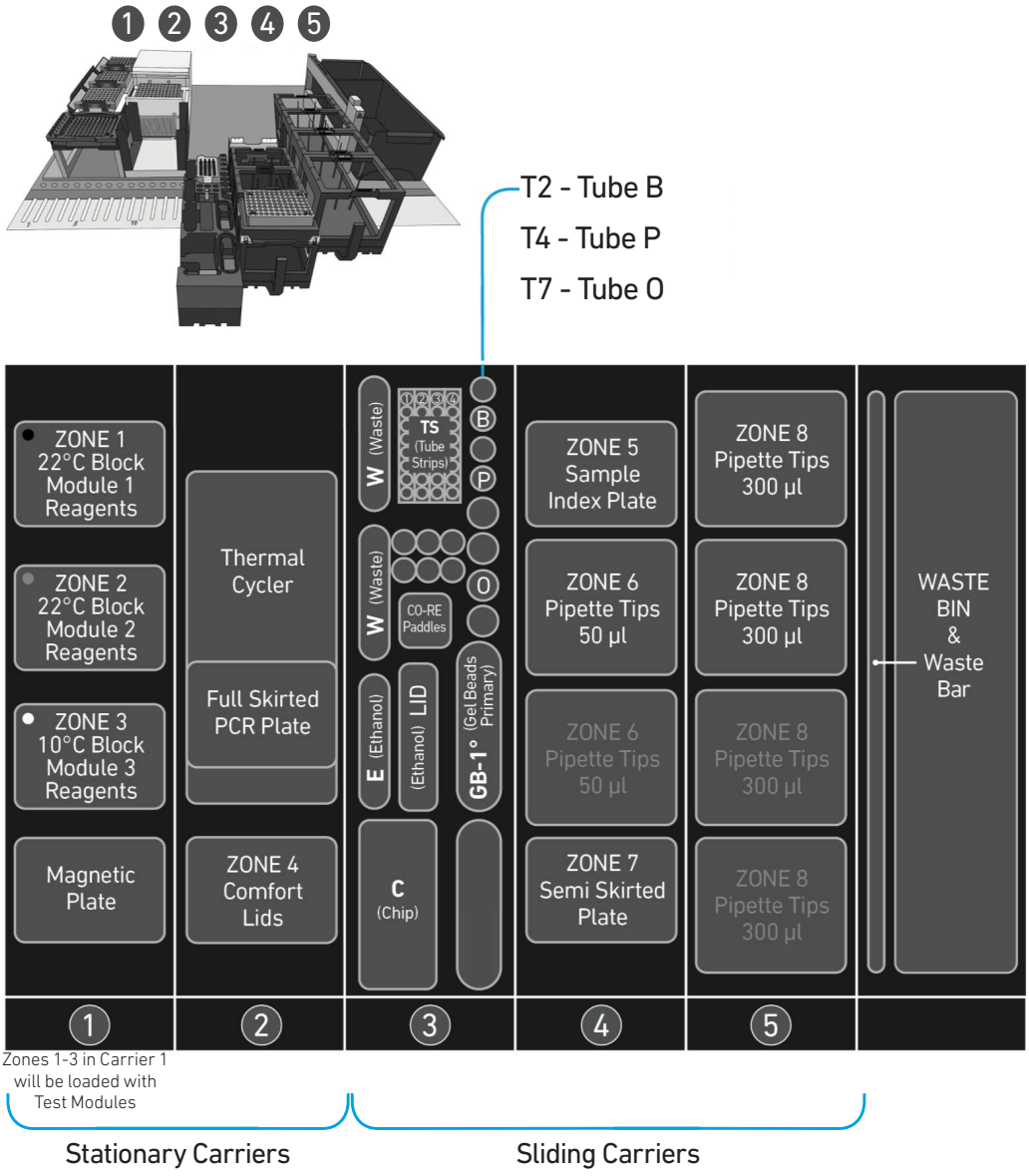
Workflow Steps

Instrument Orientation



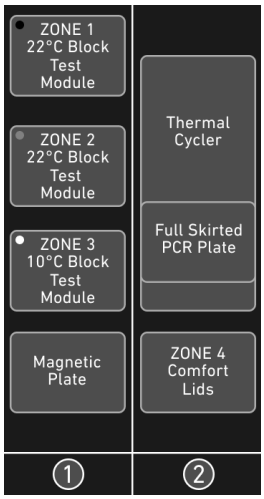
Refer to the Chromium Connect Instrument User Guide (CG000180) and Quick Reference Cards (CG000256) for more information.

