510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I. Background Information:

A. 510(k) Number:

K202304

B. Applicant

NYU Langone Health (NYU)

C. Proprietary and Established Names:

NYU Langone Genome PACT (Profiling of Actionable **C**ancer Targets)

D. Regulatory Information

Product Code	Classification	Regulation Section	Panel
PZM	**Class II (Special Controls)**	**21 CFR 866.6080**	**88 Pathology**

II. Submission/Device Overview:

A. Purpose for Submission:

New device

B. Measurand:

Somatic single nucleotide variants, and insertions, and deletions (indels) smaller than 35 bp, in human genomic DNA obtained from formalin-fixed, paraffin-embedded tumor tissue.

Refer to Appendix 1 for complete list of genes included in this assay.

C. Type of Test:

Next generation sequencing tumor profiling test

III. Intended Use/Indications for Use:

A. Indications for Use:

The NYU Langone Genome PACT assay is a qualitative *in vitro* diagnostic test that uses targeted next generation sequencing of formalin-fixed paraffin-embedded (FFPE) matched with normal specimens from patients with solid malignant neoplasms to detect tumor gene

alterations in a 607-gene panel. The test is intended to provide information on somatic mutations (point mutations and small insertions and deletions) for use by qualified health care professionals in accordance with professional guidelines, and is not conclusive or prescriptive for labeled use of any specific therapeutic product. NYU Langone Genome PACT is a single-site assay performed at NYU Langone Health.

B. Special conditions for use statement(s):

Rx - For prescription use.

For *in vitro* diagnostic use.

C. Special instrument requirements:

Illumina NextSeq 500/550 Sequencers (qualified by NYU Langone Health).

IV. Device/System Characteristics:

A. Device Description:

A description of required equipment, software, reagents, vendors, and storage conditions were provided, and are described in the product labeling (NYU Langone Genome PACT Standard Operating Procedure manual). NYU Langone Health assumes responsibility for the device.

1. Sample Preparation:
The tumor volume and minimum tumor content needed to obtain sufficient DNA for testing to achieve the necessary quality performance are shown in the Table 1 below:

Table 1. Specimen Handling and Processing for Validated Specimen Types

Tissue Type	Volume	Minimum Tumor Proportion	Macro-dissection requirements (Based on tumor proportion)	Limitations	Storage
FFPE sections	10-20 unstained sections, 5 microns thick	More than 10% of viable tumor cells;	Yes, macrodissection to minimize the % of non- neoplastic tissue in the sample	Archival paraffin-embedded material subjected to acid decalcification is unsuitable for analysis because acid decalcification severely damages nucleic acids.	Room temp

Genomic DNA is extracted from tissue specimens per protocol. DNA is quantified and concentrated if necessary. The amount of DNA required to perform the test is 100-250ng. Tumor and Normal DNA sample is run in singlicate.

2. Library Preparation:
Sequence libraries are prepared using KAPA Biosystems HyperPlus Reagents by first enzymatically fragmenting DNA at 37C for 20 minutes. This product immediately undergoes end-repair via production of blunt-ended, 5'-phosphorylated fragments. To the 3' ends of the dsDNA library fragments, dAMP is added (A-tailing). Next, dsDNA adapters with 3'dTMP is ligated to the A-tailed library fragments. Library fragments with appropriate adapter sequences are amplified via ligation-mediated pre-capture PCR. A quality control check on the amplified DNA libraries is performed: Samples should be a smear; average fragment size of ~291bp**;** and concentration >1.6ng/µL (to ensure adequate hybridization for capture.

3. Hybrid Capture NGS:
Library capture is conducted using xGen Lockdown Capture reagents. Pooled sequencing libraries are hybridized to the vendor oligo pool. Capture beads are used to pull down the complex of capture oligos and genomic DNA fragments. Unbound fragments are washed away. The enriched fragment pool is amplified by ligation-mediated PCR. The success of the enrichment is measured as a quality control step: Samples should be a smear, average fragment size with the peak at ~313bp; the molarity of the amplified DNA pool should be ≥2nM. Reactions can be stored at 4-20°C until ready for purification, up to 1 week.

4. Sequencing and Data Analysis:
Sequencing is conducted with the Illumina NextSeq 500/550 Sequencing Instruments and reagents and PhiX Control v3. The sequencing process uses multiple quality checks. Bioinformatics tools are summarized in the Table 2.

a) *Data Management System (DMS):* Automated sample tracking and archival of run-associated metadata (barcode, run name, samples accession number, patient medical record number, source (class), specimen type, and panel version) is conducted with the following key functions: Tracking sample status through various stages of data analysis; tracking iterations of analysis applied to a given sample; recording versions of databases and algorithms used in analysis; archival of selected pipeline output files (FASTQ, BAM, VCF) and sequencing run statistics (% of undetermined reads per lane, quality scores, reads per sample, number of targets with 0X and <50X coverage, average and median coverage of the Tumor and Normal sample).

b) *Demultiplexing and FASTQ generation:* The analysis pipeline uses software provided by Illumina. Two FASTQ files are generated per samples corresponding to full length forward and reverse reads. Demultiplexing quality control includes quality metrics for per-base sequence quality, sequence content, GC content and sequence length distribution, relative percentages of unmatched indices.

c) *Read alignment and BAM generation:* Spurious adapter sequences are trimmed prior to read alignment. Reads are aligned in paired-end mode to the hg19 b37 version of the human genome. Aligned reads are written to a Sequence Alignment Map (SAM) file, which is then converted into Binary Alignment Map (BAM) format. PCR duplicates are removed. Each base within a read is assigned a base quality score by the sequencing software, which reflects the probability an error was made with the

base call. To account for systemic biases that may not accurately reflect the actual error probabilities observed empirically, the analysis pipeline uses another tool to adjust the reported quality scores based on the selected covariates. Reassigned quality scores are subject to a threshold of 20, corresponding to a 1/100 chance of error.

Table 2. Summary overview of bioinformatics tools (qualified by Langone)

Bioinformatics step
Adapter and low-quality bases trimming
1st pass alignment
Removing duplicate reads
Realigning and recalibrating
Fragment size distribution, capture efficiency, depth of coverage
Analyzing Tumor and Normal samples separately for SNV, insertions and deletions
Analyzing matched Tumor – Normal pairs for SNV, insertions and deletions

d) *Sample QC checks:* The unique combination of homozygous SNPs specific to a given sample serves as a 'fingerprint' for the identity of the corresponding patient and serves to identify potential sample mix-ups and contamination between samples and barcodes. QC checks involving the use of these 'fingerprint' SNPs are detailed below:

i. *Sample mix-up check:* The analysis pipeline identifies the homozygous SNPs in all Tumor and Normal samples. All Tumor-Normal pairs are compared for the matching homozygous variants. The fidelity QC filter is set to show only homozygous variants that fit the fidelity criteria: >200 coverage, VAF >0.98. The Tumor and Normal from the same patient should show >90% overlap of these homozygous SNP variants. Conversely, the concordance between samples from different patients should be low (<25%). Pairs of samples from the same patient with >10% discordance ("unexpected mismatches") can be due to hypermutant tumors with thousands of new mutations due to sun exposure (melanoma) or alkylating chemotherapy agents (glioblastoma). However, these samples are relatively rare, mutations are usually heterozygous, and in case that a clinical run includes such sample, all other Tumor-Normal pairs should still show high fidelity.

ii. *Check for presence of tumor in normal:* Normal samples are expected to be free of known pathogenic hotspot SNVs and insertions and deletions (indels) that are commonly (somatically) recurrent in tumor samples. As a first pass check, the pipeline annotates normal samples at known 'hotspot' locations derived from somatic mutation catalogs. If a known pathogenic tumor-specific mutation (i.e. BRAF V600E, EGFR L858R) is detected with mutation frequency > 1.8% (established false positive rate) in a normal sample, the normal sample is flagged for review and possible exclusion from analysis. If the normal sample shows tumor contamination, the Tumor-Normal pair is excluded from further analysis and DNA from a Tumor sample with matched Normal control is extracted and the analysis repeated.

e) *Mutation calling – SNVs and Indels:* The analysis pipeline identifies two classes of mutations: (1) single nucleotide variants (SNVs) and (2) small insertions and deletions (indels). Paired sample mutation calling is performed on tumor samples and their respective matched normal controls. In this test, a normal DNA is always required for comparison. Filtering is performed to remove low quality sequence data, sources of sequencing artifacts, and germline results.

i. *Analysis of positive and negative controls*: data from controls is used to confirm lack of contamination as well as analytical sensitivity and specificity.

ii. *Filters on sample coverage:* A sequence coverage ≥ 100X is required to achieve 95% power to detect mutations with underlying variant frequency of 10% or greater. To ensure that at least 98% of targeted exons meet this coverage, per sample mean coverage has been conservatively set at ≥ 300X. A lower coverage threshold for the matched normal is set at 100X.

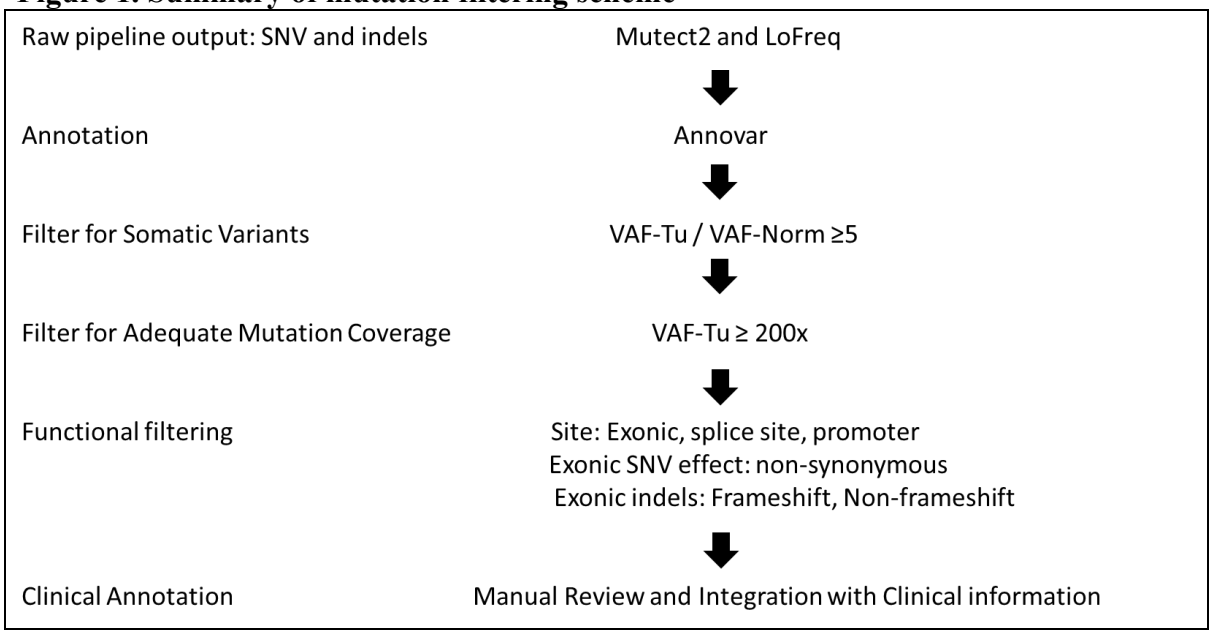
iii. *Filtering for high confidence mutations:* Raw SNV and indel calls are subjected to a series of filtering steps to ensure only high-confidence calls are admitted to the final step of manual review. These parameters include (1) evidence of it being a somatic mutation i.e., the mutation has to be present in ≥ 5% of Tumor reads and less than <1.8% of Normal reads. Mutation has to have coverage of ≥ 200 reads (≥10 mutant reads); in the Normal sample the coverage of the site should be ≥ 100x and <1.8% of Normal reads should show the mutation (<2 reads with mutation) resulting in ration between the Tumor and Normal samples to be >/= 5.0, (2) technical characteristics that use coverage depth (Depth), number of mutant reads, variant allele frequency (VAF).

iv. *Mutation functional and site annotation:* Predicted functional effect and site for each mutation is curated by automated software using information from ANNOVAR.

v. *Confirmation of variants: Variants identified in genes in which less than 20* somatic variants of that type have previously identified are confirmed by Sanger sequencing.

vi. *Reporting:* Somatic variants are reported into two FDA recommended categories. These include Level 2: "Cancer Mutations with Evidence of Clinical Significance" and Level 3: "Cancer Mutations with Potential Clinical Significance". Somatic Tier I variants listed in Oncokb as level 1 and 2 are being reported as Cancer Mutations with Evidence of Clinical Significance. Other Tier I, and all Tier II are being reported as Cancer Mutations with Potential Clinical Significance. The test does not report variants of unknown clinical significance, benign or likely benign variants. The filtering scheme and threshold are shown in Figure 1 below. The threshold values for the filtering criteria were established based on paired-sample mutation analysis on replicates of normal FFPE samples and optimized to accept high confidence mutation calls reject false positive calls.

Figure 1. Summary of mutation filtering scheme



5. Controls:

a) *Matched normal control:* Genomic DNA is extracted from patient-matched normal peripheral blood for use as a matched normal control.

b) *Positive control (PC):* A commercially available, multiplexed mixture of biosynthetic DNA targets precisely blended at 10% each with a single, well-characterized genomic background, is qualified and used by Langone as positive control material. This control is used to assess the performance of the NGS-based somatic mutation assay across a range of genes and mutation types including SNVs, insertions and deletion in 28 genes. The assay tracks 35 SNVs and indels that showed consistent performance across 8 consecutive test runs (Table 4A and 4B).

For each clinical run, the VAF and coverage of Positive Control mutations are reviewed to ensure that the mutations are detected above the coverage and VAF thresholds. Furthermore, the assay tracks the VAF and coverage of PC mutations from run to run and compares them to the pool of previously profiled PC to ensure that they are within a 2 SD range of the pool. The detection of PC mutations must stay within the established sensitivity of the assay. The Positive Control with expected variant frequency (VAF) prior to pooling are shown in Table 3.

