NovaSeq™ 6000Dx Instrument

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Package Insert

FOR IN VITRO DIAGNOSTIC USE
FOR EXPORT ONLY

Intended Use

The NovaSeq 6000Dx Instrument is intended for sequencing of DNA libraries when used with *in vitro* diagnostic (IVD) assays. The NovaSeq 6000Dx Instrument is intended for use with specific registered, certified, or approved IVD reagents and analytical software.

Principles of Procedure

The Illumina® NovaSeq 6000Dx Instrument is intended for sequencing DNA libraries with *in vitro* diagnostic assays. For its input, the NovaSeq 6000Dx uses libraries generated from DNA where sample indexes and capture sequences are added to amplified targets. Sample libraries are captured on a flow cell and sequenced on the instrument using sequencing by synthesis (SBS) chemistry. SBS chemistry uses a reversible-terminator method to detect fluorescently labeled single nucleotide bases as they are incorporated into growing DNA strands. The Real-Time Analysis (RTA) software performs image analysis and base calling and assigns a quality score to each base for each sequencing cycle. When primary analysis finishes, secondary analysis can be executed on the included and required Illumina DRAGEN Server for NovaSeq 6000Dx to process base calls. The NovaSeq 6000Dx uses different secondary analysis applications depending on the workflow. For the DRAGEN for Illumina DNA Prep with Enrichment Dx Application, processing includes demultiplexing, FASTQ file generation, alignment, variant calling, and generation of variant call format (VCF and gVCF) files. The VCF and gVCF files contain information about either germline or somatic variants (depending on selected workflow) found at specific positions in a reference genome.

Dual Mode of Operation

The NovaSeq 6000Dx includes a single boot hard drive with separate *in vitro* diagnostic (IVD) and research use only (RUO) modes. The mode is selected using a toggle on the Sequencing screen. The selected mode is clearly labeled in the interface on all screens. IVD sequencing assays, including the DRAGEN for Illumina DNA Prep with Enrichment Dx Application in either germline and or somatic workflows, are executed in IVD mode. Only IVD sequencing reagents can be used in IVD mode. Performance characteristics and limitations of procedure for the NovaSeq 6000Dx have been established using the DRAGEN for Illumina DNA Prep with Enrichment Dx Application in IVD mode.

Limitations of the Procedure

1. For *in vitro* diagnostic use only.

2. The DRAGEN for Illumina DNA Prep with Enrichment Dx Application, when used with the NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles) and NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles) is capable of delivering:

   - Sequencing output:

     - ≥ 1.0 terabases (TB) with the S2 kit

     - ≥ 3.0 TB with the S4 kit

   - Read length (in paired end run) 2 x 150 base pairs (bp).

   - Bases higher than Q30 ≥ 85% at read length of 2 x 150 bp. Equal or greater than 85% of base calls have Phred scale quality scores greater than 30, indicating base call accuracy greater than 99.9%.

3. Insertions of length > 18 bp and deletions of length > 21 bp have not been validated.

4. Large variants, including multinucleotide variants (MNVs) and large indels, might be reported as separate smaller variants in the output VCF file.

5. Small MNVs are reported as separate variants in the output VCF file.

6. Deletions are reported in the VCF file at the coordinate of the preceding base per VCF format. Therefore, consider adjacent variants before reporting that an individual base call is a homozygous reference.

7. Germline-specific limitations:

   - The NovaSeq 6000Dx using the Germline FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application is designed to deliver qualitative results for germline variant calling (eg, homozygous, heterozygous, wild type).

   - Copy number variation can affect whether a variant is identified as homozygous or heterozygous.

   - The system will not report more than two variants at a single locus, even in the presence of copy number variation.

8. Somatic-specific limitations:

   - The NovaSeq 6000Dx using the Somatic FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application is designed to deliver qualitative results for somatic variant calling (ie, presence of a somatic variant).

   - The Somatic FASTQ and VCF generation analysis workflow cannot differentiate between germline and somatic variants. The workflow is designed to detect variants across a range of variant frequencies, but variant frequency cannot be used to differentiate somatic variants from germline variants.

   - Normal tissue in the specimen impacts the detection of variants. The reported limit of detection is based on a variant frequency relative to the total DNA extracted from both tumor and normal tissue.

   - If more than one variant allele is called at the same locus, none of the alleles will be reported as passing variants. Instead, the full set of alleles will be reported but filtered via the multiallelic tag.

Quality Control Procedures

The NovaSeq 6000Dx software evaluates each run, sample, and base call against quality control metrics. Positive and negative controls are recommended in library preparation and should be evaluated. Evaluate controls as follows.

- Negative Control (No Template Control) or other negative control—Must generate the expected result. If the negative control generates a result different from what is expected, a possible error in sample tracking, incorrect recording of indexing primers, or contamination has occurred.
- Positive Control Sample—Must generate the expected result. If the positive control generates a result different from what is expected, a possible error in sample tracking or incorrect recording of indexing primers has occurred.

Product Components

The Illumina NovaSeq 6000Dx consists of the following:

1. NovaSeq 6000Dx Instrument (Catalog # 20068232)
2. Software components for the NovaSeq 6000Dx Instrument include the following:

Software Application	Install Location	Function	Description
NovaSeq Operating Software	NovaSeq 6000Dx	Controls instrument operation	The NovaSeq Operating Software (NVOS) manages operation of the instrument during sequencing and generates images for use by the Real-Time Analysis (RTA) software.
Real-Time Analysis Software (RTA)	NovaSeq 6000Dx	Performs primary analysis	The RTA software application converts the images generated by NVOS for each tile per cycle of the sequencing run into base call files. The base call files are inputs for the Application Modules on the Illumina DRAGEN Server for NovaSeq 6000Dx. The RTA software application does not include a user interface.
Illumina Run Manager	Illumina DRAGEN Server	Controls run setup and management	Illumina Run Manager provides for user and instrument management, hosts the application software, and enables use of DRAGEN hardware accelerated genomics secondary analysis modules.

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Operating Conditions

For more information on operating conditions, refer to the Environmental Considerations section in the *NovaSeq 6000Dx Instrument Product Documentation*.

Element	Specification
Temperature	Maintain a lab temperature of 19°C to 25°C (22°C ±3°C). This temperature is the operating temperature range of the instrument. During a run, do not allow the ambient temperature to vary more than ±2°C.
Humidity	Maintain a noncondensing relative humidity between 20–80%. The system should be operated in an operating elevation of 2,000 meters or lower.

Consumables and Equipment

This section lists everything needed for a NovaSeq 6000Dx sequencing run. This includes Illumina supplied consumables and ancillary consumables and equipment that you must purchase from other suppliers. These items are required to complete the protocol and to perform maintenance and troubleshooting procedures.

For information on the symbols on consumables or consumable packaging, refer to *Illumina IVD Symbol Key (document # 1000000039141)*.

Sequencing Consumables

A NovaSeq 6000Dx run requires the following components:

- Buffer cartridge

- Cluster cartridge

- Flow cell

- Library tube

- SBS cartridge

NovaSeq 6000Dx consumables are packaged in the following configurations. Each component uses radio-frequency identification (RFID) for accurate consumable tracking and compatibility.

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Table 1 Illumina-Supplied Consumables

Kit Name	Contents	Illumina Catalog Number
NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles)	S2 cluster cartridge S2 flow cell S2 SBS cartridge	20046931
NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles)	S4 cluster cartridge S4 flow cell S4 SBS cartridge	20046933
NovaSeq 6000Dx S2 Buffer Cartridge	S2 buffer cartridge	20062292
NovaSeq 6000Dx S4 Buffer Cartridge	S4 buffer cartridge	20062293
NovaSeq 6000Dx Library Tube	Single library tube	20062290
NovaSeq 6000Dx Library Tube, 24 Pack	24 library tubes	20062291

When you receive your consumables, promptly store components at the indicated temperature to ensure proper performance.

Table 2 NovaSeq 6000Dx Kit Storage

Consumable	Quantity	Storage Temperature	Length	Width	Height
Flow cell	1	2°C to 8°C	27.7 cm (10.9 in)	17 cm (6.7 in)	3.8 cm (1.5 in)
Cluster cartridge	1	-25°C to -15°C	29.5 cm (11.6 in)	13 cm (5.1 in)	9.4 cm (3.7 in)
SBS cartridge	1	-25°C to -15°C	30 cm (11.8 in)	12.4 cm (4.9 in)	11.2 cm (4.4 in)
Buffer cartridge	1	15°C to 30°C	42.2 cm (16.6 in)	20.6 cm (8.1 in)	21.1 cm (8.3 in)
Library tube	1	15°C to 30°C	4.1 cm (1.6 in)	2.3 cm (0.9 in)	12.4 (4.9 in)

Consumables Details

To identify compatible kit components, flow cells and cartridges are labeled with symbols that show the kit mode.

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Table 3 Compatibility Labeling

| Kit Mode	Marking on Label	Description
S2 kit components	S2	S2 flow cell generates up to 4.1 billion single reads passing filter with output up to 1000 Gb at 2 x 150 bp. The S2 flow cell provides fast sequencing for most high-throughput applications.
S4 kit components	S4	S4 flow cell generates up to 10 billion single reads passing filter with output up to 3000 Gb at 2 x 150 bp. The S4 flow cell is a four-lane version of the flow cell, designed for maximum output.

Flow Cell

The NovaSeq 6000Dx flow cell is a patterned flow cell encased in a cartridge. The flow cell is a glass-based substrate containing billions of nanowells in an ordered arrangement. Clusters are generated in the nanowells from which sequencing is then performed.

Each flow cell has multiple lanes for sequencing pooled libraries. The S2 flow cell has two lanes and the S4 flow cell has four. Each lane is imaged in multiple swaths, and the software then divides the image of each swath into smaller portions called tiles.

Some scratches and other minor cosmetic defects on the flow cell are normal and not expected to compromise data quality and yield. Illumina recommends using these flow cells as per normal.

Figure 1 Flow Cells
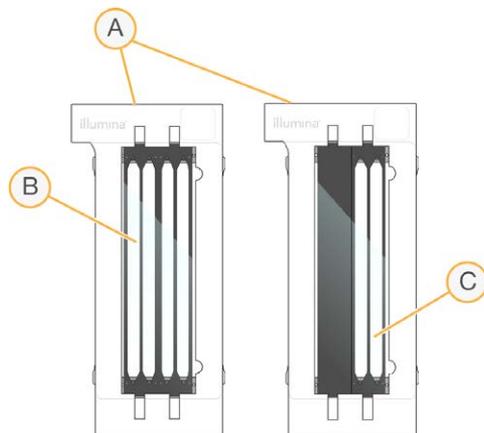
A. Flow cell cartridge
B. Four-lane flow cell (S4)
C. Two-lane flow cell (S2)

The underside of each flow cell has multiple gaskets. Libraries and reagents enter the flow cell lanes through the gaskets on the inlet end of the flow cell. Used reagents are expelled from the lanes through the gaskets at the outlet end.

⚠ CAUTION

Avoid touching the gaskets when handling the flow cell.

Figure 2   Inverted Flow Cell
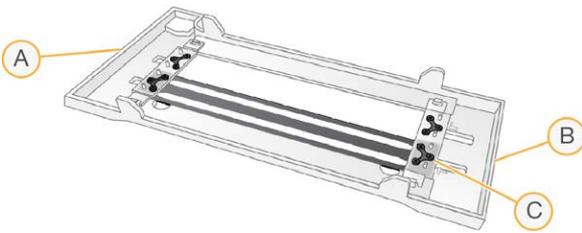
A. Outlet end
B. Inlet end
C. Gasket (one of four)

Buffer, Cluster, and SBS Cartridge Details

The NovaSeq 6000Dx buffer, cluster, and SBS cartridges have foil-seal reservoirs prefilled with reagents, buffers, and wash solution. Cluster and SBS cartridges are included with NovaSeq 6000Dx reagent kits. The buffer cartridge is sold separately.

The cartridges load directly onto the instrument and are color-coded and labeled to reduce loading errors. Guides in the reagent chiller and buffer drawers ensure proper orientation.

Table 4   NovaSeq 6000Dx Cartridges

Consumable	Description
Buffer cartridge	Prefilled with sequencing buffers and weighs up to 6.8 kg (15 lb). A plastic handle facilitates carrying, loading, and unloading. The buffer cartridge contains reagents that are sensitive to light. Keep the buffer container packaged until use.

Consumable	Description
Cluster cartridge	Prefilled with clustering, indexing, and paired-end reagents and wash solution. Includes a designated position for the library tube. Orange labeling distinguishes the cluster cartridge from the SBS cartridge. A denaturation reagent in position #30 contains formamide, which is an organic amide and reproductive toxin. To facilitate safe disposal of any unused reagent after the sequencing run, this reservoir is removable.
SBS cartridge	Prefilled with sequencing reagents at volumes specific to the number of cycles the kit supports. Each of the three reagent positions has an adjacent position reserved for the automatic post-run wash. Gray labeling distinguishes the SBS cartridge from the cluster cartridge. The SBS cartridge contains reagents that are sensitive to light. Keep the SBS container packaged until use.