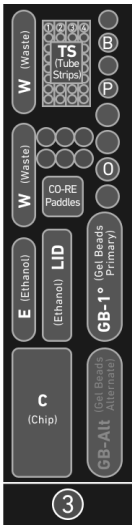
Deck Orientation

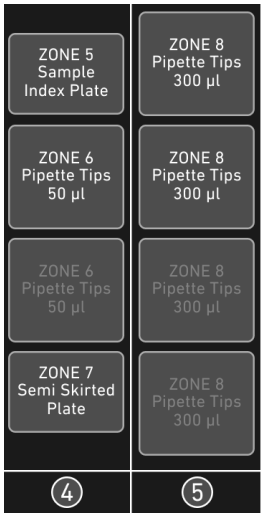


Refer to the Chromium Connect Instrument User Guide (CG000180) and Quick Reference Cards (CG000256) for more information.

Deck Layout (Reagents/ Consumables)







Chromium Automated Functional Test Run

Carrier	Zone	Item
1 Stationary *All 3 zones will be loaded with blue Test Modules	Zone 1* (Black)	22°C Block, Blue Test Module Strips
	Zone 2 *(Gray)	22°C Block, Blue Test Module Strips
	Zone 3* (White)	10°C Block, Blue Test Module Strips
	-	Magnetic Plate
2 Stationary	-	Thermal Cycler
	-	Full Skirted PCR Plate (within Thermal Cycler) *Test Sample loaded here*
	Zone 4	ComfortLids
3* Sliding Deck Rails: 15-18 Number of Lights: 4	Position W	Waste Reservoirs
	Position B	Tube B (White cap)
	Position P	Tube P (Blue cap)
	Position O	Tube O
	-	CO-RE Paddles
	Position E	Ethanol Reservoir
	Position LID	Lid for Ethanol Reservoir
	Position GB-1°	Test Gel Beads
	Position C	Test Chip
4 Sliding Deck Rails: 19-24 Number of Lights: 6	Zone 5	Test Index Plate
	Zone 6	Pipette Tips 50 µl
	Zone 7	Semi Skirted Plate
5 Sliding Deck Rails: 25-30 Number of Lights: 6	Zone 8	Pipette Tips 300 µl

Prepare Instrument

Before executing the workflow, it is required to develop a clear understanding of instrument operation and deck orientation.

Refer to the Chromium Connect Instrument User Guide (CG000180) and Quick Reference Cards (CG000256) for more information.

- Ensure that the Chromium Connect instrument is powered on.
  Ensure that the power cables are connected correctly, and the front power button is engaged, and both power switches are turned on (located at the left side of the instrument).
- The login or home screen should be displayed on the instrument touchscreen.
- Ensure that the Chromium Automated Controller located on the instrument deck is on (LED is green) and there are no errors.
  If the LED indicator on the Chromium Automated Controller is red, turn off the Chromium Automated Controller, wait for 1-2 min, and then turn it back on.

Initialize Run

- Before initializing the run, clear the instrument deck. Ensure that all consumables (tube strips, tip racks, plates, etc.), reagents, and the waste have been removed from the deck. All carriers should be placed in their respective locations on the deck and pushed back.
- Using the touchscreen, check the Chromium Automated Controller to ensure that there is no chip in the tray.
  i. Navigate through the top right selection menu to > Support > Control Panel > Starting Control Panel.
  ii. After that display, the gantry will move. Do not select any options until the door unlock click is heard. Select “Initialize” at the bottom right of the screen and wait for the system to initialize.
  iii. Navigate to Chromium Automated Controller > Get Ready > Open Tray to open the tray to check for chip. Ensure that the module lids are secure before beginning operation.
  iv. After chip check, navigate to Close Tray > Close on the bottom right of the screen to close the front panel.
- Using the touchscreen, navigate to System Verification > Functional Test.
- Follow touchscreen prompts to enter Sample Index Plate information.
- Refer to the touchscreen for an overview of the instrument and deck and follow prompts for the next steps.

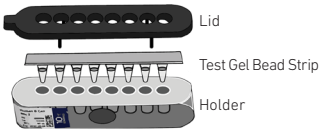
Gather Items

Follow prompts on the touchscreen to gather the listed items and reagents for loading the instrument carriers.