Table 3. Positive Controls and Expected Mutation Frequencies

Gene ID	COSMIC Identifier	Mutation Type	HGVS Nomenclature	Amino Acid	Target AF
AKT1	COSM33765	Substitution	c.49G>A	p.E17K	10%
APC	COSM13127	Substitution	c.4348C>T	p.R1450*	10%
APC	COSM18561	Insertion in HP 7N	c.4666_4667insA	p.T1556fs*3	10%
ATM	COSM21924	Deletion	c.1058_1059delGT	p.C353fs*5	10%
ERBB2	COSM682 / 20959	Insertion	c.2324_2325ins12	p.A775_G776insYVMA	10%
GNA11	COSM52969	Substitution	c.626A>T	p.Q209L	10%
GNAQ	COSM28758	SNV in HP 3N	c.626A>C	p.Q209P	10%
KIT	COSM1314	Substitution	c.2447A>T	p.D816V	10%
MPL	COSM18918	Substitution	c.1544G>T	p.W515L	10%
PDGFRA	COSM736	Substitution	c.2525A>T	p.D842V	10%
PIK3CA	COSM763	Substitution	c.1633G>A	p.E545K	10%
SMAD4	COSM14105	Insertion	c.1394_1395insT	p.A466fs*28	10%
CTNNB1	COSM5664	Substitution	c.121A>G	p.T41A	10%
EGFR	COSM6224	SNV in 3N	c.2573T>G	p.L858R	10%
GNAS	COSM27887	Substitution	c.601C>T	p.R201C	10%
JAK2	COSM12600	SNV in HP 3N	c.1849G>T	p.V617F	10%
KRAS	COSM521	Substitution	c.35G>A	p.G12D	10%
NPM1	COSM17559	Insertion	c.863_864insTCTG	p.W288fs*12	10%
NRAS	COSM584	Substitution	c.182A>G	p.Q61R	10%
PTEN	COSM4986	Insertion	c.741_742insA	p.P248fs*5	10%
PTEN	COSM5809	Deletion 6N > 5N	c.800delA	p.K267fs*9	10%
TP53	COSM10648	Substitution	c.524G>A	p.R175H	10%
TP53	COSM10660	Substitution	c.818G>A	p.R273H	10%
TP53	COSM10662	Substitution	c.743G>A	p.R248Q	10%
TP53	COSM6530	Deletion	c.723delC	p.C242fs*5	10%
BRAF	COSM476	Substitution	c.1799T>A	p.V600E	10%
EGFR	COSM12378	Insertion	c.2310_2311insGGT	p.D770_N771insG	10%
EGFR	COSM6225	Deletion	c.2236_2250del15	p.E746_A750delELREA	10%
EGFR	COSM6240	Substitution	c.2369C>T	p.T790M	10%
FGFR3	COSM715	Substitution	c.746C>G	p.S249C	10%
FLT3	COSM783	Substitution	c.2503G>T	p.D835Y	10%
PDGFRA	COSM28053	Insertion	c.1694_1695insA	p.S566fs*6	10%
PIK3CA	COSM12464	Insertion	c.3204_3205insA	p.N1068fs*4	10%
PIK3CA	COSM775	Substitution	c.3140A>G	p.H1047R	10%
RET	COSM965	Substitution	c.2753T>C	p.M918T	10%

Table 4A. Summary of VAF precision for variants in the positive controls, for n consecutive runs (events) within 2 SD.

Variant ID	n	Mean AF	Median AF	Min. AF	Max AF	2SD AF	2SD AF min	2SD AF max	n within 2SD	% within 2SD
AKT1 p.E17K	8	0.10	0.10	0.07	0.12	0.04	0.06	0.14	8	100
APC p.R1450*	8	0.10	0.09	0.09	0.11	0.02	0.08	0.12	8	100
APC p.T1556fs*3	8	0.08	0.08	0.06	0.10	0.02	0.06	0.10	8	100
ATM p.C353fs*5	8	0.09	0.10	0.08	0.11	0.02	0.08	0.11	8	100
BRAF p.V600E	8	0.10	0.10	0.08	0.12	0.02	0.08	0.13	8	100
CTNNB1 p.T41A	8	0.09	0.09	0.06	0.12	0.04	0.05	0.13	8	100
EGFR p.D770_N771insG	8	0.08	0.08	0.07	0.09	0.01	0.07	0.09	8	100
EGFR p.E746_A750delELREA	8	0.06	0.06	0.04	0.07	0.02	0.04	0.08	7	87.5
EGFR p.L858R	8	0.09	0.09	0.08	0.11	0.02	0.07	0.10	7	87.5
EGFR p.T790M	8	0.09	0.09	0.07	0.11	0.03	0.07	0.12	8	100
ERBB2 p.A775_G776insYVMA	8	0.05	0.05	0.04	0.07	0.02	0.03	0.07	7	87.5
FGFR3 p.S249C	8	0.08	0.08	0.05	0.09	0.03	0.05	0.11	7	87.5
FLT3 p.D835Y	8	0.10	0.09	0.08	0.11	0.02	0.08	0.11	8	100
GNA11 p.Q209L	8	0.09	0.10	0.06	0.11	0.04	0.05	0.13	8	100
GNAQ p.Q209P	8	0.09	0.09	0.08	0.10	0.02	0.07	0.11	8	100
GNAS p.R201C	8	0.10	0.10	0.08	0.11	0.02	0.08	0.12	7	87.5
JAK2 p.V617F	8	0.09	0.09	0.09	0.10	0.01	0.08	0.10	8	100
KIT p.D816V	8	0.10	0.10	0.07	0.12	0.03	0.07	0.13	8	100
KRAS p.G12D	8	0.10	0.10	0.07	0.11	0.03	0.07	0.12	8	100
MPL p.W515L	8	0.10	0.10	0.09	0.11	0.01	0.09	0.12	8	100
NPM1 p.W288fs*12	8	0.07	0.07	0.05	0.08	0.02	0.04	0.09	8	100
NRAS p.Q61R	8	0.09	0.09	0.06	0.10	0.02	0.06	0.11	8	100
PDGFRA p.D842V	8	0.10	0.10	0.08	0.11	0.02	0.08	0.12	8	100
PDGFRA p.S566fs*6	8	0.10	0.10	0.08	0.11	0.02	0.08	0.12	8	100
PIK3CA p.E545K	8	0.08	0.08	0.08	0.09	0.01	0.07	0.10	8	100
PIK3CA p.H1047R	8	0.09	0.09	0.08	0.10	0.01	0.07	0.10	8	100
PIK3CA p.N1068fs*4	8	0.09	0.09	0.08	0.11	0.02	0.07	0.11	8	100
PTEN p.K267fs*9	8	0.10	0.10	0.07	0.13	0.04	0.06	0.14	8	100
PTEN p.P248fs*5	8	0.09	0.09	0.07	0.11	0.02	0.07	0.12	8	100
RET p.M918T	8	0.10	0.10	0.08	0.12	0.03	0.07	0.13	8	100
SMAD4 p.A466fs*28	8	0.09	0.09	0.07	0.11	0.02	0.07	0.12	8	100
TP53 p.C242fs*5	8	0.09	0.09	0.07	0.11	0.03	0.06	0.12	8	100
TP53 p.R175H	8	0.09	0.09	0.07	0.10	0.02	0.06	0.11	8	100
TP53 p.R248Q	8	0.09	0.09	0.06	0.11	0.03	0.06	0.11	8	100
TP53 p.R273H	8	0.08	0.08	0.06	0.09	0.02	0.06	0.10	8	100

Table 4B. Summary of Depth (DP) for positive controls across 8 consecutive runs.

Variant ID	Mean DP	Median DP	Min DP	Max DP	2SD DP	2SD DP min	2SD DP max	n within 2SD	% within 2SD
AKT1 p.E17K	306	303	197	438	167	139	472	8	100
APC p.R1450*	1342	1373	888	1876	743	599	2086	8	100
APC p.T1556fs*3	1323	1290	744	2041	939	384	2262	8	100
ATM p.C353fs*5	1144	1001	599	1933	990	154	2133	8	100
BRAF p.V600E	904	855	472	1427	687	217	1591	8	100
CTNNB1 p.T41A	623	577	363	938	432	191	1055	8	100
EGFR p.D770_N771insG	1156	1192	846	1529	527	629	1684	8	100
EGFR p.E746_A750delELREA	953	848	588	1449	620	333	1573	8	100
EGFR p.L858R	1340	1361	935	1837	708	632	2047	8	100
EGFR p.T790M	1230	1263	893	1613	563	667	1792	8	100
ERBB2 p.A775_G776insYVMA	944	930	666	1371	499	445	1442	8	100
FGFR3 p.S249C	467	499	149	821	463	4	930	8	100
FLT3 p.D835Y	1359	1322	860	2084	891	468	2250	8	100
GNA11 p.Q209L	1397	1405	934	1935	786	610	2183	8	100
GNAQ p.Q209P	1118	1067	602	1772	814	304	1933	8	100
GNAS p.R201C	1348	1313	801	1996	903	444	2251	8	100
JAK2 p.V617F	897	809	435	1459	759	138	1656	8	100
KIT p.D816V	733	698	445	1126	505	228	1238	8	100
KRAS p.G12D	1534	1478	862	2439	1117	417	2652	8	100
MPL p.W515L	865	844	549	1295	531	334	1397	8	100
NPM1 p.W288fs*12	723	583	363	1330	722	0	1445	8	100
NRAS p.Q61R	818	808	493	1213	510	307	1328	8	100
PDGFRA p.D842V	1275	1317	853	1874	796	478	2071	8	100
PDGFRA p.S566fs*6	1163	1087	728	1786	780	384	1943	8	100
PIK3CA p.E545K	943	845	459	1582	811	132	1754	8	100
PIK3CA p.H1047R	1135	1150	636	1615	774	361	1909	8	100
PIK3CA p.N1068fs*4	1000	1000	592	1434	675	325	1675	8	100
PTEN p.K267fs*9	1003	879	568	1613	790	213	1794	8	100
PTEN p.P248fs*5	1104	1011	704	1791	754	351	1858	8	100
RET p.M918T	985	899	551	1575	751	235	1736	8	100
SMAD4 p.A466fs*28	999	948	550	1544	737	262	1736	8	100
TP53 p.C242fs*5	493	477	338	707	251	243	744	8	100
TP53 p.R175H	904	898	629	1245	464	439	1368	8	100
TP53 p.R248Q	533	530	365	746	257	276	791	8	100
TP53 p.R273H	851	843	604	1224	463	388	1314	8	100

c) *Negative control (NC):* DNA from a HapMap cell line (GM12878) is used as a negative control in the assay. The HapMap cell line has been generated from EBV-transformed lymphocytes. This cell line has been previously sequenced and contains germline polymorphisms but no known somatic variants. Thus, this cell line serves as a known reference sequence to compare with the published reference sequence for both sequencing accuracy using the assay and for analytical specificity for the detection of somatic variants. The number of private variants identified in each HapMap sample represents the false positive rate of~1.75% for all mutations and ~1.68% for nonsynonymous exonic mutations. A HapMap sample from each clinical run is compared to the random pool of 5 HapMap samples from the validation NC pool, thus creating a pool of known expected mutations observed in HapMap samples. The same filtering criteria are applied as for the clinical samples including overall coverage >300X; variant coverage >200X and VAF >0.05. The number of a) all private mutations and b) nonsynonymous exonic mutations private to the HapMap sample in the clinical run is reviewed to ensure <2% false positive rate. All nonsynonymous exonic mutations are screened to ensure that no known actionable cancer mutations are detected. If such mutations are observed in the HapMap sample, (e.g., EGFR exon 19/21 BRAF V600E, etc.), the run will be flagged and repeated using new reagents and controls to eliminate the possibility of cross contamination.

d) *PCR reagent control [No Template Control (NTC)]:* The NTC control should have a Qubit measurement of < 2.0ng/µL. Sequencing data from the NTC control sample will also be subjected to analysis using the pipeline, to verify that no known hotspot mutations are detected. If a hotspot mutation is detected, any samples containing that mutation in the pool will be reviewed to determine if a re-run is necessary.

6. Result Reporting:

- Upon review, results are reported out under two categories: Level 2:"Cancer Mutations with Evidence of Clinical Significance" and Level 3: "Cancer Mutations with Potential Clinical Significance" as described in the FDA Fact Sheet available here https://www.fda.gov/media/109050/download.
  The two categories are based on the supporting level of clinical evidence. Refer to the Clinical Performance Section for more information.
- Results are reported for point mutations and small insertions and deletions in protein-coding exons, promoters or splice sites of the 607 gene panel. Refer to Appendix 1b for a list of genes.
- The NYU Langone Genome PACT does not report mutations in 73 exons due to consistently low coverage (<50x) in those exons. Refer to Appendix 1c for a list of excluded exons.
- Reporting takes in account the following quality metrics in the Table 5 below.

Table 5. Sample Level Quality Control Metrics

QC Metrics	Acceptance Criteria
After Demultiplexing	<10% Undetermined Reads per Lane Base Quality Scores >30/sample >=10 million total reads/sample

After the Pipeline Analysis	>= 10 million deduplicated mapped reads per sample Average coverage of the tumor sample >300X Average coverage of the Normal Sample >100X
Coverage uniformity	≥ 98% target exons above 100X coverage < 1% of targets with 0X and <50X coverage
% Cluster passing	The percent cluster passing filter (Cluster PF) > 80%
% Reads passing filter	The percent reads passing filter (Reads PF) > 80%
Somatic mutation assessment	VAF-Tu / VAF-Norm ≥5
Somatic mutation coverage	≥ 200X
Positive Control	Detection with 94% sensitivity (33 out of 35 variants), VAF within 2SD of expected range
Negative Control	<2% of private mutations not observed in HapMap pool (false positive rate)
Sample Mix-up QC	The Tumor and Normal from the same patient should show >90% overlap of homozygous SNP variants. Conversely, the concordance between samples from different patients should be low (<25%). <1.8% of known pathogenic variants observed in any Normal Sample.
Major Contamination QC	>2% of Mutations identified as somatic in any Tumor present in a Normal sample.
Criteria for calling test failure	mean coverage across all exons < 50x

D. Test Principle:

The NYU Langone Genome PACT assay is a custom targeted sequencing platform, utilizing solution- phase exon capture and sequencing, to detect somatic alterations (point mutations, small insertions and deletions) in tumor specimens compared to matched normal control. The NYU Langone Genome PACT assay involves hybridization capture and deep sequencing of all protein-coding exons of 607 cancer- associated genes. The assay uses custom DNA probes corresponding to all exons of oncogenes and tumor suppressor genes. Probes are synthesized by a secondary manufacturer and are biotinylated to enable sequence enrichment through capture by streptavidin-conjugated beads. Probes were designed to tile the entire length of each target sequence in an overlapping fashion, typically extending 20-50 base pairs beyond the boundaries of the target.

Genomic DNA is extracted from Tumor and patient-matched blood as a Normal sample. Sequence libraries are prepared through a series of enzymatic steps including shearing of double-stranded DNA, end repair, A-base addition, ligation of barcoded sequence adaptors, and low cycle PCR amplification. Multiple barcoded sequence libraries are pooled and captured using the custom-designed biotinylated probes. Captured DNA fragments are then sequenced on an Illumina NextSeq 500/550 as paired-end reads. Sequence reads are then aligned to the reference human genome. By comparing the identity of bases from the tumor DNA to the matched normal DNA and the reference human genome, somatic alterations are identified in the tumor.

V. Substantial Equivalence Information:

A. Predicate Device Name:

MSK-IMPACT (Integrated Mutation Profiling of Actionable Cancer Targets)

B. Predicate 510(k) Number:

DEN170058

C. Comparison with Predicate:

Item	Similarities	
	New Device	**Predicate**
Indications	The test is intended to provide information on somatic mutations (point mutations and small insertions and deletions) for use by qualified health care professionals in accordance with professional guidelines, and is not conclusive or prescriptive for labeled use of any specific therapeutic product.	Same
Test principle	Custom targeted sequencing platform, utilizing solution- phase exon capture and sequencing	Same
Specimens	Matched Tumor and Normal DNA analyzed	Same
Tumor tissue type	Formalin fixed paraffin embedded tissue	Same
Type of sequencing	Massive parallel sequencing	Same
Sequencing chemistry	Illumina Exonic Hybrid Capture Sequencing-By-Synthesis chemistry	Same
Non-hotspot Mutation threshold	5% variant allele frequency	Same
Optimized and recommended starting DNA input	250ng	Same
Filter for somatic variants (Variant Allele Frequency)	VAF-Tumor / VAF-Norm ≥ 5	Same
Coverage uniformity	≥ 98% target exons above 100X coverage	Same
Cluster passing	> 80%	Same
Clinical Evidence Curation Oncopanel results are reported under one of these two categories: “Cancer Mutations with Evidence of	Same	OncoKB knowledge base

Item	Similarities	
	New Device	Predicate
Clinical Significance" or "Cancer Mutations with Potential Clinical Significance."		