Reserved Cluster Cartridge Reservoirs

Three reservoirs are reserved for custom primers and an empty position is reserved for the library tube. For sample traceability, the library tube is loaded into the cluster cartridge during run setup and remains with the cartridge through the end of the run.

Figure 3   Numbered Reservoirs
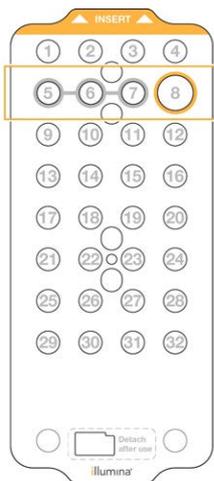
Table 5   Cluster Cartridge Reservoirs

Position	Reserved For
5, 6, and 7	Optional custom primers
8	Library tube

User-Supplied Consumables and Equipment

Table 6   Consumables

Consumable	Supplier	Purpose
Centrifuge bottle, 500 ml	General lab supplier	Diluting Tween 20 for a maintenance wash.
Centrifuge tube, 30 ml	General lab supplier	Diluting NaOCl for a maintenance wash.
Disposable gloves, powder-free	General lab supplier	General purpose.
Isopropyl alcohol wipes, 70% or Ethanol alcohol wipes, 70%	VWR, catalog # 95041-714, or equivalent General lab supplier	Cleaning components before a run and general purpose.
Lab tissue, low-lint	VWR, catalog # 21905-026, or equivalent	Drying the flow cell stage and general purpose.
Reagent grade NaOCl, 5%	Sigma-Aldrich, catalog # 239305	Performing a maintenance wash.
Pipette tips, 2 µl	General lab supplier	Pipetting for diluting and loading libraries.

Consumable	Supplier	Purpose
Pipette tips, 20 µl	General lab supplier	Pipetting for diluting and loading libraries.
Pipette tips, 200 µl	General lab supplier	Pipetting for diluting and loading libraries.
Pipette tips, 1000 µl	General lab supplier	Pipetting for diluting and loading libraries.
Reagent or spectrophotometric-grade isopropyl alcohol (99%), 100 ml bottle	General lab supplier	Cleaning optics components periodically and support the objective cleaning cartridge.
Tween 20	Sigma-Aldrich, catalog # P7949	Performing a maintenance wash.
Water, laboratory-grade	General lab supplier	Diluting Tween 20 and sodium hypochlorite for a maintenance wash.

Table 7 Equipment

Item	Source
Freezer, -25°C to -15°C	General lab supplier
Graduated cylinder, 500 ml, sterile	General lab supplier
Ice bucket	General lab supplier
Pipette, 20 µl	General lab supplier
Pipette, 200 µl	General lab supplier
Pipette, 1000 µl	General lab supplier
Refrigerator, 2°C to 8°C	General lab supplier
Tub, water baths*	General lab supplier

* Use a tub that can accommodate two reagent cartridges and the appropriate water level. For example, (61 cm × 91.4 cm × 25.4 cm)(24 in × 36 in × 10 in).

Guidelines for Laboratory-Grade Water

Always use laboratory-grade water or deionized water to perform instrument procedures. Never use tap water. Use only the following grades of water or equivalents:

- Deionized water

- Illumina PW1

- 18 Megohms (MΩ) water

- Milli-Q water

- Super-Q water

- Molecular biology grade water

Instructions for Use

The following instructions are for running the NovaSeq 6000Dx Instrument in IVD mode of operation using either the S2 or S4 kit configurations.

Create a Sequencing Run

Use the following steps to create a run using Illumina Run Manager in either IVD or RUO mode. Alternatively, select **Import Run** on the Planned tab of the Runs page and import a sample sheet. Create new runs either on the instrument or by accessing Illumina Run Manager using a browser on a networked computer.

NOTE   The exact information required by each analysis application differs, but the process to create a run includes the following steps.

1. From the Planned tab of the Runs screen, select **Create Run**.
2. Select an application, and then select **Next**.
3. Proceed through the settings screens. Depending on your application, the screens displayed can include the following:
    - **Run Settings**—Enter run parameters.
    - **Sample Data**—Enter sample data manually or by importing a CSV file containing sample information. Sample names must be unique.
    - **Analysis settings**—Enter settings for analysis.
4. On the Review screen, review run information and select **Save**.
   The run is added to the top of the runs list on the Planned tab.

Prepare Consumables

Thaw SBS and Cluster Cartridges

⚠️ CAUTION

Using hot water for thawing reagents might cause reduced data quality or run failure.

1. If a sequencing run is in progress, make sure that both sides of the instrument are available when the thaw is complete.
2. Remove the SBS and cluster cartridges from -25°C to -15°C storage.
3. Place each cartridge into a wire thaw rack.
   The racks are provided with the instrument and prevent capsizing in the water bath.

   Figure 4   Cartridges in Wire Thaw Racks
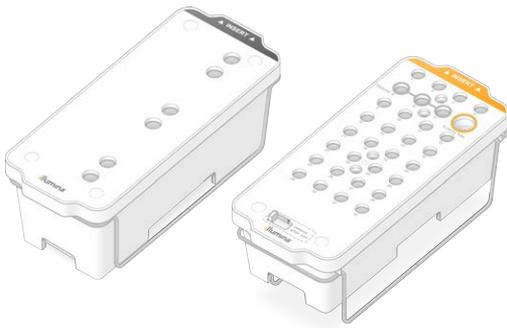
4. Use the following table to determine thaw duration.
   Thaw SBS and cluster cartridges in a room temperature (19°C to 25°C) water bath as follows. Submerge cartridges approximately halfway.

Cartridge	Duration of Thaw
S2 SBS cartridge	4 hours
S2 cluster cartridge	Up to 2 hours
S4 SBS cartridge	4 hours
S4 cluster cartridge	Up to 4 hours

⚠️ CAUTION

Failure to start sequencing within four hours of thawing reagent cartridges can result in reduced data quality.

5. Thoroughly dry the cartridge bases using paper towels. Dry between the wells so that all water is removed.

6. Inspect the foil seals for water. If water is present, blot dry with a lint-free tissue.

7. Inspect the underside of each cartridge to make sure that the reservoirs are free of ice, which indicates that the reagents are thawed.

8. Invert each cartridge 10 times to mix reagents.

⚠️ CAUTION

Failure to thoroughly invert the cartridges can result in reduced data quality.

9. Gently tap the bottom of each cartridge on the bench to reduce air bubbles.

Load Library Tube

1. Without disturbing the library at the bottom, insert the uncapped library tube containing the denatured and diluted library pool into the **Library Tube** position (#8) of the cluster cartridge.

2. Insert the library tube into position #8 of the cluster cartridge.

Figure 5   Uncapped Library Tube Loaded Into Position #8
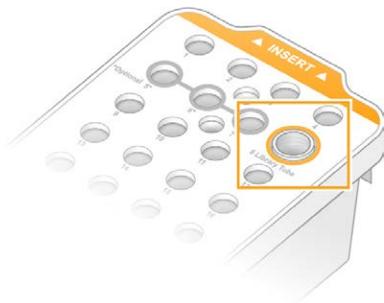

Empty Used Reagent Bottles

Use the following instructions to empty the used reagent bottles with *every* sequencing run. If your system is configured to route used reagents externally, the small bottle collects used reagents and must be emptied for each sequencing run. The large bottle must be in place.

1. Remove and empty the small used reagent bottle as follows.

   a. Raise the lever and remove the small used reagent bottle from the alcove. Grasp the bottle by the sides.

   b. Remove the threaded cap from the cap holder on the front of the bottle.

   c. Seal the bottle opening with the cap to prevent spills.

   d. Keeping the contents separate from the contents of the other bottle, discard in accordance with applicable standards for your region.

   e. Return the uncapped bottle to the alcove, and then lower the lever. Store the cap on the cap holder.

2. Remove and empty the large used reagent bottle as follows.

a. Using the top handle, remove the large used reagent bottle from the left side of the buffer drawer.

b. Remove the threaded cap from the cap holder on the front of the bottle.

c. Seal the bottle opening with the cap to prevent spills.

d. Discard the contents in accordance with applicable standards for your region. Grip both handles when emptying.

e. Return the uncapped bottle to the buffer drawer. Store the cap on the cap holder.

Figure 6   Returning the Empty Bottle

3. Put on a new pair of powder-free gloves.

⚠ CAUTION

Always put on a new pair of gloves after handling the used reagent bottle.

4. Close the buffer drawer, and then close the liquid compartment doors.

⚠ CAUTION

Failure to empty the used reagent bottles can result in a terminated run and overflow, which damages the instrument and poses a safety risk.

Prepare Flow Cell

1. Remove a new boxed flow cell package from 2°C to 8°C storage.

2. Set the sealed flow cell package aside at ambient temperature (19°C to 25°C) for 10–15 minutes.
   Use the flow cell within 12 hours of removing it from the package.

Load Consumables

Use the following instructions to start run setup and load consumables.

1. From the main menu, select **Sequence**, and then select a single or dual flow cell run as follows.

- **A+B**—Set up a dual flow cell run.

- **A**—Set up a single flow cell run on side A.

- **B**—Set up a single flow cell run on side B.

The system initiates run setup, starting with loading the flow cell.

2. Select **OK** to acknowledge the warning and open the flow cell door.

⚠️ CAUTION

Keep the surface clear during the sequencing run and avoid leaning on the instrument. Pressure to the flow cell door can cause it to open, which stops the run. Stopped runs cannot be resumed.

Load the Flow Cell

1. If present, remove the flow cell from the previous run.

2. If particulate is visible on the flow cell stage, clean the entire stage, including the fluidic interface and the glass surface of the optical alignment target, with an alcohol wipe. Dry with a lint-free tissue.

Figure 7   Flow Cell Stage
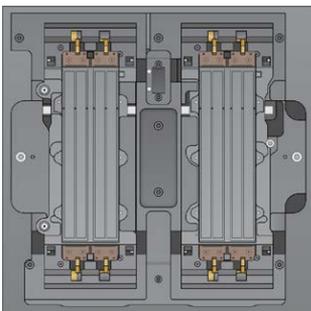
3. Remove the flow cell from the packaging as follows.

   a. Put on a new pair of powder-free gloves to avoid contaminating the glass surface of the flow cell.

   b. With the package over a flat surface, peel open the foil from the corner tab.

   c. Remove the clear plastic retainer covering the flow cell.

   d. Remove the flow cell from the package. Grasp the flow cell by the sides to avoid touching the glass or the underside gaskets.

   e. If particulate is visible on either of the glass surfaces, clean the applicable surface with a lint-free alcohol wipe and dry with a low-lint lab tissue.

   f. Discard the package appropriately.

4. Align the flow cell over the four raised clamps and place it on the flow cell stage.

Figure 8   Loaded Flow Cells Aligned Over Clamps
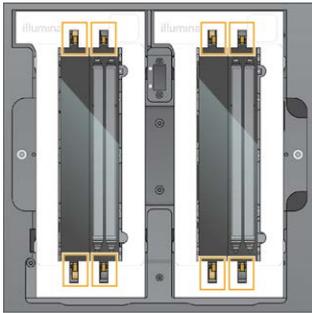
5.   Select **Close Flow Cell Door**.

The flow cell door closes, the sensors and RFID are checked, and the flow cell ID appears on the screen.

Load the SBS and Cluster Cartridges

1.   Open the liquid compartment doors, and then open the reagent chiller door.

2.   Remove the used SBS and cluster cartridges, if present from a previous run.

The used cartridges have pierced foil seals.

3.   Dispose of unused contents in accordance with applicable standards.

For safe disposal of position #30 of the cluster cartridge, refer to *Detach Position #30* on page 20.

4.   Load the prepared cartridges into the reagent chiller drawer as follows, so that the Insert labels face the back of the instrument.

   •   Place the SBS cartridge (gray label) into the left position.

   •   Place the cluster cartridge (orange label) containing the uncapped library tube into the right position.

Figure 9   Loaded Reagent Cartridges

5.   Slide the drawer into the chiller, and then close the reagent chiller door.

The sensors and RFIDs are checked. The IDs for the library tube and the two cartridges appear on the screen.

Load the Buffer Cartridge

1.   Pull the metal handle to open the buffer drawer.

2. Remove the used buffer cartridge from the right side of the buffer drawer.
   The used buffer cartridge has pierced foil seals.

3. Place a new buffer cartridge into the buffer drawer so that the Illumina label faces the front of the drawer.
   Align the cartridge with the raised guides on the drawer floor and sides.
   When properly loaded, the buffer cartridge is evenly seated and the drawer can close.

