

NextSeq™ 550Dx Instrument

FOR IN VITRO DIAGNOSTIC USE

FOR EXPORT ONLY

Catalog # 20005715

Intended Use

The NextSeq 550Dx instrument is intended for sequencing of DNA libraries when used with *in vitro* diagnostic assays. The NextSeq 550Dx instrument is to be used with specific registered, certified, or approved *in vitro* diagnostic reagents and analytical software.

Principles of Procedure

The Illumina NextSeq 550Dx instrument is intended for sequencing DNA libraries with *in vitro* diagnostic assays. For its input, the NextSeq 550Dx 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 instrument to process base calls. The NextSeq 550Dx uses different secondary analysis modules depending on the workflow. For the Germline or Somatic Variant Modules, 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 variants found at specific positions in a reference genome.

Dual Boot Configuration

The NextSeq 550Dx includes a dual boot configuration to enable the use of the instrument in either diagnostic (Dx) or research use only (RUO) mode. *In vitro* diagnostic sequencing assays, including the Germline and Somatic Variant Modules, are executed in diagnostic mode. Only IVD sequencing reagents can be used in diagnostic mode. Performance characteristics and limitations of procedure for the NextSeq 550Dx instrument have been established using the Germline and Somatic Variant Modules in diagnostic mode.

Limitations of the Procedure

- 1 For *in vitro* diagnostic use.
- 2 The Germline and Somatic Variant Modules, when used with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) or NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles), are capable of delivering:
 - ▶ Sequencing output ≥ 90 gigabases (Gb)
 - ▶ Read length (in paired end run) 2 x 150 base pairs (bp)
 - ▶ Bases equal to or greater than Q30 $\geq 75\%$ at read length of 2 x 150 bp
Equal or greater than 75% of bases have Phred scale quality scores ≥ 30 , indicating base call accuracy greater than 99.9%
- 3 Reads with indels (insertions, deletions, or combinations) where content length is > 25 bp are not aligned by the assay software. Consequently, indels of length > 25 bp are not detectable by the assay software.

- 4 The assay software might not align amplicon reads with extreme variant content, resulting in the region being reported as wild-type. Such extreme content includes:
 - ▶ Reads containing more than three indels
 - ▶ Reads of length at least 30 bp with single nucleotide variant (SNV) content > 4% of the total amplicon target length (excluding probe regions)
 - ▶ Reads of length < 30 bp with SNV content > 10% of the total amplicon length (including probe regions)
- 5 Large variants, including multinucleotide variants (MNVs) and large indels, might be reported as separate smaller variants in the output VCF file.
- 6 Deletion variants can be filtered or missed when spanning two tiled amplicons if the deletion length is greater than or equal to the overlap between the tiled amplicons.
- 7 The system cannot detect indels if they occur directly adjacent to a primer and there is no overlapping amplicon. For regions with overlapping amplicons, the assay cannot detect deletions when the region of overlap is smaller than the size of deletion to be detected. For example, if the region of overlap between two adjacent amplicons is two bases, the assay cannot detect any deletions including both of those bases. A single base deletion at either of those bases can be detected.
- 8 As with any hybridization-based library preparation workflow, underlying polymorphisms, mutations, insertions, or deletions in oligonucleotide-binding regions can affect the alleles being probed and the calls made during sequencing. For example:
 - ▶ A variant in phase with a variant in the primer region might not be amplified, resulting in a false negative.
 - ▶ Variants in the primer region could prevent the amplification of the reference allele, resulting in an incorrect homozygous variant call.
 - ▶ Indel variants in the primer region can cause a false positive call at the end of the read adjacent to the primer.
- 9 Indels can be filtered due to strand bias if they occur near the end of one read and are soft-clipped during alignment.
- 10 Small MNVs have not been validated and are only reported in the Somatic Variant Module.
- 11 Deletions are reported in the VCF 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.
- 12 Germline-specific limitations:
 - ▶ The NextSeq 550Dx instrument, using the Local Run Manager Germline Variant Module for NextSeq 550Dx, is designed to deliver qualitative results for germline variant calling (eg, homozygous, heterozygous, wild-type).
 - ▶ When used with the Germline Variant Module, the minimum coverage per amplicon needed for accurate variant calling is 150x. As a result, 150 supporting DNA fragments are required, which is equivalent to 300 overlapping paired-end reads. The number of samples and the total number of bases targeted affect coverage. GC-content and other genomic content can affect coverage.
 - ▶ Copy number variation can affect whether a variant is identified as homozygous or heterozygous.
 - ▶ Variants in certain repetitive context are filtered out in the VCF files. The RMxN repeat filter is used to filter variants if all or part of the variant sequence is present repeatedly in the reference genome adjacent to the variant position. For germline variant calling, at least nine repeats in the reference are required for a variant to be filtered. Only repeats with a length up to 5 bp are considered (R5x9).
 - ▶ An indel and an SNV at a single locus can result in only one variant being reported.
- 13 Somatic-specific limitations:
 - ▶ The NextSeq 550Dx instrument, using the Local Run Manager Somatic Variant Module for NextSeq 550Dx, is designed to deliver qualitative results for somatic variant calling (eg presence of a somatic variant with a variant frequency greater than or equal to 0.026 with a limit of detection of 0.05).
 - ▶ When used with the Somatic Variant Module, the minimum coverage per amplicon needed for accurate variant calling is 450x per oligonucleotide pool. As a result, 450 supporting DNA fragments are required

per oligonucleotide pool, which is equivalent to 900 overlapping paired-end reads. The number of samples and the total number of bases targeted affect coverage. GC-content and other genomic content can affect coverage.

- ▶ For somatic variant calling, at least six repeats in the reference are required for the variant to be filtered, and only repeats with length up to 3 bp are considered (R3x6).
- ▶ The Somatic Variant Module cannot differentiate between germline and somatic variants. The module 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.

Product Components

- 1 NextSeq 550Dx instrument (Catalog # 20005715)
- 2 Software components for the NextSeq 550Dx instrument, including the following:

| Software Application | Function | Description |
|--|--------------------------------|---|
| NextSeq 550Dx Operating Software (NOS) | Controls instrument operation | The NOS software application manages the operation of the instrument during sequencing and generates images for use by Real-Time Analysis (RTA) software. |
| Real-time Analysis Software (RTA) | Performs primary analysis | The RTA software application converts the images generated by NOS for each tile per cycle of the sequencing run into base call files, which are inputs for Local Run Manager analysis modules. The RTA software application does not contain a user interface. |
| Local Run Manager | Interface for module selection | The Local Run Manager software is an on-instrument integrated solution for user management, selecting the appropriate analysis module, and monitoring status. |
| Somatic Variant Module | Performs secondary analysis | This Local Run Manager analysis module software processes base calls through secondary analysis. The processing includes demultiplexing, FASTQ file generation, alignment, variant calling, and reporting. The variant caller (Pisces) generates VCF files that contain information about variants found at specific positions in a reference genome and includes the measured variant frequency. |
| Germline Variant Module | Performs secondary analysis | This Local Run Manager analysis module software processes base calls through secondary analysis. The processing includes demultiplexing, FASTQ file generation, alignment, variant calling, and reporting. The variant caller (Pisces) generates VCF files that contain information about variants found at specific positions in a reference genome and identifies each variant as heterozygous or homozygous. |

Operating Conditions

| Element | Specification |
|-------------|---|
| Temperature | Maintain a lab temperature of 19°C to 25°C (22°C ±3°C). This temperature is the operating temperature 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%. |

Equipment and Materials

Equipment and Materials Required, Sold Separately

NextSeq 550Dx High Output Reagent Kit v2 (300 cycles), Catalog # 20019554

NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles), Catalog # 20028871

Equipment and Materials Required, Not Provided

User-Supplied Consumables for Sequencing Runs

| Consumable | Supplier | Purpose |
|--|---|--|
| Alcohol wipes, 70% Isopropyl or Ethanol, 70% | VWR, catalog # 95041-714 (or equivalent) General lab supplier | Flow cell cleaning and general purpose |
| Lab tissue, low-lint | VWR, catalog # 21905-026 (or equivalent) | Flow cell cleaning and general purpose |

User-Supplied Consumables for Instrument Maintenance

| Consumable | Supplier | Purpose |
|------------------------------------|---|---|
| NaOCl, 5% (sodium hypochlorite) | Sigma-Aldrich, catalog # 239305 (or laboratory-grade equivalent) | Washing the instrument using the manual post-run wash; diluted to 0.12% |
| Tween 20 | Sigma-Aldrich, catalog # P7949 | Washing the instrument using manual wash options; diluted to 0.05% |
| Water, laboratory-grade | General lab supplier | Washing the instrument (manual wash) |
| Air filter | Illumina, catalog # 20022240 | Cleaning the air the instrument takes in for cooling |

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

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 he/she practices, to use or order the use of the device.

- 1 **Some components of reagents provided by Illumina for use with the NextSeq 550Dx 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, see the Safety Data Sheets (SDS) at support.illumina.com/sds.html.

- 2 Handle all blood specimens as if they are known to be infectious for human immunodeficiency virus (HIV), human hepatitis B virus (HBV), and other bloodborne pathogen agents (universal precautions).
- 3 Failure to follow the procedures as outlined can result in erroneous results or significant reduction in sample quality.
- 4 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.
- 5 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.
- 6 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).
- 7 Index to sample pairing must match the printed plate layout exactly. Local Run Manager automatically populates the index primers associated with the sample names, when entered in the module. 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.
- 8 Installation of user-supplied antivirus software is strongly recommended to protect the computer against viruses. Consult user manual for instructions on installation.
- 9 Do not operate the NextSeq 550Dx with any of the panels removed. Operating the instrument with any of the panels removed creates potential exposure to line voltage and DC voltages.
- 10 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.
- 11 The instrument weighs approximately 185 lbs. and could cause serious injury if dropped or mishandled.

Instructions for Use

The following instructions for use are for running the Germline and Somatic Variant Modules in diagnostic mode on the NextSeq 550Dx instrument using the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) or NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles).

Enter Run Information

For detailed instructions, see the NextSeq 550Dx Instrument Reference Guide (document # 1000000009513) and the applicable Local Run Manager module guide.

Set Parameters

- 1 Log in to Local Run Manager.
- 2 Select **Create Run**, and select **Somatic Variant** or **Germline Variant**.
- 3 Enter a run name that identifies the run from sequencing through analysis.
Use alphanumeric characters, spaces, underscores, or dashes.
- 4 **[Optional]** Enter a run description to help identify the run.
Use alphanumeric characters, spaces, underscores, or dashes.
- 5 Select the number of samples and index set from the drop-down list.
Consider the following information when you make a selection.
 - ▶ The drop-down list contains numbers of samples with an index set. For example, 24-Set 1 indicates 24 samples to be tested, with indexes from index set 1.
 - ▶ Index set numbers refer to different sets of i5 and i7 index pairs. Set 1 and Set 2 both provide index diversity. Two index sets are offered to help prevent depletion of a single set.
 - ▶ Choose the number of samples that is nearest to the number of samples you are testing. If the exact number of samples is not in the list, select the number nearest, but less than the number you are testing. For example, if you want to test 18 samples, select 16 samples.

- ▶ Suggested sample wells and index combinations that meet index diversity requirements are highlighted in green.

Import Manifest Files for the Run

- 1 Make sure that the manifests you want to import are available in an accessible network location or on a USB drive.
- 2 Select **Import Manifests**.
- 3 Navigate to the manifest file and select the manifests that you want to add.



NOTE


To make manifest files available for all runs using the Germline Variant or Somatic Variant analysis module, add manifests using the Module Settings feature. This feature requires admin user level permissions. For more information, see the *NextSeq 550Dx Instrument Reference Guide (document # 1000000009513)*.

Specify Samples for the Run


Specify samples for the run using one of the options and the directions that follow.

- ▶ **Enter samples manually**—Use the blank table on the Create Run screen.
- ▶ **Import samples**—Navigate to an external file in a comma-separated values (*.csv) format. A template is available for download on the Create Run screen.

Enter Samples Manually

- 1 Enter a unique sample name (**Somatic Variant analysis module**) or sample ID (**Germline Variant analysis module**).
Use alphanumeric characters, dashes, or underscores.
- 2 **[Optional]** For positive or negative control samples, right-click and select the control type.
The control in one sample well auto populates the corresponding well in the other pool with the same control.
- 3 **[Optional]** Enter a sample description in the Sample Description field.
Use alphanumeric characters, dashes, or underscores.
- 4 Select an Index 1 adapter from the Index 1 (i7) drop-down list.
When you use suggested sample wells, the software auto-populates i7 and i5 index adapters that meet diversity index requirements. If the exact number of samples you are testing is not in the list, make sure to select index adapters for extra wells.
- 5 Select an Index 2 adapter from the Index 2 (i5) drop-down list.
- 6 Select a manifest file from the Manifest drop-down list.
Samples in Pool A require a different manifest than samples in Pool B.
- 7 Choose an option to view, print, or save the plate layout as a reference for preparing libraries:
 - Select the  **Print** icon to display the plate layout. Select **Print** to print the plate layout.
 - Select **Export** to export sample information to an external file.
- 8 Select **Save Run**.

Import Samples

- 1 Select **Import Samples** and browse to the location of the sample information file. There are two types of files you can import.
 - Select **Template** on the Create Run screen to make a new plate layout. The template file contains the correct column headings for import. Enter sample information in each column for the samples in the run. Delete example information in unused cells, and then save the file.
 - Use a file of sample information that was exported from the Germline Variant or Somatic Variant module using the Export feature.
- 2 Select the  **Print** icon to display the plate layout.
- 3 Select **Print** to print the plate layout as a reference for preparing libraries.
- 4 Select **Save Run**.

Prepare the Reagent Cartridge

Make sure to follow reagent cartridge directions carefully for successful sequencing.

- 1 Remove the reagent cartridge from -25°C to -15°C storage.
- 2 Choose one of the following methods to thaw the reagents. Do not submerge the cartridge. After the cartridge is thawed, dry it before you proceed to the next step.

| Temperature | Time to Thaw | Stability Limit |
|---|--------------|-----------------------|
| 15°C to 30°C water bath | 60 minutes | Not to exceed 6 hours |
| 2°C to 8°C | 7 hours | Not to exceed 5 days |



NOTE

If more than one cartridge is thawing in the same water bath, allow for additional thawing time.

- 3 Invert the cartridge five times to mix reagents.
- 4 Inspect the bottom of the cartridge to make sure that reagents are thawed and free of precipitates. Confirm that positions 29, 30, 31, and 32 are thawed, as they are the largest and take the longest to thaw.
- 5 Gently tap on the bench to reduce air bubbles.
For best results, proceed directly to loading the sample and setting up the run.

Prepare the Flow Cell

- 1 Remove a new flow cell box from 2°C to 8°C storage.
- 2 Remove the foil package from the box and set aside at room temperature for 30 minutes.

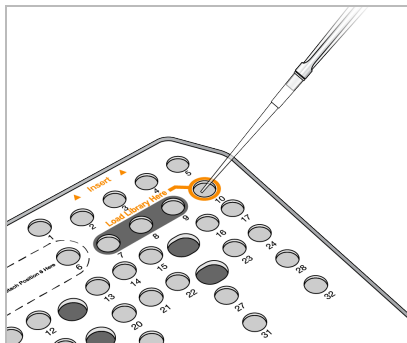
Prepare Libraries for Sequencing

Denature and dilute your libraries to a loading volume of 1.3 ml. In practice, loading concentration can vary depending on library preparation and quantification methods. Dilution of sample libraries depends on the complexity of oligonucleotide pools. For directions on how to prepare sample libraries for sequencing, including library dilution and pooling, see the Instructions for Use section for the applicable library preparation kit. Optimization of cluster density on the NextSeq 550Dx is required.

Load Libraries onto the Reagent Cartridge

- 1 Clean the foil seal covering reservoir #10 labeled **Load Library Here** using a low-lint tissue.
- 2 Pierce the seal with a clean 1 ml pipette tip.
- 3 Load 1.3 ml of prepared libraries into reservoir #10 labeled **Load Library Here**. Avoid touching the foil seal as you dispense the libraries.