Item	Differences	
	New Device	Predicate
Mutation type detection	Single nucleotide variants (SNV), small insertions and deletions (indels)	Single nucleotide variants (SNV), small insertions and deletions (indels) and Microsatellite Instability (MSI)
Sequencing instruments	Illumina NextSeq 500/550	Illumina HiSeq 2500 Sequencer
Hotspot Mutation calling threshold	5% variant allele frequency	2% variant allele frequency
Tumor analysis only (in the absence of the matched Normal)	No, the test always requires a matched Normal DNA	Yes, in the absence of the Normal, a Tumor can be compared against a pool of normal samples
Number of targeted genes	607	468
Average target coverage	>300X	>200X

VI. Standard/Guidance Document Referenced (if applicable):

Not applicable

VII. Performance Characteristics:

A. Determination of pipeline thresholds:

1. Requirements on exon coverage:
The power analysis to establish the confidence intervals for variants to be identified at the given coverage was performed. The following criteria were applied:
Calculate number of reads needed to determine if a variant is present at 95% confidence (p = 0.05)

- Null Hypothesis: No variant present; background sequencing error rate only
- Alternative Hypothesis: Variant present at the given variant allele frequency (VAF)
- background error rate: 2% (Q20)

Data are summarized in the Tables 6 and **7** below. In summary approximately 100x coverage is necessary to detect mutations present at 10% VAF with 0.95 power and 500x coverage is necessary for 95% confidence for detection of a variant with 5% frequency. The established >300X average coverage enables a detection of mutations present at 6% VAF with 0.95 power.

Table 6. Number of reads required to detect variant with variant allele frequency (VAF) for given power level ranging from 0.8 to 0.99.

VAF	0.8	0.9	0.95	0.98	0.99
0.02	Inf	Inf	Inf	Inf	Inf
0.03	1983	2606	3185	3905	4426
0.04	603	784	950	1157	1306
0.05	315	406	489	593	667
0.06	203	260	312	377	423
0.07	146	186	223	269	301
0.08	113	143	171	206	230
0.09	91	115	137	165	184
0.10	76	96	114	137	153
0.11	65	82	97	116	130
0.12	56	71	84	101	112
0.13	50	63	74	89	99
0.14	45	56	66	79	88
0.15	40	50	60	71	79
0.16	37	46	54	65	72
0.17	34	42	50	59	66
0.18	31	39	46	55	61
0.19	29	36	42	51	56
0.20	27	33	40	47	52

Table 7. 95% Confidence intervals for various VAF's at given coverages

	Coverage				
VAF	**50**	**100**	**200**	**500**	**1000**
0.02	(0.002, 0.092)	(0.004, 0.064)	(0.007, 0.047)	(0.01, 0.035)	(0.013, 0.03)
0.03	(0.004, 0.107)	(0.008, 0.078)	(0.012, 0.061)	(0.01, 8 0.048)	(0,021, 0.042)
0.04	(0.007, 0.121)	(0.013, 0.092)	(0.019, 0.074)	(0.025, 0.06)	(0.029, 0.053)
0.05	(0.011, 0.135)	(0.019. 0.105)	(0.026, 0.086)	(0.033, 0.072)	(0.038, 0.065)
0.06	(0.016, 0.149)	(0.025, 0.118)	(0.033, 0.099)	(0.041, 0.083)	(0.046, 0.076)
0.07	(0.0, 2 0.162)	(0.031, 0.131)	(0.04, 0.111)	(0.05, 0.095)	(0.05, 5 0.087)
0.08	(0.026, 0.175)	(0.037, 0.144)	(0.048, 0.123)	(0.05, 8 0.106)	(0.06, 4 0.098)
0.09	(0.031,0.188)	(0.044, 0.156)	(0.055, 0.135)	(0.06, 7 0.117)	(0.073, 0.109)
0.10	(0.037, 0.201)	(0.051, 0.168)	(0.063, 0.146)	(0.076, 0.128)	(0.082, 0.12)
0.11	(0.043, 0.213)	(0.058, 0 18)	(0.071, 0.158)	(0 .084 , 0 .139)	(0,092 , 0.13)
0.12	(0.049, 0.226)	(0.066, 0.192)	(0.08, 0.169)	(0.09, 3 0.15)	(0.1,01 0.141)
0.13	(0.055, 0.238)	(0.073. 0.204)	(0.088, 0.181)	(010,2 0.161)	(0.1, 1 0.152)
0.14	(0.062, 0.25)	(0.081, 0.215)	(0.096, 0.192)	(0.11, 1 0.172)	(0.11, 9 0.162)
0.15	(0.068, 0.262)	(0.088, 0.227)	(0.105, 0.203)	(0.12, 0.183)	(0.129, 0.173)
0.16	(0.075, 0.274)	(0.096, 0.238)	(0.113, 0.214)	(0.129, 0.194)	(0.138, 0.183)
0.17	(0.082, 0.285)	(0.104, 0.25)	(0.122, 0.225)	(0.139, 0.204)	(0.147, 0.194)
0.18	(0.089, 0.297)	(0.112. 0.261)	(0.13, 0.236)	(0.148, 0.215)	(0.157, 0.204)
0.19	(0.096, 0.308)	(0.12, 0.272)	(0.139, 0.247)	(0.15, 7 0.226)	(0.16, 6 0.215)
0.20	(0.103, 0.32)	(0.128, 0.283)	(0.148, 0.258)	(0.16, 6 0.236)	(0176, 0.225)

The Average sample coverage of NYU Langone Genome PACT is >700X and Median sample coverage is>600X (Table 8), representing approximately 0.99 power for detection of variants at 0.05 allele frequency on average (coverage 667X, Table 6). However, regions with <500x coverage would be below 95% confidence interval for VAF 5% detection and if no mutation was identified they can represent a false negative finding.

Table 8. Summary of sample coverage across ~410 samples

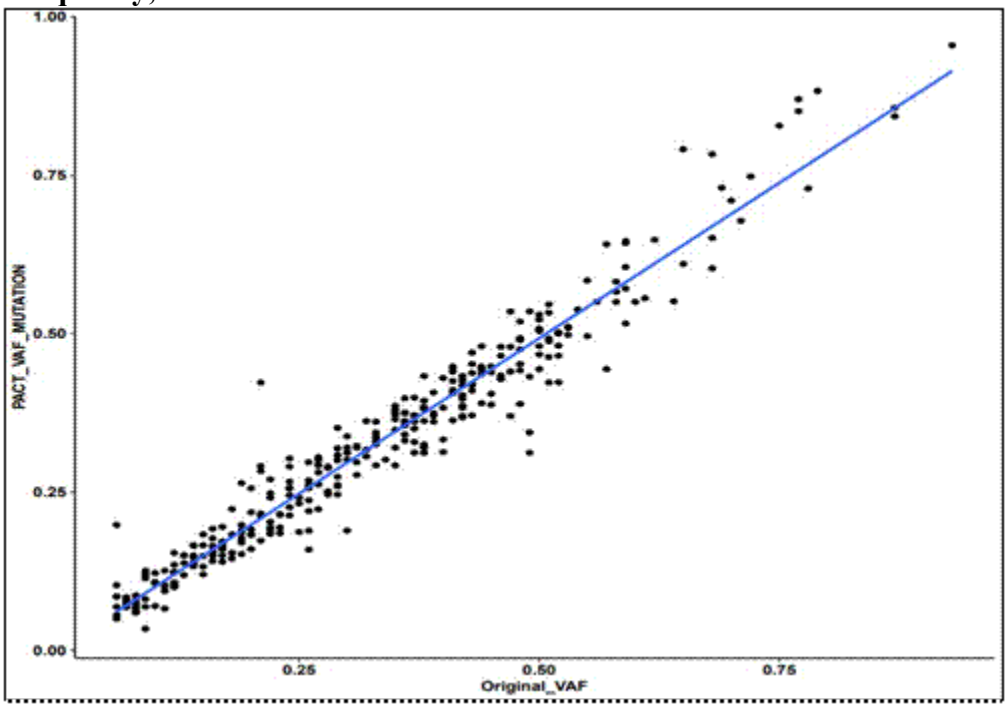
Metric	Cumulative Average*	Cumulative Median**	Range
Tumor and Normal samples			
Average sample coverage	720	711	286-1307
Median sample coverage	620	613	172-1000
Positive Control (Commercially available reference material qualified by Langone)			
Average sample coverage	788	799	612-940
Median sample coverage	780	791	605-938
Negative Control (HapMap)			

Metric	Cumulative Average*	Cumulative Median**	Range
Average sample coverage	851	847	729-1006
Median sample coverage	823	828	676-982
No template control (water)			
Average sample coverage	0.545	0.545	0.46-0.63
Median sample coverage	1	1	1-1

* Cumulative Average coverage (Average of all samples' Averages)
**Cumulative Median coverage (Median of all the samples' Medians)

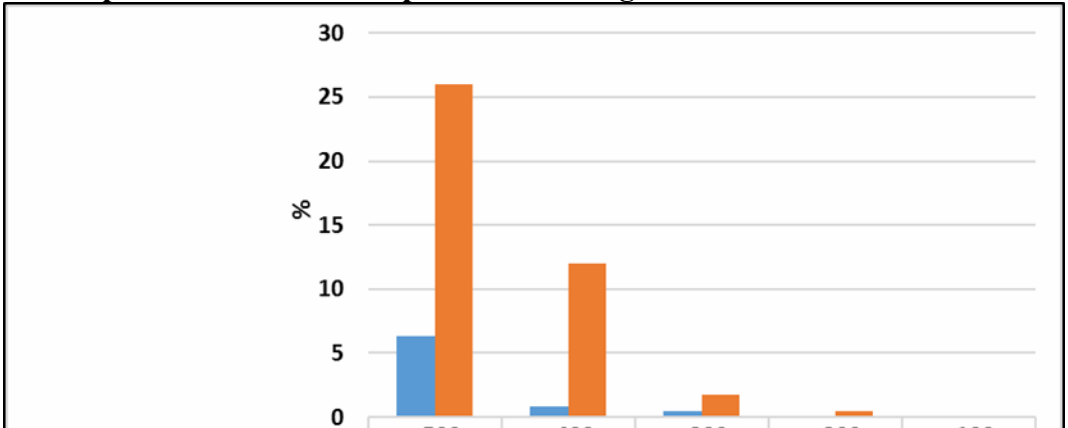
These estimates were confirmed by profiling 325 unique mutations and SNPs for which VAF was available from an orthogonal study with expected frequencies as low as 5% and average coverage highlighted in Table 7.

Figure 3. Observed vs. Expected (shown by orthogonal NGS method) Variant Frequency, Pearson correlation coefficient: 0.974



2. Requirements on sample coverage:
Coverage analysis data from 10 consecutive runs and 238 samples, including samples, as well as positive and negative control samples included in the runs, shows that approximately ~99.6% of cases had on average a coverage >300X (only 0.42 had coverage <300X) and 98.3% of cases had Median coverage >300X, see Figure 3 below. Therefore, we established 300X as Average coverage cutoff for a sample to be acceptable for analysis.

Figure 3: Cumulative presentation of average and median coverage below coverage cutoffs. X axis indicates the coverage, Y axis represents % of cases <the cutoff. N = 238 samples. >98% of the samples have Average



	<500	<400	<300	<200	<100
% of Sampleswith Average Coverage Below the Cutoff	6.3	0.84	0.42	0	0
% of Sampleswith Median Coverage Below the Cutoff	26	12	1.7	0.42	0

There were exons that presented with consistently low coverage values. None of the exons of the clinically validated genes are among those with consistently low coverage. To assess the effect of GC content on the metrics and the quality of coverage, correlation analysis from 10 independent runs was carried out. Correlation between coverage and GC content shows that poor coverage is associated with both high and low GC content. The coverage is optimal for target regions of moderate GC content (35 to 65 %).

Based on the validation, >99% of exons can be expected to be sequenced with average and median coverage greater than 100X. A 100X minimum coverage threshold per exon is required based on the power analysis calculations, which showed 100X coverage was necessary to call mutations with true underlying mutation frequency 10% or greater, with 95% power at an alpha level of 0.05). To be conservative, a threshold of 300X on mean and median sample coverage is used to determine if a sample is sequenced to sufficient depth for subsequent analysis. A sample is flagged as being at increased risk of false negatives if its mean coverage is below 300X.

3. Requirements on mutation coverage, DNA input and variant allele frequency for positive calls: Based on the validation, requirements on mutation coverage are ≥ 200X with ≥ 5% mutant reads with Normal coverage at minimum of ≥100X and <2% mutant reads. This results in ratio between the Tumor and Normal sample mutant reads to be ≥ 5.0. Calls with <50X coverage are not reported. Mutation calls with coverage between 50X and 200X need to be validated by an independent method if Level 2, see Table 9 for summary.

Table 9. Summary of coverage criteria for reporting

Coverage and VAF of the somatic mutation	Result
<50x, any finding	Fail
Somatic mutations identified with VAF > 0.05; coverage 50X-199X	Indeterminate
Somatic mutations identified with VAF > 0.05 coverage >200X	Pass
<500X, no mutations identified	No mutation reported.

B. Pre-Analytical performance:

Minimum DNA requirements were established by measuring assay performance based on different inputs and validated on precision studies. DNA was extracted from FFPE samples. The % of targets below the coverage cutoff did not vary between different sources of DNA. DNA samples are normalized to yield 100 – 250 ng input and maximized to 35 ul prior to library preparation. 100 ng is the minimal required input. The normalization and DNA quantification are performed.

Based on the established clinical VAF cutoff of 5%, samples with <10% tumor cell content are insufficient to detect any driver heterozygous mutations (estimated normalized VAF of ~50%). Therefore, only samples with >10% tumor cell content are accepted for the analysis. From the same sample a section with higher tumor cell content is preferred. When feasible, tumor content can be enriched by scalpel macrodissection. The data demonstrated that the DNA extraction has been optimized across tumor types to reasonably conclude that the analytical performance presented is representative across FFPE tumor types (Table 10).

Table 10. Pre-analytical performance summary

Tumor Type	Original samples (N)	DNA Pass (>2.9ng/uL) %	Pass Library Qubit (>2ng/µL) %	Frag Pass (>70%) %	All failed unique samples*	Fail %	Final Pass samples
All Samples (%)	455	96.3	97.5	95.1	46	10.11	409
Brain tumors	77	96.1	98.6	92.2	6	7.8	71
Lung Non-small cell carcinoma (Adenocarcinoma and Squamous Cell Carcinoma)	98	95.9	98.9	96.7	8	8.1	90
Melanoma	59	94.9	98.2	89.8	6	10.2	53
Colorectal Adenocarcinoma	54	100.0	100.0	96.3	2	3.7	52
Breast carcinoma	26	100.0	100.0	88.5	3	11.5	23
Prostate adenocarcinoma	12	91.7	90.9	83.3	2	16.7	10
Urothelial carcinoma	9	88.9	100.0	77.8	2	22.2	7
Testicular stromal tumor	1	100.0	100.0	100.0	0	0.0	1
Small intestine carcinoma	1	100.0	100.0	100.0	0	0.0	1
Soft tissue tumors (Sarcoma and GIST)	10	100.0	80	75	4	40	6
Pancreatic adenocarcinoma	11	81.8	88.9	63.6	4	36.4	7
Ovarian stromal tumor	19	94.7	94.4	89.5	2	10.5	17
GYN carcinomas (Ovarian and Uterine)	30	96.6	93.1	96.3	4	13.3	26

Tumor Type	Original samples (N)	DNA Pass (>2.9ng/uL) %	Pass Library Qubit (>2ng/µL) %	Frag Pass (>70%) %	All failed unique samples*	Fail %	Final Pass samples
Kidney tumor	40	100.0	97.5	97.5	1	2.5	39
Hepato-billiary carcinoma	7	85.7	100.0	71.4	2	28.6	5
Large Cell neuroendocrine	1	100.0	100.0	100.0	0	0.0	1

C. Analytical Performance:

The hybridization-capture-based targeted re-sequencing assay is designed to detect point mutations [single nucleotide variants (SNVs)] as well as small insertions/deletions (indels) < 35bp in length in the coding exons of 607 genes **(**Appendix 1). A total of 10,577 exons are sequenced, 94 targets were excluded during assay development due to low sequence coverage and high GC content (Appendix 2). A paired-sample analysis pipeline (tumor vs. matched normal) is used to identify somatic mutations in the targeted exons. NYU took a representative approach to validation of the SNVs and indels targeted in this panel, which is appropriate for variants of this type.

