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Large-Scale Analytical Validation of the Genexus™ Dx Sequencer

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INTRODUCTION

The Ion Torrent™ Genexus™ Dx Integrated Sequencer is part of the Genexus™ Dx System, a turnkey next-generation sequencing (NGS) solution. Comprised of two instruments, the Genexus™ Dx Purification System and the Genexus Dx Integrated Sequencer, the Genexus Dx System enables a workflow from biological specimen all the way to the final report as shown in Figure 1. The Ion Torrent Genexus Purification System automates nucleic acid extraction, purification, and quantitation on a single platform to provide a consistent and efficient workflow solution for next-generation sequencing (NGS) sample preparation. The Genexus Dx Integrated Sequencer automates NGS library preparation, templating and sequencing. Genexus Dx software links the two instruments, tracks the sample information and provides a report.

The Ion Torrent Oncomine™ Dx Express Test (ODxET) is a newly FDA-approved qualitative *in vitro* diagnostic test that uses targeted next-generation sequencing (NGS) technology to detect deletions, insertions, substitutions and copy number gain in 42 genes, and fusions and structural/splice variants in 18 genes from DNA and RNA extracted from FFPE tumor tissue samples. ODxET uses the Genexus Dx Integrated System.

The implementation of automated NGS platforms in clinical molecular pathology requires rigorous validation of analytical accuracy and reproducibility. This study evaluates the Genexus Dx Integrated Sequencer's performance for FFPE clinical samples.

MATERIALS AND METHODS

A retrospective blinded analytical accuracy study was conducted using 462 formalin-fixed paraffin embedded (FFPE) tumor samples representing 16 tumor types conducted across two investigative test sites. Each site tested the same set of 462 FFPE tumor samples. The sample set included 280 variant-positive samples for single nucleotide variants (SNVs) and insertion-deletion variants (INDELs), 60 for copy number variants (CNVs), 64 for fusions/RNA splice variants, and 58 wild-type (WT) samples. Variant calling results from the Genexus Dx Integrated Sequencer were compared to those from a well-characterized reference NGS assay.

The assay reproducibility study tested the repeatability and reproducibility of the assay across multiple reagent lots (3), sites (3), operators (2 per site), and instruments (4 per site). The study samples consisted of 21 variant positive DNA blends, 19 variant positive RNA blends, and 9 WT FFPE samples from 11 tumor types. Eleven SNVs, 3 insertions, 5 deletions, 11 CNVs, 17 RNA fusions, and 2 alternative RNA splicing variants were selected for the study.

Clinical samples containing targeted variants, as well as DNA and RNA from WT samples, were used to create sample blends for testing of each variant at 1 target level (1x−1.5x) the limit of detection (LoD).

In total, the study produced 195 valid system runs (180 planned runs and 15 runs for sample repeats) with each DNA and RNA sample blend producing 72 data points for each target variant at targeted level. The sequencing data generated from all valid system runs was used to establish the reproducibility performance of the ODxET with respect to each sample blend and each target variant tested. The statistical analysis plan included the following metrics: call rates, within run repeatability, percent agreements, system run and sample accountability, and variant component analyses.

An accredited Institutional Review Board (IRB) reviewed and approved the study protocols prior to the start of any testing activities. All study approval documentations from the IRB were forwarded to the Principal Investigator and Study Coordinator at each Test Site. The study protocol approval documentation was kept on file at the Test Site prior to initiation of the study and testing activities.

All patients provided informed consent for the use of their biopsy specimens in this clinical validation study. The informed consent was kept on file with the pharmaceutical partner, or the commercial vendor and model consent forms were submitted to the IRB as part of the submission documentation for study review.