Figure 1 Load Libraries



Set Up a Sequencing Run

- 1 Log in to the NextSeq 550Dx with your Local Run Manager software password.

- 2 From the Home screen of the NOS software, select **Sequence**.
- 3 Select a run from the list, and then select **Next**.
A series of run setup screens open in the following order: Load Flow Cell, Load Buffer Cartridge, Load Reagent Cartridge, and Pre-run Check.
- 4 When the Load Flow Cell screen appears, clean and then load the flow cell.
 - ▶ Remove the flow cell from the foil package.
 - ▶ Open the clear plastic clamshell package and remove the flow cell
 - ▶ Clean the glass surface of the flow cell with a lint-free alcohol wipe. Dry the glass with a low-lint lab tissue
 - ▶ Make sure that the glass surface of the flow cell is clean. If necessary, repeat the cleaning step.
 - ▶ Remove the used flow cell from a previous run.
 - ▶ Align the flow cell over the alignment pins and place the flow cell on the stage.
- 5 Select **Load**.
The door closes automatically, the flow cell ID appears on the screen, and the sensors are checked.
- 6 Follow the software prompts to empty the spent reagents container, load the NextSeq 550Dx buffer cartridge, and load the NextSeq 550Dx reagent cartridge.
When the NextSeq 550Dx buffer and reagent cartridges are loaded, the software reads and records the RFID. The buffer and reagent cartridge IDs appear on the screen and the sensors are checked.
- 7 When the automated pre-run check is complete, select **Start**. (Not required if configured to start automatically.)
- 8 The Sequencing screen opens when the run begins. This screen provides a visual representation of the run in progress, including intensities and quality scores (Q-scores).

Results

Real-Time Analysis (RTA) is an integrated software that performs image analysis and base calling and assigns a quality score to each base for each sequencing cycle. When primary analysis finishes, the selected Local Run Manager module on the NextSeq 550Dx instrument begins secondary analysis automatically. The secondary analysis processes described here are for the Germline and Somatic Variant Modules.

Demultiplexing

Demultiplexing compares each Index Read sequence to the index sequences specified for the run. No quality values are considered in this step.

Index reads are identified using the following steps:

- ▶ Samples are numbered starting from 1 based on the order they are listed for the run.
- ▶ Sample number 0 is reserved for clusters that were not assigned to a sample.
- ▶ Clusters are assigned to a sample when the index sequence matches exactly or when there is up to a single mismatch per Index Read.

FASTQ File Generation

After demultiplexing, the software generates intermediate analysis files in the FASTQ format, which is a text format used to represent sequences. FASTQ files contain reads for each sample and the associated quality scores. Clusters that did not pass filter are excluded.

Each FASTQ file contains reads for only one sample, and the name of that sample is included in the FASTQ file name. In the Germline and Somatic Variant Modules, eight FASTQ files are generated per sample per oligo pool, four from Read 1 and four from Read 2. This output results in a total of 8 and 16 FASTQ files per sample for Germline and Somatic, respectively. FASTQ files are the primary input for alignment.

Alignment

During the alignment step, the banded Smith-Waterman algorithm aligns clusters from each sample against amplicon sequences specified in the manifest file.

The banded Smith-Waterman algorithm performs semiglobal sequence alignments to determine similar regions between two sequences. Instead of comparing the total sequence, the Smith-Waterman algorithm compares segments of all possible lengths.

Each paired-end read is evaluated in terms of its alignment to the relevant probe sequences for that read.

- ▶ Read 1 is evaluated against the reverse complement of the Downstream Locus-Specific Oligos (DLSO).
- ▶ Read 2 is evaluated against the Upstream Locus-Specific Oligos (ULSO).
- ▶ If the start of a read matches a probe sequence with no more than one mismatch, the full length of the read is aligned against the amplicon target for that sequence.
- ▶ If the start of a read matches a probe sequence with no more than three differences (mismatches or shifts due to leading indels), the full length of the read is aligned against the amplicon target for that sequence.
- ▶ Indels within the DLSO and ULSO are not observed given the assay chemistry.

Alignments are filtered from alignment results based on mismatch rates over either the region of interest or the full amplicon, depending on the amplicon length. Filtered alignments are written in alignment files as unaligned and are not used in variant calling.

Variant Calling

The variant caller Pisces is designed to make SNV and indel variant calls from libraries prepared for the instrument.

Reports and Additional Output Files

The variant analysis modules produce PDF and tab-delimited (*.txt) reports that display metrics such as sequencing depth and variant counts. The modules also produce output files such as VCF and genome Variant Call Format (gVCF) files for variant-calling applications.

Quality Control Procedures

The NextSeq 550Dx software evaluates each run, sample, and base call against quality control metrics. Positive and negative controls are also recommended in library preparation and need to 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, then 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, then a possible error in sample tracking or incorrect recording of indexing primers has occurred.

Performance Characteristics

Performance characteristics for the NextSeq 550Dx instrument were established using the Germline and Somatic Variant Modules with the TruSeq Custom Amplicon Kit Dx and NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) and confirmed using the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles). Studies included Sample Indexing, Sample Carryover, DNA Input, Analytical Sensitivity (Limit of Blank / Limit of Detection), Accuracy, Precision, Method Comparison, and Reproducibility.

The analytical studies using the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) were designed to evaluate performance claims previously established with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles). Results demonstrate that reagent kits (v2 and v2.5) have comparable performance using the TruSeq Custom Amplicon Kit Dx. See the *TruSeq Custom Amplicon Kit 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 the assay correctly reports.
 - ▶ $(\# \text{ variant loci correctly reported by the assay}) / (\text{total \# of variant loci})$
 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 the assay correctly reports.
 - ▶ $(\# \text{ wild-type loci correctly reported by the assay}) / (\text{total \# of wild-type loci})$
 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.
 - ▶ $((\# \text{ variant loci correctly reported by the assay}) + (\# \text{ wild-type loci correctly reported by the assay})) / ((\text{total \# of variant loci}) + (\text{total \# of wild-type loci}))$
- 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 Autosomal call rate is calculated as total number of loci passing filters divided by the total number of positions sequenced for chromosomes 1-22; chromosomes X and Y are excluded. This metric does not consider the agreement of the calls with the reference method.

NextSeq 550Dx High Output Reagent Kit v2 (300 Cycles) Performance

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 (8) and maximum (96) number of samples that can be processed in a single sequencing run by the NextSeq 550Dx instrument. Eight unique Platinum Genome samples were tested with 12 different indexing primer combinations per sample. Sample results from four sequencing runs using the Germline Variant Module were compared to Platinum Genomes version 2016-1.0.

For the first set of runs, 96 uniquely indexed sample libraries were tested with a representative assay designed to query a variety of genes covering 12,588 bases per strand across all 23 human chromosomes to verify the ability of the assay to make a genotyping call consistently for a given sample across different indexing primer combinations. For the second set of runs, eight uniquely indexed sample libraries were sequenced in two sequencing runs to verify the minimum number of indexes supported.

For the 96-index runs, PPA for SNVs ranged from 98.7% to 100%, PPA for insertions and deletions was 100% and NPA was 100% for each of the 96 index combinations. The 8-index runs had PPA values of 100% (SNVs, insertions, and deletions) and NPA of 100% for each of the eight index combinations.

Sample Carryover

The NextSeq 550Dx 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). Two Platinum Genome samples, one male and one female, were tested with a representative assay designed to query various genes covering 12,588 bases (150 amplicons) across 23 different chromosomes, including both sex chromosomes. Libraries were

sequenced on the NextSeq 550Dx instrument using the Germline Variant Module. Carryover of male samples into female samples was observed by the presence of Y chromosome amplicon reads in female samples.

Within-run carryover can 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 46 replicates each of male and female samples plus four no template controls was sequenced one time on the NextSeq 550Dx instrument. Within-run sample carryover was assessed by comparing Y chromosome amplicon coverage of each female replicate to the average Y chromosome amplicon coverage of all male replicates in the pool. The median observed within-run carryover was 0.084%.

For testing of run-to-run sample carryover, two library pools were prepared and sequenced consecutively on one NextSeq 550Dx instrument. The first pool contained 46 replicates of female sample plus two no template controls. The second pool contained 46 replicates of male sample plus two no template controls. 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 by comparing Y chromosome amplicon coverage between corresponding replicates of the female pool repeat run and male pool run. The median observed run-to-run carryover was 0.0076%.

DNA Input

Blood (Germline)

The blood DNA input range for the TruSeq Custom Amplicon Kit Dx library preparation using the Germline Variant Module workflow was established for the NextSeq 550Dx instrument. This range was evaluated by performing a serial dilution study using 13 Platinum Genome samples with a representative assay designed to query various genes covering 12,588 bases across 23 different chromosomes. The library was sequenced on two NextSeq 550Dx instruments using one lot of NextSeq 550Dx High Output Reagent Kit v2 (300 cycles).

Five samples were tested in duplicate at five DNA input levels ranging from 250 ng to 12 ng (250 ng, 100 ng, 50 ng, 25 ng, and 12 ng). Eight samples were tested in as a single replicate at each of the five DNA 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 [Table 1](#); NPA is presented in [Table 2](#). All input levels had similar accuracy. The recommended DNA input for TruSeq Custom Amplicon Kit Dx is 50 ng with 25 ng and 100 ng providing a lower and upper limit to meet performance characteristics.

Table 1 PPA Results for Each DNA Input by Variant Type

| DNA Input (ng) | Variant Type | Expected Variants | TP | FN | Variant No Calls | PPA (%) |
|----------------|--------------|-------------------|------|----|------------------|---------|
| 12 | SNV | 2412 | 2381 | 31 | 0 | 98.7 |
| 25 | | | 2404 | 8 | 0 | 99.7 |
| 50 | | | 2403 | 9 | 0 | 99.6 |
| 100 | | | 2412 | 0 | 0 | 100 |
| 250 | | | 2412 | 0 | 0 | 100 |
| 12 | Insertion | 808 | 784 | 3 | 21 | 99.6 |
| 25 | | | 781 | 5 | 22 | 99.4 |
| 50 | | | 786 | 2 | 20 | 99.8 |
| 100 | | | 786 | 0 | 22 | 100 |
| 250 | | | 786 | 0 | 22 | 100 |

| | | | | | | |
|-----|----------|-----|-----|----|----|------|
| 12 | Deletion | 758 | 732 | 12 | 14 | 98.4 |
| 25 | | | 737 | 7 | 14 | 99.1 |
| 50 | | | 742 | 2 | 14 | 99.7 |
| 100 | | | 744 | 0 | 14 | 100 |
| 250 | | | 744 | 0 | 14 | 100 |

Table 2 NPA for Each DNA Input

| DNA Input (ng) | TN | FP | Reference No Calls | NPA (%) |
|----------------|--------|----|--------------------|---------|
| 12 | 430940 | 4 | 26 | >99.9 |
| 25 | 430936 | 0 | 34 | 100 |
| 50 | 430936 | 2 | 32 | >99.9 |
| 100 | 430942 | 0 | 28 | 100 |
| 250 | 430942 | 0 | 28 | 100 |

FFPE (Somatic)

The formalin-fixed paraffin-embedded (FFPE) DNA input range for TruSeq Custom Amplicon Kit Dx library preparation using the Somatic Variant Module workflow was established for the NextSeq 550Dx instrument. The DNA input range was evaluated by performing a serial dilution study using three Platinum Genome samples with a representative assay designed to query various genes covering 12,588 bases across 23 different chromosomes. Platinum Genome cell lines GM12878 and GM12877 were formalin fixed and embedded in paraffin followed by DNA extraction. GM12878 was diluted with GM12877 such that the variant allele frequencies (VAFs) of 81 variants (55 SNVs, 10 insertions, and 16 deletions) were near 0.025, 0.05, or 0.10. In addition, each sample had 91 variants with higher variant frequencies of up to 1.0 VAF. Samples were processed in duplicate at five DNA input levels with mean delta quantitative cycle (dCq) of 2.1, 3.6, 4.6, 6.0, and 7.8 as measured by the TruSeq Custom Amplicon Dx - FFPE QC Kit. Each library was sequenced on two NextSeq 550Dx instruments using two lots of NextSeq 550Dx High Output Reagent Kit v2 (300 cycles). For determination of accuracy, sample variant calls were compared to Platinum Genomes version 2016-1.0. PPA for each variant type (SNVs, insertions, and deletions) is presented in Table 3; NPA is presented in Table 4. The recommended DNA input for variants at 0.05 VAF or above is dCq \leq 4 with 4.6 providing a lower limit to meet the performance characteristics.

Table 3 PPA Results for Each DNA Input by Variant Type

| Mean dCq | Variant Type | Expected Variants | Expected No Calls | Target Dilution VAF | | | | | |
|----------|--------------|-------------------|-------------------|---------------------|---------|------------------|---------|------------------|---------|
| | | | | 0.025 | | 0.05 | | 0.10 | |
| | | | | Variant No Calls | PPA (%) | Variant No Calls | PPA (%) | Variant No Calls | PPA (%) |
| 2.1 | SNV | 808 | Not applicable. | 196 | 100 | 0 | 100 | 0 | 100 |
| 3.6 | | | | 250 | 99.3 | 4 | 100 | 0 | 100 |
| 4.6 | | | | 251 | 94.6 | 51 | 99.2 | 5 | 100 |
| 6.0 | | | | 257 | 65.3 | 213 | 91.4 | 100 | 100 |
| 7.8 | | | | 254 | 69.3 | 185 | 90.7 | 100 | 100 |
| 2.1 | Insertion | 264 | 8 | 66 | 96.5 | 8 | 100 | 8 | 100 |
| 3.6 | | | | 62 | 97.0 | 8 | 100 | 8 | 100 |
| 4.6 | | | | 48 | 96.3 | 21 | 100 | 8 | 100 |
| 6.0 | | | | 40 | 80.4 | 47 | 98.2 | 24 | 95.8 |
| 7.8 | | | | 57 | 87.0 | 56 | 96.2 | 31 | 100 |

| | | | | | | | | | |
|-----|----------|-----|----|----|------|-----|------|----|------|
| 2.1 | Deletion | 304 | 16 | 58 | 100 | 16 | 100 | 16 | 100 |
| 3.6 | | | | 80 | 100 | 16 | 100 | 16 | 100 |
| 4.6 | | | | 65 | 95.4 | 28 | 100 | 16 | 100 |
| 6.0 | | | | 78 | 74.8 | 105 | 94.0 | 36 | 100 |
| 7.8 | | | | 76 | 75.0 | 79 | 95.1 | 57 | 98.8 |

Table 4 NPA for Each DNA Input

| Mean dCq | Expected Wild Type | Target Dilution VAF | | | | | |
|----------|--------------------|---------------------|---------|--------------------|---------|--------------------|---------|
| | | 0.025 | | 0.05 | | 0.10 | |
| | | Reference No Calls | NPA (%) | Reference No Calls | NPA (%) | Reference No Calls | NPA (%) |
| 2.1 | 93688 | 344 | 100 | 260 | 100 | 324 | 100 |
| 3.6 | | 400 | 100 | 332 | 100 | 380 | 100 |
| 4.6 | | 1308 | 100 | 1336 | 100 | 784 | 100 |
| 6.0 | | 3900 | >99.9 | 3296 | >99.9 | 2996 | 100 |
| 7.8 | | 3020 | >99.9 | 2880 | >99.9 | 2448 | >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 Variant Module on the NextSeq 550Dx instrument. This was performed using a representative assay designed to query various genes covering 12,588 bases across 23 different chromosomes. Platinum Genome cell lines GM12878 and GM12877 were formalin fixed and embedded in paraffin followed by DNA extraction. GM12878 was diluted with GM12877 such that the variant frequencies of 74 variants (53 SNVs, 7 insertions, and 14 deletions) were 0.05 ± 0.02 . GM12877 and diluted GM12878 (GM12878-D) were tested over six consecutive start days with a single instrument, alternating between two lots of NextSeq 550Dx High Output Reagent Kit v2 (300 cycles), for a total of six sequencing runs. This test resulted in 60 replicates for each variant in GM12878-D and 72 replicates for each corresponding wild-type coordinate in GM12877 for each reagent lot. LoB and LoD were calculated using the classical approach stated in CLSI EP17-A2 using the nonparametric option. LoB and LoD were calculated for SNVs, insertions, and deletions separately by pooling the variant frequencies for a given variant type. The Type I error was defined as 0.01, and the Type II error was defined as 0.05.