   Figure 10   Load the Buffer Cartridge
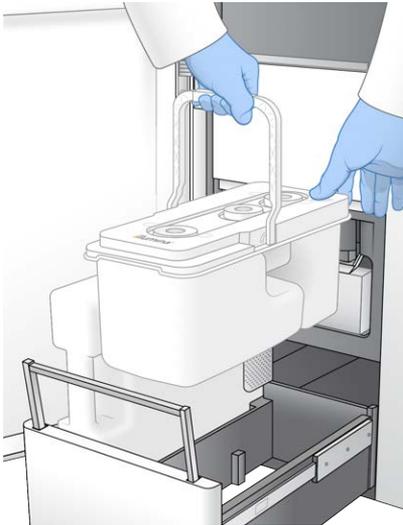
4. If both used reagents bottles have been emptied, select the checkbox acknowledging that both used reagent bottles are empty.

   NOTE   Failure to empty the used reagent bottles can result in a terminated run and overflow, which damages the instrument and poses a safety risk.

5. When consumables have been added, select **Run Selection** to proceed.

Select and Start Run

The instrument scans the library tube ID and searches for a matching planned run.

1. If a planned run matching the library tube ID is found for each side being used, run selection is skipped.
   Select **Review** to proceed.

2. If there is no matching run for one or either side, select **Run Selection**, and then select one or more planned runs.
   The same planned run cannot be selected on both sides.

3. When one or more runs are selected, select **Pre-Run Checks**.

4. Wait approximately 5 minutes for the pre-run check to complete.
   The run starts automatically after successful completion.

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NOTE   To avoid overfilling the hard drive, do not copy any data to C:\ after the run starts.

Pre-Run Check Errors

1. If pre-run checks fail due to a sensor error, such as flow cell not detected, you must exit and restart the workflow.
2. For other pre-run check failures, select **Retry** to restart the failed check or **Retry All** to restart all checks. Errors require resolution before the run can start.
3. Select the **Error** icon to see error details.
4. If the alignment check fails, resolve the error as follows.
    a. Select **Reload**, and then select **OK** to return to the Load screen.
    b. Remove any items from the top of the instrument, and then select **OK**. The flow cell door opens.
    c. Reload the flow cell, and then select **Run Setup**.
    d. Proceed through each screen to reread each RFID and return to the Pre-Run Checks screen.
    e. Redo the check.

Monitor Run Progress

The following details are displayed on the Sequencing screen while the run is in progress. The Sequencing screen is accessed via the main menu.

*   **Status of individual run steps**
*   **Time to completion**—The run completion date and time (yyyy-mm-dd hh:mm).
*   **Run progress**—The current run step. The size of the progress bar is not proportional to the run rate of each step.
*   **Q-scores**—The distribution of quality scores (Q-scores).
*   **Intensity**—The value of cluster intensities of the 90th percentile for each tile. Plot colors indicate the red and green channels.
*   **Clusters passing filter (%)**—The percentage of clusters passing filter.
*   **Projected Total Yield (GB)**—The projected yield for the flow cell run. If the per lane metrics are selected (H), the numbers displayed are the current yield per lane and update per cycle throughout the run.
*   **Q30**—The percentage of base calls for the run that have a Q-score of ≥ 30.

Status Icons

A status icon on the NVOS interface indicates run status. A number on the icon indicates the number of conditions for a status.

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When a run status changes, the icon blinks. Select the icon to view a description of the condition. Select **Acknowledge** to clear the message, and then select **Close** to close the dialog box.

Status Icon	Status Name	Description
	Status okay	System is normal.
	Processing	System is processing.
	Warning	A warning has occurred and attention is required. Warnings do not stop a run or require action before proceeding.
	Error	An error has occurred. Errors require action before proceeding with the run.
	Information	A noncritical message is available.

Run Metrics

The software displays metrics generated during the run. Metrics appear in the form of plots, graphs, and tables based on data generated by RTA3 and written to InterOp files.

Clustering takes approximately 2 hours, then sequencing begins with cycle 1. Metrics are updated as sequencing progresses. Clusters passing filter, yield, and quality scores are available after cycle 26. Prior to cycle 26, no values are populated and are designated as not applicable.

After Sequencing

The following sections provide instructions on steps that happen after sequencing has been completed.

Automatic Post-Run Wash

When sequencing is complete, the software initiates an automatic post-run wash that takes approximately 80 minutes. The system pumps 0.24% sodium hypochlorite (NaOCl) from position #17 and dilutes it to 0.12%. The 0.12% NaOCl is pumped to the ExAmp reagent and library positions, through the flow cell, and then to the used reagent bottles. The wash flushes template from the system to prevent cross-contamination.

When the wash is complete, the system is placed in a safe state and the Home button becomes active. Leave consumables in place until the next run. After the wash, the sippers remain in the SBS and cluster cartridges to prevent air from entering the system. The sippers in the buffer cartridge are raised so the used reagent bottles can be emptied. Wash buffer is then pumped through all lines to clear away NaOCl and reagents from the system.

NOTE   If an error occurs during an automatic post-run wash, and the post-run wash is incomplete, a maintenance wash is required.

Detach Position #30

The reservoir in position #30 of the cluster cartridge contains formamide. It is removed from the used cluster cartridge and discarded separately.

⚠ CAUTION

This set of reagents contains potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations. For additional environmental, health, and safety information, refer to the SDS at support.illumina.com/sds.html.

1. While wearing gloves, push the white plastic tab labeled **Detach after use** to the right.
2. Place a hand or solid surface under the reservoir and press the clear plastic tab toward the Illumina label to release the reservoir from under the cluster cartridge.

NOTE   Avoid stacking cluster cartridges when storing. Stacking might cause accidental detachment of the reservoir.

Figure 11   Removable Position #30
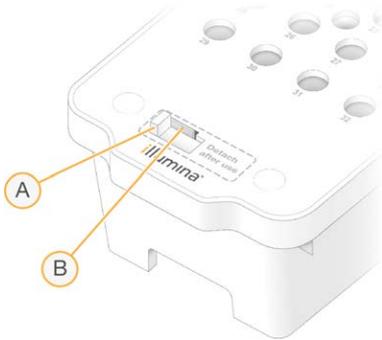
A. White plastic tab to detach
B. Clear plastic tab to release

3. Dispose of the reservoir in accordance with applicable standards.

Sequencing Output

During sequencing, data is automatically transferred from the NovaSeq 6000Dx Instrument to the Illumina DRAGEN Server. When primary analysis finishes, and transfer of data is complete, the secondary analysis on the Illumina DRAGEN Server can begin automatically using the analysis options defined by the application selected in Illumina Run Manager. The results produced depend on the options chosen during run setup. To view results from a run, select the desired run name in the Completed tab on the Runs screen. You can also find output files in the location specified on the Instrument Settings screen.

Real-Time Analysis

The NovaSeq 6000Dx Instrument runs RTA3, an implementation of Real-Time Analysis software, on the instrument Compute Engine (CE). RTA3 extracts intensities from images received from the camera, performs base calling, assigns a quality score to base calls, aligns to PhiX, and reports data in InterOp files.

To optimize processing time, RTA3 stores information in memory. If RTA3 is terminated, processing does not resume and any run data being processed in memory is lost.

RTA3 Inputs

RTA3 requires tile images contained in local system memory for processing. RTA3 receives run information and commands from the NVOS.

RTA3 Outputs

Images for each color channel are passed in memory to RTA3 as tiles. From these images, RTA3 outputs a set of quality-scored base call files and filter files. All other outputs are supporting output files.

File Type	Description
Base call files	Each tile that is analyzed is included in a concatenated base call (*.cbcl) file. Tiles from the same lane and surface are aggregated into one CBCL file for each lane and surface.
Filter files	Each tile produces a filter file (*.filter) that specifies whether a cluster passes filters.

RTA3 provides real-time metrics of run quality stored as InterOp files, which are a binary output containing tile, cycle, and read-level metrics.

Error Handling

RTA3 creates log files and writes them to the Logs folder. Errors are recorded in a text file in *.log file format.

The following log files are transferred to the final output destination at the end of processing:

- `info_00000.log` summarizes important run events.
- `error_00000.log` lists errors that occurred during a run.
- `warning_00000.log` lists warnings that occurred during a run.

Flow Cell Tiles

Tiles are small imaging areas on the flow cell. The camera takes one image of each swath, which the software divides into tiles for RTA3 processing. The total number of tiles depends on how many lanes, swaths, and surfaces are imaged on the flow cell.

- S2 flow cells have a total of 1408 tiles.
- S4 flow cells have a total of 3744 tiles.

Flow Cell Component	S2	S4	Description
Lanes	2	4	A lane is a physical channel with input and output ports.
Surfaces	2	2	The S2 and S4 flow cells are imaged on two surfaces: the top and bottom. The top surface of a tile is imaged first.
Swaths per lane	4	6	A swath is a column in a flow cell lane that the camera captures as one scanned image.
Tiles per swath	88	78	A tile is a portion of a swath and depicts an imaged area on the flow cell.
Total tiles generated	1408	3744	Lanes × surfaces × swaths × tiles per swath equals the total number of tiles.

The tile name is a five-digit number that represents the tile position on the flow cell. For example, tile name 1_1205 indicates lane 1, top surface, swath 2, tile 5.

- The first digit is the lane number:
  - 1 or 2 for an S2 flow cell.
  - 1, 2, 3, or 4 for an S4 flow cell.
- The second digit represents the surface: 1 for top or 2 for bottom.
- The third digit represents the swath number:
  - 1, 2, 3, or 4 for an S2 flow cell.
  - 1, 2, 3, 4, 5, or 6 for an S4 flow cell.
- The last two digits represent the tile number. The numbering starts with 01 at the outlet end of the flow cell through 88 or 78 at the inlet end.
  - 01 through 88 for an S2 flow cell.
  - 01 through 78 for an S4 flow cell.

Real-Time Analysis Workflow

Registration ↓	Records the location of each cluster on the patterned flow cell.
Intensity extraction ↓	Determines an intensity value for each cluster.
Phasing correction ↓	Corrects the effects of phasing and prephasing.
Base calling ↓	Determines a base call per cycle for every cluster.
Quality scoring	Assigns a quality score to every base call.
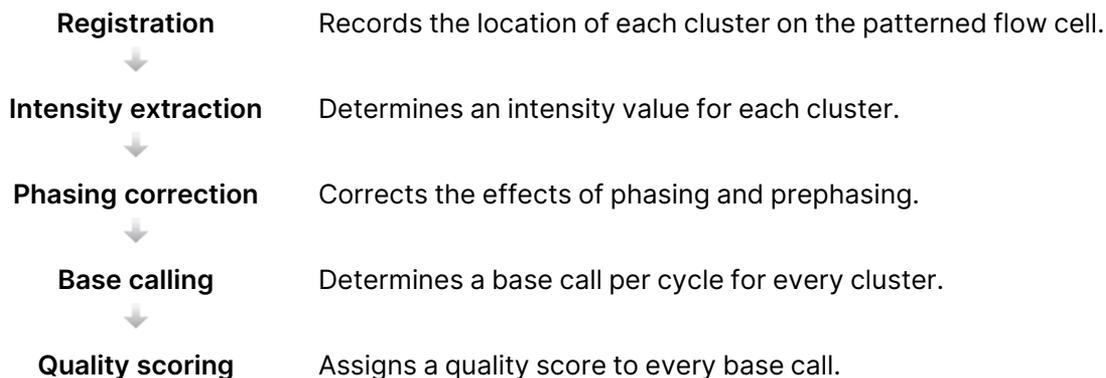
Registration

Registration aligns an image to the rotated square array of nanowells on the patterned flow cell. Because of the ordered arrangement of nanowells, the X and Y coordinates for each cluster in a tile are predetermined. Cluster positions are written to a cluster location (s.locs) file for each run.

If registration fails for any images in a cycle, no base calls are generated for that tile in that cycle.

Intensity Extraction

After registration, intensity extraction calculates an intensity value for each nanowell in a given image. If registration failed, the intensity for that tile cannot be extracted.

Phasing Correction

During the sequencing reaction, each DNA strand in a cluster extends by one base per cycle. Phasing and prephasing occurs when a strand becomes out of phase with the current incorporation cycle.

Phasing occurs when a base incorporation falls behind.

Prephasing occurs when a base incorporation jumps ahead.

Figure 12   Phasing and Prephasing
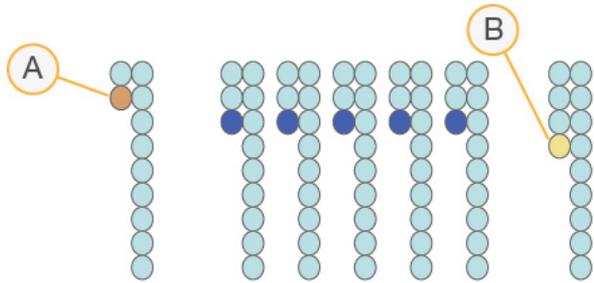
A.   Read with a base that is phasing
B.   Read with a base that is prephasing.