1. Precision Studies

a) Intra-assay reproducibility

The intra-assay reproducibility was demonstrated by analyzing a set of 9 patient samples in triplicates within the same run. Three (3) samples were selected to represent each variant type: SNV, Insertion, and Deletion. The results, samples selection and critical parameters (quality, depth of coverage and variant allele frequency) and statistical analysis are summarized in Table 11 and 12, respectively. All replicates were performed with 100 ng DNA input (lowest required input).

Table 11. Intra-assay reproducibility: SNV, insertions, and deletions samples

Sample-replicate	Gene coding mutation amino acid change	Qual	Depth	Freq
1-1	NPM1 c.859_860insTCTG:p.L287fs	359	215	0.08
1-2		727	214	0.107
1-3		375	161	0.081
2-1	ERBB2 c.2265_2266insGCATACGTGATG:p.E755delinsEAYVM	3756	659	0.162
2-2		3229	622	0.15
2-3		2796	664	0.123
3-1	EGFR c.2300_2301insCAGCGTGGA:p.A767delinsASVD	1970	456	0.129
3-2		2130	452	0.142
3-3		1630	470	0.106
4-1	EGFR c.2236_2250del:p.746_750del	1134	612	0.074
4-2		567	386	0.057
4-3		1044	734	0.056
5-1	EGFR c.2309_2310insCAACCCCCACGG:p.D770delinsDNPHG	1695	610	0.084
5-2		945	621	0.055
5-3		1639	555	0.088
6-1	BRAF c.1798_1799delGTinsAA:p.V600K	1072	236	0.208
6-2		2028	387	0.23

Sample-replicate	Gene coding mutation amino acid change	Qual	Depth	Freq
6-3		1172	350	0.157
7-1	TP53 c.G197A:p.G66D	404	466	0.056
7-2		305	255	0.071
7-3		459	506	0.059
8-1	PIK3CA c.G1633A:p.E545K	390	291	0.076
8-2		156	255	0.059
8-3		268	280	0.061
9-1	ATM c.A5071C:p.S1691R	3359	293	0.478
9-2		3334	328	0.448
9-3		4417	364	0.508

Table 12. Intra-assay reproducibility: SNV, insertions, and deletions statistical analysis

	mean	sd	median	minimum	maximum	positive call	positive call rate(95% CI)
NPM1.c.859_860insTCTG.p.L287fs	0.09	0.02	0.08	0.08	0.11	3 out of 3	100%(31%,100%)
ERBB2.c.2265_2266insGCATACGTGATG.p.E755delinsEAYVM	0.15	0.02	0.15	0.12	0.16	3 out of 3	100%(31%,100%)
EGFR.c.2300_2301insCAGCGTGGA.p.A767delinsASVD	0.13	0.02	0.13	0.11	0.14	3 out of 3	100%(31%,100%)
EGFR.c.2236_2250del.p.746_750del	0.06	0.01	0.06	0.06	0.07	3 out of 3	100%(31%,100%)
EGFR.c.2309_2310insCAACCCCCACGG.p.D770delinsDNPHG	0.08	0.02	0.08	0.06	0.09	3 out of 3	100%(31%,100%)
BRAF.c.1798_1799delGTinsAA.p.V600K	0.20	0.04	0.21	0.16	0.23	3 out of 3	100%(31%,100%)
TP53.c.G197A.p.G66D	0.06	0.01	0.06	0.06	0.07	3 out of 3	100%(31%,100%)
PIK3CA.c.G1633A.p.E545K	0.07	0.01	0.06	0.06	0.08	3 out of 3	100%(31%,100%)
ATM.c.A5071C.p.S1691R	0.48	0.03	0.48	0.45	0.51	3 out of 3	100%(31%,100%)

b) *Inter-assay panel-wide reproducibility: SNVs, insertions, deletions*

The SNV, indel inter-assay reproducibility was assessed testing 16 patients selected for 28 previously known unique mutations detected with orthogonal NGS methods (Table 13). All sixteen samples were FFPE derived. All reproducibility samples were prepared independently for each run and carried a different molecular barcode to account for the reproducibility of all steps of the procedure. All replicates were performed with 100 ng DNA input (lowest required input). Critical parameters including variant allele frequency (VAF), depth of coverage and quality between runs were assessed and correlated. All runs passed QC metric criteria. All expected mutations showed 100% Positive call rate, (Table 14A).

Table 13. Inter-assay panel-wide reproducibility (16 unique patients) – select variants

Tumor type	Gene	Coding	AA change	Variant type
Colon adenocarcinoma	ATM	c.A5071C	p.S1691R	nonsynonymous SNV
Colon adenocarcinoma	KRAS	c.G34T	p.G12C	nonsynonymous SNV
Lung	SAMD9	c.1800_1801del	p.E600fs	frameshift deletion

Tumor type	Gene	Coding	AA change	Variant type
adenocarcinoma				
Lung adenocarcinoma	ERBB2	c.2220_2221insGCATACGTGATG	p.E740delinsEAYVM	nonframeshift insertion
Colon adenocarcinoma	MET	c.T1085C	p.M362T	nonsynonymous SNV
Colon adenocarcinoma	NRAS	c.G34T	p.G12C	nonsynonymous SNV
Colon adenocarcinoma	KMT2D	c.11750_11758del:p.3917_3920del	p.3917_3920del	nonframeshift deletion
Colon adenocarcinoma	HRAS	c.488_507del	p.L163fs	frameshift deletion
Pancreatic adenocarcinoma	PIK3CA	c.G1633A	p.E545K	nonsynonymous SNV
Pancreatic adenocarcinoma	KRAS	c.G35T	p.G12V	nonsynonymous SNV
Pancreatic adenocarcinoma	SMAD4	c.1239_1241del:p.413_414del	p.413_414del	nonframeshift deletion
PNET	HSP90AA1	c.1108_1109del	p.E370fs	frameshift deletion
Medulloblastoma	AURKC	c.20_27del	p.T7fs	frameshift deletion
Medulloblastoma	KMT2B	c.1126_1128del:p.376_376del	p.376_376del	nonframeshift deletion
Pediatric glioma	SPEN	c.535_561del:p.179_187del	p.179_187del	nonframeshift deletion
Pediatric glioma	HIST1H1C	c.590_604del:p.197_202del	p.197_202del	nonframeshift deletion
Pediatric glioma	TAF1L	c.2927_2928del	p.T976fs	frameshift deletion
Lung adenocarcinoma	EGFR	c.2309_2310insCAACCCCCACGG	p.D770delinsDNPHG	nonframeshift insertion
Glioblastoma	EPHA7	c.72_73del	p.T24fs	frameshift deletion
Glioblastoma	FANCD2	c.1278_1278del	p.L426fs	frameshift deletion
Glioblastoma	ICK	c.1106_1117del:p.369_373del	p.369_373del	nonframeshift deletion
Lung adenocarcinoma	EGFR	c.2236_2250del:p.746_750del	p.746_750del	nonframeshift deletion
Melanoma	BRAF	c.G1798_1799delinsAA	p.V600K	nonsynonymous DNV
Glioblastoma	PTEN	c.1649_1652del	p.Y550fs	frameshift deletion
Lung adenocarcinoma	SDHA	c.1944_1945del	p.T648fs	frameshift deletion
Lung adenocarcinoma	EGFR	c.2300_2301insCAGCGTGGA	p.A767delinsASVD	nonframeshift insertion
Lung adenocarcinoma	CYP2D6	c.775delA	p.R259fs	frameshift deletion

Table 14A. Inter-assay reproducibility SNV -variant allele frequency

Gene	AA Change	Mean	SD	Median	Minimum	Maximum	Positive Call	Positive Call Rate (95% CI)
BRAF	p.V600K	0.33	0.02	0.33	0.29	0.35	5 out of 5	100% (46.3%, 100%)
KRAS	p.G12V	0.07	0.01	0.07	0.06	0.09	5 out of 5	100% (46.3%, 100%)
PIK3CA	p.E545K	0.08	0.02	0.08	0.06	0.11	5 out of 5	100% (46.3%, 100%)
MET	p.M362T	0.45	0.01	0.45	0.44	0.46	5 out of 5	100% (46.3%, 100%)
NRAS	p.G12C	0.14	0.01	0.14	0.12	0.15	5 out of 5	100% (46.3%, 100%)
ATM	p.S1691R	0.50	0.02	0.50	0.47	0.53	5 out of 5	100% (46.3%, 100%)
KRAS	p.G12C	0.06	0.01	0.06	0.06	0.07	5 out of 5	100% (46.3%, 100%)

a) Deletions

Gene	AA change	Mean	SD	Median	Min	Max	Positive Call	Positive Call Rate(95% Ci)
FANCD2	p.L426fs	0.35	0.02	0.35	0.32	0.38	5 out of 5	100% (46.3%, 100%)
CYP2D6	p.R259fs	0.21	0.01	0.21	0.19	0.22	5 out of 5	100% (46.3%, 100%)
SDHA	p.T648fs	0.15	0.01	0.15	0.14	0.17	5 out of 5	100% (46.3%, 100%)
PTEN	p.Y550fs	0.46	0.03	0.45	0.42	0.50	5 out of 5	100% (46.3%, 100%)
EGFR	p.746_750del	0.09	0.01	0.10	0.08	0.10	5 out of 5	100% (46.3%, 100%)
ICK	p.369_373del	0.34	0.05	0.36	0.26	0.38	5 out of 5	100% (46.3%, 100%)
EPHA7	p.T24fs	0.50	0.03	0.49	0.47	0.54	5 out of 5	100% (46.3%, 100%)
HIST1H1C	p.197_202del	0.27	0.02	0.27	0.25	0.30	5 out of 5	100% (46.3%, 100%)
TAF1L	p.T976fs	0.44	0.04	0.43	0.38	0.49	5 out of 5	100% (46.3%, 100%)
SPEN	p.179_187del	0.19	0.01	0.19	0.18	0.19	5 out of 5	100% (46.3%, 100%)
AURKC	p.T7fs	0.51	0.03	0.50	0.47	0.55	5 out of 5	100% (46.3%, 100%)
KMT2B	p.376_376del	0.64	0.02	0.63	0.62	0.67	5 out of 5	100% (46.3%, 100%)
HSP90AA1	p.E370fs	0.48	0.02	0.48	0.44	0.50	5 out of 5	100% (46.3%, 100%)
SMAD4	p.413_414del	0.17	0.02	0.17	0.14	0.19	5 out of 5	100% (46.3%, 100%)
HRAS	p.L163fs	0.36	0.02	0.35	0.34	0.39	5 out of 5	100% (46.3%, 100%)
KMT2D	p.3917_3920del	0.36	0.02	0.37	0.34	0.38	5 out of 5	100% (46.3%, 100%)
SAMD9	p.E600fs	0.44	0.02	0.44	0.42	0.48	5 out of 5	100% (46.3%, 100%)

b) Insertions

Gene	AA Change	Mean	SD	Median	Min	Max	Positive Call	Positive Call Rate(95% CI)
NPM1	p.L287fs	0.13	0.02	0.14	0.11	0.16	5 out of 5	100% (46.3%, 100%)
EGFR	p.D770delinsDNPHG	0.07	0.01	0.06	0.06	0.08	5 out of 5	100% (46.3%, 100%)
EGFR	p.A767delinsASVD	0.14	0.01	0.15	0.13	0.16	5 out of 5	100% (46.3%, 100%)
ERBB2	p.E740delinsEAYVM	0.17	0.01	0.17	0.16	0.19	5 out of 5	100% (46.3%, 100%)

c) *Panel-wide Run-to-Run Precision:*
The precision analysis was performed for the known mutations, and also performed for all additional mutations identified in each specimen in any of the test replicates. Testing identified 26 additional somatic mutations that were not known before the

start of the precision studies, likely due to the size of the panel/s used. All were subsequently confirmed by orthogonal testing. Mutations showed reproducibility across all replicates with only one deletion, which was missed on one of the replicates, likely due to low VAF (median VAF: 0.06). In total, 54 mutations were tested with a positive call rate 269 out of 270 replicates. Precision per specimen across all mutation types, and precision per type of mutation are summarized in Table 14B. One discordant was observed for AFF3 deletion. All runs passed the quality metrics criteria.