For the LoB, the pooled variant frequencies were sorted from lowest to highest, and the 99th rank position for each reagent lot for each variant type was calculated (Table 5). The Somatic Variant Module uses a cutoff (the effective LoB) of 0.026 VAF for determining the qualitative detection of variants. The calculated LoB verified that this cutoff results in a Type I error of no more than 0.01.

Table 5 Limit of Blank

| Variant Type | Total Observations | LoB Reagent Lot 1 (%) | LoB Reagent Lot 2 (%) |
|--------------|--------------------|-----------------------|-----------------------|
| SNV | 3816 | 0.77 | 0.77 |
| Insertion | 504 | 0.56 | 0.56 |
| Deletion | 1008 | 1.20 | 1.20 |

For the LoD, the percentage of individual mutation frequency for each reagent lot for each variant type falling below the cutoff of 0.026 was calculated Table 6. Because the percentages were less than the Type II error of 5% (0.05), the median of the combined variant frequencies was calculated as the LoD (Table 6). The LoD for each variant type was taken as the larger of the two values calculated for the two reagents lots - 4.97% for SNVs, 5.12% for insertions, and 5.26% for deletions.

Table 6 Limit of Detection

| Reagent Lot | Variant Type | Total Observations | # of VAF measurements < 2.6% | % of VAF measurements < 2.6% | Limit of Detection (%) |
|-------------|--------------|--------------------|------------------------------|------------------------------|------------------------|
| 1 | SNV | 3180 | 53 | 1.7 | 4.94 |
| | Insertion | 420 | 6 | 1.4 | 5.08 |
| | Deletion | 840 | 7 | 0.8 | 5.22 |
| 2 | SNV | 3180 | 51 | 1.6 | 4.97 |
| | Insertion | 420 | 5 | 1.2 | 5.12 |
| | Deletion | 840 | 7 | 0.80 | 5.26 |

Accuracy

Germline

The following study was conducted to assess the variant calling accuracy of the Germline Variant Module on the NextSeq 550Dx instrument using the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles). 13 unique Platinum Genome samples were tested using a representative assay designed to query a variety of genes covering 12,588 bases (150 amplicons) across 23 different chromosomes. A total of nine runs were performed using three sequencing instruments, three reagent lots, and three operators over five start days. Accuracy was determined for SNVs, insertions, and deletions by comparing the results to a well-characterized composite reference method, Platinum Genomes version 2016-1.0. Confident genomic regions were defined based on this reference method unless specified otherwise.

Table 7 Summary of Germline Agreement

| Criteria | Total Observations ¹ | Result by Observation ² | Result by Run ³ |
|--------------------|---------------------------------|------------------------------------|----------------------------|
| PPA for SNV | 819 | 98.7 | >99.9 |
| PPA for insertions | 819 | 95.0 | 98.9 |
| PPA for deletions | 819 | 100 | 100 |
| NPA | 819 | 100 | 100 |
| OPA | 819 | >99.9 | >99.9 |

¹Calculated as number of samples per run (91) x number of Runs (9) = 819.

²Lowest observed value by sample replicate across all 9 runs.

³Lowest value when data from each run are analyzed in aggregate.

Table 8 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 8 Germline Agreement per Sample

| Sample | Mean Call Rate | Expected Variants ¹ | TP | FN | Variant No Calls | TN | FP | PPA | NPA | OPA |
|---------|----------------|--------------------------------|------|----|------------------|--------|----|-------|-----|-------|
| NA12877 | >99.9 | 4788 | 4788 | 0 | 0 | 756762 | 0 | 100 | 100 | 100 |
| NA12878 | >99.9 | 8505 | 8379 | 1 | 125 | 751464 | 0 | >99.9 | 100 | >99.9 |
| NA12879 | >99.9 | 6048 | 5985 | 5 | 58 | 757701 | 0 | 99.9 | 100 | >99.9 |
| NA12880 | >99.9 | 6993 | 6930 | 0 | 63 | 757638 | 0 | 100 | 100 | 100 |

| Sample | Mean Call Rate | Expected Variants ¹ | TP | FN | Variant No Calls | TN | FP | PPA | NPA | OPA |
|---------|----------------|--------------------------------|------|----|------------------|--------|----|-------|-----|-------|
| NA12881 | >99.9 | 7875 | 7811 | 3 | 61 | 751653 | 0 | >99.9 | 100 | >99.9 |
| NA12882 | >99.9 | 6300 | 6174 | 3 | 123 | 754803 | 0 | >99.9 | 100 | >99.9 |
| NA12883 | >99.9 | 7119 | 7056 | 0 | 63 | 751905 | 0 | 100 | 100 | 100 |
| NA12884 | >99.9 | 7182 | 7119 | 6 | 57 | 754146 | 0 | 99.9 | 100 | >99.9 |
| NA12885 | >99.9 | 7686 | 7560 | 2 | 124 | 754173 | 0 | >99.9 | 100 | >99.9 |
| NA12886 | >99.9 | 7245 | 7182 | 7 | 56 | 752469 | 0 | 99.9 | 100 | >99.9 |
| NA12887 | >99.9 | 7119 | 7119 | 0 | 0 | 750645 | 0 | 100 | 100 | 100 |
| NA12888 | >99.9 | 6804 | 6804 | 0 | 0 | 756065 | 0 | 100 | 100 | 100 |
| NA12893 | >99.9 | 7434 | 7371 | 1 | 62 | 750015 | 0 | >99.9 | 100 | >99.9 |

¹ Total number of variants in all sample replicates across 9 runs.

Table 9 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 9 Germline Agreement per Sample by Variant Type

| >Sample | SNVs | | | Insertions | | | Deletions | | |
|---------|-----------|------|-----|------------|------|-----|-----------|------|----|
| | >Expected | >TP | >FN | >Expected | >TP | >FN | Expected | TP | FN |
| NA12877 | 2331 | 2331 | 0 | 1323 | 1323 | 0 | 1134 | 1134 | 0 |
| NA12878 | 5733 | 5733 | 0 | 1260 | 1197 | 1 | 1512 | 1449 | 0 |
| NA12879 | 3591 | 3591 | 0 | 1323 | 1260 | 5 | 1134 | 1134 | 0 |
| NA12880 | 4221 | 4221 | 0 | 1512 | 1512 | 0 | 1260 | 1197 | 0 |
| NA12881 | 4914 | 4913 | 1 | 1512 | 1449 | 2 | 1449 | 1449 | 0 |
| NA12882 | 3717 | 3717 | 0 | 1386 | 1323 | 3 | 1197 | 1134 | 0 |
| NA12883 | 4284 | 4284 | 0 | 1449 | 1449 | 0 | 1386 | 1323 | 0 |
| NA12884 | 4284 | 4284 | 0 | 1575 | 1512 | 6 | 1323 | 1323 | 0 |
| NA12885 | 4725 | 4725 | 0 | 1575 | 1512 | 2 | 1386 | 1323 | 0 |
| NA12886 | 4347 | 4347 | 0 | 1449 | 1386 | 7 | 1449 | 1449 | 0 |
| NA12887 | 4284 | 4284 | 0 | 1323 | 1323 | 0 | 1512 | 1512 | 0 |
| NA12888 | 4158 | 4158 | 0 | 1449 | 1449 | 0 | 1197 | 1197 | 0 |
| NA12893 | 4599 | 4599 | 0 | 1386 | 1323 | 1 | 1449 | 1449 | 0 |

The samples were further analyzed for calling small insertions and deletions (indels). An overall summary is presented in Table 10. There were a total of 71 indels ranging in size from 1-24 bp for insertions and 1-25 bp for deletions.

Table 10 Summary of Germline Indel Detection

| Variant Type | Expected Variants | TP | FN | Variant No Calls | PPA |
|--------------|-------------------|-------|----|------------------|------|
| Insertion | 18522 | 18018 | 27 | 477 | 99.9 |
| Deletion | 17388 | 17073 | 0 | 315 | 100 |

The representative assay consisted of 150 amplicons designed to cover various genomic content. The GC content of the amplicons ranged from 0.19-0.87. Amplicons also had a range of single nucleotide (eg, PolyA, PolyT), dinucleotide, and trinucleotide repeats. Data were compiled on a per amplicon basis (Table 11) to determine the effect of genomic content on percent correct calls. Percent correct calls consists of variant and reference calls and is less than 100% if there are either incorrect or no calls.