RTA3 corrects the effects of phasing and prephasing, which maximizes the data quality at every cycle throughout the run.

Base Calling

Base calling determines a base (A, C, G, or T) for every cluster of a given tile at a specific cycle. The NovaSeq 6000Dx Instrument uses two-channel sequencing, which requires only two images to encode the data for four DNA bases, one image from the green channel and one from the red channel.

A no call is identified as N. No calls occur when a cluster does not pass filter, registration fails, or a cluster is shifted off the image.

Intensities for each cluster are extracted from the red and green images and compared against each other, which results in four distinct populations. Each population corresponds to a base. The base calling process determines to which population each cluster belongs.

Figure 13   Visualization of Cluster Intensities
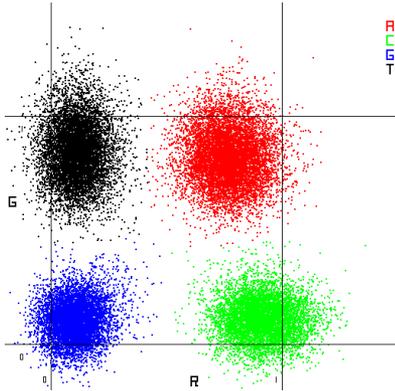
Table 8   Base Calls in 2-Channel Sequencing

Base	Red Channel	Green Channel	Result
A	1 (on)	1 (on)	Clusters that show intensity in both the red and green channels.
C	1 (on)	0 (off)	Clusters that show intensity in the red channel only.
G	0 (off)	0 (off)	Clusters that show no intensity at a known cluster location.
T	0 (off)	1 (on)	Clusters that show intensity in the green channel only.

Clusters Passing Filter

During the run, RTA3 filters raw data to remove reads that do not meet the data quality threshold. Overlapping and low-quality clusters are removed.

For two-channel analysis, RTA3 uses a population-based system to determine the chastity (intensity purity measurement) of a base call. Clusters pass filter (PF) when no more than one base call in the first 25 cycles has a chastity below a fixed threshold. When included, PhiX alignment is performed at cycle 26 on a subset of tiles for clusters that passed filter. Clusters that do not pass filter are not base called and not aligned.

Quality Scores

A quality score (Q-score) is a prediction of the probability of an incorrect base call. A higher Q-score implies that a base call is higher quality and more likely to be correct. After the Q-score is determined, results are recorded in CBCL files.

The Q-score succinctly communicates small error probabilities. Quality scores are represented as Q(X), where X is the score. The following table shows the relationship between a quality score and error probability.

Q-Score Q(X)	Error Probability
Q40	0.0001 (1 in 10,000)
Q30	0.001 (1 in 1000)
Q20	0.01 (1 in 100)
Q10	0.1 (1 in 10)

Quality Scoring and Reporting

Quality scoring calculates a set of predictors for each base call, and then uses the predictor values to look up the Q-score in a quality table. Quality tables are created to provide optimally accurate quality predictions for runs generated by a specific configuration of sequencing platform and version of chemistry.

Quality scoring is based on a modified version of the Phred algorithm.

To generate the Q-table for the NovaSeq 6000Dx Instrument, three groups of base calls were determined, based on the clustering of these specific predictive features. Following grouping of the base calls, the mean error rate was empirically calculated for each of the three groups and the corresponding Q-scores were recorded in the Q-table alongside the predictive features correlating to that group. As such, only three Q-scores are possible with RTA3 and these Q-scores represent the average error rate of the group. Overall, this results in simplified, yet highly accurate quality scoring. The three groups in the quality table correspond to marginal (< Q15), medium (~Q20), and high-quality (> Q30) base calls, and are assigned the specific scores of 12, 26, and 34 respectively. Additionally, a null score of 2 is assigned to any no-calls. This Q-score reporting model reduces storage space and bandwidth requirements without affecting accuracy or performance.

Figure 14   Simplified Q-Scoring with RTA3

FOR IN VITRO DIAGNOSTIC USE
FOR EXPORT ONLY
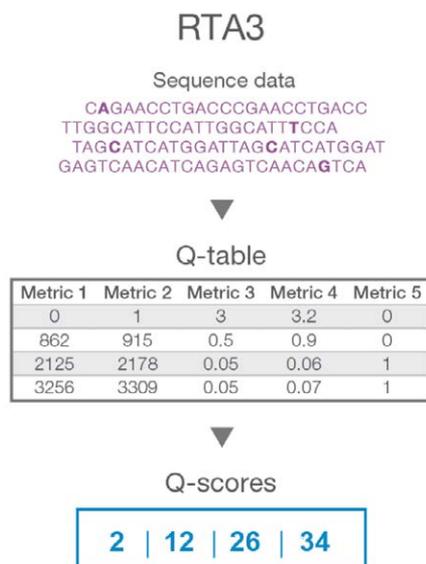
Sequencing Output Files

File Type	File Description, Location, and Name

Base call files	Each cluster analyzed is included in a base call file, aggregated in one file per cycle, lane, and surface. The aggregated file contains the base call and encoded quality score for every cluster.
	`Data\Intensities\BaseCalls\L001\C1.1`
	`L[lane]_[surface].cbcl`, for example `L001_1.cbcl`

Cluster location files	For each flow cell, a binary cluster location file contains the XY coordinates for clusters in a tile. A hexagonal layout that matches the nanowell layout of the flow cell predefines the coordinates.
	`Data\Intensities`
	`s_[lane].locs`

Filter files	The filter file specifies whether a cluster passed filters. Filter files are generated at cycle 26 using 25 cycles of data. For each tile, one filter file is generated.
	`Data\Intensities\BaseCalls\L001`
	`s_[lane]_[tile].filter`

Run information file	Lists the run name, number of cycles in each read, whether the read is an Index Read, and the number of swaths and tiles on the flow cell. The run info file is created at the beginning of the run. `[Root folder],RunInfo.xml`
Thumbnail files	Thumbnail images for the first cycle of each sequencing read. `Thumbnail_Images\L001\C[X.1]`—Files are stored in a subfolder for each cycle. `s_[lane]_[tile]_[channel].jpg`—The thumbnail image includes the tile number.

Sequencing Output Folder Structure

The NVOS generates the output folder name automatically.

📁 **Config**—Configuration settings for the run.

📁 **Logs**—Log files describing operational steps, instrument analytics, and RTA3 events.

   📄 SampleSheet.csv—Sample sheet or other attached file, if applicable.

📁 **Data**

  📁 **Intensities**

    📁 **BaseCalls**

      📁 **L00[X]**—Base call files (*.cbcl) aggregated in one file per lane, surface, and cycle.

    📄 s.locs—The cluster locations file for the run.

📁 **InterOp**—Binary files.

📁 **Recipe**—Run-specific recipe file.

📁 **Thumbnail Images**—Thumbnail images for every 10th tile.

📁 **LIMS**—The run setup file (*.json), if applicable.

📁**Audit**

    📄 AuditInfo.xml

📄 RTA3.cfg

📄 RunInfo.xml

📄 RunParameters.xml

📄 RTAComplete.txt

📄 CopyComplete.txt

📄 SequenceComplete.txt

📄 IlluminaRunManagerCopyComplete.txt

📄 Manifest.tsv

Warnings and Precautions

⚠️ CAUTION

Federal law restricts this device to sale by or on the order of a physician or other practitioner licensed by the law of the State in which they practice, to use or order the use of the device.

- **Some components of reagents provided by Illumina for use with the NovaSeq 6000Dx Instrument contain potentially hazardous chemicals. Personal injury can occur through inhalation, ingestion, skin contact, and eye contact. Wear protective equipment, including eye protection, gloves, and laboratory coat appropriate for risk of exposure. Handle used reagents as chemical waste and discard in accordance with applicable regional, national, and local laws and regulations.** For additional environmental, health, and safety information, refer to the Safety Data Sheets (SDS) at support.illumina.com/sds.html.

- Failure to follow the procedures as outlined can result in erroneous results or significant reduction in sample quality.

- Use routine laboratory precautions. Do not pipette by mouth. Do not eat, drink, or smoke in designated work areas. Wear disposable gloves and laboratory coats when handling specimens and kit reagents. Wash hands thoroughly after handling specimens and kit reagents.

- Proper laboratory practices and good laboratory hygiene are required to prevent PCR products from contaminating reagents, instrumentation, and genomic DNA samples. PCR contamination can cause inaccurate and unreliable results.

- To prevent contamination, make sure that pre-amplification and post-amplification areas have dedicated equipment and consumables (eg, pipettes, pipette tips, heat blocks, vortexers, and centrifuges).

- Index to sample pairing requires matching the index plate layout exactly. The DNA Prep with Enrichment Application automatically populates the index primers associated with the sample names, when entered during run setup. The user is advised to verify the index primers associate with samples before starting the sequencing run. Mismatches between the sample and plate layout results in loss of positive sample identification and incorrect result reporting.

- Installation of user-supplied antivirus software is strongly recommended to protect the computer against viruses.

- Do not operate the NovaSeq 6000Dx with any of the panels removed. Operating the instrument with any of the panels removed creates potential exposure to line voltage and DC voltages.

- Do not touch the flow cell stage in the flow cell compartment. The heater in this compartment operates between 22°C and 95°C and can result in burns.

- The instrument weighs approximately 1059 lbs. and could cause serious injury if dropped or mishandled.

Performance Characteristics

Performance characteristics for the NovaSeq 6000Dx instrument were established using the Illumina DNA Prep with Enrichment Dx for library preparation, the NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles) and NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles) for sequencing, and the DRAGEN for Illumina DNA Prep with Enrichment Dx Application for secondary analysis including germline and somatic variant detection. Studies included Sample Indexing, Sample Carryover, DNA Input, Analytical Sensitivity (Limit of Blank / Limit of Detection), Accuracy, Precision, Method Comparison, and Reproducibility. See the *Illumina DNA Prep with Enrichment Dx Package Insert* for performance characteristics related to pre-analytical factors, such as extraction methods or interfering substances.

Definitions of Calculations Used in Performance Characteristics

1. Positive Percent Agreement (PPA) is calculated as the proportion of loci classified as variants by a reference method that are correctly reported by the assay.

   - (# variant loci correctly reported by the assay) / (total # of variant loci)

   Variant loci reported by the assay that are concordant with the reference method are true positives (TPs). Variant loci reported as reference calls or as different variant calls by the assay are false negatives (FNs).

2. Negative Percent Agreement (NPA) is calculated as the proportion of loci classified as wild type by a reference method that are correctly reported by the assay.

   - (# wild type loci correctly reported by the assay)/ (total # of wild type loci)

   Wild type loci reported by the assay that are concordant with the reference method are true negatives (TNs). Wild type loci reported as variants by the assay are false positives (FPs).

3. Overall percent agreement (OPA) is calculated as the proportion of loci correctly reported by the assay relative to a reference method.

   - ((# variant loci correctly reported by the assay) + (# wild type loci correctly reported by the assay)) / ((total # of variant loci) + (total # of wild type loci))

4. The calculations of PPA, NPA, and OPA do not include no calls (variant or reference loci not meeting one or more quality filters).

5. Percent Positive Calls (PPC) is number of observations with the variant detected divided by the total number of observations tested excluding any invalid observations or those filtered as low depth.

6. Percent Negative Calls (PNC) is calculated as number of observations with passing reference as the outcome at a position divided by the total number of observations tested excluding any invalid observations or those filtered as low depth.

7. Percent Autosome Callability is calculated as the percent of non-N reference positions in targeted regions in autosomal chromosomes with a passing genotype call.

Sample Indexing

Sample index primers, added during library preparation, assign a unique sequence to each sample DNA. These unique sequences allow multiple samples to be pooled together into a single sequencing run. Sample indexing is used for both germline and somatic workflows. The purpose of this study was to establish the minimum (12) and maximum (192) number of samples that can be processed in a single sequencing run by the NovaSeq 6000Dx Instrument. Twelve unique Platinum Genome DNA samples (NA12877–NA12888) were tested with at least 12 different indexing primer combinations per sample. Sample libraries were prepared using a representative assay designed to query a variety of genes covering 1,970,505 bases across all 23 human chromosomes. Sample results from four sequencing runs using the Germline FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application were compared to Platinum Genomes version 2016-1.0.

For the first set of runs, 192 uniquely indexed sample libraries were sequenced in two sequencing runs, one each with S2 and S4 reagents, to verify both the maximum number of indexes supported and the ability of the assay to consistently make a genotyping call for a given sample across different indexing primer combinations. For the second set of runs, 12 uniquely indexed sample libraries were sequenced in two sequencing runs, one each with S2 and S4 reagents, to verify the minimum number of indexes supported.

For the 192-index runs, PPA for SNVs ranged from 99.7% to 100%, PPA for insertions was 100%, PPA for deletions ranged from 96.7% to 100% and NPA was 100%. For the 12-index runs, PPA for SNVs ranged from 99.7% to 100%, PPA for insertions ranged from 89.6% to 100%, PPA for deletions ranged from 94.6% to 100% and NPA was 100%.