Table 14B. Panel-wide precision summary for all 5 replicates for 16 unique patient samples

Gene	Mutation Type	Exon, coding, amino acid change	Median Coverage (range)	VAF mean	SD	Median	Min	Max	Call rate
Colon adenocarcinoma									
ATM	nonsynonymous SNV	exon34, c.A5071C, p.S1691R	853 (762-1417)	0.5	0.02	0.5	0.47	0.53	5 / 5
KRAS	nonsynonymous SNV	exon2, c.G34T, p.G12C	1211 (1211-1855)	0.06	0.01	0.06	0.06	0.07	5 / 5
APC	nonsynonymous SNV	exon16, c.T3920A, p.I1307K	492 (377-661)	0.45	0.05	0.47	0.4	0.49	5 / 5
SDHA	frameshift deletion	exon15, c.1944_1945del, p.L649Efs*4	1442 (1185-1774)	0.15	0.01	0.15	0.14	0.17	5 / 5
Lung adenocarcinoma									
SAMD9	frameshift deletion	exon3, c.1800_1801del, p.E600fs	228 (202-297)	0.44	0.02	0.44	0.42	0.48	5 / 5
ERBB2	nonframeshift deletion	exon20, c.2220_2221insGCATACGTGATG, p.E740delinsEAYVM	1112 (804-1379	0.17	0.01	0.17	0.16	0.19	5 / 5
TP53	nonsynonymous SNV	exon4, c.G314A, p.G105D	363 (357-396)	0.41	0.04	0.42	0.36	0.45	5 / 5
Colon adenocarcinoma									
MET	nonsynonymous SNV	exon14, c.T1085C, p.M362T	926 (663-952)	0.45	0.01	0.45	0.44	0.46	5 / 5
NRAS	nonsynonymous SNV	exon2, c.G34T, p.G12C	720 (472-769)	0.14	0.01	0.14	0.12	0.15	5 / 5
HRAS	frameshift deletion	exon5, c.488_507del, p.L163fs	818 (445-1084)	0.36	0.02	0.35	0.34	0.39	5 / 5
KMT2D	nonframeshift deletion	exon39, c.11750_11758del, p.3917_3920del	1593 (1238-1876)	0.36	0.02	0.37	0.34	0.38	5 / 5
HNF1A	nonsynonymous SNV	exon1, c.G92A, p.G31D	570 (406-721)	0.16	0.02	0.17	0.14	0.2	5 / 5
MLH3	nonsynonymous SNV	exon2, c.G2221T, p.V741F	1067 (883-1756)	0.16	0.01	0.17	0.15	0.17	5 / 5
CHD7	nonframeshift insertion	exon3, c.2049_2050insAAAGCA, p.A685_K686dup	923 (857-1380)	0.41	0.02	0.42	0.39	0.42	5 / 5
SETD2	frameshift	exon18, c.7330dupT,	824	0.15	0.02	0.15	0.14	0.17	5 / 5

Gene	Mutation Type	Exon, coding, amino acid change	Median Coverage (range)	VAF mean	SD	Median	Min	Max	Call rate
	insertion	p.Y2444Lfs*2	(726-1159)						
CREB3L2	nonframeshift deletion	exon2, c.299_301del, p.100_101del	458 (352-597)	0.3	0.03	0.29	0.26	0.34	5 / 5
Pancreatic adenocarcinoma									
PIK3CA	nonsynonymous SNV	exon10, c.G1633A, p.E545K	638 (503-972)	0.08	0.02	0.08	0.06	0.11	5 / 5
KRAS	nonsynonymous SNV	exon2, c.G35T, p.G12V	823 (726-1295)	0.07	0.01	0.07	0.06	0.09	5 / 5
SMAD4	nonframeshift deletion	exon10, c.1239_1241del, p.413_414del	511 (455-841)	0.17	0.02	0.17	0.14	0.19	5 / 5
AR	nonframeshift deletion	exon1, c.171_179del, p.57_60del	696 (243-821)	0.1	0.04	0.09	0.06	0.15	5 / 5
PNET									
HSP90AA1	frameshift deletion	exon6, c.1108_1109del, p.E370fs	425 (416-711)	0.48	0.02	0.48	0.44	0.5	5 / 5
FGFR1	nonsynonymous SNV	exon13, c.A1729G, p.N577D	642 (431-799)	0.44	0.02	0.45	0.41	0.46	5 / 5
IL7R	nonsynonymous SNV	exon2, c.T197C, p.I66T	401 (326-567)	0.46	0.04	0.48	0.41	0.49	5 / 5
Medulloblastoma									
AURKC	frameshift deletion	exon3, c.20_27del, p.T7fs	470 (418-641)	0.51	0.03	0.5	0.47	0.55	5 / 5
KMT2B	nonframeshift deletion	exon3, c.1126_1128del, p.376_376del	1034 (846-1475)	0.64	0.02	0.63	0.62	0.67	5 / 5
v	Pediatric glioma								
SPEN	nonframeshift deletion	exon3, c.535_561del, p.Q179_R187del	1599 (1476-1951)	0.19	0.01	0.19	0.18	0.19	5 / 5
IDH1	nonsynonymous SNV	exon4, c.G395A, p.R132H	1240 (1138-1918)	0.18	0.01	0.19	0.17	0.2	5 / 5
Pediatric glioma									
HIST1H1C	nonframeshift deletion	exon1, c.590_604del, p.A197_K201del	1236 (881-1520)	0.27	0.02	0.27	0.25	0.3	5 / 5
TAF1L	frameshift deletion	exon1, c.2927_2928del, p.T976fs	383 (376-622)	0.44	0.04	0.43	0.38	0.49	5 / 5
FGFR4	nonsynonymous SNV	exon9, c.G1162A, p.G388R	1066 (470-1842)	0.42	0.03	0.43	0.38	0.44	5 / 5
Lung adenocarcinoma									
EGFR	nonframeshift insertion	exon20, c.2309_2310insCAACCCCCACGG, p.D770delinsDNPHG	1058 (873-1297)	0.07	0.01	0.06	0.06	0.08	5 / 5
TP53	nonsynonymous SNV	exon8, c.A838G, p.R280G	566 (410-732)	0.08	0.01	0.08	0.06	0.1	5 / 5
AFF3	nonframeshift deletion	exon13, c.1330_1332del, p.444_444del	1918 (1610-2509)	0.06	0.01	0.06	0.05	0.07	4/5
Glioblastoma									
EPHA7	frameshift	exon1, c.72_73del,	518	0.5	0.03	0.49	0.47	0.54	5 / 5

Gene	Mutation Type	Exon, coding, amino acid change	Median Coverage (range)	VAF mean	SD	Median	Min	Max	Call rate
	deletion	p.T24fs	(476-807)						
ADGRD1	frameshift insertion	exon6, c.499dupA, p.V168Sfs*57	690 (482-754)	0.43	0.02	0.43	0.41	0.46	5 / 5
Glioblastoma									
FANCD2	frameshift deletion	exon15, c.1278_1278del, p.L426fs	931 (871-1571)	0.35	0.02	0.35	0.32	0.38	5 / 5
ICK	nonframeshift deletion	exon10, c.1106_1117del, p.369_373del	249 (209-281)	0.34	0.05	0.36	0.26	0.38	5 / 5
PTPN11	nonsynonymous SNV	exon13, c.G1508T, p.G503V	799 (576-1006)	0.31	0.02	0.31	0.28	0.33	5 / 5
Lung adenocarcinoma									
EGFR	nonframeshift deletion	exon19, c.2236_2250del, p.746_750del	1133 (987-1680)	0.09	0.01	0.1	0.08	0.1	5 / 5
TNK2	nonsynonymous SNV	exon13, c.G2864A, p.R955H	479 (368-833)	0.14	0.03	0.14	0.1	0.17	5 / 5
NOTCH2	frameshift deletion	exon1:c.17_18del:p.P6fs	750 (341-1963)	0.13	0.02	0.13	0.11	0.15	5 / 5
Melanoma									
BRAF	nonsynonymous DNV	exon15, c.G1798_1799delinsAA, p.V600K	720 (653-1190)	0.33	0.02	0.33	0.29	0.35	5 / 5
JAK3	nonsynonymous SNV	exon16, c.G2164A, p.V722I	1579 (1044-2069)	0.48	0.01	0.48	0.46	0.5	5 / 5
SDHD	nonsynonymous SNV	exon2, c.G34A, p.G12S	813 (696-966)	0.45	0.02	0.45	0.42	0.47	5 / 5
IRS2	nonframeshift deletion	exon1, c.82_84del, p.28_28del	1317 (943-2215)	0.34	0.01	0.34	0.33	0.35	5 / 5
KAT6A	nonframeshift insertion	exon17, c.4982_4983insACAGCAGCCACAGCC, p.Q1657_P1661dup	552 (374-746)	0.18	0.03	0.18	0.13	0.22	5 / 5
	Glioblastoma								
PTEN	frameshift deletion	exon10, c.1649_1652del, p.Y550fs	431 (332-491)	0.46	0.03	0.45	0.42	0.5	5 / 5
TERT	nonsynonymous SNV	exon6, c.C2177T, p.T726M	1363 (1066-1680)	0.47	0.02	0.47	0.43	0.5	5 / 5
Lung adenocarcinoma									
EGFR	nonframeshift insertion	exon20, c.2300_2301insCAGCGTGGA, p.A767delinsASVD	1228 (1042-1372)	0.14	0.01	0.15	0.13	0.16	5 / 5
CYP2D6	frameshift deletion	exon5, c.775delA, p.R259fs	1090 (871-1214)	0.21	0.01	0.21	0.19	0.22	5 / 5
SDHA	frameshift deletion	exon15, c.1944_1945del, p.T648fs	1442 (1185-1774)	0.15	0.01	0.15	0.14	0.17	5 / 5
MN1	nonframeshift insertion	exon1, c.1619_1620insGCA, p.Q550dup	627 (430-782)	0.16	0.02	0.17	0.14	0.18	5 / 5

Gene	Mutation Type	Exon, coding, amino acid change	Median Coverage (range)	VAF mean	SD	Median	Min	Max	Call rate
Sarcoma									
NPM1	frameshift insertion	exon11, c.859_860insTCTG, p.L287fs	795 (629-1095)	0.13	0.02	0.14	0.11	0.16	5 / 5
DNMT3A	nonsynonymous SNV	exon16, c.C1903T, p.R635W	1291 (857-1587)	0.13	0.02	0.14	0.11	0.15	5 / 5

d) *Per Specimen Precision:*
Results of the precision studies were combined and precision across all reportable genes was determined for each specimen. The positive call rate based on the total number of mutations along with the 2-sides 95% confidence interval were calculated (Table 14C).

Table 14C: Precision per specimen across all reportable mutations (N – 5 replicates)

Tumor type	Total No Unique Mutations detected across all 5 replicates	Positive call rate per mutation	Positive call rate (two- sided 95% CI)
Colon adenocarcinoma	4	5/5	20/20 100% (83.9%, 100.0%)
Lung adenocarcinoma	3	5/5	15/15 100.0% (79.6%, 100.0%)
Colon adenocarcinoma	9	5/5	45/45 100.0% (92.1%, 100.0%)
Pancreatic adenocarcinoma	4	5/5	20/20 100% (83.9%, 100.0%)
PNET	3	5/5	15/15 100.0% (79.6%, 100.0%)
Medulloblastoma	2	5/5	10/10 100.0 (72.2%, 100.0%)
Pediatric glioma	2	5/5	10/10 100.0 (72.2%, 100.0%)
Pediatric glioma	3	5/5	15/15 100.0% (79.6%, 100.0%)
Lung adenocarcinoma	**3**	**5/5 for 2 4/5 for 1**	**14/15 93.3% (70.2%, 98.8%)**
Glioblastoma	2	5/5	10/10 100.0 (72.2%, 100.0%)
Glioblastoma	3	5/5	15/15 100.0% (79.6%, 100.0%)
Lung adenocarcinoma	3	5/5	15/15 100.0% (79.6%, 100.0%)

Melanoma	5	5/5	25/25 100.0% (86.7%, 100.0%)
Glioblastoma	2	5/5	10/10 100.0 (72.2%, 100.0%)
Lung adenocarcinoma	4	5/5	20/20 100% (83.9%, 100.0%)
Myeloid sarcoma	2	5/5	10/10 100.0 (72.2%, 100.0%)
Total mutations	**5** **4**	**5/5 for 53** **4/5 for 1**	**269/270** **99.6% (97.9%, 99.9%)**
SNV	2 2	5/5	110/110 100.0% (96.6%, 100.0%)
Insertion	8	5/5 for 7 4/5 for 1	39/40 97.5% (87.1%, 99.6%)
Deletion	2 4	5/5	120/120 100.0% (96.9%, 100.0%)

2. Analytical Sensitivity – Limit of Detection (LoD)
The LoD of the NYU Langone PACT assay is defined as the mutant allele fraction at which 95% of replicates across all replicates for a variant type are reliably detected. Studies were conducted to demonstrate a putative LoD for each variant type. Two studies were conducted (1) with commercially available reference materials and (2) with clinical specimens.

a) *Reference Material:*
Commercially available reference material consisting of mutant DNA blended in genomic DNA was used to obtain a preliminary LoD. For positive calls. The control material provides a mix of mutations (SNV, insertions and deletions) The list of mutations is shown in **Table 15**.

Table 15. Limit of Detection - Reference Material DNA Mix

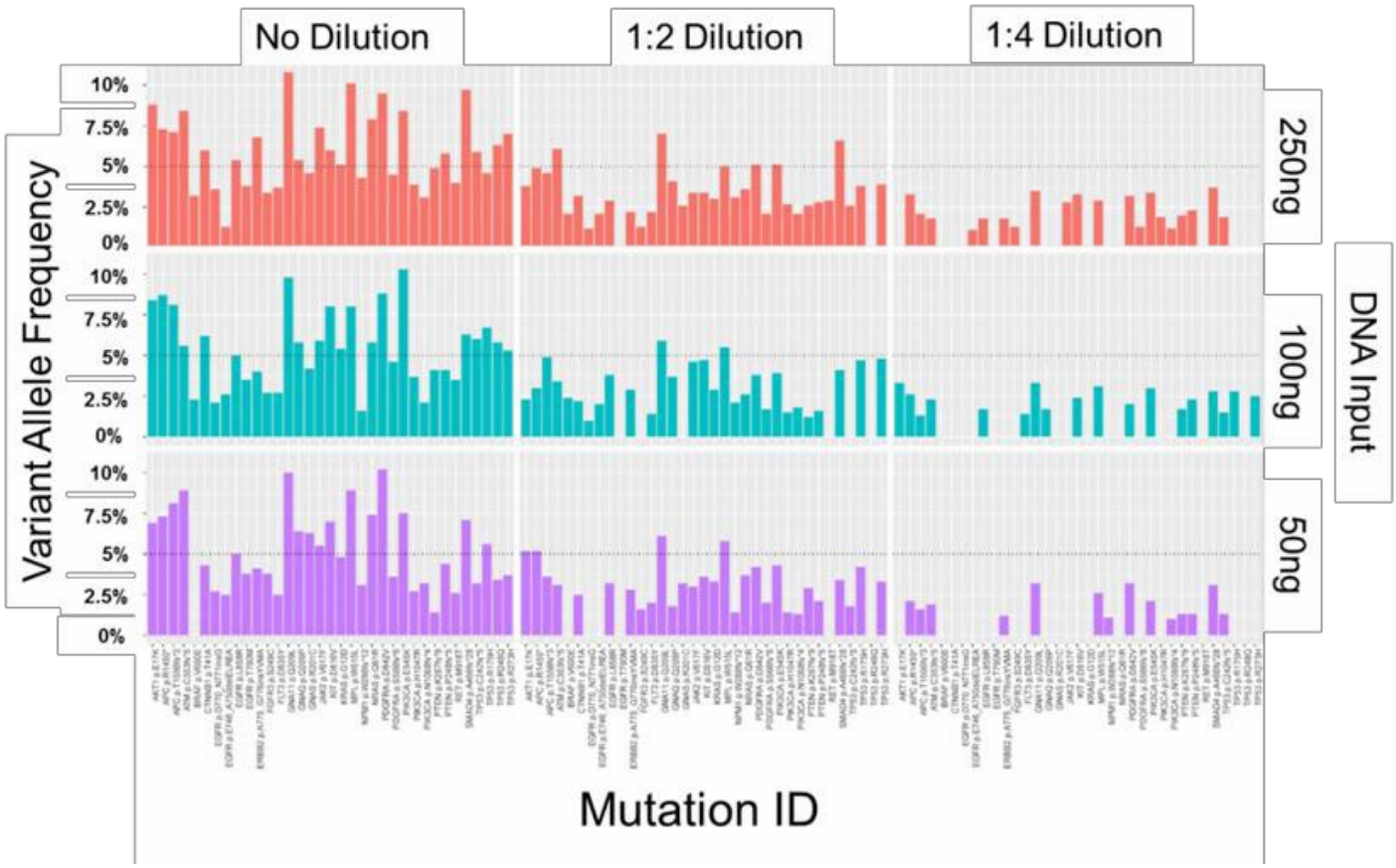
Gene ID	COSMIC Identifier	Mutation Type	HGVS Nomenclature	Amino Acid	Target AF
AKT1	COSM33765	Substitution	c.49G>A	p.E17K	10%
APC	COSM13127	Substitution	c.4348C>T	p.R1450*	10%
APC	COSM18561	Insertion in HP 7N	c.4666_4667insA	p.T1556fs*3	10%
ATM	COSM21924	Deletion	c.1058_1059delGT	p.C353fs*5	10%
ERBB2	COSM682/ 20959	Insertion	c.2324_2325ins12	p.A775_G776insYVMA	10%
GNA11	COSM5296	Substitution	c.626A>T	p.Q209L	10%