Table 11 Germline Amplicon-level Accuracy

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|----------------------------------|------------|---------------|-----------------|----------|-----------------|
| 1 | 1 | 36450499 | 36450591 | 93 | 93 | Indel | 0.22 | 76167 | 0 | 0 | 100 |
| 2 | 1 | 109465122 | 109465200 | 79 | 79 | Poly A (5), Poly C (5), indel | 0.38 | 64701 | 0 | 0 | 100 |
| 3 | 1 | 218353867 | 218353957 | 91 | 91 | Indel | 0.4 | 74529 | 0 | 0 | 100 |
| 4 | 1 | 223906657 | 223906748 | 92 | 92 | Indel | 0.49 | 75348 | 0 | 0 | 100 |
| 5 | 1 | 228526602 | 228526682 | 81 | 81 | Poly G (5) | 0.69 | 66339 | 0 | 0 | 100 |
| 6 | 1 | 236372039 | 236372108 | 70 | 70 | Poly T (10), indel | 0.39 | 57330 | 0 | 0 | 100 |
| 7 | 1 | 247812041 | 247812128 | 88 | 88 | Poly A (5), CT(3), TAA(3), indel | 0.27 | 72072 | 0 | 0 | 100 |
| 8 | 2 | 55862774 | 55862863 | 90 | 90 | Indel | 0.28 | 73710 | 0 | 0 | 100 |
| 9 | 2 | 87003930 | 87004009 | 80 | 80 | Indel | 0.38 | 65520 | 0 | 0 | 100 |
| 10 | 2 | 177016721 | 177016805 | 85 | 81 | N/A | 0.65 | 66339 | 0 | 0 | 100 |
| 11 | 2 | 186625727 | 186625801 | 75 | 75 | Poly A (8) | 0.35 | 61425 | 0 | 0 | 100 |
| 12 | 2 | 190323504 | 190323591 | 88 | 88 | Poly T (5) | 0.42 | 72072 | 0 | 0 | 100 |
| 13 | 2 | 200796740 | 200796826 | 87 | 87 | Poly T (5), indel | 0.31 | 71253 | 0 | 0 | 100 |
| 14 | 2 | 212245049 | 212245139 | 91 | 91 | Poly T (5), Poly A (6), indel | 0.3 | 74529 | 0 | 0 | 100 |
| 15 | 2 | 228147052 | 228147144 | 93 | 93 | Indel | 0.43 | 76167 | 0 | 0 | 100 |
| 16 | 2 | 235016350 | 235016422 | 73 | 73 | Poly T (5), indel | 0.42 | 59787 | 0 | 0 | 100 |
| 17 | 3 | 4466229 | 4466321 | 93 | 93 | AT(3), indel | 0.27 | 74823 | 0 | 1344 | 98.2 |
| 18 | 3 | 46620561 | 46620643 | 83 | 83 | N/A | 0.43 | 67977 | 0 | 0 | 100 |
| 19 | 3 | 49851331 | 49851400 | 70 | 70 | CT(3), indel | 0.49 | 57330 | 0 | 0 | 100 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|---|------------|---------------|-----------------|----------|-----------------|
| 20 | 3 | 189713161 | 189713248 | 88 | 88 | Poly A (5), Poly T (5), Poly A (9), TG(3) | 0.41 | 72072 | 0 | 0 | 100 |
| 21 | 3 | 190106030 | 190106104 | 75 | 74 | Indel | 0.57 | 60543 | 0 | 63 | 99.9 |
| 22 | 4 | 2233667 | 2233744 | 78 | 78 | Poly A (6) | 0.26 | 63882 | 0 | 0 | 100 |
| 23 | 4 | 7780541 | 7780637 | 97 | 97 | Poly G (6), Poly T (5), Poly A (5) | 0.42 | 79443 | 0 | 0 | 100 |
| 24 | 4 | 15688604 | 15688681 | 78 | 78 | N/A | 0.29 | 63882 | 0 | 0 | 100 |
| 25 | 4 | 56236521 | 56236586 | 66 | 62 | Poly A (5), indel | 0.36 | 50778 | 0 | 0 | 100 |
| 26 | 4 | 102839244 | 102839314 | 71 | 69 | Poly A (5) | 0.46 | 56511 | 0 | 0 | 100 |
| 27 | 4 | 164446743 | 164446804 | 62 | 62 | Poly A (7), indel | 0.27 | 50778 | 0 | 0 | 100 |
| 28 | 5 | 1882081 | 1882158 | 78 | 75 | N/A | 0.78 | 61425 | 0 | 0 | 100 |
| 29 | 5 | 14769061 | 14769144 | 84 | 84 | GT(3), CCA(3) | 0.62 | 68796 | 0 | 0 | 100 |
| 30 | 5 | 41069808 | 41069871 | 64 | 64 | N/A | 0.39 | 52416 | 0 | 0 | 100 |
| 31 | 5 | 74077114 | 74077196 | 83 | 83 | Poly A (6), indel | 0.3 | 67977 | 0 | 0 | 100 |
| 32 | 5 | 147475343 | 147475409 | 67 | 67 | Poly T (5) | 0.37 | 54873 | 0 | 0 | 100 |
| 33 | 5 | 149323731 | 149323821 | 91 | 91 | CT(4), AG(3) | 0.55 | 74529 | 0 | 0 | 100 |
| 34 | 5 | 155662213 | 155662287 | 75 | 75 | Indel | 0.43 | 61425 | 0 | 0 | 100 |
| 35 | 6 | 6318713 | 6318814 | 102 | 102 | Poly G (6) | 0.68 | 83538 | 0 | 0 | 100 |
| 36 | 6 | 24949983 | 24950074 | 92 | 92 | Indel | 0.63 | 75348 | 0 | 0 | 100 |
| 37 | 6 | 31084900 | 31084999 | 100 | 94 | GCT(5), indel | 0.61 | 76608 | 0 | 378 | 99.5 |
| 38 | 6 | 32147987 | 32148084 | 98 | 98 | Poly T (5), TCT(3), CTT(3) | 0.55 | 80262 | 0 | 0 | 100 |
| 39 | 6 | 32986864 | 32986958 | 95 | 95 | Indel | 0.53 | 77805 | 0 | 0 | 100 |
| 40 | 6 | 33408498 | 33408583 | 86 | 86 | Poly C (6) | 0.7 | 70434 | 0 | 0 | 100 |
| 41 | 6 | 41647401 | 41647495 | 95 | 94 | Poly G (5), indel | 0.61 | 76986 | 0 | 0 | 100 |
| 42 | 6 | 112435865 | 112435955 | 91 | 91 | Poly A (5) | 0.44 | 74529 | 0 | 0 | 100 |
| 43 | 7 | 22202076 | 22202148 | 73 | 73 | N/A | 0.44 | 59787 | 0 | 0 | 100 |
| 44 | 7 | 66276100 | 66276187 | 88 | 88 | Indel | 0.35 | 72072 | 0 | 0 | 100 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|-------------------------------|------------|---------------|-----------------|----------|-----------------|
| 45 | 7 | 77365735 | 77365821 | 87 | 87 | Poly A (7), AG(4) | 0.26 | 71253 | 0 | 0 | 100 |
| 46 | 7 | 110939946 | 110940030 | 85 | 85 | Indel | 0.38 | 69615 | 0 | 0 | 100 |
| 47 | 7 | 128533468 | 128533557 | 90 | 90 | Poly G (5), indel | 0.62 | 73710 | 0 | 0 | 100 |
| 48 | 7 | 149503875 | 149503965 | 91 | 91 | Poly G (6), Poly C (6), indel | 0.71 | 74529 | 0 | 0 | 100 |
| 49 | 7 | 154404519 | 154404599 | 81 | 66 | N/A | 0.31 | 54054 | 0 | 0 | 100 |
| 50 | 7 | 156476507 | 156476599 | 93 | 93 | Indel | 0.35 | 76167 | 0 | 0 | 100 |
| 51 | 8 | 1817312 | 1817394 | 83 | 83 | N/A | 0.42 | 67977 | 0 | 0 | 100 |
| 52 | 8 | 24811020 | 24811109 | 90 | 89 | Poly G (7), CTC(4), indel | 0.61 | 72171 | 0 | 720 | 99.0 |
| 53 | 8 | 76518625 | 76518691 | 67 | 67 | Indel | 0.3 | 54873 | 0 | 0 | 100 |
| 54 | 9 | 103054909 | 103055006 | 98 | 98 | Poly G (6) | 0.67 | 80262 | 0 | 0 | 100 |
| 55 | 9 | 105586150 | 105586214 | 65 | 65 | Indel | 0.32 | 53235 | 0 | 0 | 100 |
| 56 | 9 | 107620823 | 107620918 | 96 | 96 | N/A | 0.49 | 78624 | 0 | 0 | 100 |
| 57 | 9 | 123769149 | 123769231 | 83 | 83 | AT(3) | 0.37 | 67977 | 0 | 0 | 100 |
| 58 | 9 | 138995345 | 138995441 | 97 | 97 | Poly C (6), indel | 0.68 | 79443 | 0 | 0 | 100 |
| 59 | 10 | 5987120 | 5987198 | 79 | 78 | Poly G (5), indel | 0.47 | 63882 | 0 | 0 | 100 |
| 60 | 10 | 11784629 | 11784726 | 98 | 91 | GC(3) | 0.87 | 74529 | 0 | 0 | 100 |
| 61 | 10 | 27317777 | 27317855 | 79 | 79 | Poly T (5) | 0.3 | 64701 | 0 | 0 | 100 |
| 62 | 10 | 33018351 | 33018440 | 90 | 90 | Poly A (5), Poly T (5) | 0.2 | 73710 | 0 | 0 | 100 |
| 63 | 10 | 45084159 | 45084253 | 95 | 95 | Indel | 0.35 | 77805 | 0 | 0 | 100 |
| 64 | 10 | 55892599 | 55892687 | 89 | 88 | AC(11), indel | 0.42 | 71747 | 0 | 325 | 99.5 |
| 65 | 10 | 101611250 | 101611329 | 80 | 80 | N/A | 0.49 | 65520 | 0 | 0 | 100 |
| 66 | 10 | 118351373 | 118351453 | 81 | 81 | N/A | 0.51 | 66339 | 0 | 0 | 100 |
| 67 | 11 | 8159816 | 8159912 | 97 | 96 | N/A | 0.45 | 78624 | 0 | 0 | 100 |
| 68 | 11 | 30177648 | 30177717 | 70 | 70 | Indel | 0.46 | 57330 | 0 | 0 | 100 |
| 69 | 11 | 47470345 | 47470444 | 100 | 100 | N/A | 0.65 | 81900 | 0 | 0 | 100 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|---|------------|---------------|-----------------|----------|-----------------|
| 70 | 11 | 59837679 | 59837740 | 62 | 62 | Indel | 0.37 | 50778 | 0 | 0 | 100 |
| 71 | 11 | 64418856 | 64418957 | 102 | 102 | N/A | 0.59 | 83538 | 0 | 0 | 100 |
| 72 | 11 | 93529612 | 93529684 | 73 | 73 | Poly A (5) | 0.4 | 59787 | 0 | 0 | 100 |
| 73 | 11 | 101347052 | 101347136 | 85 | 85 | N/A | 0.42 | 69615 | 0 | 0 | 100 |
| 74 | 11 | 102477336 | 102477426 | 91 | 91 | Poly G (6) | 0.55 | 74529 | 0 | 0 | 100 |
| 75 | 11 | 118406285 | 118406369 | 85 | 85 | Indel | 0.53 | 69615 | 0 | 0 | 100 |
| 76 | 11 | 120357801 | 120357885 | 85 | 85 | Poly A (5), CA(3), indel | 0.34 | 69615 | 0 | 0 | 100 |
| 77 | 11 | 125769313 | 125769397 | 85 | 85 | GA(3) | 0.52 | 69615 | 0 | 0 | 100 |
| 78 | 12 | 2834770 | 2834853 | 84 | 84 | Poly C (5), indel | 0.52 | 68796 | 0 | 0 | 100 |
| 79 | 12 | 26811004 | 26811096 | 93 | 93 | Poly A (7), AC(4) | 0.33 | 76167 | 0 | 0 | 100 |
| 80 | 12 | 30881766 | 30881846 | 81 | 81 | N/A | 0.49 | 66339 | 0 | 0 | 100 |
| 81 | 12 | 88474105 | 88474175 | 71 | 71 | Poly A (6) | 0.35 | 58149 | 0 | 0 | 100 |
| 82 | 12 | 120966872 | 120966966 | 95 | 95 | Poly G (5) | 0.68 | 77805 | 0 | 0 | 100 |
| 83 | 13 | 24167504 | 24167576 | 73 | 73 | N/A | 0.52 | 59787 | 0 | 0 | 100 |
| 84 | 13 | 25816961 | 25817049 | 89 | 88 | Poly A (5), Poly T (7), Poly A (7), indel | 0.22 | 72072 | 0 | 0 | 100 |
| 85 | 13 | 44880112 | 44880200 | 89 | 89 | Indel | 0.49 | 72891 | 0 | 0 | 100 |
| 86 | 13 | 77665218 | 77665294 | 77 | 77 | Indel | 0.39 | 63063 | 0 | 0 | 100 |
| 87 | 14 | 31619327 | 31619393 | 67 | 67 | GA(3),TA(3) | 0.39 | 54873 | 0 | 0 | 100 |
| 88 | 14 | 39517884 | 39517966 | 83 | 83 | N/A | 0.25 | 67977 | 0 | 0 | 100 |
| 89 | 14 | 46958962 | 46959034 | 73 | 72 | Poly T (5), indel | 0.19 | 58642 | 0 | 326 | 99.4 |
| 90 | 14 | 58050030 | 58050110 | 81 | 81 | Indel | 0.38 | 66339 | 0 | 0 | 100 |
| 91 | 14 | 82390559 | 82390649 | 91 | 91 | Indel | 0.35 | 74529 | 0 | 0 | 100 |
| 92 | 14 | 92549544 | 92549609 | 66 | 66 | Poly A (5) | 0.41 | 54054 | 0 | 0 | 100 |
| 93 | 14 | 102808496 | 102808589 | 94 | 94 | Indel | 0.62 | 76986 | 0 | 0 | 100 |
| 94 | 15 | 43170751 | 43170848 | 98 | 96 | Poly C (5) | 0.45 | 78624 | 0 | 0 | 100 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|---|------------|---------------|-----------------|----------|-----------------|
| 95 | 15 | 63446149 | 63446216 | 68 | 68 | Indel | 0.25 | 55692 | 0 | 0 | 100 |
| 96 | 15 | 77879807 | 77879901 | 95 | 93 | Poly G (5), indel | 0.68 | 76167 | 0 | 0 | 100 |
| 97 | 15 | 81625334 | 81625428 | 95 | 95 | Poly T (6) | 0.43 | 77805 | 0 | 0 | 100 |
| 98 | 15 | 85438263 | 85438334 | 72 | 71 | Indel | 0.65 | 58149 | 0 | 0 | 100 |
| 99 | 15 | 89817413 | 89817503 | 91 | 91 | N/A | 0.36 | 74529 | 0 | 0 | 100 |
| 100 | 15 | 89864274 | 89864343 | 70 | 70 | Indel | 0.56 | 57330 | 0 | 0 | 100 |
| 101 | 16 | 1894910 | 1894972 | 63 | 63 | N/A | 0.27 | 51597 | 0 | 0 | 100 |
| 102 | 16 | 28997904 | 28997998 | 95 | 95 | Poly C (5) | 0.67 | 77805 | 0 | 0 | 100 |
| 103 | 16 | 53682908 | 53682994 | 87 | 87 | TA(3) | 0.41 | 71253 | 0 | 0 | 100 |
| 104 | 16 | 57954406 | 57954509 | 104 | 104 | Poly C (5) | 0.67 | 85176 | 0 | 0 | 100 |
| 105 | 16 | 85706375 | 85706465 | 91 | 91 | Poly T (5), indel | 0.37 | 74529 | 0 | 0 | 100 |
| 106 | 17 | 3563920 | 3564008 | 89 | 89 | GC(3) | 0.64 | 72891 | 0 | 0 | 100 |
| 107 | 17 | 3594191 | 3594277 | 87 | 87 | Poly C (5), indel | 0.67 | 71247 | 0 | 6 | 100 |
| 108 | 17 | 3970090 | 3970180 | 91 | 91 | Indel | 0.46 | 74529 | 0 | 0 | 100 |
| 109 | 17 | 16084945 | 16085037 | 93 | 93 | Indel | 0.26 | 76167 | 0 | 0 | 100 |
| 110 | 17 | 33998759 | 33998849 | 91 | 89 | Poly T (5) | 0.54 | 72891 | 0 | 0 | 100 |
| 111 | 17 | 39589691 | 39589774 | 84 | 82 | Poly A (13), indel (x2) | 0.29 | 66343 | 27 | 788 | 98.8 |
| 112 | 17 | 41244394 | 41244484 | 91 | 91 | Poly A (5) | 0.34 | 74529 | 0 | 0 | 100 |
| 113 | 17 | 45438866 | 45438957 | 92 | 92 | Poly A (7), AT(3), AT (4), AT(4), indel | 0.26 | 75348 | 0 | 0 | 100 |
| 114 | 17 | 61502432 | 61502510 | 79 | 79 | Indel | 0.41 | 64413 | 0 | 288 | 99.6 |
| 115 | 17 | 64023582 | 64023667 | 86 | 86 | Poly T (7) | 0.22 | 70434 | 0 | 0 | 100 |
| 116 | 17 | 72308237 | 72308320 | 84 | 84 | GAG(3) | 0.62 | 68796 | 0 | 0 | 100 |
| 117 | 18 | 2616456 | 2616522 | 67 | 67 | GA(3) | 0.31 | 54873 | 0 | 0 | 100 |
| 118 | 18 | 6980478 | 6980568 | 91 | 91 | N/A | 0.37 | 74529 | 0 | 0 | 100 |
| 119 | 18 | 9888026 | 9888094 | 69 | 69 | Poly A (6), TG(3) | 0.43 | 56511 | 0 | 0 | 100 |
| 120 | 18 | 38836999 | 38837073 | 75 | 75 | Poly A (5), indel | 0.37 | 61425 | 0 | 0 | 100 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|--------------------------|------------|---------------|-----------------|----------|-----------------|
| 121 | 18 | 47405382 | 47405462 | 81 | 81 | CTC(3), indel | 0.47 | 66339 | 0 | 0 | 100 |
| 122 | 18 | 54815665 | 54815749 | 85 | 85 | CT(3), indel | 0.45 | 69615 | 0 | 0 | 100 |
| 123 | 18 | 59773996 | 59774060 | 65 | 65 | N/A | 0.48 | 53235 | 0 | 0 | 100 |
| 124 | 19 | 625143 | 625241 | 99 | 99 | N/A | 0.59 | 81081 | 0 | 0 | 100 |
| 125 | 19 | 18121418 | 18121491 | 74 | 74 | N/A | 0.68 | 60605 | 1 | 0 | 100 |
| 126 | 19 | 18186574 | 18186643 | 70 | 70 | N/A | 0.64 | 57330 | 0 | 0 | 100 |
| 127 | 20 | 746056 | 746149 | 94 | 94 | N/A | 0.61 | 76986 | 0 | 0 | 100 |
| 128 | 20 | 10633195 | 10633276 | 82 | 82 | AC(3) | 0.59 | 67158 | 0 | 0 | 100 |
| 129 | 20 | 17705633 | 17705708 | 76 | 76 | CT(3) | 0.58 | 62244 | 0 | 0 | 100 |
| 130 | 20 | 21766821 | 21766890 | 70 | 70 | GT(3),TG(4), indel | 0.46 | 57330 | 0 | 0 | 100 |
| 131 | 20 | 25278421 | 25278521 | 101 | 101 | Indel | 0.63 | 82719 | 0 | 0 | 100 |
| 132 | 20 | 50897302 | 50897368 | 67 | 67 | Indel | 0.36 | 54873 | 0 | 0 | 100 |
| 133 | 20 | 62331904 | 62331994 | 91 | 88 | Poly G (6) | 0.73 | 72072 | 0 | 0 | 100 |
| 134 | 20 | 62690860 | 62690946 | 87 | 87 | Indel | 0.57 | 71253 | 0 | 0 | 100 |
| 135 | 21 | 30300823 | 30300888 | 66 | 66 | Indel | 0.35 | 54054 | 0 | 0 | 100 |
| 136 | 21 | 33694176 | 33694273 | 98 | 98 | Poly T (6), CA(3) | 0.54 | 80262 | 0 | 0 | 100 |
| 137 | 21 | 36710706 | 36710792 | 87 | 87 | GT(3), indel | 0.39 | 71253 | 0 | 0 | 100 |
| 138 | 21 | 46644924 | 46644992 | 69 | 69 | Poly A (6), AG(3), indel | 0.32 | 56439 | 0 | 72 | 99.9 |
| 139 | 21 | 46705575 | 46705664 | 90 | 90 | Poly T (5), Poly A (6) | 0.5 | 73710 | 0 | 0 | 100 |
| 140 | 22 | 25750774 | 25750873 | 100 | 100 | Indel | 0.63 | 81900 | 0 | 0 | 100 |
| 141 | 22 | 32439233 | 32439329 | 97 | 97 | N/A | 0.68 | 79443 | 0 | 0 | 100 |
| 142 | 22 | 37409844 | 37409940 | 97 | 97 | Indel | 0.46 | 79443 | 0 | 0 | 100 |
| 143 | 22 | 37637596 | 37637694 | 99 | 99 | N/A | 0.6 | 81081 | 0 | 0 | 100 |
| 144 | 22 | 47081347 | 47081438 | 92 | 92 | Indel | 0.66 | 75348 | 0 | 0 | 100 |
| 145 | X | 15870424 | 15870492 | 69 | 69 | Poly T (5) | 0.26 | 56511 | 0 | 0 | 100 |
| 146 | X | 135288543 | 135288611 | 69 | 69 | Poly C (5) | 0.62 | 56511 | 0 | 0 | 100 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|--------------------------|------------|---------------|-----------------|----------|-----------------|
| 147 | X | 135290777 | 135290847 | 71 | 71 | N/A | 0.52 | 58149 | 0 | 0 | 100 |
| 148 | Y | 2655397 | 2655461 | 65 | 0 | N/A | 0.55 | 0 | 0 | 0 | N/A |
| 149 | Y | 2655519 | 2655609 | 91 | 0 | N/A | 0.48 | 0 | 0 | 0 | N/A |
| 150 | Y | 2655609 | 2655679 | 71 | 0 | Poly A (5) | 0.37 | 0 | 0 | 0 | N/A |

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 150 amplicons, 92 amplicons were fully contained within the highly confident genomic regions, 41 amplicons had partial overlap, and 17 amplicons had no overlap in the NIST sequence. This outcome resulted in 10,000 coordinates per replicate for comparison. Non-variant base calls were compared to human genome reference sequence build hg19. The accuracy results are shown in [Table 12](#).