Sample Carryover

The NovaSeq 6000Dx Instrument allows for sequencing of multiple samples plus controls in a single sequencing run. A study was conducted to evaluate the extent of sample carryover within a sequencing run (within-run) and between sequencing runs (run-to-run). Twelve Platinum Genome DNA samples, six male and six female, were tested with a representative assay designed to query a variety of genes covering 1,970,505 bases across all 23 human chromosomes, including both sex chromosomes. Libraries were sequenced on the NovaSeq 6000Dx Instrument using the Germline FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application. Carryover of male samples into female samples was observed by the presence of Y chromosome target reads in female samples.

Within-run carryover may be introduced during cluster generation, index cycle base calling, and sample demultiplexing. For testing of sample carryover within a sequencing run, a library pool consisting of at least twelve replicates of each unique male and female sample plus two no template controls, for a total of 192 uniquely indexed libraries, was sequenced on the NovaSeq 6000Dx Instrument in two sequencing runs, one each with S2 and S4 reagents. Within-run sample carryover was assessed by comparing Y chromosome target coverage of each female replicate to the average Y chromosome target coverage of all male replicates in the pool. The 95th percentile of observed within-run carryover was 0.0090% and 0.041% for S2 and S4 reagents, respectively.

For testing of run-to-run sample carryover, two library pools were prepared and sequenced consecutively on one NovaSeq 6000Dx Instrument, with side A using S4 reagents and side B using S2 reagents. The first pool contained at least twelve replicates of six unique female sample plus two no template controls, for a total of 96 uniquely indexed libraries. The second pool contained at least twelve replicates of six unique male sample plus two no template controls, for a total of 96 uniquely indexed libraries. Both pools used the same set of index adapters. The female pool was sequenced first followed by a subsequent sequencing run with the male pool, followed by another repeat sequencing run of the female pool. Run-to-run sample carryover was assessed per reagent type, S2 and S4, by comparing Y chromosome target coverage between corresponding replicates of the female pool repeat run and male pool run. The 95th percentile of observed run-to-run carryover was 0.0089% and 0.012% for S2 and S4 reagents, respectively.

DNA Input

Blood (Germline)

The blood DNA input range for the Illumina DNA Prep with Enrichment Dx Kit using the DRAGEN for Illumina DNA Prep with Enrichment Dx Application was established for the NovaSeq 6000Dx. This was evaluated by performing a serial dilution study using eight Platinum Genome DNA samples (NA12877 – NA12884) with a representative assay designed to query a variety of genes covering 1,970,505 bases across all 23 human chromosomes. Libraries were sequenced on one NovaSeq 6000Dx Instrument using one lot each of NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles) and NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles).

Seven samples were tested in duplicate at six DNA input levels ranging from 1000 ng to 10 ng (1000 ng, 250 ng, 100 ng, 50 ng, 25 ng, and 10 ng). An eighth sample (NA12884) was tested as a single replicate at 10 ng input and in duplicate for all other input levels. For determination of accuracy, sample genotypes were compared to Platinum Genomes version 2016-1.0. Results were determined for each input level. PPA for each variant type (SNVs, insertions, and deletions) is presented in *PPA Results for Each Blood DNA Input by Variant Type* on page 32. NPA is presented in *NPA for Each Blood DNA Input* on page 32. All input levels had similar accuracy. The recommended blood DNA input for the Illumina DNA Prep with Enrichment Dx is 50–1000 ng with 1000 ng and 10 ng providing an upper and lower limit to meet performance characteristics when sequenced on the NovaSeq 6000Dx.

Table 9 PPA Results for Each Blood DNA Input by Variant Type

DNA Input (ng)	Variant Type	Expected Variants	TP	FN	Variant No Calls	PPA (%)
10	SNV	69612	69538	68	6	99.9
25		74192	74105	75	12	99.9
50			74105	74	13	99.9
100			74116	72	4	99.9
250			74113	72	7	99.9
1000			74112	73	7	99.9
10	Insertion	2732	2732	0	0	100
25		2928	2916	6	6	99.8
50			2914	8	6	99.7
100			2917	6	5	99.8
250			2928	0	0	100
1000			2921	5	2	99.8
10	Deletion	2084	2049	4	31	99.8
25		2240	2200	9	31	99.6
50			2207	3	30	99.9
100			2199	1	40	>99.9
250			2201	0	39	100
1000			2195	2	43	99.9

Table 10 NPA for Each Blood DNA Input

DNA Input (ng)	TN	FP	Reference No Calls	NPA (%)
10	115449045	384	285751	>99.9

DNA Input (ng)	TN	FP	Reference No Calls	NPA (%)
25	123012157	415	438153	>99.9
50	122985299	369	465043	>99.9
100	122976660	321	473730	>99.9
250	122971099	331	479289	>99.9
1000	122978527	324	471882	>99.9

FFPE (Somatic)

The formalin-fixed paraffin-embedded (FFPE) DNA input range for the Illumina DNA Prep with Enrichment Dx Kit using the DRAGEN for Illumina DNA Prep with Enrichment Dx Application was established for the NovaSeq 6000Dx. This was evaluated by performing a serial dilution study using two Platinum Genome samples with a representative assay designed to query a variety of genes covering 1,970,505 bases across all 23 human chromosomes. Libraries were sequenced on one NovaSeq 6000Dx Instrument using one lot each of NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles) and NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles).

Sample GM12877 DNA was diluted with sample GM12878 DNA to create GM12877-13 with unique GM12877 heterozygous and homozygous variants at frequencies near 6.5% and 13%, respectively. Undiluted GM12877 was also tested. GM12877-13 was tested in duplicate at four DNA input levels ranging from 1000 ng to 25 ng (1000 ng, 250 ng, 50 ng and 25 ng). GM12877 was tested as a single replicate at 250 ng and in duplicate for all other input levels. For determination of accuracy, sample variant calls were compared to Platinum Genomes version 2016-1.0. Results were determined for each input level. PPA for each variant type (SNVs, insertions, and deletions) is presented in *PPA Results for Each FFPE DNA Input by Variant Type and Target VAF* on page 33. NPA is presented in *NPA for Each FFPE DNA Input* on page 34. All input levels had similar accuracy. For FFPE samples with ΔCq value of ≤ 5, the recommended DNA input is 50–1000 ng for the Illumina DNA Prep with Enrichment Dx Kit with 1000 ng and 25 ng providing an upper and lower limit to meet performance characteristics when sequenced on the NovaSeq 6000Dx.

Table 11 PPA Results for Each FFPE DNA Input by Variant Type and Target VAF

		Target Dilution VAF									
		0.065					0.13				
DNA Input (ng)	Variant Type	Expected Variants	TP	FN	Variant No Calls	PPA (%)	Expected Variants	TP	FN	Variant No Calls	PPA (%)
25	SNV	3000	2931	8	61	99.7	624	624	0	0	100
50		3000	2930	8	62	99.7	624	622	0	2	100
250		3000	2927	8	65	99.7	624	624	0	0	100
1000		3000	2921	8	71	99.7	624	624	0	0	100

| Target Dilution VAF | | | | | | | | | | | |
| | | 0.065 | | | | | 0.13 | | | | |
DNA Input (ng)	Variant Type	Expected Variants	TP	FN	Variant No Calls	PPA (%)	Expected Variants	TP	FN	Variant No Calls	PPA (%)
25	Insertion	96	96	0	0	100	48	48	0	0	100
50		96	96	0	0	100	48	48	0	0	100
250		96	96	0	0	100	48	48	0	0	100
1000		96	96	0	0	100	48	48	0	0	100
25	Deletion	88	88	0	0	100	32	32	0	0	100
50		88	88	0	0	100	32	31	0	1	100
250		88	88	0	0	100	32	32	0	0	100
1000		88	88	0	0	100	32	32	0	0	100

Table 12   NPA for Each FFPE DNA Input

DNA Input (ng)	Expected Wild Type	TN	FP	Reference No Calls	NPA (%)
25	25354119	25353706	413	5499498	>99.9
50	27538269	27538013	256	3315421	>99.9
250	21562303	21561983	320	1577958	>99.9
1000	29030903	29030596	307	1822781	>99.9

Analytical Sensitivity (Limit of Blank [LoB] and Limit of Detection [LoD])

This study was conducted to evaluate the Limit of Blank (LoB) and Limit of Detection (LoD) for the Somatic FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application on the NovaSeq 6000Dx Instrument. The study was performed using a representative assay designed to query a variety of genes covering 1,970,505 bases across all 23 human chromosomes. Platinum Genome cell lines GM12878 and GM12877 were formalin fixed and embedded in paraffin followed by DNA extraction. Dilutions of GM12877 into GM12878 were prepared to make samples consisting of 0%, 4%, 6.5% and 13% GM12877 by volume, such that the variant frequencies of 489 unique GM12877 variants (454 SNVs, 17 insertions, and 18 deletions) ranged between 0 and 0.13. Sample libraries were prepared using two lots of Illumina DNA Prep with Enrichment Dx Kit reagents and sequenced over six consecutive start days with two NovaSeq 6000Dx Instruments and two lots each of NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles) and NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles), for a total of twelve sequencing runs. This resulted in 288 observations for each variant in each of the sample dilutions. LoB and LoD were calculated using the classical approach stated in CLSI EP17-A2. LoB and LoD were calculated for S2 and S4 reagents separately by pooling the variant frequencies of all variants in the sequencing run for each reagent type. The Type I error was defined as 0.01, and the Type II error was defined as 0.05.

The LoB was evaluated for 489 loci independently across two sequencing lots for each reagent type (S2 or S4) and library preparation. For S2 reagents, the 95th percentile LoB was 2.9%. For S4 reagents, the 95th percentile LoB was 2.2%.

LoD was successfully calculated for 478 of 489 variants for S2 and 485 of 489 variants for S4. The variants where no LoD was determined for one or both library preparations were excluded from final assigned of LoD for the NovaSeq 6000Dx system. The LoD of the NovaSeq 6000Dx system with S2 and S4 reagents was determined by taking the 95th percentile of the individual variant LoDs. For S2 reagents, the 95th percentile across 478 variant LoDs was 4.8%. For S4 reagents, the 95th percentile across 485 variant LODs was 3.9%.

Accuracy

Germline

The following study was conducted to assess the variant calling accuracy of the Germline FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application on the NovaSeq 6000Dx Instrument using the NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles). Four unique Platinum Genome DNA samples were tested using a representative assay designed to query a variety of genes covering 1,970,505 bases (9,232 targets) across all 23 human chromosomes. Each of the samples was tested in replicates of 12 except for NA12880, which were tested in replicates of 11. A total of 18 runs were performed using three sequencing instruments, three lots of S2 reagents, and two operators over six start days. Accuracy was determined for SNVs, insertions, and deletions by comparing the results to Platinum Genomes version 2016-1.0.

Table 13 Summary of Germline Agreement

Criteria	Total Observations[1]	Result of Observation[2]	Result by Run[3]
PPA for SNV	846	99.8	99.9
PPA for insertions	846	97.9	>99.9
PPA for deletions	846	96.9	99.9
NPA	846	>99.9	>99.9
OPA	846	>99.9	>99.9

[1]Calculated as number of samples per run (47) x number of Runs (18) = 846.
[2]Lowest observed value by sample replicate across all 18 runs.
[3]Lowest value when data from each run are analyzed in aggregate.

Germline Agreement per Sample on page 37 contains the study data presented with positive and negative percent agreement on a per sample basis, where the variant results are compared to Platinum Genomes version 2016-1.0 for PPA calculations. The three variant types (SNVs, insertions, and deletions) are combined. Because the reference method only provides results for the single nucleotide variants and insertions/deletions, non-variant base results are compared to human genome reference sequence build hg19 for NPA calculations.

Table 14   Germline Agreement per Sample

Sample	Autosome Callability	Expected Variants[1]	TP	FN	Variant No Calls	TN	FP	PPA	NPA	OPA
NA12877	99.4	273672	273452	220	0	414765131	931	99.9	>99.9	>99.9
NA12878	99.4	265680	265208	234	238	414803691	1193	99.9	>99.9	>99.9
NA12879	99.4	261792	261792	0	0	414746986	1429	100	>99.9	>99.9
NA12880	99.4	246114	245551	399	164	380157538	1458	99.8	>99.9	>99.9

[1] Total number of variants in all sample replicates across 18 runs.

Germline Agreement per Sample by Variant Type on page 37 contains the study data presented on a per sample basis, where the variant results are compared to the well—characterized composite reference method. Detection is evaluated for each variant type—SNVs, insertions, and deletions—separately. Reference positions are excluded.