Gene ID	COSMIC Identifier	Mutation Type	HGVS Nomenclature	Amino Acid	Target AF
	9				
GNAQ	COSM28758	SNV in HP 3N	c.626A>C	p.Q209P	10%
KIT	COSM1314	Substitution	c.2447A>T	p.D816V	10%
MPL	COSM18918	Substitution	c.1544G>T	p.W515L	10%
PDGFRA	COSM736	Substitution	c.2525A>T	p.D842V	10%
PIK3CA	COSM763	Substitution	c.1633G>A	p.E545K	10%
SMAD4	COSM14105	Insertion	c.1394_1395insT	p.A466fs*28	10%
CTNNB1	COSM5664	Substitution	c.121A>G	p.T41A	7%
EGFR	COSM6224	SNV in 3N	c.2573T>G	p.L858R	7%
GNAS	COSM27887	Substitution	c.601C>T	p.R201C	7%
JAK2	COSM12600	SNV in HP 3N	c.1849G>T	p.V617F	7%
KRAS	COSM521	Substitution	c.35G>A	p.G12D	7%
NPM1	COSM17559	Insertion	c.863_864insTCTG	p.W288fs*12	7%
NRAS/CSDE1	COSM584	Substitution	c.182A>G	p.Q61R	7%
PTEN	COSM4986	Insertion	c.741_742insA	p.P248fs*5	7%
PTEN	COSM5809	Deletion 6N > 5N	c.800delA	p.K267fs*9	7%
TP53	COSM10648	Substitution	c.524G>A	p.R175H	7%
TP53	COSM10660	Substitution	c.818G>A	p.R273H	7%
TP53	COSM10662	Substitution	c.743G>A	p.R248Q	7%
TP53	COSM6530	Deletion	c.723delC	p.C242fs*5	7%
BRAF	COSM476	Substitution	c.1799T>A	p.V600E	4%
EGFR	COSM12378	Insertion	c.2310_2311insGGT	p.D770_N771insG	4%
EGFR	COSM6225	Deletion	c.2236_2250del15	p.E746_A750delELREA	4%
EGFR	COSM6240	Substitution	c.2369C>T	p.T790M	4%
FGFR3	COSM715	Substitution	c.746C>G	p.S249C	4%
FLT3	COSM783	Substitution	c.2503G>T	p.D835Y	4%
PDGFRA	COSM28053	Insertion	c.1694_1695insA	p.S566fs*6	4%
PIK3CA	COSM12464	Insertion	c.3204_3205insA	p.N1068fs*4	4%

Gene ID	COSMIC Identifier	Mutation Type	HGVS Nomenclature	Amino Acid	Target AF
PIK3CA	COSM775	Substitution	c.3140A>G	p.H1047R	4%
RET	COSM965	Substitution	c.2753T>C	p.M918T	4%

Of note, variant allele frequency (VAF) detected by NYU Langone PACT assay in non-diluted conditions was below the manufacturer's advertised uniform content (Figure 4, No Dilution, 250ng). Control material was diluted at 1:2 and 1:4 ratio with the negative control HapMap DNA. In addition, different DNA inputs (250ng, 100ng and 50ng) for each dilution were tested. There was expected decrease of VAF with increasing sample dilution. However, the VAF remained relatively stable down to 100ng of DNA input in the non-diluted control, particularly for all mutations with VAF>5%. However, 50ng input leads to loss of some mutations, most pronounced when VAF is <5%. The drop out of mutations is most pronounced at the 1:4 Dilution. Variants with low frequency are particularly sensitive to decreasing DNA input while variants with VAF >5% are reliably detected even with low DNA input. Dashed line highlights the 5% VAF threshold established in this study.

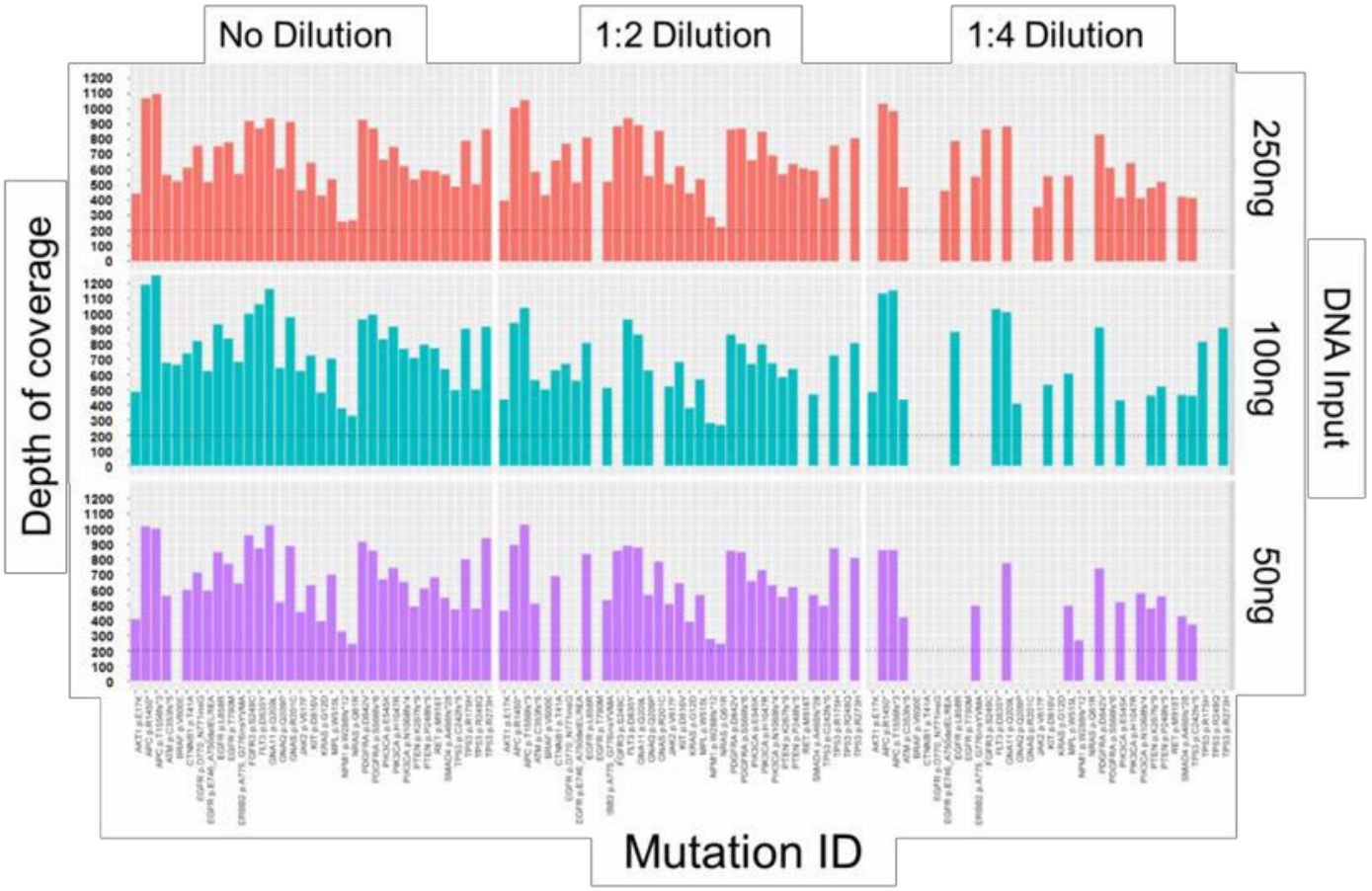
Figure 4: Variant allele frequency and DNA input:



A similar pattern was observed when variant coverage was assessed under these

conditions. While depth of coverage of the non-diluted sample remains robust (>200X) even at 50 ng of input (with an exception of one SNV), increased dilutions results in a dropout of low level variants Based on this analysis it was concluded that SNVs, insertions and deletions with >5% VAF can be reliably detected with >200X coverage with 100ng of the DNA input, Figures 4 and 5. The data demonstrates that low DNA input produces >200x coverage in samples with no dilution. Samples with high dilution show marked dropout of the coverage of predicted variants. Dashed line highlights 200x coverage threshold established in this study. Table with all VAF and Depth data for all dilutions and DNA inputs is provided in Appendix 5

Figure 5: Depth of coverage and DNA input as a function of DNA input



Based on this analysis it was concluded that SNVs, insertions and deletions with >5% VAF can be reliably detected with >200X coverage with 100ng of the DNA input. All reproducibility studies were performed with 100ng input.

The analysis shows that mutations with at least 5-10% VAF, whether due to a low prevalence of the variant in the tumor OR a highly prevalent mutation but in a sample with a low tumor cell content, can be expected to be sequenced with sufficient coverage to enable confident mutation calling. These lower limits of detection thresholds (5% VAF, 200X coverage and 100 ng DNA input) were

subsequently tested both in intra- and inter- assay reproducibility studies as detailed above. This analysis correlates with the Accuracy cohort in which~95% of all confirmed mutations with VAF >5% had coverage >200X. Applying these criteria to the dataset, the sensitivity of 94% for SNVs and indels and a specificity of 98% using a VAF of 0.05 and coverage >200X was established. Cutoff values for variant frequency and coverage were selected to minimize false positive rates.

False negative rates for SNV and indels will be minimized by requiring at least 10% estimated tumor content and by manual inspection of all detected actionable somatic nonsynonymous actionable mutations even those with coverage 50X-200X (see section on Coverage).

b) *Clinical Specimens:* Limit of detection was confirmed by profiling 16 FFPE samples including 6 SNV, 5 insertions and 5 deletions with VAF at the LOD in 5 separate runs (80 replicates total). All replicates were performed with 100 ng DNA input (lowest required input). Mutations were accurately called in 80 out of 80 times, 5 out of 5 per mutation, with 100% Positive call rate see Table 16 below.

Table 16. Inter-assay reproducibility at the LOD for VAF

Gene	Mutation type	AA change	mean	SD	med	min	max	positive call	positive call rate(95% CI)
SNV									
AHI1	SNV	p.S1123F	0.08	0.02	0.07	0.06	0.10	5 out of 5	100%(46.3%, 100%)
TP53	SNV	p.R280G	0.08	0.01	0.08	0.06	0.10	5 out of 5	100%(46.3%, 100%)
KRAS	SNV	p.G12V	0.07	0.01	0.07	0.06	0.09	5 out of 5	100%(46.3%, 100%)
PIK3CA	SNV	p.E545K	0.08	0.02	0.08	0.06	0.11	5 out of 5	100%(46.3%, 100%)
KRAS	SNV	p.G12C	0.06	0.01	0.06	0.06	0.07	5 out of 5	100%(46.3%, 100%)
BRAF	SNV	p.V600E	0.06	0.01	0.06	0.05	0.07	5 out of 5	100%(46.3%, 100%)
Insertions									
EGFR	Insertion	p.D770delinsDNPHG	0.07	0.01	0.06	0.06	0.08	5 out of 5	100%(46.3%, 100%)
FOXO3	frameshift insertion	p.D380fs	0.08	0.01	0.08	0.06	0.08	5 out of 5	100%(46.3%, 100%)
EP400	nonframeshift insertion	p.R2719delinsRQ	0.07	0.01	0.07	0.06	0.08	5 out of 5	100%(46.3%, 100%)
IL21R	frameshift insertion	p.S383Lfs*5	0.07	0.01	0.07	0.06	0.09	5 out of 5	100%(46.3%, 100%)
BCR	frameshift insertion	BCR:NM_021574:exon18:	0.08	0.01	0.08	0.07	0.09	5 out of 5	100%(46.3%, 100%)
Deletions									
EGFR	Deletion	p.746_750del	0.09	0.01	0.10	0.08	0.10	5 out of 5	100%(46.3%,

									100%)
KMT2B	frameshift deletion	p.R965Dfs*21	0.08	0.01	0.07	0.07	0.082814	5 out of 5	100%(46.3%, 100%)
FIP1L1	frameshift deletion	FIP1L1:NM_001134938:ex	0.07	0.02	0.06	0.05	0.089783	5 out of 5	100%(46.3%, 100%)
ASXL1	frameshift deletion	p.G643fs	0.07	0.01	0.07	0.06	0.091612	5 out of 5	100%(46.3%, 100%)
ACVR2A	frameshift deletion	p.K437Rfs*5	0.08	0.02	0.08	0.07	0.110333	5 out of 5	100%(46.3%, 100%)

c) *DNA-Input:* The validated DNA concentration is the amount at which the average read depth over the exon regions was maintained at the criteria established including (e.g., Number of targets with 0 and <50X coverage < 1%, Average coverage of the sample >300X) and have 100% positive mutation call rate based on reproducibility and sensitivity studies. The recommended DNA concentration for the assay is 250ng with the lowest acceptable DNA input of 100 ng. The DNA input range 50-250ng was assessed for accuracy and sequencing failures as a function of the input DNA concentration, see Figures 4 and 5 above.

3. Linearity/assay reportable range**:**

Not applicable

4. Traceability (controls, calibrators, or methods):
The NYU Langone Genome PACT is not traceable to any known standard. Controls and quality metrics are described in the device description section.

5. Stability
Reagent stability is based on manufacturer expiration dating, and supported by NYU molecular pathology laboratory verification. Stability of the reagents is monitored through the use of consistent controls.

6. Expected values:
The laboratory follows protocols for the use of controls consistent with CLIA regulation. The NYU Langone Genome PACT does not use calibrators; however, the verification of mutant allele frequency is maintained by analysis of a positive control sample with expected allele frequencies.

7. Analytical specificity:
High analytical specificity is maintained by paired tumor/matched normal sequencing and was established during assay optimization.

Interference:
The NYU Langone Genome PACT assay pre-analytic steps are designed to minimize interference. Provided the quality of the FFPE specimen received for testing complies with standard fixation and embedding conditions (e.g.: no degradation, not decalcified), no interfering substances were identified during the validation, using DNA extracted by

protocol standard procedures.

8. Assay cut-off:

The NYU Langone PACT does not report mutations below 5% variant allele frequency.

9. Comparison studies: Accuracy studies

a) *Method comparison:*

The NYU Langone Genome PACT assay is designed to detect SNVs and small insertions and deletions in the entire coding sequence of 607 genes. The accuracy of the assay was assessed by comparison of the NYU Langone Genome PACT result to the original results obtained with the validated orthogonal methods. Testing was conducted per protocol on 455 unique FFP cancer samples, see Table 17. A total of 777 unique mutations in 409 tumor specimens that passed QC criteria, from 19 different cancer types were tested and are listed in Table 17 and Figure 6 and 7 below.