ANALYTICAL ACCURACY RESULTS

To evaluate the analytical accuracy of ODxET for detecting SNVs, INDELs, CNVs, fusions, and RNA splice variants in FFPE clinical tumor samples, ODxET results were compared with those obtained from validated orthogonal methods. In this study, Level 2 and Level 3 SNVs, INDELs, CNVs, fusions, and RNA splice variants across various tumor types were evaluated. The samples were prescreened for target variants using an orthogonal method similar to ODxET. The study included 397 SNVs/multi nucleotide variants (MNVs), 5 insertions, 23 deletions, 114 CNVs, and 47 fusion or splice samples. Additionally, 58 unique WT samples and 42 samples pre-identified as negative for entire variant classes were evaluated. Of these, 36 WT samples and 40 variant class-negative samples were included in the concordance analysis. A total of 462 FFPE tumor samples representing 15 tumor types was tested.
Concordance analysis was performed at the variant level using results from both ODxET and the orthogonal test. PPA and NPA estimates, along with their respective two-sided 95% CIs (confidence intervals), were calculated using the orthogonal method as the reference. The accuracy results, excluding unknown results, are summarized by variant type and further stratified according to the FDA's Biomarker Class Level for tumor profiling NGS tests, as shown in Table 1.

Table 1. Accuracy results for ODxET tumor profiling variants by variant type and clinical significance

Variant Type[1]	Total Unique Variants/ Genes	ODxET+, Comparator+	ODxET+, Comparator-	ODxET-, Comparator+	ODxET-, Comparator-	PPA (n/N) [95% CI][2]	NPA (n/N) [95% CI][2]
All Variants (SNV + CNV + Fusion)	2944/46	586	112	11	800035	98.2% (586/597) 95% CI: (96.7%, 99.0%)	100.0% (800035/800147) 95% CI: (100.0%, 100.0%)
Level 2 Variants	1544/22	190	19	0	116800	100.0% (190/190) 95% CI: (98.0%, 100.0%)	100.0% (116800/116819) 95% CI: (100.0%, 100.0%)
Level 3 Variants	1400/43	396	93	11	683235	97.3% (396/407) 95% CI: (95.2%, 98.5%)	100.0% (683235/683328) 95% CI: (100.0%, 100.0%)
All SNVs	1324/42	397	47	2	387643	99.5% (397/399) 95% CI: (98.2%, 99.9%)	100.0% (387643/387690) 95% CI: (100.0%, 100.0%)
Level 2 SNV	265/17	137	4	0	10594	100.0% (137/137) 95% CI: (97.3%, 100.0%)	100.0% (10594/10598) 95% CI: (99.9%, 100.0%)
Level 3 SNV	1059/38	260	43	2	377049	99.2% (260/262) 95% CI: (97.3%, 99.8%)	100.0% (377049/377092) 95% CI: (100.0%, 100.0%)
All MNVs + Complex	287/14	0	0	0	6466	N/E[3]	100.0% (6466/6466) 95% CI: (99.9%, 100.0%)
Level 2 MNVs + Complex	210/12	0	0	0	343	N/E	100.0% (343/343) 95% CI: (98.9%, 100.0%)
Level 3 MNVs + Complex	76/19	0	0	0	6123	N/E	100.0% (6123/6123) 95% CI: (99.9%, 100.0%)
All Insertions	7/11	5	1	0	61874	100.0% (5/5) 95% CI: (56.6%, 100.0%)	100.0% (61874/61875) 95% CI: (100.0%, 100.0%)
Level 2 Insertions	52/3	0	0	0	2772	N/E	100.0% (2772/2772) 95% CI: (99.9%, 100.0%)
Level 3 Insertions	24/9	5	1	0	59102	100.0% (5/5) 95% CI: (56.6%, 100.0%)	100.0% (59102/59103) 95% CI: (100.0%, 100.0%)
All Deletions	258/14	23	2	0	78635	100.0% (23/23) 95% CI: (85.7%, 100.0%)	100.0% (78635/78637) 95% CI: (100.0%, 100.0%)
Level 2 Deletions	206/5	14	1	0	9292	100.0% (14/14) 95% CI: (78.5%, 100.0%)	100.0% (9292/9293) 95% CI: (99.9%, 100.0%)
Level 3 Deletions	52/13	9	1	0	69343	100.0% (9/9) 95% CI: (70.1%, 100.0%)	100.0% (69343/69344) 95% CI: (100.0%, 100.0%)
All CNV	10/10	114	32	7	4294	94.2% (114/121) 95% CI: (88.5%, 97.2%)	99.3% (4294/4326) 95% CI: (99.0%, 99.5%)
Level 2 CNV	2/2	14	3	0	238	100.0% (14/14) 95% CI: (78.5%, 100.0%)	98.8% (238/241) 95% CI: (96.4%, 99.6%)
Level 3 CNV	8/8	100	29	7	4056	93.5% (100/107) 95% CI: (87.1%, 96.8%)	99.3% (4056/4085) 95% CI: (99.0%, 99.5%)
All RNA Variants	990/18	47	30	2	261123	95.9% (47/49) 95% CI: (86.3%, 98.9%)	100.0% (261123/261153) 95% CI: (100.0%, 100.0%)
Level 2 RNA Variants	809/10	25	11	0	93561	100.0% (25/25) 95% CI: (86.7%, 100.0%)	100.0% (93561/93572) 95% CI: (100.0%, 100.0%)
Level 3 RNA Variants	181/16	22	19	2	167562	91.7% (22/24) 95% CI: (74.2%, 97.7%)	100.0% (167562/167581) 95% CI: (100.0%, 100.0%)