Table 15   Germline Agreement per Sample by Variant Type

	SVNs			Insertions			Deletions		
Sample	Expected	TP	FN	Expected	TP	FN	Expected	TP	FN
NA12877	255096	254877	219	10368	10367	1	8208	8208	0
NA12878	250344	250077	221	8424	8424	0	6912	6707	13
NA12879	246024	246024	0	8856	8856	0	6912	6912	0
NA12880	229482	229086	396	9306	9306	0	7326	7159	3

The samples were further analyzed for calling small insertions and deletions (indels). An overall summary is presented in Summary of Germline Indel Detection on page 37. There were a total of 210 indels ranging in size from 1–18 bp for insertions and 1–21 bp for deletions.

Table 16   Summary of Germline Indel Detection

Variant Type	Expected Variants	TP	FN	Variant No Calls	PPA
Insertion	36954	36953	1	0	>99.9
Deletion	29358	28986	16	356	99.9

The representative assay consisted of 9,232 targets covering a variety of genomic content. The GC content of the targets ranged from 0.20-0.86. Targets also had a range of single nucleotide (eg, PolyA, PolyT), dinucleotide, and trinucleotide repeats. Data compiled on a per chromosome basis to determine the effect of genomic content on percent correct calls is presented in *Germline Chromosome-level Accuracy* on page 38. Percent correct calls consists of variant and reference calls and is less than 100% if there are either incorrect or no calls.

Table 17   Germline Chromosome-level Accuracy

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr1	47	728	138328	Poly A (12), Poly C (7), Poly T (14), Poly G (7), Dinucleotide (22), Trinucleotide (8), Insertion (18), Deletion (4)	[0.22 - 0.8]; Median: 0.51	114888718	34	966860	>99.9	0.83
chr2	39	628	159588	Poly A (46), Poly C (8), Poly T (23), Poly G (7), Dinucleotide (22), Trinucleotide (8), Insertion (5), Deletion (2)	[0.24 - 0.81]; Median: 0.44	132293464	798	460345	>99.9	0.35

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr3	38	650	137627	Poly A (18), Poly C (6), Poly T (18), Poly G (7), Dinucleotide (12), Trinucleotide (6), Insertion (11), Deletion (1)	[0.25 - 0.86]; Median: 0.45	114625053	2	226461	>99.9	0.20
chr4	17	370	73766	Poly A (9), Poly C (7), Poly T (25), Poly G (6), Dinucleotide (5), Trinucleotide (5), Insertion (2), Deletion (2)	[0.27 - 0.77]; Median: 0.45	61872303	0	66741	100	0.11

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr5	25	507	90008	Poly A (10), Poly C (6), Poly T (12), Poly G (7), Dinucleotide (10), Trinucleotide (8), Insertion (8), Deletion (18)	[0.29 - 0.79]; Median: 0.46	75314497	912	153061	>99.9	0.20
chr6	39	453	126721	Poly A (28), Poly C (7), Poly T (33), Poly G (7), Dinucleotide (18), Trinucleotide (11), Insertion (4), Deletion (2)	[0.24 - 0.79]; Median: 0.48	103412695	1	182361	>99.9	0.18

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr7	21	450	161501	Poly A (27), Poly C (8), Poly T (21), Poly G (7), Dinucleotide (31), Trinucleotide (5), Insertion (1), Deletion (4)	[0.2 - 0.77]; Median: 0.46	132534074	19	246884	>99.9	0.19
chr8	18	381	67775	Poly A (19), Poly C (7), Poly T (13), Poly G (7), Dinucleotide (5), Trinucleotide (9), Insertion (4), Deletion (1)	[0.26 - 0.78]; Median: 0.47	56247612	411	170925	>99.9	0.30

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr9	23	347	87100	Poly A (12), Poly C (7), Poly T (27), Poly G (8), Dinucleotide (9), Trinucleotide (9), Insertion (4), Deletion (1)	[0.27 - 0.83]; Median: 0.49	72650800	20	241991	>99.9	0.33
chr10	14	317	66723	Poly A (26), Poly C (7), Poly T (15), Poly G (6), Dinucleotide (16), Trinucleotide (6), Insertion (1), Deletion (1)	[0.23 - 0.78]; Median: 0.44	55539058	1	188216	>99.9	0.34

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr11	29	511	91786	Poly A (28), Poly C (8), Poly T (21), Poly G (7), Dinucleotide (26), Trinucleotide (7), Insertion (2), Deletion (2)	[0.28 - 0.8]; Median: 0.47	75744222	742	259258	>99.9	0.34
chr12	29	577	120365	Poly A (19), Poly C (8), Poly T (40), Poly G (7), Dinucleotide (7), Trinucleotide (7), Insertion (1), Deletion (5)	[0.26 - 0.77]; Median: 0.49	99972530	1	542005	>99.9	0.54

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr13	13	283	58639	Poly A (24), Poly C (6), Poly T (12), Poly G (7), Dinucleotide (6), Trinucleotide (8), Insertion (14), Deletion (0)	[0.28 - 0.79]; Median: 0.42	48503179	1	45666	>99.9	0.09
chr14	11	147	26980	Poly A (21), Poly C (6), Poly T (18), Poly G (11), Dinucleotide (6), Trinucleotide (6), Insertion (4), Deletion (1)	[0.29 - 0.77]; Median: 0.47	22286153	198	147895	>99.9	0.66

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr15	15	266	52091	Poly A (26), Poly C (7), Poly T (13), Poly G (6), Trinucleotide (8), Insertion (4), Deletion (6)	[0.29 - 0.76]; Median: 0.46	43600279	0	99041	100	0.23
chr16	21	366	80030	Poly A (7), Poly C (7), Poly T (15), Poly G (7), Dinucleotide (5), Trinucleotide (10), Insertion (15), Deletion (21)	[0.3 - 0.76]; Median: 0.54	65490245	16	1438278	>99.9	2.15

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr17	36	645	118062	Poly A (19), Poly C (7), Poly T (18), Poly G (8), Dinucleotide (13), Trinucleotide (6), Insertion (18), Deletion (16)	[0.28 - 0.82]; Median: 0.49	97929929	417	335905	>99.9	0.34
chr18	9	99	19195	Poly A (7), Poly C (7), Poly T (15), Poly G (6), Trinucleotide (10), Insertion (4), Deletion (0)	[0.22 - 0.78]; Median: 0.44	15967171	312	42077	>99.9	0.26

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr19	30	605	104004	Poly A (19), Poly C (7), Poly T (31), Poly G (7), Dinucleotide (5), Trinucleotide (7), Insertion (2), Deletion (21)	[0.33 - 0.83]; Median: 0.59	85642066	3	678213	>99.9	0.79
chr20	12	179	33795	Poly A (6), Poly C (6), Poly T (7), Poly G (8), Trinucleotide (9), Insertion (5), Deletion (0)	[0.31 - 0.84]; Median: 0.53	28108712	0	38374	100	0.14
chr21	5	63	30642	Poly A (28), Poly C (6), Poly T (24), Poly G (7), Dinucleotide (5), Insertion (2), Deletion (5)	[0.22 - 0.78]; Median: 0.52	25319736	50	57434	>99.9	0.23

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr22	10	187	36727	Poly A (26), Poly C (7), Poly T (19), Poly G (7), Dinucleotide (5), Trinucleotide (6), Insertion (6), Deletion (0)	[0.27 - 0.74]; Median: 0.51	30258131	0	42673	100	0.14
chrX	23	433	83576	Poly A (18), Poly C (8), Poly T (23), Poly G (9), Dinucleotide (5), Trinucleotide (23), Insertion (3), Deletion (0)	[0.2 - 0.72]; Median: 0.48	67318722	0	770544	100	1.13
chrY	0	40	5476	Poly A (11), Poly C (8), Poly T (11), Poly G (5), Insertion (0), Deletion (0)	[0.4 - 0.59]; Median: 0.45	0	0	0	N/A	N/A

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The sequencing results for sample NA12878 were compared to a highly confident genotype for NA12878, established by the National Institutes of Standards and Technology (NIST) (v.2.19). Out of the 9,232 targets, 8,009 targets were fully contained within the highly confident genomic regions, 776 targets had partial overlap, and 447 targets had no overlap in the NIST sequence. This resulted in 1,831,483 coordinates per replicate for comparison. Non-variant base calls were compared to human genome reference sequence build hg19. The accuracy results are shown in *Germline Agreement of NA12878 Sample with NIST Database* on page 49.

Table 18   Germline Agreement of NA12878 Sample with NIST Database

Sample	# Targets covered	Autosome Callability	TP	FN	TN	FP	PPA	NPA	OPA
NA12878	8785	99.4	247709	218	394262149	4584	>99.9	>99.9	>99.9

Based on the data provided by this 18-run Germline study, the NovaSeq 6000Dx Instrument can consistently sequence:

- GC content ≥ 20% (all called bases in 1692 sequenced target regions with 20% GC content called correctly with no-call rate of 0%)

- GC content ≤ 86% (all called bases in 846 sequenced target regions with 86% GC content called correctly with no-call rate of 0%)

- PolyA lengths ≤ 46 (all called bases in 846 sequenced target regions with 46 PolyA repeat called correctly with no-call rate of 0.27%)

- PolyT lengths ≤ 40 (13384074 out of 13384321 called bases in 846 sequenced target regions with 40 PolyT repeat called correctly with no-call rate of 0.26%)

- PolyG lengths ≤ 11 (all called bases in 846 sequenced target regions with 11 PolyG repeat called correctly with no-call rate of 0%)

- PolyC lengths ≤ 8 (9815030 out of 9815035 called bases in 5922 sequenced target regions with 8 PolyC repeat called correctly with no-call rate of 0.53%)

- Dinucleotide repeat lengths ≤ 31x (32233922 out of 32233926 called bases in 846 sequenced target regions with 31 Dinucleotide repeat called correctly with no-call rate of 0.21%)

- Trinucleotide repeat lengths ≤ 23x (all called bases in 846 sequenced target regions with 23 Trinucleotide repeat called correctly with no-call rate of 0.21%)

- Insertion lengths ≤ 18 (all called bases in 1692 sequenced target regions with 18 Insertion called correctly with no-call rate of 7.71%)

- Deletion lengths ≤ 21 (all called bases in 1692 sequenced target regions with 21 Deletion called correctly with no-call rate of 1.14%)

Somatic

The study described here was used to assess variant calling accuracy of the Somatic FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application on the NovaSeq 6000Dx Instrument using the NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles).

This study used a representative assay designed to query a variety of genes covering 1,970,505 bases (9,232 targets) across all 23 human chromosomes. Platinum Genome DNA was extracted from FFPE treated blocks to generate four unique samples for evaluation in the study.

Sample GM12877 DNA was diluted with sample GM12878 DNA to create GM12877-13 with unique GM12877 heterozygous and homozygous variants at frequencies near 6.5% and 13%, respectively. Sample GM12878 DNA was similarly diluted with sample GM12877 DNA to create GM12878-13 with unique GM12878 heterozygous and homozygous variants at frequencies near 6.5% and 13%, respectively. Undiluted GM12877 and GM12878 were also tested. Each of the samples was tested in replicates of 12 except for undiluted GM12878, which were tested in replicates of eleven. A total of eighteen runs were performed using three sequencing instruments, three lots of S4 reagents, and two operators over six start days. Accuracy was determined for SNVs, insertions, and deletions by comparing the results to Platinum Genomes version 2016-1.0.

Table 19 Summary of Somatic Agreement

Criteria	No. of Observations[1]	Result of Observations[2]	Result by Run[3]
PPA for somatic SNVs	846	99.8	98.9
PPA for somatic insertions	846	100	100
PPA for somatic deletions	846	100	100
NPA	846	>99.9	>99.9
OPA	846	>99.9	>99.9

[1] Calculated as = No. of samples per run (47) x No. of Runs (18) = 846.
[2] Lowest observed value by sample replicate across all 18 runs.
[3] Lowest value when data from each run are analyzed in aggregate.

Somatic Agreement per Sample on page 51 contains the study data presented with positive and negative percent agreement on a per sample basis, where the variant results are compared to the well-characterized composite reference method for PPA calculations. The

three variant types (SNVs, insertions, and deletions) are combined. Because the reference method only provides results for the single nucleotide variants and insertions/deletions, non-variant base results are compared to human genome reference sequence build hg19 for NPA calculations.

Table 20 Somatic Agreement per Sample

Sample	Autosome Callability	Expected Variants	TP	FN	Variant No Calls	TN	FP	PPA	NPA	OPA
GM12877	95.4	96228	95022	198	1008	365425810	1203	99.8	>99.9	>99.9
GM12878	94.5	96768	96278	0	490	395002023	1278	100	>99.9	>99.9
GM12877-13	94.7	104976	103029	216	1731	395989324	1286	99.8	>99.9	>99.9
GM12878-13	95.2	96768	96027	0	741	397900884	1218	100	>99.9	>99.9

Somatic Agreement per Sample by Variant Type on page 51 contains the study data presented on a per sample basis, where the variant results are compared to the well-characterized composite reference method. Detection is evaluated for each variant type—SNVs, insertions, and deletions—separately. Reference positions are excluded.