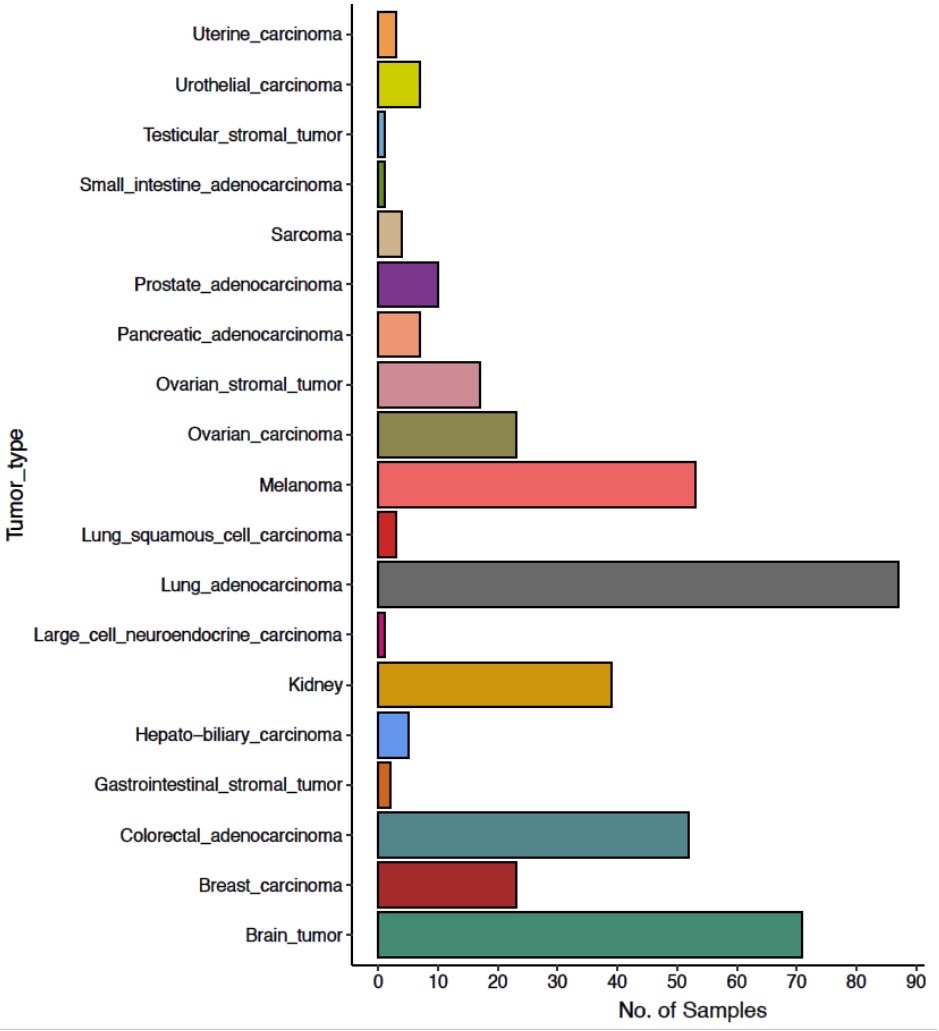


Figure 6. Distribution of all tumor types in the Accuracy cohort.

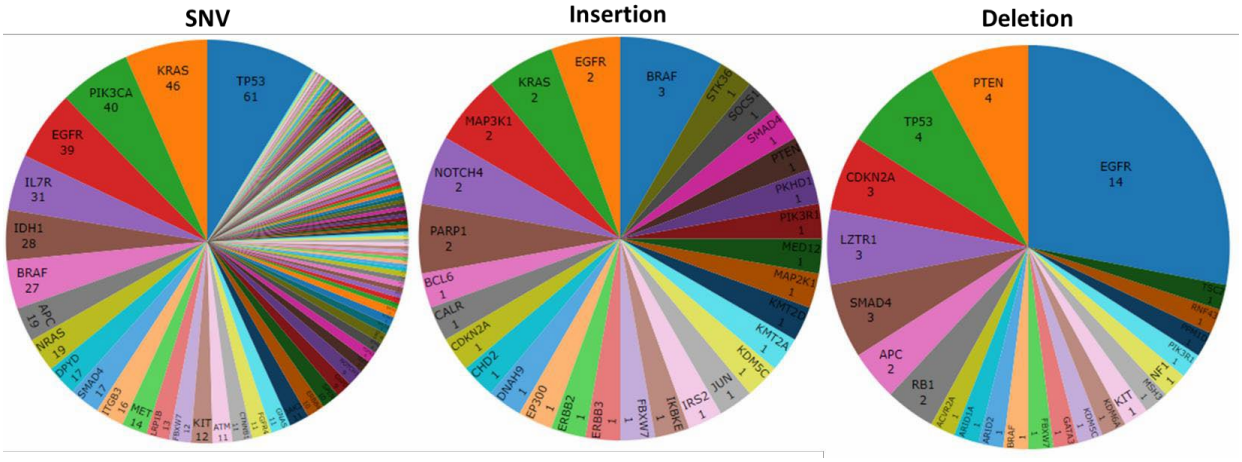


Figure 7. Distribution of all confirmed SNVs (A), insertions (B) and deletions (C).

Table 17. Unique SNVs, Insertions and Deletions Represented in the Accuracy Summary Per Gene, Exon and AA change

Gene	Mutation type	Exon	AA change	Number of Samples
ABL1	SNV	exon6	N350D	1
AKT1	SNV	exon3	D46E	1
AKT1	SNV	exon3	E17K	1
APC	SNV	exon16	E1317Q	2
APC	Deletion	exon16	E1464fs	2
APC	SNV	exon16	R1114X	2
APC	SNV	exon16	V1125A	2
APC	SNV	exon6	R876X	5
APC	SNV	exon16	E1309K	1
APC	SNV	exon16	E1353X	1
APC	SNV	exon16	G131X	1
APC	SNV	exon16	I1307K	1
APC	SNV	exon16	Q1429X	1
APC	SNV	exon16	R1450X	1
AR	SNV	exon5	W742C	1
ARID1A	Deletion	exon1	D1850fs*4	1
ARID2	Deletion	exon19	K1716fs	1
ARID2	SNV	exon19	Q1717X	1
ATM	SNV	exon17	F858L	2
ATM	SNV	exon12	P604S	2
ATM	SNV	exon34	S1691R	2

Gene	Mutation type	Exon	AA change	Number of Samples
ATM	SNV	exon63	G3051X	1
ATM	SNV	exon56	N2736T	1
ATM	SNV	exon63	R3008C	1
ATM	SNV	exon23	G1130X	1
ATM	SNV	exon22	W1058X	1
ATR	SNV	exon32	E1840Q	1
ATRX	SNV	exon2	N12S	1
BARD1	SNV	exon4	M383I	1
BCL6	Insertion	exon5	F497_P498insS	1
BCL6	SNV	exon4	R98Q	1
BRAF	Insertion	exon15	V600K	2
BRAF	Insertion	exon15	VK600_601EN	2
BRAF	SNV	exon15	V600E	21
BRAF	SNV	exon15	G596R	1
BRAF	SNV	exon15	V600M	1
BRAF	SNV	exon15	W604L	1
CALR	Deletion	exon9	E364fs	2
CALR	Deletion	exon9	Q365fs	2
CALR	Insertion	exon9	K385fs	4
CDK12	SNV	exon1	M1K	1
CDKN2A	Deletion	exon2	A60fs	1
CDKN2A	Deletion	exon2	L62fs	1
CDKN2A	Insertion	exon2	R80X	1
CDKN2A	SNV	exon2	V59L	1
CDKN2A	SNV	exon2	W110X	1
CDKN2A	SNV	exon2	G55D	1
CIC	SNV	exon18	D1440N	1
CSF1R	SNV	exon20	E916K	1
CTNNB1	SNV	exon4	S33F	4
CTNNB1	SNV	exon4	D25A	1
CTNNB1	SNV	exon4	D32H	1
CTNNB1	SNV	exon4	S26F	1
CTNNB1	SNV	exon4	S45F	1
CTNNB1	SNV	exon11	V534A	1
DDX3X	SNV	exon5	D98V	1
EGFR	SNV	exon19	V742F	2
EGFR	SNV	exon20	V769M	2

Gene	Mutation type	Exon	AA change	Number of Samples
EGFR	SNV	exon20	T790M	6
EGFR	Deletion	exon19	E746_A750del	12
EGFR	SNV	exon21	L858R	13
EGFR	Deletion	exon19	745_750del	1
EGFR	SNV	exon7	A289V	1
EGFR	Insertion	exon20	A767delinsASVD	1
EGFR	SNV	exon18	A864V	1
EGFR	Deletion	exon20	D770delinsDNPHG	1
EGFR	SNV	exon3	E114K	1
EGFR	SNV	exon18	E709A	1
EGFR	SNV	exon18	G719A	1
EGFR	SNV	exon18	K708E	1
EGFR	SNV	exon21	L861R	1
EGFR	SNV	exon3	Q105H	1
EGFR	SNV	exon3	R108K	1
EGFR	SNV	exon6	S227F	1
ERBB2	SNV	exon20	D769Y	1
ERBB2	Insertion	exon20	E755delinsEAYVM	1
ERBB3	Insertion	exon23	A913fs	1
ERBB3	SNV	exon3	V104L	1
ERBB4	SNV	exon15	R612Q	2
ERBB4	SNV	exon28	E1261X	1
ERBB4	SNV	exon28	L1296Q	1
ERBB4	SNV	exon27	P1132L	1
ERBB4	SNV	exon6	T209K	1
ERBB4	SNV	exon11	T422A	1
ERBB4	SNV	exon11	Y429H	1
ERBB4	SNV	exon1	A17V	1
ERBB4	SNV	exon3	L91R	1
ERG	SNV	exon4	R196K	1
ESR1	SNV	exon6	S341T	1
EZH2	SNV	exon16	Y646C	2
FANCA	SNV	exon25	R770C	1
FANCE	SNV	exon2	E276K	1
FANCF	SNV	exon1	R55G	1
FAS	SNV	exon7	T214N	1
FBXW7	SNV	exon11	R465H	3

Gene	Mutation type	Exon	AA change	Number of Samples
FBXW7	Insertion	exon10	R505C	3
FBXW7	SNV	exon5	Q277X	1
FBXW7	Deletion	exon8	Q388fs	1
FBXW7	SNV	exon5	R278X	1
FBXW7	SNV	exon11	R465C	1
FBXW7	SNV	exon11	R465H	1
FBXW7	SNV	exon11	S582L	1
FBXW7	SNV	exon13	S596F	1
FGF19	SNV	exon3	R127C	1
FGF3	SNV	exon3	M190I	1
FGF4	SNV	exon2	G143S	1
FGFR2	SNV	exon7	R255Q	1
FGFR3	SNV	exon16	A719T	1
FGFR3	SNV	exon14	H645R	1
FGFR3	SNV	exon13	R566W	1
FGFR3	SNV	exon7	S249C	1
FGFR3	SNV	exon13	P584L	1
FGFR3	SNV	exon17	R750C	1
FGFR4	SNV	exon9	G388R	1
FLT1	SNV	exon16	A769V	1
FLT3	Insertion	exon14	E598delinsETGSSDNEYFYVDFREYE	2
FLT3	SNV	exon20	D835Y	1
FLT3	Insertion	exon14	F612delinsYEYDLKWEFPRENLEF	1
FLT3	Insertion	exon14	K602delinsFREYEYDLK	1
FLT3	SNV	exon11	P460S	1
FLT4	SNV	exon29	E1283X	1
FLT4	SNV	exon29	Q1286E	1
FLT4	SNV	exon13	E586Q	1
GATA3	SNV	exon6	M357I	1
GATA3	Deletion	exon3	S237fs*67	1
GNAQ	SNV	exon5	Q209P	2
GNAQ	SNV	exon5	S267F	1
GNAS	SNV	exon8	R844H	3
GNAS	SNV	exon8	R844C	4
GNAS	SNV	exon1	P349L	1
GNAS	SNV	exon1	P376L	1

Gene	Mutation type	Exon	AA change	Number of Samples
GNAS	SNV	exon8	R201C	1
HRAS	SNV	exon3	G48E	1
HSP90AA1	SNV	exon1	P13S	1
IDH1	SNV	exon4	R132C	3
IDH1	SNV	exon4	R132H	25
IDH2	SNV	exon4	R172K	4
IDH2	SNV	exon4	R172M	1
IDH2	SNV	exon4	R172W	1
IGF1R	SNV	exon14	A943T	1
IGF1R	SNV	exon1	S20F	1
IRS2	Insertion	exon1	A701_V702insA	1
IRS2	SNV	exon1	D1324G	1
IRS2	SNV	exon1	K118R	1
JAK2	Deletion	exon12	541_543del	2
JAK2	SNV	exon14	V617F	6
JAK2	SNV	exon14	V617E	3
JAK2	SNV	splice site	splice site 1642-2A>C	1
JAK3	SNV	exon16	R697W	2
JAK3	SNV	exon16	V722I	5
JAK3	SNV	exon19	G845S	1
JAK3	SNV	exon4	Q140K	1
JAK3	SNV	exon16	V718L	1
JAK3	SNV	exon3	L73M	1
KDM5C	Deletion	exon26	P1508fs*36	1
KDM5C	SNV	splice site	splice site 352-1G>T	1
KDM6A	SNV	exon17	Q835X	1
KDR	SNV	exon30	L1327X	1
KEAP1	SNV	exon3	V369A	1
KEL	SNV	exon1	M1T	1
KIT	SNV	exon11	G565V	2
KIT	SNV	exon17	R804Q	2
KIT	SNV	exon19	D872N	1
KIT	SNV	exon11	L576P	1
KIT	SNV	exon10	M536T	1
KIT	SNV	exon10	M537L	1
KIT	SNV	exon14	S684T	1
KIT	SNV	exon10	V532I	1

Gene	Mutation type	Exon	AA change	Number of Samples
KIT	SNV	exon11	V555D	1
KIT	Deletion	exon11	V559_E561del	1
KIT	SNV	exon13	V654A	1
KMT2D	Insertion	exon33	I2760delinsRMGPLSLLGGNPTRLLSGPI	1
KMT2D	SNV	exon5	P196L	1
KMT2D	SNV	exon42	S4617C	1
KRAS	SNV	exon2	G12A	2
KRAS	SNV	exon2	G12C	2
KRAS	Insertion	exon2	G12F	2
KRAS	SNV	exon2	G13C	2
KRAS	SNV	exon2	G13D	4
KRAS	SNV	exon2	G12D	6
KRAS	SNV	exon2	G12V	9
KRAS	SNV	exon2	A146T	1
KRAS	SNV	exon5	K185R	1
LRP1B	SNV	exon23	K1223M	2
LRP1B	SNV	exon66	C3449Y	1
LRP1B	SNV	exon49	K2639E	1
LRP1B	SNV	exon41	L2157V	1
LRP1B	SNV	exon44	P2450T	1
LRP1B	SNV	exon2	Q48R	1
LRP1B	SNV	exon37	W1969C	1
LRP1B	SNV	exon56	C2978X	1
LRP1B	SNV	exon25	E1338K	1
LRP1B	SNV	exon16	I844M	1
LRP1B	SNV	exon48	P2605S	1
LZTR1	Deletion	exon8	F258fs	2
LZTR1	Deletion	splice site	splice site 401-2delA	1
MET	SNV	exon14	R988C	2
MET	SNV	exon14	T1010I	10
MET	SNV	exon2	M362T	1
MLH1	SNV	exon12	A395T	1
MPL	SNV	exon10	G509S	1
MRE11A	SNV	exon6	A177T	1
MRE11A	SNV	exon17	V618A	1
MTOR	SNV	exon43	R1987Q	1