[1] Level 2 = Cancer Mutations with Evidence of Clinical Significance, Level 3 = Cancer Mutations with Potential Clinical Significance.
[2] 95% 2-sided confidence interval calculated via the Wilson Score method.
[3] N/E: Not evaluable. The statistic cannot be calculated as there were no sample variant results in this category.

REPRODUCIBILITY RESULTS

A total of 67 across 58 samples were evaluated (with some samples containing 2 variants), including 41 representative DNA variants (15 SNVs, 6 insertions, 5 deletions, 4 indels, and 11 CNVs), 22 RNA fusions, 3 alternate RNA splice variants, and one imbalance variant. All samples were derived from FFPE tissue samples representing 11 cancer types: bladder, breast, cholangiocarcinoma, colorectal, endometrial, glioma, melanoma, NSCLC, pancreatic, prostate, and thyroid. The study was designed to assess reproducibility (variability across 3 test sites, operators, and instruments) and repeatability (within-run variability) using three lots of ODxET reagents, controls, and Genexus Dx Integrated Sequencer reagents and consumables. Each test site included 2 operators and 2 instruments.
Positive and negative call rates were evaluated for samples containing targeted variants across all variant types detected by ODxET. Results are summarized by variant type in Table 2 and Table 3, respectively. The positive call rate was >97% for all variant types tested near or above the LoD, both including and excluding no calls. The negative call rate was >97% for all variant types, both including and excluding no calls, except for deletions and fusion imbalance at 1–1.5X LoD. For these exceptions, the negative call rates including no calls were 95.27% for deletions and 49.10% for fusion imbalance.

Table 2. Positive call rate for tumor profiling variants by variant class

Variant Type	LoD Level	Total calls	Positive calls	Negative calls	No Calls	Positive Call Rate (No Calls included) 95% Score CI[1]	Positive Call Rate (No Calls Excluded) 95% Score CI[1]
SNV	1–1.5X	1368	1329	26	13	97.15% (96.13%, 97.91%)	98.08% (97.20%, 98.69%)
SNV	2–3X	576	575	0	1	99.83% (99.02%, 99.97%)	100.00% (99.34%, 100.00%)
INSERTION	1–1.5X	504	503	0	1	99.80% (98.88%, 99.96%)	100.00% (99.24%, 100.00%)
INSERTION	2–3X	144	144	0	0	100.00% (97.40%,100.00%)	100.00% (97.40%, 100.00%)
DELETION	1–1.5X	576	561	9	6	97.40% (95.75%, 98.42%)	98.42% (97.03%, 99.17%)
DELETION	2–3X	216	216	0	0	100.00% (98.25%, 100.00%)	100.00% (98.25%, 100.00%)
CNV	1–1.5X	792	789	3	0	99.62% (98.89%, 99.87%)	99.62% (98.89%, 99.87%)
FUSION	1–1.5X	1584	1555	29	0	98.17% (97.38%, 98.72%)	98.17% (97.38%, 98.72%)
FUSION	2–3X	432	432	0	0	100.00% (99.12%, 100.00%)	100.00% (99.12%, 100.00%)
SPLICE VARIANT	1–1.5X	288	287	1	0	99.65% (98.06%, 99.94%)	99.65% (98.06%, 99.94%)
SPLICE VARIANT	2–3X	144	144	0	0	100.00% (97.40%, 100.00%)	100.00% (97.40%, 100.00%)
FUSION IMBALANCE	1–1.5X	72	72	0	0	100.00% (94.93%, 100.00%)	100.00% (94.93%, 100.00%)