Item	Qty
Nuclease-free Water	10 ml
Ethanol, Pure (200 Proof, anhydrous)	40 ml
Hamilton	
ComfortLids	3
50 µl CO-RE/CO-RE II Pipette Tips, with filter (Black, Conductive)	1 rack
300 µl CO-RE/ CO-RE II Pipette Tips, with filter (Black, Conductive)	2 racks
Reagent Reservoir, 60 ml	3
Eppendorf	
96-well Semi Skirted Plate	1
96-well Full Skirted Plate	1
Thermo Fisher Scientific	
MicroAmp 8-Tube Strip, 0.2 ml	1
10x Genomics	
Automated Test Chip *(keep sealed)*	**1**
Test Module *(blue tube strip)*	24 tube strips/run
Tubes Tube B *(white cap)* Tube P *(blue cap)* Tube O Test Sample	 1 tube/run 1 tube/run 1 tube/run 1 tube/run
Test Index Plate	1 plate
Test Gel Bead Strip	1 tube strip/run

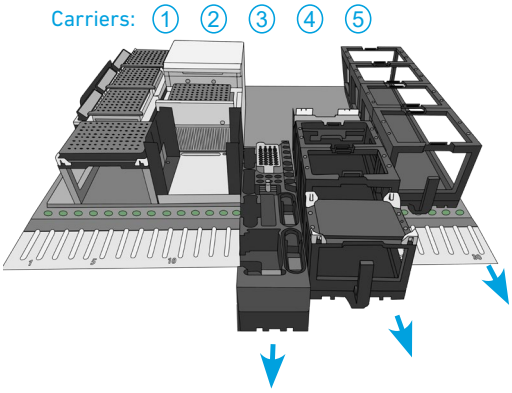
Prepare Reagents

Follow prompts on the touchscreen to prepare reagents before loading the instrument deck. Some important guidelines are highlighted below.

ACTION	GUIDELINES *Follow touchscreen prompts for specifics and timing*
Reagents	• Centrifuge all reagent tubes to collect liquid at the bottom of the tubes. Remove all caps when loading onto the carriers.
Prepare Ethanol	• Prepare **50 ml** 80% Ethanol in Nuclease-free water and dispense in Ethanol Reservoir when prompted.
Sample Index Test Plate	• Vortex Sample Index Plate for **15 sec** at maximum speed and centrifuge at **300 rcf** for **1 min** at **22˚C**.
Test Modules	• Centrifuge all test modules at **300 rcf** for **1 min** at **22°C**. • Confirm there are no bubbles at the bottoms of any module tubes.
Prepare Test Gel Beads	• Snap the tube strip holder with the Test Gel Bead strip into a 10x Vortex Adapter. Vortex **30 sec**. • Centrifuge the Test Gel Bead strip for **~5 sec** after removing from the holder. Confirm there are no bubbles at the bottoms of the tubes and the liquid levels look even. • Place the Test Gel Bead strip back in the holder and secure the holder lid.  

Load Instrument

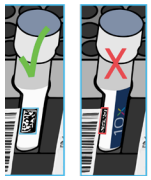
Follow the instructions on the touchscreen to load all reagents and consumables on the carriers. Some key guidelines are provided below:



Carriers

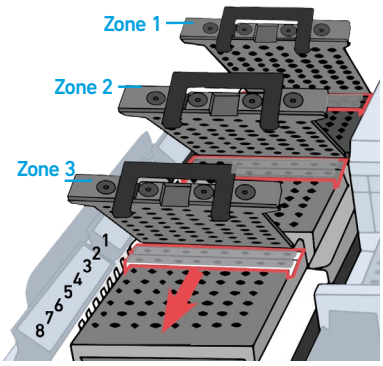
- Handle the carriers as prompted.
- Ensure that Carriers 3, 4, and 5 are completely slid out and placed on an off-deck workspace before loading.
- Align the carriers to the corresponding Deck Rails when sliding them in or out of the deck.
- Remove tube caps and ensure correct orientation of tube labels with barcodes to enable scanning (as shown on the touchscreen).
- Follow the instrument touchscreen prompts to load the Test Chip and the Test Sample.