Table 21 Somatic Agreement per Sample by Variant Type

	SNVs			Insertions			Deletions		
Sample	Expected	TP	FN	Expected	TP	FN	Expected	TP	FN
GM12877	89694	88488	198	3564	3564	0	2970	2970	0
GM12878	92664	92390	0	2160	2160	0	1944	1728	0
GM12877-13	97848	95901	216	3888	3888	0	3240	3240	0
GM12878-13	92664	92139	0	2160	2160	0	1944	1728	0

The four samples were further analyzed for calling small insertions and deletions (indels). An overall summary is presented in *Summary of Somatic Indel Detection* on page 52. There were a total of 210 indels ranging in size from 1–18 bp for insertions and 1–21 bp for deletions.

Table 22   Summary of Somatic Indel Detection

Variant Type	Expected Variants	TP	FN	Variant No Calls	PPA
Insertion	11772	11772	0	0	100
Deletion	10098	9666	0	432	100

The representative assay consisted of 9,232 targets covering a variety of genomic content. The GC content of the targets ranged from 0.20–0.86. Targets also had a range of single nucleotide (eg, PolyA, PolyT), dinucleotide, and trinucleotide repeats. Data compiled on a per chromosome basis to determine the effect of genomic content on percent correct calls is presented in *Somatic Chromosome-level Accuracy* on page 52. Percent correct calls consists of variant and reference calls and is less than 100% if there are either incorrect or no calls.

Table 23   Somatic Chromosome-level Accuracy

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr1	47	728	138328	Poly A (12), Poly C (7), Poly T (14), Poly G (7), Dinucleotide (22), Trinucleotide (8), Insertion (3), Deletion (0)	[0.22 - 0.8]; Median: 0.51	110145939	52	5642613	>99.9	4.9

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr2	39	628	159588	Poly A (46), Poly C (8), Poly T (23), Poly G (7), Dinucleotide (22), Trinucleotide (8), Insertion (5), Deletion (1)	[0.24 - 0.81]; Median: 0.44	126795713	842	5850393	>99.9	4.4
chr3	38	650	137627	Poly A (18), Poly C (6), Poly T (18), Poly G (7), Dinucleotide (12), Trinucleotide (6), Insertion (1), Deletion (1)	[0.25 - 0.86]; Median: 0.45	109902527	593	4889226	>99.9	4.3

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr4	17	370	73766	Poly A (9), Poly C (7), Poly T (25), Poly G (6), Dinucleotide (5), Trinucleotide (5), Insertion (0), Deletion (1)	[0.27 - 0.77]; Median: 0.45	59373461	16	2517412	>99.9	4.1
chr5	25	507	90008	Poly A (10), Poly C (6), Poly T (12), Poly G (7), Dinucleotide (10), Trinucleotide (8), Insertion (8), Deletion (18)	[0.29 - 0.79]; Median: 0.46	72261191	723	3116981	>99.9	4.1

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr6	39	453	126721	Poly A (28), Poly C (7), Poly T (33), Poly G (7), Dinucleotide (18), Trinucleotide (11), Insertion (0), Deletion (1)	[0.24 - 0.79]; Median: 0.48	98593101	687	4890221	>99.9	4.7
chr7	21	450	161501	Poly A (27), Poly C (8), Poly T (21), Poly G (7), Dinucleotide (31), Trinucleotide (5), Insertion (1), Deletion (4)	[0.2 - 0.77]; Median: 0.46	126913574	104	5773856	>99.9	4.4

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr8	18	381	67775	Poly A (19), Poly C (7), Poly T (13), Poly G (7), Dinucleotide (5), Trinucleotide (9), Insertion (4), Deletion (0)	[0.26 - 0.78]; Median: 0.47	53430489	175	2958909	>99.9	5.2
chr9	23	347	87100	Poly A (12), Poly C (7), Poly T (27), Poly G (8), Dinucleotide (9), Trinucleotide (9), Insertion (0), Deletion (1)	[0.27 - 0.83]; Median: 0.49	69594586	74	3260257	>99.9	4.5

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr10	14	317	66723	Poly A (26), Poly C (7), Poly T (15), Poly G (6), Dinucleotide (16), Trinucleotide (6), Insertion (0), Deletion (0)	[0.23 - 0.78]; Median: 0.44	53209592	90	2469444	>99.9	4.4
chr11	29	511	91786	Poly A (28), Poly C (8), Poly T (21), Poly G (7), Dinucleotide (26), Trinucleotide (7), Insertion (2), Deletion (2)	[0.28 - 0.8]; Median: 0.47	72291795	150	3665560	>99.9	4.8

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr12	29	577	120365	Poly A (19), Poly C (8), Poly T (40), Poly G (7), Dinucleotide (7), Trinucleotide (7), Insertion (0), Deletion (3)	[0.26 - 0.77]; Median: 0.49	96109352	101	4331932	>99.9	4.3
chr13	13	283	58639	Poly A (24), Poly C (6), Poly T (12), Poly G (7), Dinucleotide (6), Trinucleotide (8), Insertion (14), Deletion (0)	[0.28 - 0.79]; Median: 0.42	46130028	44	2384839	>99.9	4.9

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr14	11	147	26980	Poly A (21), Poly C (6), Poly T (18), Poly G (11), Dinucleotide (6), Trinucleotide (6), Insertion (4), Deletion (0)	[0.29 - 0.77]; Median: 0.47	21336891	0	1078329	100	4.8
chr15	15	266	52091	Poly A (26), Poly C (7), Poly T (13), Poly G (6), Trinucleotide (8), Insertion (4), Deletion (0)	[0.29 - 0.76]; Median: 0.46	41918631	184	1753300	>99.9	4.0

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr16	21	366	80030	Poly A (7), Poly C (7), Poly T (15), Poly G (7), Dinucleotide (5), Trinucleotide (10), Insertion (15), Deletion (21)	[0.3 - 0.76]; Median: 0.54	62344351	18	4540539	>99.9	6.8
chr17	36	645	118062	Poly A (19), Poly C (7), Poly T (18), Poly G (8), Dinucleotide (13), Trinucleotide (6), Insertion (18), Deletion (1)	[0.28 - 0.82]; Median: 0.49	93811318	414	4403622	>99.9	4.5

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr18	9	99	19195	Poly A (7), Poly C (7), Poly T (15), Poly G (6), Trinucleotide (10), Insertion (0), Deletion (0)	[0.22 - 0.78]; Median: 0.44	15007653	6	990633	>99.9	6.2
chr19	30	605	104004	Poly A (19), Poly C (7), Poly T (31), Poly G (7), Dinucleotide (5), Trinucleotide (7), Insertion (2), Deletion (3)	[0.33 - 0.83]; Median: 0.59	81416722	455	4860311	>99.9	5.6
chr20	12	179	33795	Poly A (6), Poly C (6), Poly T (7), Poly G (8), Trinucleotide (9), Insertion (5), Deletion (0)	[0.31 - 0.84]; Median: 0.53	26833936	7	1301905	>99.9	4.6

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chr21	5	63	30642	Poly A (28), Poly C (6), Poly T (24), Poly G (7), Dinucleotide (5), Insertion (1), Deletion (0)	[0.22 - 0.78]; Median: 0.52	24169250	44	1172087	>99.9	4.6
chr22	10	187	36727	Poly A (26), Poly C (7), Poly T (19), Poly G (7), Dinucleotide (5), Trinucleotide (6), Insertion (6), Deletion (0)	[0.27 - 0.74]; Median: 0.51	28887217	86	1392179	>99.9	4.6

Chromosome	# Genes	# Targets	# Bases	Genomic Content	GC Range	Correct Calls	Incorrect Calls	No calls	% Correct Calls	%No Calls
chrX	23	433	83576	Poly A (18), Poly C (8), Poly T (23), Poly G (9), Dinucleotide (5), Trinucleotide (23), Insertion (3), Deletion (0)	[0.2 - 0.72]; Median: 0.48	64231080	241	3852253	>99.9	5.7
chrY	0	40	5476	Poly A (11), Poly C (8), Poly T (11), Poly G (5), Insertion (0), Deletion (0)	[0.4 - 0.59]; Median: 0.45	0	0	0	N/A	N/A

The sequencing results for sample GM12878 were compared to a highly confident genotype for NA12878, established by the National Institute of Standards and Technology (NIST) (v.2.19). Out of the 9,232 targets, 8,009 targets were fully contained within the highly confident genomic regions, 776 targets had partial overlap, and 447 targets had no overlap in the NIST sequence. This resulted in 1,831,483 coordinates per replicate for comparison. Non-variant base calls were compared to human genome reference sequence build hg19. The accuracy results are shown in *Somatic Agreement of GM12878 Sample with NIST Database* on page 63.

Table 24   Somatic Agreement of GM12878 Sample with NIST Database

Sample	# Targets covered	Autosome Callability	TP	FN	TN	FP	PPA	NPA	OPA
GM12878	8785	94.5	247228	0	375073821	2043	100	>99.9	>99.9

Based on the data provided by this 18-run Somatic study, the NovaSeq 6000Dx Instrument can consistently sequence:

- GC content ≥ 20% (all called bases in 1692 sequenced target regions with 20% GC content called correctly with no-call rate of 0.34%)

- GC content ≤ 86% (all called bases in 846 sequenced target regions with 86% GC content called correctly with no-call rate of 4.21%)

- PolyA lengths ≤ 46 (14550082 out of 14550083 called bases in 846 sequenced target regions with 46 PolyA repeat called correctly with no-call rate of 4.18%)

- PolyT lengths ≤ 40 (12833489 out of 12833491 called bases in 846 sequenced target regions with 40 PolyT repeat called correctly with no-call rate of 4.37%)

- PolyG lengths ≤ 11 (all called bases in 846 sequenced target regions with 11 PolyG repeat called correctly with no-call rate of 7.59%)

- PolyC lengths ≤ 8 (9405604 out of 9405615 called bases in 5922 sequenced target regions with 8 PolyC repeat called correctly with no-call rate of 4.68%)

- Dinucleotide repeat lengths ≤ 31x (30996684 out of 30996712 called bases in 846 sequenced target regions with 31 Dinucleotide repeat called correctly with no-call rate of 4.04%)

- Trinucleotide repeat lengths ≤ 23x (all called bases in 846 sequenced target regions with 23 Trinucleotide repeat called correctly with no-call rate of 5.39%)

- Insertion lengths ≤ 18 (all called bases in 846 sequenced target regions with 18 Insertion called correctly with no-call rate of 1.44%)

- Deletion lengths ≤ 21 (all called bases in 846 sequenced target regions with 21 Deletion called correctly with no-call rate of 7.86%)

Precision

The precision of the NovaSeq 6000Dx Instrument was evaluated using Platinum Genome samples with a representative assay designed to query a variety of genes covering 1,970,505 bases across 23 different chromosomes using 9,232 target oligos. A total of 1723 targeted small variants (SNVs, insertions and deletions) were evaluated. Germline testing consisted of eleven or twelve replicates of four unique Platinum Genome samples. Somatic testing consisted of eleven or twelve replicates of four unique FFPE-treated Platinum Genome samples at different VAF levels. Sample libraries were prepared using Illumina DNA Prep with Enrichment Dx Kit reagents.

Testing was performed at one internal site using three NovaSeq 6000Dx Instruments, three lots each of NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles) and NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles), and two operators over six start days. For each start day, germline sample libraries were sequenced on one instrument side using S2 reagents and the Germline FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application, and somatic sample libraries were sequenced on the other instrument side using S4 reagents and the Somatic FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application. This testing resulted in 18 flow cells for each the germline and somatic workflows.

Germline

For germline runs, genomic locations where a targeted germline variant is detected are reported as positive (variant). For expected positive germline variants, the data were evaluated for no call rate and percent positive call (PPC) within each variant type (SNV, insertion, deletion). *Within-Lab Precision Germline Call Observations for Expected Positive Results by Variant Type* on page 65 summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method, for each variant type.

Table 25   Within-Lab Precision Germline Call Observations for Expected Positive Results by Variant Type

Variant Type	No Calls Observed[1]	Total Calls	Percent No Calls	Positive Calls Observed[2]	Total Evaluable Calls	PPC	95% LCL[3]	95% UCL
SNV	6	980316	<0.01	979854	980310	99.95	99.95	99.96
Insertion	0	36738	0	36738	36738	100	>99.99	100
Deletion	18	34434	0.05	32160	34416	93.44	93.18	93.70

[1] No Call defined as targeted chromosomal position where a variant cannot be determined (due to low coverage depth).
[2] Positive call defined as targeted chromosomal positions where a variant is detected.
[3] Two-sided 95% confidence intervals calculated using the Wilson Score method.

Genomic locations where a targeted variant is not detected are reported as negative (wild type). For expected negative locations, the data were evaluated for no call rate and percent negative call (PNC). *Within-Lab*

summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method.