Table 12 Germline Agreement of NA12878 Sample with NIST Database

| Sample | # Amplicons | Mean Call Rate | TP | FN | TN | FP | PPA | NPA | OPA |
|---------|-------------|----------------|------|----|--------|----|-------|-----|-------|
| NA12878 | 133 | >99.9 | 6552 | 1 | 610470 | 0 | >99.9 | 100 | >99.9 |

Based on the data provided by this nine-run Germline study, the NextSeq 550Dx instrument can consistently sequence:

- ▶ GC content $\geq 19\%$ (all called bases in 819 sequenced amplicons with 19% GC content called correctly with no-call rate of 0.6%)
- ▶ GC content $\leq 87\%$ (all called bases in 819 sequenced amplicons with 87% GC content called correctly with zero no-calls)
- ▶ PolyA lengths ≤ 9 (all called bases in 819 sequenced amplicons containing a PolyA repeat of nine nucleotides called correctly with zero no calls)
- ▶ PolyT lengths ≤ 10 (all called bases in 819 sequenced amplicons containing a PolyT repeat of ten nucleotides called correctly with zero no calls)
- ▶ PolyG lengths ≤ 7 (all called bases in 819 sequenced amplicons containing a PolyG repeat of seven nucleotides called correctly with no-call rate of 1.0%)
- ▶ PolyC lengths ≤ 6 (all called bases in 2457 sequenced amplicons containing a PolyC repeat of six nucleotides were called correctly with zero no calls)
- ▶ Dinucleotide repeat lengths $\leq 11x$ (all called bases in 819 sequenced amplicons containing an 11x dinucleotide repeat were called correctly with no-call rate of 0.5%)
- ▶ Trinucleotide repeat lengths $\leq 5x$ (all called bases in 819 sequenced amplicons containing a 5x trinucleotide repeat were called correctly with no-call rate of 0.5%)
- ▶ Insertion lengths ≤ 24 (66343 out of 66370 called bases in 819 sequenced amplicons containing a 24-nucleotide insertion called correctly with no-call rate of 1.2%; no incorrect calls occurred in region containing 24-nucleotide insertion)
- ▶ Deletion lengths ≤ 25 (all called bases in 2457 sequenced amplicons containing a 25-nucleotide deletion called correctly with zero no-calls)

Somatic

The study described here was used to assess variant calling accuracy of the Somatic Variant Module on the NextSeq 550Dx instrument using the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles).

This study used a representative assay designed to query a variety of genes covering 12,588 bases (150 amplicons) across 23 different chromosomes. Platinum Genome DNA was extracted from FFPE treated blocks to generate six unique samples for evaluation in the study.

Sample GM12877 DNA was diluted with sample GM12878 DNA to create GM12877-D5 and GM12877-D7 as a set of unique heterozygous variants with variant frequencies near 5% and 7%. Sample GM12878 DNA was similarly diluted with sample GM12877 DNA to create GM12878-D5 and GM12878-D7. Each of the samples was tested in triplicate except the diluted samples, which were tested in replicates of six. A total of nine runs were performed using three sequencing instruments, three reagent lots, and three operators over five start days. Accuracy was determined for SNVs, insertions, and deletions by comparing results to the well-characterized composite reference method, Platinum Genomes version 2016- 1.0. Confident genomic regions were defined based on this reference method unless otherwise specified.

Table 13 Summary of Somatic Agreement

| Criteria | Total Observations ¹ | Result by Observation ² | Result by Run ³ |
|--------------------|---------------------------------|------------------------------------|----------------------------|
| PPA for SNV | 378 | 98.9 | 99.9 |
| PPA for insertions | 378 | 96.9 | 99.9 |
| PPA for deletions | 378 | 97.1 | 99.9 |
| NPA | 378 | >99.9 | >99.9 |
| OPA | 378 | >99.9 | >99.9 |

¹Calculated as number of samples per run (42) x number of Runs (9) = 378.

²Lowest observed value by sample replicate across all 9 runs.

³Lowest value when data from each run are analyzed in aggregate.

Table 14 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 14 Somatic Agreement per Sample

| Sample | Mean Call Rate | Expected | TP | FN | Variant No Calls | TN | FP | PPA | NPA | OPA |
|------------|----------------|----------|------|----|------------------|--------|----|------|-------|-------|
| GM12877 | 98.7 | 2052 | 2025 | 0 | 27 | 318682 | 15 | 100 | >99.9 | >99.9 |
| GM12878 | 98.8 | 3645 | 3564 | 0 | 81 | 317645 | 0 | 100 | 100 | 100 |
| GM12879 | 99.8 | 2592 | 2538 | 0 | 54 | 323614 | 2 | 100 | >99.9 | >99.9 |
| GM12884 | 99.8 | 3078 | 3024 | 0 | 54 | 322038 | 5 | 100 | >99.9 | >99.9 |
| GM12885 | 99.8 | 3294 | 3213 | 0 | 81 | 322121 | 0 | 100 | 100 | 100 |
| GM12888 | 99.8 | 2916 | 2889 | 0 | 27 | 323048 | 2 | 100 | >99.9 | >99.9 |
| GM12877-D5 | 99.8 | 9288 | 8930 | 0 | 358 | 630621 | 0 | 100 | 100 | 100 |
| GM12877-D7 | 99.7 | 9288 | 9032 | 0 | 256 | 629719 | 0 | 100 | 100 | 100 |
| GM12878-D5 | 99.5 | 9288 | 8699 | 42 | 547 | 628582 | 0 | 99.5 | 100 | >99.9 |
| GM12878-D7 | 99.7 | 9288 | 9108 | 0 | 180 | 629803 | 0 | 100 | 100 | 100 |

Table 15 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 Somatic Agreement per Sample by Variant Type

| Sample | SNVs | | | Insertions | | | Deletions | | |
|---------|----------|------|----|------------|-----|----|-----------|-----|----|
| | Expected | TP | FN | Expected | TP | FN | Expected | TP | FN |
| GM12877 | 999 | 999 | 0 | 567 | 567 | 0 | 486 | 459 | 0 |
| GM12878 | 2457 | 2457 | 0 | 540 | 513 | 0 | 648 | 594 | 0 |
| GM12879 | 1539 | 1539 | 0 | 567 | 540 | 0 | 486 | 459 | 0 |
| GM12884 | 1836 | 1836 | 0 | 675 | 648 | 0 | 567 | 540 | 0 |
| GM12885 | 2025 | 2025 | 0 | 675 | 648 | 0 | 594 | 540 | 0 |

| Sample | SNVs | | | Insertions | | | Deletions | | |
|------------|----------|------|----|------------|------|----|-----------|------|----|
| | Expected | TP | FN | Expected | TP | FN | Expected | TP | FN |
| GM12888 | 1782 | 1782 | 0 | 621 | 621 | 0 | 513 | 486 | 0 |
| GM12877-D5 | 5454 | 5392 | 0 | 1782 | 1647 | 0 | 2052 | 1891 | 0 |
| GM12877-D7 | 5454 | 5406 | 0 | 1782 | 1728 | 0 | 2052 | 1898 | 0 |
| GM12878-D5 | 5454 | 5192 | 28 | 1782 | 1651 | 9 | 2052 | 1856 | 5 |
| GM12878-D7 | 5454 | 5445 | 0 | 1782 | 1719 | 0 | 2052 | 1944 | 0 |

The ten samples were further analyzed for calling small insertions and deletions (indels) (Table 16). There were a total of 71 indels ranging in size from 1-24 bp for insertions and 1-25 bp for deletions.

Table 16 Summary of Somatic Indel Detection

| Variant Type | Expected Variants | TP | FN | Variant No Calls | PPA |
|--------------|-------------------|-------|----|------------------|-------|
| Insertion | 10773 | 10282 | 9 | 482 | 99.2 |
| Deletion | 11502 | 10667 | 5 | 830 | >99.9 |

The 150 amplicons were designed to cover various genomic content. The GC content of the amplicons ranged from 0.19-0.87%. Amplicons also had a range of single nucleotide (e.g. PolyA, PolyT), dinucleotide, and trinucleotide repeats. Data were compiled on a per amplicon basis (Table 17) to determine the effect of genomic content on percent correct calls. Percent correct calls consists of variant and reference calls and is less than 100% if there are either incorrect or no calls.