Table 3. Negative call rate for tumor profiling variants by variant class

Variant Type	LoD Level	Total Calls	Negative Calls	Positive Calls	No Calls	Negative Call Rate (No Calls Included) 95% Score CI[1]	Negative Call Rate (No Calls excluded) 95% Score CI[1]
SNV	1–1.5X	2964168	2945554	1319	17295	99.37% (99.36%, 99.38%)	99.96% (99.95%, 99.96%)
SNV	2–3X	1164456	1160937	455	3064	99.70% (99.69%, 99.71%)	99.96% (99.96%, 99.96%)
INSERTION	1–1.5X	593064	589125	1	3938	99.34% (99.31%, 99.36%)	100.00% (100.00%, 100.00%)
INSERTION	2–3X	233424	232810	0	614	99.74% (99.72%, 99.76%)	100.00% (100.00%, 100.00%)
DELETION	1–1.5X	783648	746619	0	37029	95.27% (95.23%, 95.32%)	100.00% (100.00%, 100.00%)
DELETION	2–3X	307872	300911	0	6961	97.74% (97.69%, 97.79%)	100.00% (100.00%, 100.00%)
CNV	1–1.5X	6408	6408	0	0	100.00% (99.94%, 100.00%)	100.00% (99.94%, 100.00%)
FUSION	1–1.5X	1825056	1821714	50	3292	99.82% (99.81%, 99.82%)	100.00% (100.00%, 100.00%)
FUSION	2–3X	564984	561681	88	3215	99.42% (99.40%, 99.43%)	99.98% (99.98%, 99.99%)
SPLICE VARIANT	1–1.5X	7200	7099	101	0	98.60% (98.30%, 98.84%)	98.60% (98.30%, 98.84%)
SPLICE VARIANT	2–3X	2160	2160	0	0	100.00% (99.82%, 100.00%)	100.00% (99.82%, 100.00%)
FUSION IMBALANCE	1–1.5X	2880	1414	0	1466	49.10% (47.27%, 50.92%)	100.00% (99.73%, 100.00%)

[1] 95% 2-sided confidence interval calculated via the Wilson Score method

CONCLUSIONS

- This large scale analytical validation of the Genexus Dx Integrated Sequencer produced robust analytical accuracy and assay reproducibility data using FFPE clinical samples. These findings support its suitability for rapid next-generation sequencing.
- The Genexus Dx Integrated Sequencer demonstrated high concordance with the reference assay across all variant classes.
  - For SNV/INDEL detection (n=280), PPA was 99.5% for SNVs and 100% for INDELs and NPA was 100% for both SNVs and INDELs.
  - For CNV detection (n=60), PPA was 94.2% and NPA was 99.3%. For fusion and RNA splice variant detection (n=64), PPA was 95.9% and NPA was 100%. For the PPA estimates, the data were aggregated at the panel wide variant-level for SNV and INDELs, and at the gene-level for CNVs and RNA variants. The NPA was estimated using the samples predefined as WT in the study protocol.
- In the assay reproducibility study, both the positive and negative call rate was >97% for all variant types tested near or above the LoD, both including and excluding no calls. However, there was an exception for deletions and fusion imbalance at 1–1.5X LoD. For these exceptions, the negative call rates including no calls were 95.27% for deletions and 49.10% for fusion imbalance.

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ACKNOWLEDGEMENTS

We are deeply grateful to all the individuals involved in the development of the Genexus Dx system and Oncomine Dx Express Test.

DISCLAIMER

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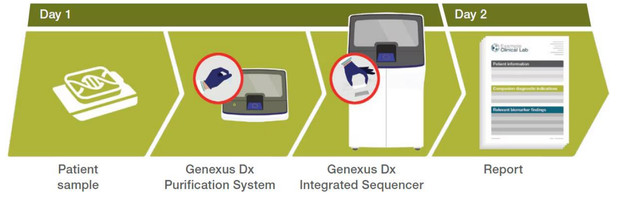

Figure 1. Overview of the Genexus Dx System workflow

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