Table 26   Within-Lab Precision Germline Call Observations for Expected Negative Results

Variant Type	No Calls Observed[1]	Total Calls	Percent No Calls	Negative Calls Observed[2]	Total Evaluable Calls	PNC	95% LCL[3]	95% UCL
Wild type	0	406170	0	406170	406170	100	>99.99	100

[1] No Call defined as targeted chromosomal position where a variant cannot be determined (due to low coverage depth).
[2] Negative call defined as targeted chromosomal positions where a variant is not detected.
[3] Two-sided 95% confidence intervals calculated using the Wilson Score method.

The contribution of each parameter (instrument, reagent lot, day, library replicate) to overall variability was determined by variance component analysis using variant frequency as the response variable. The overall standard deviation had a mean of 0.0370. The largest contributor to variant frequency variability was from library prep replicates, which contributed to 17.1% of the overall variability. Day contributed to 1%, while instrument and reagent lot each contributed to less than 1% of the total variability *Within-Lab Precision Variance Components Estimates for Germline Sample Variant Frequencies* on page 66 (SD = standard deviation).

Table 27   Within-Lab Precision Variance Components Estimates for Germline Sample Variant Frequencies

Component	Mean SD	Mean % of total SD
Day	0.0020	1.028
Instrument	0.0018	0.837
Consumable lot	0.0016	0.712
Library replicate	0.0143	17.110
Total	0.0370	100

Somatic

For somatic runs, genomic locations where a targeted somatic variant is detected are reported as positive (variant). For diluted samples GM12877-13 and GM12878-13 with expected positive somatic variants at VAFs between 6.5% and 13%, the data were evaluated for no call rate and percent positive call (PPC) within each variant type (SNV, insertion, deletion). *Within-Lab Precision Somatic Call Observations for Expected Positive Results by Variant Type (VAF is ≥ 6.5% and ≤ 13%)* on page 67 summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method, for each variant type.

Table 28 Within-Lab Precision Somatic Call Observations for Expected Positive Results by Variant Type (VAF is ≥ 6.5% and ≤ 13%)

Variant Type	No Calls Observed[1]	Total Calls	Percent No Calls	Positive Calls Observed[2]	Total Evaluable Calls	PPC	95% LCL[3]	95% UCL
SNV	0	96939	0	96069	96939	99.10	99.04	99.16
Insertion	0	3004	0	3004	3004	100	99.87	100
Deletion	0	2912	0	2907	2912	99.83	99.60	99.93

[1] No Call defined as targeted chromosomal position where a variant cannot be determined (due to low coverage depth).
[2] Positive call defined as targeted chromosomal positions where a variant is detected.
[3] Two-sided 95% confidence intervals calculated using the Wilson Score method.

Genomic locations where a targeted somatic variant is not detected are reported as negative (wild type). For expected negative locations, the data were evaluated for no call rate and percent negative calls. *Within-Lab Precision Somatic Call Observations for Expected Negative Results* on page 67 summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method, for each variant type.

Table 29 Within-Lab Precision Somatic Call Observations for Expected Negative Results

Variant Type	No Calls Observed[1]	Total Calls	Percent No Calls	Negative Calls Observed[2]	Total Evaluable Calls	PNC	95% LCL[3]	95% UCL
Wild type	0	194922	0	194919	194922	>99.99	>99.99	100

[1] No Call defined as targeted chromosomal position where a variant cannot be determined (due to low coverage depth).
[2] Negative call defined as targeted chromosomal positions where a variant is not detected.
[3] Two-sided 95% confidence intervals calculated using the Wilson Score method.

The contribution of each parameter (instrument, reagent lot, day, library replicate) to overall variability was determined by variance component analysis using variant frequency as the response variable. The overall standard deviation had a mean of 0.0062. Library prep replicates remained the most significant source of variability, accounting for 50.7% of the total. Day, instrument, and consumable lot all contributed to less than 1% of the total variability *Within-Lab Precision Variance Components Estimates for Somatic Sample Variant Frequencies* on page 67 (SD = standard deviation).

Table 30 Within-Lab Precision Variance Components Estimates for Somatic Sample Variant Frequencies

Component	Mean SD	Mean % of total SD
Day	0.0002	0.41
Instrument	0.0002	0.40

Component	Mean SD	Mean % of total SD
Consumable lot	0.0002	0.35
Library replicate	0.0044	50.7
Total	0.0062	100

Method Comparison

A study was performed to compare performance between the NovaSeq 6000Dx and NextSeq 550Dx Instruments. Variant frequency agreement for blood samples were evaluated using a representative assay designed to query a variety of genes covering 1,970,505 bases across all 23 human chromosomes. Eight Platinum Genome DNA samples were tested, seven in replicates of six and one (NA12881) in replicates of five. Libraries were sequenced on the NovaSeq 6000Dx Instrument using the Germline FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application, and on the NextSeq 550Dx Instrument using the DNA Generate FASTQ Dx Local Run Manager module. *Variant Frequency Correlation Plots (Points are colored by unique variant. Variants may be colored differently in each individual plot.)* on page 69 plots the VAF correlation between the two instruments for each sample. Based on the strong correlation between NovaSeq 6000Dx Instrument and NextSeq 550Dx Instrument, performance characteristics related to pre-analytical factors (eg, extraction methods or interfering substances) are determined to be applicable to both instruments. See the Illumina DNA Prep with Enrichment Dx package insert for additional details.

Figure 15   Variant Frequency Correlation Plots (Points are colored by unique variant. Variants may be colored differently in each individual plot.)
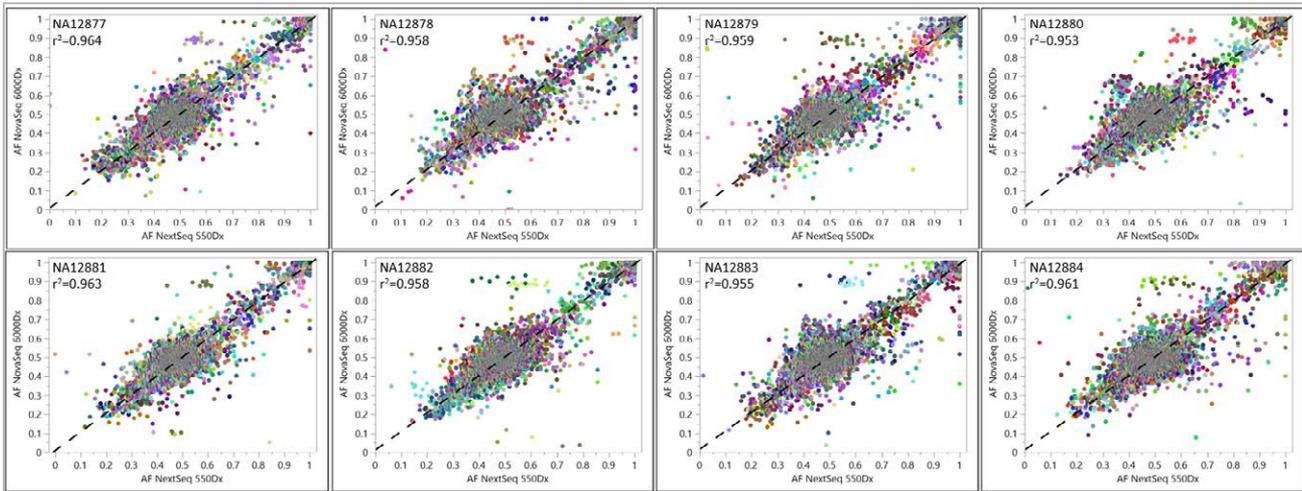
Reproducibility

The reproducibility of the NovaSeq 6000Dx Instrument was evaluated using Platinum Genome samples with a representative assay designed to query a variety of genes covering 1,970,505 bases across 23 different chromosomes using 9,232 target oligos. A total of 1723 targeted small variants (SNVs, insertions and deletions) were evaluated. Germline testing consisted of three or four replicates of twelve unique Platinum samples. Somatic testing consisted of five or six replicates of eight unique FFPE-treated Platinum Genome samples at different VAF levels. Sample libraries were prepared using Illumina DNA Prep with Enrichment Dx Kit reagents.

Testing was performed at three external sites using one lot each of NovaSeq 6000Dx S2 Reagent v1.5 Kit (300 cycles) and NovaSeq 6000Dx S4 Reagent v1.5 Kit (300 cycles). A single NovaSeq 6000Dx Instrument was used at each site. Two operators conducted the testing at each site. Each operator performed testing on three nonconsecutive start days for each sample type for a total of 36 flow cells across the three sites. For each start day, germline sample libraries were sequenced on instrument side A using S2 reagents and the Germline FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application, and somatic sample libraries were sequenced on instrument side B using S4 reagents and the Somatic FASTQ and VCF generation analysis workflow of the DRAGEN for Illumina DNA Prep with Enrichment Dx Application. This testing resulted in 18 flow cells for each the germline and somatic workflows.

Germline

For germline runs, genomic locations where a targeted germline variant is detected are reported as positive (variant). For expected positive germline variants, the data were evaluated for no call rate and percent positive call (PPC) within each variant type (SNV, insertion, deletion). *Germline Call Observations for Expected Positive Results by Variant Type* on page 70 summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method, for each variant type.

Table 31 Germline Call Observations for Expected Positive Results by Variant Type

Variant Type	No Calls Observed[1]	Total Calls	Percent No Calls	Positive Calls Observed[2]	Total Evaluable Calls	PPC	95% LCL[3]	95% UCL
SNV	0	991026	0	990276	991026	99.92	99.92	99.93
Insertion	0	38358	0	38358	38358	100	99.99	100
Deletion	0	34758	0	32228	34758	92.72	92.44	92.99

[1] No Call defined as targeted chromosomal position where a variant cannot be determined (due to low coverage depth).
[2] Positive call defined as targeted chromosomal positions where a variant is detected.
[3] Two-sided 95% confidence intervals calculated using the Wilson Score method.

Genomic locations where a targeted variant is not detected are reported as negative (wild type). For expected negative locations, the data were evaluated for no call rate and percent negative call (PNC). *Germline Call Observations for Expected Negative Results* summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method.

Table 32 Germline Call Observations for Expected Negative Results

Variant Type	No Calls Observed[1]	Total Calls	Percent No Calls	Negative Calls Observed[2]	Total Evaluable Calls	PNC	95% LCL[3]	95% UCL
Wild type	0	393516	0	393516	393516	100	>99.99	100

[1] No Call defined as targeted chromosomal position where a variant cannot be determined (due to low coverage depth).
[2] Negative call defined as targeted chromosomal positions where a variant is not detected.
[3] Two-sided 95% confidence intervals calculated using the Wilson Score method.

Somatic

For somatic runs, genomic locations where a targeted somatic variant is detected are reported as positive (variant). For expected positive somatic variants where the mean variant allele frequency (VAF) is greater than or equal to 14% and less than or equal to 28%, the data were evaluated for no call rate and percent positive call (PPC) within each variant type (SNV, insertion, deletion). *Somatic Call Observations for Expected Positive Results by Variant Type (Mean VAF ≥ 14% and ≤ 28%)* summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method, for each variant type.

Table 33 Somatic Call Observations for Expected Positive Results by Variant Type (Mean VAF ≥ 14% and ≤ 28%)

Variant Type	No Calls Observed[1]	Total Calls	Percent No Calls	Positive Calls Observed[2]	Total Evaluable Calls	PPC	95% LCL[3]	95% UCL
SNV	0	71028	0	70314	71028	98.99	98.92	99.07
Insertion	0	1962	0	1962	1962	100	99.80	100
Deletion	0	2142	0	2098	2142	97.95	97.25	98.47

[1] No Call defined as targeted chromosomal position where a variant cannot be determined (due to low coverage depth).
[2] Positive call defined as targeted chromosomal positions where a variant is detected.
[3] Two-sided 95% confidence intervals calculated using the Wilson Score method.

Genomic locations where a targeted somatic variant is not detected are reported as negative (wild type). For expected negative locations, the data were evaluated for no call rate and percent negative calls. *Somatic Call Observations for Expected Negative Results* on page 71 summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method, for each variant type.

Table 34 Somatic Call Observations for Expected Negative Results

Variant Type	No Calls Observed[1]	Total Calls	Percent No Calls	Negative Calls Observed[2]	Total Evaluable Calls	PNC	95% LCL[3]	95% UCL
Wild type	0	92718	0	92714	92718	>99.99	99.99	100

[1] No Call defined as targeted chromosomal position where a variant cannot be determined (due to low coverage depth).
[2] Negative call defined as targeted chromosomal positions where a variant is not detected.
[3] Two-sided 95% confidence intervals calculated using the Wilson Score method.

Revision History

Document	Date	Description of Change
Document # 200025276 v01	September 2022	Updated precision data for germline call observations.
Document # 200025276 v00	August 2022	Initial release.

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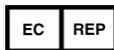
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