Table 17 Somatic Amplicon-level Accuracy

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|----------------------------------|------------|---------------|-----------------|----------|-----------------|
| 1 | 1 | 36450499 | 36450591 | 93 | 93 | Indel | 0.22 | 35066 | 0 | 88 | 99.7 |
| 2 | 1 | 109465122 | 109465200 | 79 | 79 | Poly A (5), Poly C (5), indel | 0.38 | 29827 | 0 | 35 | 99.9 |
| 3 | 1 | 218353867 | 218353957 | 91 | 91 | Indel | 0.4 | 34202 | 0 | 283 | 99.2 |
| 4 | 1 | 223906657 | 223906748 | 92 | 92 | Indel | 0.49 | 34613 | 0 | 163 | 99.5 |
| 5 | 1 | 228526602 | 228526682 | 81 | 81 | Poly G (5) | 0.69 | 30571 | 0 | 47 | 99.8 |
| 6 | 1 | 236372039 | 236372108 | 70 | 70 | Poly T (10), indel | 0.39 | 26452 | 0 | 8 | 100.0 |
| 7 | 1 | 247812041 | 247812128 | 88 | 88 | Poly A (5), CT(3), TAA(3), indel | 0.27 | 33148 | 0 | 116 | 99.7 |
| 8 | 2 | 55862774 | 55862863 | 90 | 90 | Indel | 0.28 | 33928 | 0 | 92 | 99.7 |
| 9 | 2 | 87003930 | 87004009 | 80 | 80 | Indel | 0.38 | 30218 | 0 | 22 | 99.9 |
| 10 | 2 | 177016721 | 177016805 | 85 | 81 | N/A | 0.65 | 30616 | 0 | 2 | >99.9 |
| 11 | 2 | 186625727 | 186625801 | 75 | 75 | Poly A (8) | 0.35 | 28017 | 0 | 499 | 98.3 |
| 12 | 2 | 190323504 | 190323591 | 88 | 88 | Poly T (5) | 0.42 | 33207 | 0 | 57 | 99.8 |
| 13 | 2 | 200796740 | 200796826 | 87 | 87 | Poly T (5), indel | 0.31 | 32524 | 9 | 718 | 97.8 |
| 14 | 2 | 212245049 | 212245139 | 91 | 91 | Poly T (5), Poly A (6), indel | 0.3 | 33972 | 0 | 456 | 98.7 |
| 15 | 2 | 228147052 | 228147144 | 93 | 93 | N/A | 0.43 | 35051 | 0 | 103 | 99.7 |
| 16 | 2 | 235016350 | 235016422 | 73 | 73 | Poly T (5), indel | 0.42 | 27459 | 0 | 136 | 99.5 |
| 17 | 3 | 4466229 | 4466321 | 93 | 93 | AT(3), indel | 0.27 | 34534 | 0 | 620 | 98.2 |
| 18 | 3 | 46620561 | 46620643 | 83 | 83 | N/A | 0.43 | 31339 | 0 | 44 | 99.9 |
| 19 | 3 | 49851331 | 49851400 | 70 | 70 | CT(3), indel | 0.49 | 26373 | 0 | 87 | 99.7 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|--|------------|---------------|-----------------|----------|-----------------|
| 20 | 3 | 189713161 | 189713248 | 88 | 88 | Poly A (5), Poly T (5), Poly A (9), TG (3) | 0.41 | 32829 | 0 | 857 | 97.5 |
| 21 | 3 | 190106030 | 190106104 | 75 | 74 | Indel | 0.57 | 27925 | 0 | 47 | 99.8 |
| 22 | 4 | 2233667 | 2233744 | 78 | 78 | Poly A (6) | 0.26 | 29327 | 4 | 162 | 99.4 |
| 23 | 4 | 7780541 | 7780637 | 97 | 97 | Poly G (6), Poly T (5), Poly A (5) | 0.42 | 36585 | 0 | 117 | 99.7 |
| 24 | 4 | 15688604 | 15688681 | 78 | 78 | N/A | 0.29 | 29427 | 0 | 57 | 99.8 |
| 25 | 4 | 56236521 | 56236586 | 66 | 62 | Poly A (5), indel | 0.36 | 23356 | 5 | 75 | 99.7 |
| 26 | 4 | 102839244 | 102839314 | 71 | 69 | Poly A (5) | 0.46 | 25942 | 0 | 140 | 99.5 |
| 27 | 4 | 164446743 | 164446804 | 62 | 62 | Poly A (7), indel | 0.27 | 22944 | 0 | 560 | 97.6 |
| 28 | 5 | 1882081 | 1882158 | 78 | 75 | N/A | 0.78 | 28299 | 0 | 53 | 99.8 |
| 29 | 5 | 14769061 | 14769144 | 84 | 84 | GT(3), CCA(3) | 0.62 | 31658 | 0 | 94 | 99.7 |
| 30 | 5 | 41069808 | 41069871 | 64 | 64 | N/A | 0.39 | 24120 | 0 | 72 | 99.7 |
| 31 | 5 | 74077114 | 74077196 | 83 | 83 | Poly A (6), indel | 0.3 | 31297 | 0 | 77 | 99.8 |
| 32 | 5 | 147475343 | 147475409 | 67 | 67 | Poly T (5) | 0.37 | 25277 | 0 | 55 | 99.8 |
| 33 | 5 | 149323731 | 149323821 | 91 | 91 | CT(4), AG(3) | 0.55 | 34308 | 0 | 90 | 99.7 |
| 34 | 5 | 155662213 | 155662287 | 75 | 75 | Indel | 0.43 | 28266 | 0 | 163 | 99.4 |
| 35 | 6 | 6318713 | 6318814 | 102 | 102 | Poly G (6) | 0.68 | 38489 | 0 | 67 | 99.8 |
| 36 | 6 | 24949983 | 24950074 | 92 | 92 | Indel | 0.63 | 34730 | 0 | 46 | 99.9 |
| 37 | 6 | 31084900 | 31084999 | 100 | 94 | GCT(5), indel | 0.61 | 35057 | 0 | 483 | 98.6 |
| 38 | 6 | 32147987 | 32148084 | 98 | 98 | Poly T (5), TCT(3), CTT(3) | 0.55 | 36647 | 0 | 406 | 98.9 |
| 39 | 6 | 32986864 | 32986958 | 95 | 95 | Indel | 0.53 | 35681 | 0 | 238 | 99.3 |
| 40 | 6 | 33408498 | 33408583 | 86 | 86 | Poly C (6) | 0.7 | 32438 | 0 | 70 | 99.8 |
| 41 | 6 | 41647401 | 41647495 | 95 | 94 | Poly G (5), indel | 0.61 | 35441 | 0 | 91 | 99.7 |
| 42 | 6 | 112435865 | 112435955 | 91 | 91 | Poly A (5) | 0.44 | 34354 | 0 | 44 | 99.9 |
| 43 | 7 | 22202076 | 22202148 | 73 | 73 | N/A | 0.44 | 27575 | 0 | 28 | 99.9 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|-------------------------------|------------|---------------|-----------------|----------|-----------------|
| 44 | 7 | 66276100 | 66276187 | 88 | 88 | Indel | 0.35 | 33060 | 0 | 213 | 99.4 |
| 45 | 7 | 77365735 | 77365821 | 87 | 87 | Poly A (7), AG(4) | 0.26 | 32423 | 0 | 489 | 98.5 |
| 46 | 7 | 110939946 | 110940030 | 85 | 85 | Indel | 0.38 | 32074 | 0 | 56 | 99.8 |
| 47 | 7 | 128533468 | 128533557 | 90 | 90 | Poly G (5), indel | 0.62 | 33791 | 0 | 281 | 99.2 |
| 48 | 7 | 149503875 | 149503965 | 91 | 91 | Poly G (6), Poly C (6), indel | 0.71 | 34316 | 0 | 82 | 99.8 |
| 49 | 7 | 154404519 | 154404599 | 81 | 66 | N/A | 0.31 | 24901 | 0 | 47 | 99.8 |
| 50 | 7 | 156476507 | 156476599 | 93 | 93 | Indel | 0.35 | 35067 | 0 | 87 | 99.8 |
| 51 | 8 | 1817312 | 1817394 | 83 | 83 | N/A | 0.42 | 31365 | 0 | 9 | >99.9 |
| 52 | 8 | 24811020 | 24811109 | 90 | 89 | Poly G (7), CTC(4), indel | 0.61 | 32781 | 0 | 890 | 97.4 |
| 53 | 8 | 76518625 | 76518691 | 67 | 67 | Indel | 0.3 | 25228 | 0 | 146 | 99.4 |
| 54 | 9 | 103054909 | 103055006 | 98 | 98 | Poly G (6) | 0.67 | 36968 | 0 | 76 | 99.8 |
| 55 | 9 | 105586150 | 105586214 | 65 | 65 | Indel | 0.32 | 24472 | 0 | 100 | 99.6 |
| 56 | 9 | 107620823 | 107620918 | 96 | 96 | N/A | 0.49 | 36203 | 0 | 85 | 99.8 |
| 57 | 9 | 123769149 | 123769231 | 83 | 83 | AT(3) | 0.37 | 31329 | 0 | 45 | 99.9 |
| 58 | 9 | 138995345 | 138995441 | 97 | 97 | Poly C (6), indel | 0.68 | 36472 | 0 | 201 | 99.5 |
| 59 | 10 | 5987120 | 5987198 | 79 | 78 | Poly G (5), indel | 0.47 | 29473 | 0 | 11 | >99.9 |
| 60 | 10 | 11784629 | 11784726 | 98 | 91 | GC(3) | 0.87 | 34188 | 0 | 213 | 99.4 |
| 61 | 10 | 27317777 | 27317855 | 79 | 79 | Poly T (5) | 0.3 | 29843 | 0 | 19 | 99.9 |
| 62 | 10 | 33018351 | 33018440 | 90 | 90 | Poly A (5), Poly T (5) | 0.2 | 33968 | 0 | 68 | 99.8 |
| 63 | 10 | 45084159 | 45084253 | 95 | 95 | Indel | 0.35 | 35829 | 0 | 81 | 99.8 |
| 64 | 10 | 55892599 | 55892687 | 89 | 88 | AC(11), indel | 0.42 | 32098 | 88 | 2048 | 93.8 |
| 65 | 10 | 101611250 | 101611329 | 80 | 80 | N/A | 0.49 | 30217 | 0 | 28 | 99.9 |
| 66 | 10 | 118351373 | 118351453 | 81 | 81 | N/A | 0.51 | 30531 | 0 | 96 | 99.7 |
| 67 | 11 | 8159816 | 8159912 | 97 | 96 | N/A | 0.45 | 36105 | 0 | 192 | 99.5 |
| 68 | 11 | 30177648 | 30177717 | 70 | 70 | Indel | 0.46 | 26318 | 0 | 153 | 99.4 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|---|------------|---------------|-----------------|----------|-----------------|
| 69 | 11 | 47470345 | 47470444 | 100 | 100 | N/A | 0.65 | 37785 | 0 | 24 | 99.9 |
| 70 | 11 | 59837679 | 59837740 | 62 | 62 | Indel | 0.37 | 23368 | 0 | 68 | 99.7 |
| 71 | 11 | 64418856 | 64418957 | 102 | 102 | N/A | 0.59 | 38546 | 0 | 10 | >99.9 |
| 72 | 11 | 93529612 | 93529684 | 73 | 73 | Poly A (5) | 0.4 | 27516 | 0 | 78 | 99.7 |
| 73 | 11 | 101347052 | 101347136 | 85 | 85 | N/A | 0.42 | 32083 | 0 | 48 | 99.9 |
| 74 | 11 | 102477336 | 102477426 | 91 | 91 | Poly G (6) | 0.55 | 34047 | 0 | 369 | 98.9 |
| 75 | 11 | 118406285 | 118406369 | 85 | 85 | Indel | 0.53 | 32065 | 0 | 74 | 99.8 |
| 76 | 11 | 120357801 | 120357885 | 85 | 85 | Poly A (5), CA(3), indel | 0.34 | 32083 | 0 | 47 | 99.9 |
| 77 | 11 | 125769313 | 125769397 | 85 | 85 | GA(3) | 0.52 | 32103 | 0 | 27 | 99.9 |
| 78 | 12 | 2834770 | 2834853 | 84 | 84 | Poly C (5), indel | 0.52 | 31645 | 16 | 525 | 98.3 |
| 79 | 12 | 26811004 | 26811096 | 93 | 93 | Poly A (7), AC(4) | 0.33 | 34824 | 0 | 330 | 99.1 |
| 80 | 12 | 30881766 | 30881846 | 81 | 81 | N/A | 0.49 | 30497 | 0 | 121 | 99.6 |
| 81 | 12 | 88474105 | 88474175 | 71 | 71 | Poly A (6) | 0.35 | 26773 | 0 | 65 | 99.8 |
| 82 | 12 | 120966872 | 120966966 | 95 | 95 | Poly G (5) | 0.68 | 35830 | 9 | 72 | 99.8 |
| 83 | 13 | 24167504 | 24167576 | 73 | 73 | N/A | 0.52 | 27498 | 0 | 114 | 99.6 |
| 84 | 13 | 25816961 | 25817049 | 89 | 88 | Poly A (5), Poly T (7), Poly A (7), indel | 0.22 | 32824 | 0 | 566 | 98.3 |
| 85 | 13 | 44880112 | 44880200 | 89 | 89 | Indel | 0.49 | 33574 | 0 | 77 | 99.8 |
| 86 | 13 | 77665218 | 77665294 | 77 | 77 | Indel | 0.39 | 29075 | 0 | 31 | 99.9 |
| 87 | 14 | 31619327 | 31619393 | 67 | 67 | GA(3),TA(3) | 0.39 | 25313 | 0 | 13 | 99.9 |
| 88 | 14 | 39517884 | 39517966 | 83 | 83 | N/A | 0.25 | 31360 | 0 | 22 | 99.9 |
| 89 | 14 | 46958962 | 46959034 | 73 | 72 | Poly T (5), indel | 0.19 | 26499 | 0 | 717 | 97.4 |
| 90 | 14 | 58050030 | 58050110 | 81 | 81 | Indel | 0.38 | 30494 | 0 | 133 | 99.6 |
| 91 | 14 | 82390559 | 82390649 | 91 | 91 | Indel | 0.35 | 34313 | 0 | 86 | 99.7 |
| 92 | 14 | 92549544 | 92549609 | 66 | 66 | Poly A (5) | 0.41 | 24555 | 0 | 1527 | 94.1 |
| 93 | 14 | 102808496 | 102808589 | 94 | 94 | Indel | 0.62 | 35472 | 0 | 69 | 99.8 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|--|------------|---------------|-----------------|----------|-----------------|
| 94 | 15 | 43170751 | 43170848 | 98 | 96 | Poly C (5) | 0.45 | 36264 | 0 | 24 | 99.9 |
| 95 | 15 | 63446149 | 63446216 | 68 | 68 | Indel | 0.25 | 25667 | 0 | 37 | 99.9 |
| 96 | 15 | 77879807 | 77879901 | 95 | 93 | Poly G (5), indel | 0.68 | 34745 | 0 | 432 | 98.8 |
| 97 | 15 | 81625334 | 81625428 | 95 | 95 | Poly T (6) | 0.43 | 35870 | 0 | 40 | 99.9 |
| 98 | 15 | 85438263 | 85438334 | 72 | 71 | Indel | 0.65 | 26762 | 0 | 76 | 99.7 |
| 99 | 15 | 89817413 | 89817503 | 91 | 91 | N/A | 0.36 | 34286 | 0 | 112 | 99.7 |
| 100 | 15 | 89864274 | 89864343 | 70 | 70 | Indel | 0.56 | 26449 | 0 | 11 | >99.9 |
| 101 | 16 | 1894910 | 1894972 | 63 | 63 | N/A | 0.27 | 23809 | 0 | 5 | >99.9 |
| 102 | 16 | 28997904 | 28997998 | 95 | 95 | Poly C (5) | 0.67 | 35860 | 0 | 50 | 99.9 |
| 103 | 16 | 53682908 | 53682994 | 87 | 87 | TA(3) | 0.41 | 32835 | 0 | 60 | 99.8 |
| 104 | 16 | 57954406 | 57954509 | 104 | 104 | Poly C (5) | 0.67 | 39177 | 0 | 144 | 99.6 |
| 105 | 16 | 85706375 | 85706465 | 91 | 91 | Poly T (5), indel | 0.37 | 34075 | 0 | 323 | 99.1 |
| 106 | 17 | 3563920 | 3564008 | 89 | 89 | GC(3) | 0.64 | 33632 | 0 | 11 | >99.9 |
| 107 | 17 | 3594191 | 3594277 | 87 | 87 | Poly C (5), indel | 0.67 | 32752 | 0 | 134 | 99.6 |
| 108 | 17 | 3970090 | 3970180 | 91 | 91 | Indel | 0.46 | 34343 | 0 | 82 | 99.8 |
| 109 | 17 | 16084945 | 16085037 | 93 | 93 | Indel | 0.26 | 35077 | 0 | 78 | 99.8 |
| 110 | 17 | 33998759 | 33998849 | 91 | 89 | Poly T (5) | 0.54 | 33553 | 0 | 89 | 99.7 |
| 111 | 17 | 39589691 | 39589774 | 84 | 82 | Poly A (13), indel (x2) | 0.29 | 30554 | 53 | 2296 | 92.9 |
| 112 | 17 | 41244394 | 41244484 | 91 | 91 | Poly A (5) | 0.34 | 34360 | 0 | 38 | 99.9 |
| 113 | 17 | 45438866 | 45438957 | 92 | 92 | Poly A (7), AT(3), AT(4), AT(4), indel | 0.26 | 34367 | 0 | 418 | 98.8 |
| 114 | 17 | 61502432 | 61502510 | 79 | 79 | Indel | 0.41 | 29751 | 0 | 119 | 99.6 |
| 115 | 17 | 64023582 | 64023667 | 86 | 86 | Poly T (7) | 0.22 | 32176 | 0 | 340 | 99.0 |
| 116 | 17 | 72308237 | 72308320 | 84 | 84 | GAG(3) | 0.62 | 31604 | 7 | 141 | 99.5 |
| 117 | 18 | 2616456 | 2616522 | 67 | 67 | GA(3) | 0.31 | 25273 | 8 | 45 | 99.8 |
| 118 | 18 | 6980478 | 6980568 | 91 | 91 | N/A | 0.37 | 34386 | 0 | 12 | >99.9 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|--------------------------|------------|---------------|-----------------|----------|-----------------|
| 119 | 18 | 9888026 | 9888094 | 69 | 69 | Poly A (6), TG(3) | 0.43 | 25692 | 0 | 399 | 98.5 |
| 120 | 18 | 38836999 | 38837073 | 75 | 75 | Poly A (5), indel | 0.37 | 27923 | 0 | 893 | 96.9 |
| 121 | 18 | 47405382 | 47405462 | 81 | 81 | CTC(3), indel | 0.47 | 30598 | 0 | 20 | 99.9 |
| 122 | 18 | 54815665 | 54815749 | 85 | 85 | CT(3), indel | 0.45 | 31969 | 0 | 161 | 99.5 |
| 123 | 18 | 59773996 | 59774060 | 65 | 65 | N/A | 0.48 | 24531 | 0 | 48 | 99.8 |
| 124 | 19 | 625143 | 625241 | 99 | 99 | N/A | 0.59 | 37298 | 0 | 124 | 99.7 |
| 125 | 19 | 18121418 | 18121491 | 74 | 74 | N/A | 0.68 | 27881 | 0 | 109 | 99.6 |
| 126 | 19 | 18186574 | 18186643 | 70 | 70 | N/A | 0.64 | 26442 | 0 | 26 | 99.9 |
| 127 | 20 | 746056 | 746149 | 94 | 94 | N/A | 0.61 | 35501 | 0 | 31 | 99.9 |
| 128 | 20 | 10633195 | 10633276 | 82 | 82 | AC(3) | 0.59 | 30951 | 0 | 72 | 99.8 |
| 129 | 20 | 17705633 | 17705708 | 76 | 76 | CT(3) | 0.58 | 28686 | 0 | 42 | 99.9 |
| 130 | 20 | 21766821 | 21766890 | 70 | 70 | GT(3),TG(4), indel | 0.46 | 26372 | 0 | 88 | 99.7 |
| 131 | 20 | 25278421 | 25278521 | 101 | 101 | Indel | 0.63 | 38159 | 0 | 20 | 99.9 |
| 132 | 20 | 50897302 | 50897368 | 67 | 67 | Indel | 0.36 | 25188 | 0 | 544 | 97.9 |
| 133 | 20 | 62331904 | 62331994 | 91 | 88 | Poly G (6) | 0.73 | 32969 | 0 | 309 | 99.1 |
| 134 | 20 | 62690860 | 62690946 | 87 | 87 | Indel | 0.57 | 32818 | 0 | 77 | 99.8 |
| 135 | 21 | 30300823 | 30300888 | 66 | 66 | Indel | 0.35 | 24758 | 9 | 181 | 99.2 |
| 136 | 21 | 33694176 | 33694273 | 98 | 98 | Poly T (6), CA(3) | 0.54 | 36902 | 0 | 160 | 99.6 |
| 137 | 21 | 36710706 | 36710792 | 87 | 87 | GT(3), indel | 0.39 | 32841 | 0 | 48 | 99.9 |
| 138 | 21 | 46644924 | 46644992 | 69 | 69 | Poly A (6), AG(3), indel | 0.32 | 25939 | 0 | 280 | 98.9 |
| 139 | 21 | 46705575 | 46705664 | 90 | 90 | Poly T (5), Poly A (6) | 0.5 | 33942 | 0 | 78 | 99.8 |
| 140 | 22 | 25750774 | 25750873 | 100 | 100 | Indel | 0.63 | 37733 | 0 | 86 | 99.8 |
| 141 | 22 | 32439233 | 32439329 | 97 | 97 | N/A | 0.68 | 36617 | 0 | 49 | 99.9 |
| 142 | 22 | 37409844 | 37409940 | 97 | 97 | Indel | 0.46 | 36525 | 0 | 162 | 99.6 |
| 143 | 22 | 37637596 | 37637694 | 99 | 99 | N/A | 0.6 | 37398 | 0 | 24 | 99.9 |

| Amplicon | Chromosome | Amplicon Start | Amplicon End | Analyzed Fragment Size | Bases in Confident Regions | Amplicon Genomic Content | GC content | Correct Calls | Incorrect Calls | No Calls | % Correct Calls |
|----------|------------|----------------|--------------|------------------------|----------------------------|--------------------------|------------|---------------|-----------------|----------|-----------------|
| 144 | 22 | 47081347 | 47081438 | 92 | 92 | Indel | 0.66 | 34754 | 0 | 22 | 99.9 |
| 145 | X | 15870424 | 15870492 | 69 | 69 | Poly T (5) | 0.26 | 26046 | 0 | 36 | 99.9 |
| 146 | X | 135288543 | 135288611 | 69 | 69 | Poly C (5) | 0.62 | 26019 | 0 | 63 | 99.8 |
| 147 | X | 135290777 | 135290847 | 71 | 71 | N/A | 0.52 | 26780 | 0 | 58 | 99.8 |
| 148 | Y | 2655397 | 2655461 | 65 | 0 | N/A | 0.55 | 0 | 0 | 0 | NA |
| 149 | Y | 2655519 | 2655609 | 91 | 0 | N/A | 0.48 | 0 | 0 | 0 | NA |
| 150 | Y | 2655609 | 2655679 | 71 | 0 | Poly A (5) | 0.37 | 0 | 0 | 0 | NA |

The sequencing results for sample GM12878 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 150 amplicons, 92 amplicons were fully contained within the highly confident genomic regions, 41 amplicons had partial overlap, and 17 amplicons had no overlap in the NIST sequence. This outcome resulted in 10,000 coordinates per replicate for comparison. Non-variant base calls were compared to human genome reference sequence build hg19. The accuracy results are shown in [Table 18](#).