Barcode Orientation



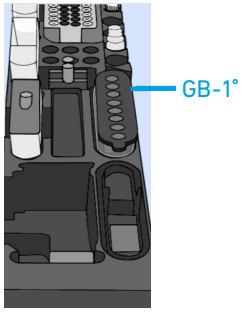
Modules

- Load test module tube strips in the corresponding positions on the Carrier, starting from back to front row. All three zones receive the same blue Test Modules for this automated functional test run.
- DO NOT skip any rows when loading.
- Use pinhole alignment to place module tube strips in the correct orientation (as shown on the touchscreen).



Load Test Gel Beads

- Load Test Gel Bead at Position GB-1˚ as shown on the touchscreen.



Consult the Chromium Connect User Guide (CG000180) for more information.

Scan

After loading is complete, follow prompts on the touchscreen to select the “SCAN” button to initiate scanning.

Gantry will move during scanning.

Run

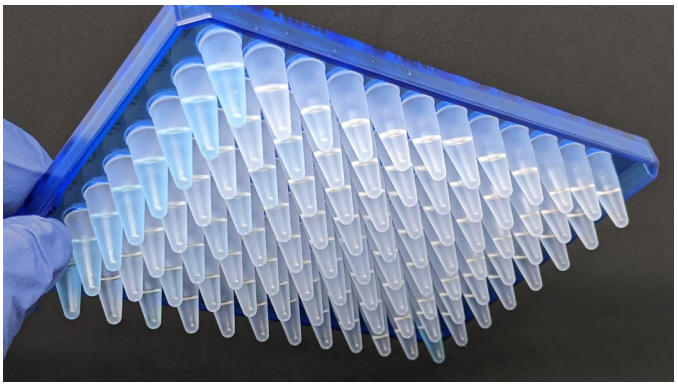
- After scanning is complete, follow prompts on the touchscreen to select the “PLAY” button to start the run. The run time is ~1 h 45 min.

Unload & Check

- After run completion, unload the instrument as per prompts on the instrument touchscreen.
- While unloading, check and document (photograph) the liquid levels in the items shown below. The representative images below show the expected liquid levels after a successful functional test run.

If these items do not look similar to the representative images, refer to the Troubleshooting section for additional guidance.

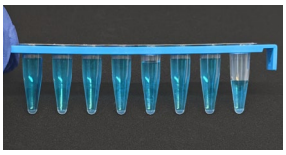
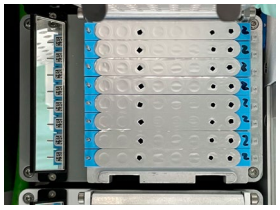
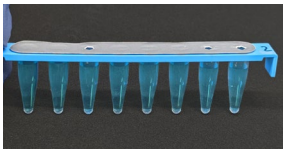
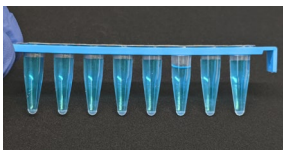
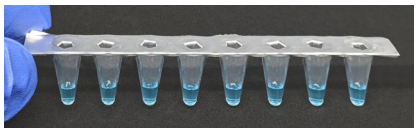
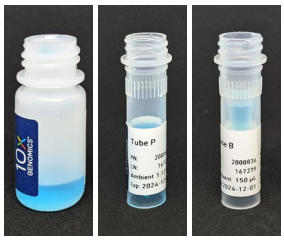
- A run report can be generated and exported as per prompts on the instrument touchscreen.

Check Items	Representative Images After a Successful Functional Test Run
Carrier 2	
Full Skirted Plate	
Carrier 3	
Collection Tube Strip	
Carrier 4	
Semi Skirted Plate	

Troubleshooting

Troubleshooting

- After run completion, if the results are not are not as described in the Unload & Check section, check and document (photograph) the additional items in carriers 1 and 4 (representative images shown below).
- Contact support@10xgenomics.com with all the photographed items for guidance.

Check Items	Representative Images
Carrier 1	
Zone 1-Blue Tube Strip Piercing Pattern & Volumes	 
Zone 2-Blue Tube Strip Piercing Pattern & Volumes	 
Zone 3- Blue Tube Strip Piercing Pattern & Volumes	 
Carrier 3	
Test Gel Beads	
Tubes	

If the functional test run was completed as part of an open 10x Genomics Support case, all above mentioned photographs and requested run logs should be submitted along with the case email.