Table 18 Somatic Agreement of GM12878 Sample with NIST Database

| Sample | # Amplicons | Mean Call Rate | TP | FN | TN | FP | PPA | NPA | OPA |
|---------|-------------|----------------|------|----|--------|----|-----|-----|-----|
| GM12878 | 133 | 98.8 | 2808 | 0 | 258488 | 0 | 100 | 100 | 100 |

Based on the data provided by this nine-run Somatic study, the NextSeq 550Dx instrument can consistently sequence:

- ▶ GC content $\geq 19\%$ (all called bases in 378 sequenced amplicons with 19% GC content called correctly with no-call rate of 2.6%)
- ▶ GC content $\leq 87\%$ (all called bases in 378 sequenced amplicons with 87% GC content called correctly with no-call rate of 0.6%)
- ▶ PolyA lengths ≤ 9 (all called bases in 378 sequenced amplicons containing a PolyA repeat of nine nucleotides called correctly with no-call rate of 2.5%)
- ▶ PolyT lengths ≤ 10 (all called bases in 378 sequenced amplicons containing a PolyT repeat of ten nucleotides called correctly with no-call rate of less than 0.1%)
- ▶ PolyG lengths ≤ 6 (all called bases in 2268 sequenced amplicons containing a PolyG repeat of six nucleotides called correctly with no-call rate of 0.5%)
- ▶ PolyC lengths ≤ 6 (all called bases in 756 sequenced amplicons containing a PolyC repeat of six nucleotides called correctly with no-call rate of 0.4%)
- ▶ Dinucleotide repeat lengths $\leq 4x$ (all called bases in 1890 sequenced amplicons containing a 4x dinucleotide repeat were called correctly with no-call rate of 0.9%)
- ▶ Trinucleotide repeat lengths $\leq 5x$ (all called bases in 378 sequenced amplicons containing a 5x trinucleotide repeat were called correctly with no-call rate of 1.4%)
- ▶ Insertion lengths ≤ 23 (all called bases in 378 sequenced amplicons containing a 23-nucleotide insertion called correctly with no-call rate of 0.8%)
- ▶ Deletion lengths ≤ 25 (all called bases in 1134 sequenced amplicons containing a 25-nucleotide deletion called correctly with no-call rate of 0.7%)

Precision

The NextSeq 550Dx instrument precision was determined by testing 13 unique Platinum Genome samples using three instruments, three reagent lots, and three operators to generate nine sequencing runs over five start days. The representative assay, samples, and reference method are the same as described for the germline accuracy study. Precision contributions were determined by variance component analysis using VAF as the response variable and calculating the standard deviations at the component level for the instrument, reagent lot, operator, and start day ([Table 19](#)). The total number of observations used in the analysis for each component of instrument, operator, or reagent lot variability was 699, 176, and 235 for SNVs, insertions, and deletions, respectively.

Table 19 Precision Results for the NextSeq 550Dx Instrument (Standard Deviation)

| Component | Variant Type | Component SD | | Total SD | |
|-----------|--------------|--------------|--------|----------|--------|
| | | Max | Median | Max | Median |
| Lot | SNV | 0.0076 | 0.0002 | 0.0833 | 0.0154 |
| | Insertion | 0.0104 | 0.0000 | 0.0410 | 0.0157 |
| | Deletion | 0.0046 | 0.0005 | 0.0560 | 0.0187 |

| Component | Variant Type | Component SD | | Total SD | |
|------------|--------------|--------------|--------|----------|--------|
| | | Max | Median | Max | Median |
| Instrument | SNV | 0.0114 | 0.0003 | 0.0840 | 0.0153 |
| | Insertion | 0.0138 | 0.0009 | 0.0407 | 0.0161 |
| | Deletion | 0.0079 | 0.0008 | 0.0549 | 0.0187 |
| Operator | SNV | 0.0226 | 0.0008 | 0.0841 | 0.0155 |
| | Insertion | 0.0344 | 0.0010 | 0.0417 | 0.0164 |
| | Deletion | 0.0083 | 0.0013 | 0.0547 | 0.0187 |
| Day | SNV | 0.0277 | 0.0012 | 0.0825 | 0.0160 |
| | Insertion | 0.0235 | 0.0012 | 0.0409 | 0.0169 |
| | Deletion | 0.0271 | 0.0014 | 0.0548 | 0.0188 |

Method Comparison (Sequencing Platform)

Whole blood and FFPE samples were assessed on the NextSeq 550Dx instrument and MiSeqDx instrument using the TruSeq Custom Amplicon Kit Dx germline and somatic workflows. Variant frequency agreement for blood and FFPE samples were evaluated using multiple representative assays. Figure 2 plots the VAF correlation between the two instruments for one representative assay and Table 20 summarizes this correlation by assay panel. Based on the strong correlation between MiSeqDx 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 TruSeq Custom Amplicon Kit Dx package insert for additional details.

Figure 2 VAF Correlation of MiSeqDx to NextSeq 550Dx Instruments for FFPE (Left) and Blood (Right) Samples Using Assay 1

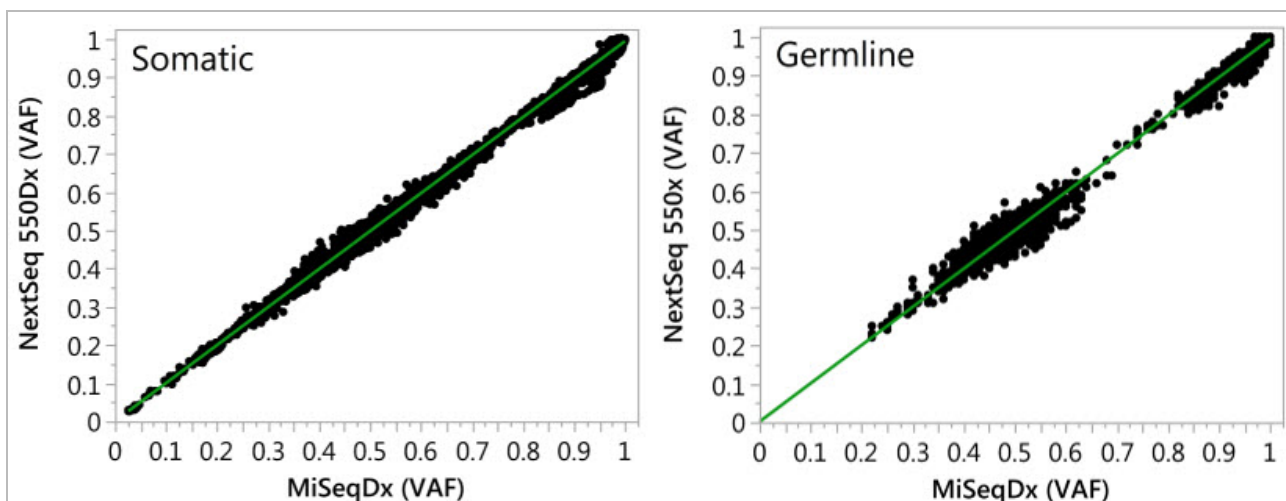


Table 20 Method Comparison Results Using Unique Blood and FFPE Samples

| Source of gDNA | Assay (Oligo Panel) | Biological Replicates (Samples) | Technical Replicates (per Sample) | Observations (# of Variants) | Slope | Intercept | Correlation (R ²) |
|----------------|---------------------|---------------------------------|-----------------------------------|------------------------------|-------|-----------|-------------------------------|
| Blood | Assay 1 | 45 | 2 | 8369 ¹ | 0.992 | 0.002 | 0.995 ² |
| Blood | Assay 2 | 45 | 2 | 5457 | 0.995 | 0.005 | 0.981 |
| FFPE | Assay 1 | 46 | 2 | 8319 | 0.993 | 0.000 | 0.997 ² |
| FFPE | Assay 3 | 40 | 1 | 280 | 0.969 | 0.015 | 0.978 |

¹Two data points were removed based on stated limitation for the Germline Variant module.

²Coefficient of determination for the VAF plots as illustrated in Figure 2.

Reproducibility

The reproducibility of the NextSeq 550Dx instrument was evaluated using Platinum Genome samples with a representative assay designed to query a variety of genes covering 12,588 bases across 23 different chromosomes using 150 amplicons. Germline testing consisted of seven replicates of 13 samples; somatic testing consisted of six replicates of seven samples at different VAF levels. Samples were prepared using the TruSeq Custom Amplicon Kit Dx.

Testing was performed at three external sites using one lot of NextSeq 550Dx High Output Reagent Kit v2 (300 cycles). A single NextSeq 550Dx 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 runs across the three sites. This testing resulted in 18 runs for each the germline and somatic workflows.

Germline

Germline variants with VAF level of ≥ 0.2 are reported as positive (variant). For expected positive germline variants, the data were evaluated for no call rate and correct positive call rate within each variant type (SNV, insertion, deletion). [Table 21](#) 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 21 Germline Call Observations for Expected Positive Results by Variant Type

| Variant Type | No Call | | | Correct Positive Call | | | | |
|--------------|----------|---------|---------|-----------------------|---------|---------|---------|---------|
| | Observed | Total | Percent | Observed | Total | Percent | 95% LCL | 95% UCL |
| SNV | 16 | 110,376 | 0.014 | 110,349 | 110,360 | 99.99 | 99.98 | 99.99 |
| Insertions | 1026 | 37,044 | 2.77 | 36,018 | 36,018 | 100 | 99.99 | 100.00 |
| Deletions | 648 | 34,776 | 1.86 | 34,128 | 34,128 | 100 | 99.99 | 100.00 |

Germline variants with VAF level of < 0.2 are reported as negative (wild-type). For expected negative germline locations, the data were evaluated for no call and correct wild-type call rates. [Table 22](#) summarizes the observed rates, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method.

Table 22 Germline Call Observations for Expected Negative Results

| Variant Type | No Call | | | Correct Negative Call | | | | |
|--------------|----------|------------|---------|-----------------------|------------|---------|---------|---------|
| | Observed | Total | Percent | Observed | Total | Percent | 95% LCL | 95% UCL |
| Wild-type | 4883 | 19,600,182 | 0.025 | 19,595,299 | 19,595,299 | 100 | 100.00 | 100.00 |

Germline variants with VAF level ≥ 0.2 and < 0.7 are called positive heterozygous for the variant, and variants with VAF level ≥ 0.7 are called positive homozygous for the variant. Germline samples with heterozygous variants were used to determine if the inherent variability of the assay would affect the genotype call. The Cx was determined for both cutoffs (0.2 for heterozygous and 0.7 for homozygous genotypes), where x is the proportion of repeated tests that exceed the cutoff. For the lower cutoff of 0.2 VAF, the Cx was $\geq 99.999\%$, indicating that $\geq 99.999\%$ of heterozygous variants would be called heterozygous. With respect to the upper cutoff of 0.7 VAF, the Cx was $\leq 0.001\%$ thereby indicating that $\leq 0.001\%$ of heterozygous variants would be called homozygous. [Table 23](#) summarizes the results by variant type.

Germline variants with VAF level ≥ 0.2 and < 0.7 are called positive heterozygous for the variant, and variants with VAF level ≥ 0.7 are called positive homozygous for the variant. Germline samples with heterozygous variants were used to determine if the inherent variability of the assay would affect the genotype call. The Cx was determined for both cutoffs (0.2 for heterozygous and 0.7 for homozygous genotypes), where x is the proportion of repeated tests that exceed the cut off. With respect to the lower cutoff of 0.2 VAF, the Cx was $\geq 99.999\%$ thereby indicating that \geq

99.999% of heterozygous variants would be called heterozygous. For the upper cutoff of 0.7 VAF, the Cx was $\leq 0.001\%$, indicating that $\leq 0.001\%$ of heterozygous variants would be called homozygous. Table 23 summarizes the results by variant type.

Table 23 Germline Cx Values for Heterozygous Variants

| Variant Type | Cutoff At 0.2 VAF | Cutoff At 0.7 VAF |
|--------------|-------------------|-------------------|
| | $\geq C99.999\%$ | $\leq C0.001\%$ |
| SNV | 94/94 | 94/94 |
| Insertions | 24/24 | 24/24 |
| Deletions | 35/35 | 35/35 |
| Total | 153 | 153 |

Somatic

Somatic variants with VAF levels ≥ 0.026 are reported as positive (variant). Observations with VAF levels ≥ 0.01 and < 0.026 were considered as equivocal for the purposes of this analysis (neither positive nor negative, flagged as low variant frequency). To assess the performance, the results were calculated in three ways:

- ▶ Best case: Any equivocal result was considered a correct positive call (agreement with the expected results)
- ▶ Worst case: Any equivocal result was considered an incorrect call (disagreement with the expected results)
- ▶ Exclusion case: Any equivocal result was excluded from the analysis

Three tables, Table 24, Table 25, and Table 26, summarize the call results for the best case, worst case and exclusion case, respectively, along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method.

Table 24 Somatic Call Observations for Expected Positive Results by Variant Type (Best Case)

| Variant type | Correct Positive Call | | | | |
|--------------|-----------------------|--------|---------|---------|---------|
| | Observed | Total | Percent | 95% LCL | 95% UCL |
| SNV | 54,346 | 54,346 | 100 | 99.99 | 100.00 |
| Insertions | 18,036 | 18,036 | 100 | 99.98 | 100.00 |
| Deletions | 18,381 | 18,381 | 100 | 99.98 | 100.00 |

Table 25 Somatic Call Observations for Expected Positive Results by Variant Type (Worst Case)

| Variant Type | Correct Positive Call | | | | |
|--------------|-----------------------|--------|---------|---------|---------|
| | Observed | Total | Percent | 95% LCL | 95% UCL |
| SNV | 54,346 | 54,346 | 100 | 99.99 | 100.00 |
| Insertions | 18,000 | 18,036 | 99.8 | 99.72 | 99.86 |
| Deletions | 18,381 | 18,381 | 100 | 99.98 | 100.00 |

Table 26 Somatic Call Observations for Expected Positive Results by Variant Type (Equivocal Calls Removed)

| Variant Type | Correct Positive Call | | | | |
|--------------|-----------------------|--------|---------|---------|---------|
| | Observed | Total | Percent | 95% LCL | 95% UCL |
| SNV | 54,346 | 54,346 | 100 | 99.99 | 100.00 |
| Insertions | 18,000 | 18,000 | 100 | 99.98 | 100.00 |
| Deletions | 18,381 | 18,381 | 100 | 99.98 | 100.00 |

Somatic variants with VAF level < 0.01 are reported as negative (wild-type) calls. For expected negative somatic locations, the data were evaluated for no call rate and correct wild-type call rate. Correct wild-type calls were determined by excluding the no calls and subtracting the observed calls that fell into the equivocal zone (VAF levels ≥ 0.01 and < 0.026) as well as the incorrect calls that were above the cutoff (VAF levels ≥ 0.026) from the

total. [Table 27](#) summarizes the observed, total, and percentage results for negative somatic locations for no call rate and correct wild-type call rate along with the lower and upper 95% confidence levels (LCL/UCL) calculated using the Wilson Score method.

Table 27 Somatic Call Observations for Expected Negative Results

| Variant Type | No Call | | | Correct Call | | | | | | |
|--------------|----------|-----------|---------|--------------|-----------|-----------|-----------|---------|---------|---------|
| | Observed | Total | Percent | Equivocal | Incorrect | Correct | Total | Percent | 95% LCL | 95% UCL |
| Wild-type | 36,326 | 8,909,676 | 0.408 | 2254 | 121 | 8,870,975 | 8,873,350 | 99.97 | 99.972 | 99.974 |

Somatic samples at different VAF levels for the same variant were evaluated to determine the C95 of the assay (within each variant type). To evaluate variability near assay cutoff, samples that had expected VAF levels between 0.02 and 0.07 were used. The C95 was determined for each variant, with the highest C95 for each variant type reported in [Table 28](#).

Table 28 Somatic C95 Summary

| Variant Type | N | C95 |
|--------------|----|--------|
| SNV | 74 | 0.0613 |
| Insertion | 24 | 0.0573 |
| Deletion | 33 | 0.0575 |

NextSeq 550Dx High Output Reagent Kit v2.5 (300 Cycle) Performance

Overview

The NextSeq 550Dx is supported by two reagent kits: the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) and the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles). To demonstrate that the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) can meet analytical performance requirements verified and validated with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles), studies were conducted with the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles). Two library preparations using the TruSeq Custom Amplicon Kit Dx were performed, one with the Germline workflow and the other with the Somatic workflow. Libraries from each workflow were tested with three lots of NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) using three NextSeq 550Dx instruments. In addition, testing for each workflow included a single run with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles).

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

Verification with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) demonstrated that the NextSeq 550Dx instrument could detect variants at 0.05 VAF with a Type II error ≤ 0.05 and that the 0.026 VAF cutoff used by the Somatic Variant Module (effective LoB) supports a Type I error ≤ 0.01 . Based on these claims it is expected that a variant at 0.05 VAF is greater than or equal to 0.026 VAF 95% of the time and that a wild-type position is less than 0.026 VAF 99% of the time. To make sure that these claims were met with the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles), repeated measurements were conducted on the NextSeq 550Dx instrument with wild-type samples (LoB samples) and with samples containing variants at 0.05 VAF (LoD Samples) using the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles). The proportion of calls above and below the 0.026 cutoff were then compared to the claims established with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles).

Testing included two LoD samples each with a unique set of variants targeted to 0.05 VAF and corresponding LoB samples that were wild-type for targeted variants. For library preparation, LoD and LoB samples were processed in replicates of eight and seven, respectively, using the TruSeq Custom Amplicon Kit Dx. Libraries were initially sequenced using the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) to identify variants/genomic coordinates for LoB/LoD evaluation with the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles). All variants

with average VAF between 0.045 - 0.055 (LoD Variants) based on the results from the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) were used for LoD analysis (N = 51 variants). For LoB analysis, the 51 corresponding genomic coordinates were assessed.

For evaluation of the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles), libraries were sequenced in three runs over three consecutive days using the same instrument and reagent kit lot. This testing amounted to 24 replicates for each of the 51 LoD variants and 21 replicates for each of the corresponding wild-type positions. The proportion of wild-type calls with VAF < 0.026 are provided in [Table 29](#). The proportion of LoD variant calls with VAF greater than or equal to 0.026 are provided in [Table 30](#).

Table 29 Proportion of Calls < 0.026 for Wild-type Positions (LoB Claim Evaluation)

| Variant Type | Positions Evaluated | Total Observations | # of VAF Measurements $\geq 2.6\%$ | Proportion < 2.6% | Proportion 95% Confidence Interval |
|--------------|---------------------|--------------------|------------------------------------|-------------------|------------------------------------|
| SNV | 32 | 672 | 0 | 1 | 0.994 - 1 |
| Insertion | 11 | 231 | 0 | 1 | 0.984 - 1 |
| Deletion | 8 | 168 | 0 | 1 | 0.978 - 1 |

Table 30 Proportion of Calls ≥ 0.026 VAF for LoD Variants (LoD Claim Evaluation)

| Variant Type | Positions Evaluated | Total Observations | # of VAF Measurements < 2.6% | # of VAF Measurements $\geq 2.6\%$ | Proportion $\geq 2.6\%$ | Proportion 95% Confidence Interval |
|--------------|---------------------|--------------------|------------------------------|------------------------------------|-------------------------|------------------------------------|
| SNV | 32 | 768 | 1 | 767 | 0.999 | 0.993 - 1 |
| Insertion | 11 | 264 | 3 | 261 | 0.989 | 0.967 - 0.996 |
| Deletion | 8 | 192 | 2 | 190 | 0.99 | 0.963 - 0.997 |

Accuracy

Germline

The following study was conducted to assess the variant call accuracy with the Germline Variant Module using the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles). Twelve unique Platinum Genome samples were tested using a representative assay. A total of 11 runs were performed using three NextSeq 550Dx instruments and three of the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles).

Accuracy was determined for SNVs, insertions, and deletions by comparing the results to a well-characterized composite reference method, Platinum Genomes version 2016-1.0. Accuracy results from a single sequencing run with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) are provided for reference. A summary of the results is provided in [Table 31](#).

Table 31 Summary of Germline Agreement

| Criteria | Total Observations (v2.5) ¹ | Result by Observation (v2.5) ² | Result by Observation (v2) ³ | Result by Run (v2.5) ⁴ | Result by Run (v2) ⁴ |
|--------------------|--|---|---|-----------------------------------|---------------------------------|
| PPA for SNV | 1056 | 98.7 | 98.7 | >99.9 | >99.9 |
| PPA for insertions | 1056 | 100 | 100 | 100 | 98.9 |
| PPA for deletions | 1056 | 95.2 | 95.2 | >99.9 | 100 |
| NPA | 1056 | 100 | 100 | 100 | 100 |
| OPA | 1056 | >99.9 | >99.9 | >99.9 | >99.9 |

¹Calculated as number of samples per run x number of runs (96 samples per run x 11 runs = 1056 observations).

²Lowest observed value by sample replicate across all runs (based on 11 runs for NextSeq 550Dx High Output Reagent Kit v2.5).

³Lowest observed value by sample replicate across 1 run (96 total observations).

⁴ Lowest value when data from each run are analyzed in aggregate.

Somatic

The following study was conducted to assess the variant calling accuracy of the Somatic Variant Module on the NextSeq 550Dx instrument using the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles). Ten Platinum Genome FFPE samples (two with variants diluted down to 0.05 VAF) were tested using a representative assay. A total of 11 runs were performed using three NextSeq 550Dx instruments and three lots of the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles).

Accuracy was determined for SNVs, insertions, and deletions by comparing the results to a well-characterized composite reference method, Platinum Genomes version 2016-1.0. Accuracy results from a single sequencing run with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) are provided for reference. A summary of the results is provided in [Table 32](#).

Table 32 Summary of Somatic Agreement

| Criteria | Total Observations (v2.5) ¹ | Result by Observation (v2.5) ² | Result by Observation (v2) ³ | Result by Run (v2.5) ⁴ | Result by Run (v2) ⁴ |
|--------------------|--|---|---|-----------------------------------|---------------------------------|
| PPA for SNV | 528 | 100 | 100 | 100 | 100 |
| PPA for insertions | 528 | 96.9 | 96.9 | > 99.9 | > 99.9 |
| PPA for deletions | 528 | 100 | 100 | 100 | 100 |
| NPA | 528 | > 99.9 | > 99.9 | > 99.9 | > 99.9 |
| OPA | 528 | > 99.9 | > 99.9 | > 99.9 | > 99.9 |

¹ Calculated as number of samples per run x number of runs (48 samples per run x 11 runs = 528 observations).

² Lowest observed value by sample replicate across all runs (based on 11 runs for NextSeq 550Dx High Output Reagent Kit v2.5).

³ Lowest observed value by sample replicate across 1 run (96 total observations).

⁴ Lowest value when data from each run are analyzed in aggregate.

Precision

Germline

The precision of the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) with the Germline Variant Module was evaluated using Platinum Genome samples and a representative assay. Testing consisted of a single library preparation using the TruSeq Custom Amplicon Kit Dx and included 12 samples processed with eight replicates each. Libraries were sequenced with three lots of the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) and three NextSeq 550Dx instruments for a total of nine sequencing runs.

Samples with heterozygous variants were used to determine if the inherent variability of the assay would affect the genotype call (N = 153 unique heterozygous variants). The Cx was determined for both Germline Variant Module cutoffs (0.2 for heterozygous and 0.7 for homozygous genotypes), where x is the proportion of repeated tests that exceed the cutoff. For the lower cutoff of 0.2 VAF, the variant with the minimum Cx for the NextSeq 550Dx Reagent Kit v2.5 (300 cycles) was > 99.9%, indicating that > 99.9% of heterozygous variants would be called heterozygous. For the upper cutoff of 0.7 VAF, the variant with the maximum Cx for the NextSeq 550Dx Reagent Kit v2.5 (300 cycles) was < 1.5%, indicating that ≤ 1.5% of heterozygous variants would be called homozygous. [Table 33](#) summarizes the results by variant type. Cx values from the single sequencing run using the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) are provided for reference.

Table 33 Germline Cx Values for Heterozygous Variants

| Variant Type | N | Cutoff at 0.2 VAF | | Cutoff at 0.7 VAF | |
|--------------|----|----------------------------|--------------------------|----------------------------|--------------------------|
| | | Min Cx (v2.5) ¹ | Min Cx (v2) ² | Max Cx (v2.5) ¹ | Max Cx (v2) ² |
| SNV | 94 | >99.9% | >99.9% | 1.5% | 1.0% |
| Insertions | 24 | 100% | 100% | 0% | <0.1% |
| Deletions | 35 | 100% | >99.9% | <0.1% | <0.1% |

¹Cx values based on Total Standard Deviation estimates from variance component analysis.

²Cx values based on Sample Standard Deviations.

Somatic

The precision of the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) with the Somatic Variant Module was evaluated using Platinum Genome FFPE samples and a representative assay. Testing consisted of a single library preparation using the TruSeq Custom Amplicon Kit Dx and included two samples with eight replicates each. Libraries were sequenced using three lots of NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) and three NextSeq 550Dx instruments for a total of nine sequencing runs.

Somatic variants with expected VAF levels ≤ 0.10 VAF (N = 131 unique variants) were used to evaluate instrument variability near the Somatic Variant Module VAF cutoff (somatic variants with VAF level ≥ 0.026 are called positive for the variant). C95 values were determined for each of the somatic variants. C95 values represent the VAF at which the probability of being greater than the Somatic Variant Module VAF cutoff is 95%. The highest C95 values by variant type are reported in [Table 34](#). C95 results from a single sequencing run using the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) are provided for reference.

Table 34 Somatic C95 Summary

| Variant Type | # of Variants Evaluated | C95 (v2.5) ¹ | C95 (v2) ² |
|--------------|-------------------------|-------------------------|-----------------------|
| SNV | 74 | 0.064 | 0.063 |
| Insertions | 24 | 0.062 | 0.061 |
| Deletions | 33 | 0.060 | 0.060 |

¹C95 values based on Total Standard Deviation estimates from variance component analysis.

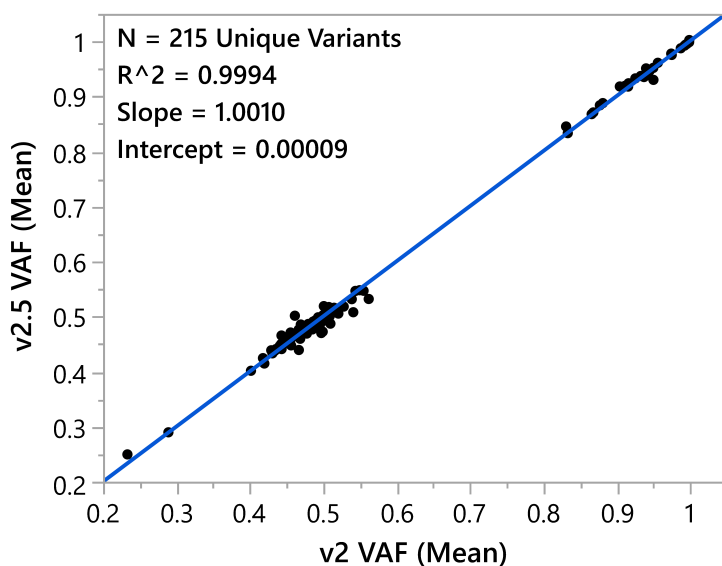
²C95 values based on Sample Standard Deviations.

Method Comparison (Reagent Kit)

Germline

The average VAFs from 215 unique variants were evaluated across the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) and the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) using results generated from the Germline Variant Module. VAF averages were calculated from 11 sequencing runs (v2.5) and one sequencing run (v2). At least eight replicates were used to calculate the average for each variant. [Figure 3](#) plots the VAF correlation between the two reagent kits. Based on the strong linear VAF correlation and the similarity in results between reagent kits, performance characteristics initially verified and validated with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) with the Germline Variant Module are determined to be applicable to the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles).

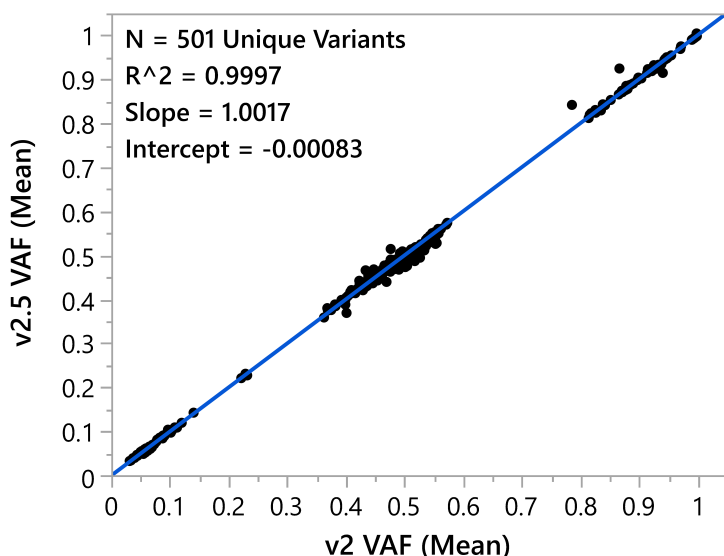
Figure 3 Germline Variant Module Variant Allele Frequency (VAF) correlation between NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) and the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles).



Somatic

The average VAFs for 501 unique variants were evaluated across the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) and the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles) using results generated from the Somatic Variant Module. VAF averages were calculated from 11 sequencing runs (v2.5) and one sequencing run (v2). At least three replicates were used to calculate the average for each unique variant. Figure 4 plots the VAF correlation between the two reagent kits. Based on the VAF correlation and the similarity in results between reagent kits, performance characteristics verified and validated with the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) with the Somatic Variant Module are determined to be applicable to the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles).

Figure 4 Somatic Variant Module Variant Allele Frequency (VAF) correlation between the NextSeq 550Dx High Output Reagent Kit v2 (300 cycles) and the NextSeq 550Dx High Output Reagent Kit v2.5 (300 cycles).



Revision History

| Document | Date | Description of Change |
|------------------------------|-------------|---|
| Document # 1000000030326 v04 | August 2021 | Added Revision History table. Updated EU Authorized Representative address. |

Patents and Trademarks

This document and its contents are proprietary to Illumina, Inc. and its affiliates ("Illumina"), and are intended solely for the contractual use of its customer in connection with the use of the product(s) described herein and for no other purpose. This document and its contents shall not be used or distributed for any other purpose and/or otherwise communicated, disclosed, or reproduced in any way whatsoever without the prior written consent of Illumina. Illumina does not convey any license under its patent, trademark, copyright, or common-law rights nor similar rights of any third parties by this document.

The instructions in this document must be strictly and explicitly followed by qualified and properly trained personnel in order to ensure the proper and safe use of the product(s) described herein. All of the contents of this document must be fully read and understood prior to using such product(s).

FAILURE TO COMPLETELY READ AND EXPLICITLY FOLLOW ALL OF THE INSTRUCTIONS CONTAINED HEREIN MAY RESULT IN DAMAGE TO THE PRODUCT(S), INJURY TO PERSONS, INCLUDING TO USERS OR OTHERS, AND DAMAGE TO OTHER PROPERTY, AND WILL VOID ANY WARRANTY APPLICABLE TO THE PRODUCT(S).

ILLUMINA DOES NOT ASSUME ANY LIABILITY ARISING OUT OF THE IMPROPER USE OF THE PRODUCT(S) DESCRIBED HEREIN (INCLUDING PARTS THEREOF OR SOFTWARE).

© 2020 Illumina, Inc. All rights reserved.

All trademarks are the property of Illumina, Inc. or their respective owners. For specific trademark information, see www.illumina.com/company/legal.html.

Contact Information



Illumina
5200 Illumina Way
San Diego, California 92122 U.S.A.
+1.800.809.ILMN (4566)
+1.858.202.4566 (outside North
America)
techsupport@illumina.com
www.illumina.com



Illumina Netherlands B.V.
Steenoven 19
5626 DK Eindhoven
The Netherlands

Australian Sponsor

Illumina Australia Pty Ltd
Nursing Association Building
Level 3, 535 Elizabeth Street
Melbourne, VIC 3000
Australia

Product Labeling

For a complete reference to symbols that may appear on product packaging and labeling, refer to the symbol key at support.illumina.com on the *Documentation and Literature* tab for your kit.