Gene	Mutation type	Exon	AA change	Number of Samples
MYC	SNV	exon2	S82F	1
MYCN	SNV	exon2	G144S	1
NF1	SNV	exon28	L1274P	1
NF1	Deletion	exon46	T2284fs	1
NFE2L2	SNV	exon2	D13H	3
NFE2L2	SNV	exon2	L14V	1
NFE2L2	SNV	exon2	R18G	1
NOTCH1	SNV	exon34	L2457V	3
NOTCH1	SNV	exon9	C514W	1
NOTCH1	SNV	exon34	L244P	1
NOTCH1	SNV	exon34	S2467L	1
NOTCH1	SNV	exon27	V1721M	1
NOTCH1	SNV	exon34	V2200M	1
NOTCH1	SNV	exon26	G1673S	1
NOTCH1	SNV	exon13	G691R	1
NOTCH4	Insertion	exon13	G704fs	1
NPM1	Insertion	exon11	L287fs	11
NRAS	SNV	exon3	Q61H	2
NRAS	SNV	exon3	Q61R	4
NRAS	SNV	exon3	Q61K	6
NRAS	SNV	exon2	G12C	1
NRAS	SNV	exon2	G12D	1
NRAS	SNV	exon2	G13D	1
NRAS	SNV	exon2	G13R	1
NRAS	SNV	exon3	S65R	1
NTRK1	SNV	exon14	S550P	1
NTRK1	SNV	exon12	E492K	1
PALB2	SNV	exon4	V78I	1
PIK3C2B	SNV	exon3	E209Q	1
PIK3C2B	SNV	exon3	R260Q	1
PIK3CA	SNV	exon2	R108H	2
PIK3CA	SNV	exon21	H1047L	4
PIK3CA	SNV	exon10	E545K	5
PIK3CA	SNV	exon10	E542K	6
PIK3CA	SNV	exon21	H1047R	8
PIK3CA	SNV	exon21	A1035V	1
PIK3CA	SNV	exon21	M1043I	1

Gene	Mutation type	Exon	AA change	Number of Samples
PIK3CA	SNV	exon2	R88Q	1
PIK3CA	SNV	exon10	E545Q	1
PIK3CG	SNV	exon2	R579I	1
PIK3R1	Deletion	exon10	422_427del	1
POLD1	SNV	exon22	D911N	1
PTCH1	SNV	exon18	F976L	1
PTEN	Insertion	exon9	E18fs	1
PTEN	Deletion	exon5	G127fs	1
PTEN	SNV	exon5	Q110E	1
PTEN	SNV	exon6	R173H	1
PTEN	Deletion	exon9	R378fs	1
PTEN	Deletion	exon10	Y550fs	1
PTPN11	SNV	exon13	E523K	1
PTPN11	SNV	exon13	G503V	1
RAF1	SNV	exon10	Y364C	1
RB1	Deletion	exon11	S350fs	1
RET	SNV	exon15	I880N	1
RET	SNV	exon14	R813W	1
RNF43	Deletion	exon1	G659fs*41	1
RNF43	SNV	exon4	R145Q	1
ROS1	SNV	exon4	C80F	1
ROS1	SNV	exon43	L2292V	1
RPTOR	SNV	exon20	E776Q	1
SMAD4	Deletion	exon9	330_331del	2
SMAD4	SNV	exon12	R497H	2
SMAD4	Deletion	exon10	413_414del	1
SMAD4	SNV	exon12	D537H	1
SMAD4	SNV	exon12	I525V	1
SMAD4	SNV	exon3	K88R	1
SMAD4	Insertion	exon3	L121fs	1
SMAD4	SNV	exon2	M4T	1
SMAD4	SNV	exon6	P257L	1
SMAD4	SNV	exon9	P320T	1
SMAD4	SNV	exon3	P91S	1
SMAD4	SNV	exon5	T222A	1
SMAD4	SNV	exon9	V333I	1
SMARCA4	SNV	exon26	E1144X	1

Gene	Mutation type	Exon	AA change	Number of Samples
SMARCB1	SNV	exon4	P146S	1
SMO	SNV	exon6	R400H	2
SMO	SNV	exon11	P641A	1
SMO	SNV	exon5	V329G	1
SOX10	SNV	exon4	S314L	1
SPEN	SNV	exon11	A970V	1
SPEN	SNV	exon2	I84T	1
SPEN	SNV	exon11	K1293E	1
SPEN	SNV	exon11	S1540F	1
SPEN	SNV	exon11	E2264X	2
SPEN	SNV	exon11	D2266N	1
SPEN	SNV	exon11	E1962K	1
SPEN	SNV	exon4	E330K	1
SPEN	SNV	exon11	L1934S	1
SPTA1	SNV	exon3	Q95E	1
STAT4	SNV	exon5	A148T	1
STK11	SNV	exon5	A205V	1
STK11	SNV	exon6	E256X	1
TERT	SNV	promoter	TERT promoter -124C>T	4
TERT	SNV	promoter	TERT promoter -146C>T	1
TET2	SNV	exon3	P712S	1
TGFBR2	SNV	exon7	D522Y	1
TGFBR2	SNV	exon4	I180V	1
TP53	SNV	exon8	G266R	2
TP53	SNV	exon5	H179R	2
TP53	SNV	exon4	R110L	2
TP53	SNV	exon6	R196X	2
TP53	SNV	exon6	R234C	2
TP53	SNV	exon7	R249M	2
TP53	Deletion	exon8	R282fs	2
TP53	SNV	exon6	R213X	3
TP53	SNV	exon10	R342X	3
TP53	SNV	exon7	G245S	4
TP53	SNV	exon5	R175H	4
TP53	SNV	exon8	R282W	4
TP53	SNV	exon7	R248Q	5
TP53	Deletion	exon4	A76fs	1

Gene	Mutation type	Exon	AA change	Number of Samples
TP53	SNV	exon7	C242F	1
TP53	SNV	exon4	G105D	1
TP53	SNV	exon4	G66D	1
TP53	SNV	exon5	H179Y	1
TP53	SNV	exon6	H214R	1
TP53	SNV	exon7	I251S	1
TP53	SNV	exon7	M246L	1
TP53	SNV	exon5	N92S	1
TP53	Deletion	exon5	P152fs	1
TP53	SNV	exon5	R158H	1
TP53	SNV	exon6	R196X	1
TP53	SNV	exon7	R209W	1
TP53	SNV	exon8	R267P	1
TP53	SNV	exon8	R267W	1
TP53	SNV	exon8	R273C	1
TP53	SNV	exon8	R273H	1
TP53	SNV	exon8	R306X	1
TP53	SNV	exon5	S127Y	1
TP53	SNV	exon7	S241F	1
TP53	SNV	exon5	V157F	1
TP53	SNV	exon4	Y107H	1
TSC1	SNV	exon15	K587R	1
TSC1	SNV	exon14	L456V	1
TSC2	Deletion	exon34	E1344del	1
TSC2	Deletion	exon35	F1510del	1
VHL	SNV	exon1	G104D	1
ZNF703	Deletion	exon1	H402_D403>PTHLGGSSCSTCSAHD	1

b) *Undetected variants*

Out of 777 total investigated mutations, 770 (99.1%) were detected and 7 known mutations (SNV or indels) (~1%) were not detected during the validation, (Table 18) and are considered false negative. These include:

- Four MYD88 L265P mutations, which were previously detected by outside quantitative PCR analysis. The limit of detection for this test is 0.2 percent mutant alleles. Upon review of the outside reports, all missed MYD88 cases were below the LOD.
- One CEBPA case showed suboptimal DNA fragmentation, which could be

the reason for suboptimal results.

- Mutations in KIT and STK11 genes with VAFs close to the LOD were were identified not upon re-review of the data. The depth of coverage is higher in the comparator hotspot panel for these regions, which could be the reason why these mutations were missed. In addition, amplicon based NGS, may have had slightly higher sensitivity, or higher false positive rate than hybrid capture (PACT) particularly at low frequency events.

Table 18. Missed variants

Gene	Genetic Aberration	Originally detected VAF / CN	Lower Limit of Detection of the original assay	Potential reasons for failure to detect the aberration
CEBPA	c.926_985dup	Not known	40% of mutant cells	Poor DNA quality / poor library quality
FLT3	D835	Not known	5%	SNV near homopolymeric region, therefore may not have been detected
KIT	D816V	5.5%	5%	Close to the LLD, coverage (?),different sequencing chemistry, age of blocks
MYD88	L265P	3.5	0.2%	Below the LLD
MYD88	L265P	1.7	0.2%	Below the LLD
MYD88	L265P	2.5	0.2%	Below the LLD
MYD88	L265P	3.2	0.2%	Below the LLD
STK11	E256Ter	5.2	5%	Close to the LLD, different sequencing chemistry with increased coverage,

c) *Insertions and deletions size distribution*

The Accuracy cohort included 50 deletions and 36 insertions. For distributions across different genes see Figure 10. Deletion size in our Accuracy cohort ranged from 1bp to 22bp, while insertion size ranged from 1bp to 36bp. The number of cases in each category size is shown in Figure 9 below. The only missed mutation in insertion/deletion category is noted in Table 18 above.

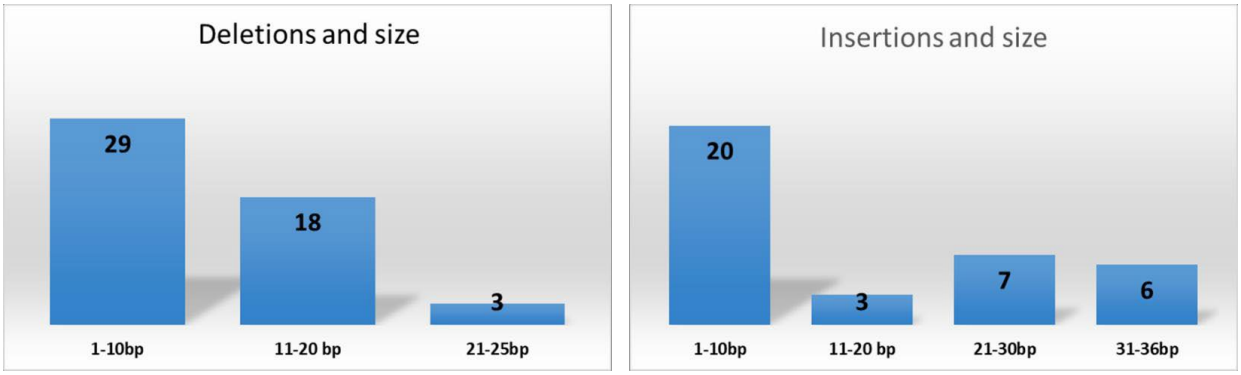


Figure 9: Distribution of deletions and insertions size. The number in the bar represents the number of detected cases in given size range.

d) *Positive percent agreement (PPA)*

PPA was based on a) the entire clinical Accuracy cohort, for which known positive

results come from various orthogonal assays, in-house and industry, and b) on a targeted comparison of 100 tumors which were profiled by PACT and an orthogonal NGS comparator that uses a different chemistry and examination of predefined 62 exons. PPA derived from the Accuracy cohort is summarized in Table 19.

Table 19. Positive percent agreement (PPA) assessment summary

	Positive call	**Positive call rate (95% CI)**
all mutations	770 out of 777	99.1% (98.2%,99.6%)
SNV	684 out of 690	99.1% (98.1%,99.6%)
Insertions	36 out of 37	97.3% (86.2%,99.5%)
Deletions	50 out of 50	100% (92.9%, 100.0%)
Level 2 mutations	140 out of 140	100% (97.3%, 100.0%)
Level 3 mutations	630 out of 637	98.9% (97.7%, 99.5%)

e) *Accuracy assessment of the wild-type sequence and negative percent agreement (NPA)*

To assess the concordance between NYU Genome PACT and orthogonal targeted hotspot 50 gene NGS method, we compared 100 tumors and pre-specified 62 exons in which at least one of the regions previously showed a mutation. All selected exons had coverage >500x by the orthogonal method, which was the requirement for reporting lack of detected mutation as negative result on the orthogonal NGS test (exons with coverage <500x would be reported as indeterminate in the orthogonal test). All 62 regions were analyzed by PACT in all 100 tumors, with a total of 6200 exons. Data are summarized in the Table 20 below.

In total, the cohort contained 236 known mutations (Expected positive value) across 62 exons in 100 tumors. All 236 known mutations were detected by PACT assay. Out of 5964 exons that were wild-type on the original orthogonal testing, one showed a SNV mutation in a TP53 exon8, not detected by the orthogonal method previously and therefore can be considered false positive, see Table 20.

Therefore, in this analysis, positive percent agreement (PPA) is 100% and negative percent agreement (NPA) is ~99% with one out of 100 cases showing a false positive result (Level 3 SNV mutation). No false positive Level 2 SNV mutations were detected (Level 2 NPA: 100%), for insertions and deletions (Level 2 and 3) NPA: 100%. Level 3 SNV NPA: ~99%

Based our Accuracy data, the PACT test can accurately detect insertions up to 35bp and deletions up to 25bp.

Table 20. Comparison of orthogonal NGS testing platform and NYU PACT:

Target Area (N=62)	Expected Positives	False Positives	Target Area (N=62)	Expected Positives	False Positives
AKT1 exon 3	1	0	KIT exon 11	4	0
APC exon 16	10	0	KIT exon 13	1	0
APC exon 6	1	0	KIT exon 17	2	0
ATM exon 12	1	0	KRAS exon 2	19	0
ATM exon 17	2	0	MET exon 14	10	0
ATM exon 34	1	0	MLH1 exon 12	1	0
ATM exon 56	1	0	NOTCH1 exon 34	1	0
BRAF exon 15	18	0	NRAS exon 2	2	0
CDKN2A exon 2	2	0	NRAS exon 3	7	0
CTNNB1 exon 4	5	0	PIK3CA exon 10	5	0
EGFR exon 19	10	0	PIK3CA exon 2	3	0
EGFR exon 20	6	0	PIK3CA exon 21	10	0
EGFR exon 21	13	0	PTEN exon 3	1	0
EGFR exon 3	1	0	PTEN exon 6	1	0
ERBB2 exon 20	1	0	PTEN exon 9	1	0
ERBB4 exon 15	2	0	PTPN11 exon 13	2	0
EZH2 exon 16	2	0	RB1 exon 11	1	0
FBXW7 exon 10	2	0	RET exon 15	1	0
FBXW7 exon 11	2	0	SMAD4 exon 12	2	0
FBXW7 exon 5	1	0	SMO exon 11	1	0
FGFR2 exon 7	1	0	SMO exon 5	1	0
FGFR3 exon 14	1	0	STK11 exon 5	1	0
FGFR3 exon 16	1	0	STK11 exon 6	1	0
FLT3 exon 11	1	0	TP53 exon 10	1	0
GNAQ exon 5	1	0	TP53 exon 4	4	0
GNAS exon 8	5	0	TP53 exon 5	10	0
HRAS exon 3	1	0	TP53 exon 6	5	0

IDH1 exon 4	15	0		TP53 exon 7	8	0
IDH2 exon 4	4	0		TP53 exon 8	11	**1**
JAK3 exon 16	3	0		VHL exon 1	1	0
KDR exon 30	1	0		KIT exon 11	4	0
KIT exon 10	2	0				

f) *Negative percent agreement (NPA)*

A single case showed an extra TP53 mutation in a head-to-head comparison between the NYU Langone Genome PACT and the orthogonal hotspot NGS assay. This results in an estimated 100% NPA for Level 2 and ~99% NPA for Level 3 mutations (SNV). No false positive insertions or deletions were detected with 100% NPA for both types of mutation in both Level 2 and Level 3 categories.

This NPA on Accuracy samples is similar to validated false positive rate/ specificity of 1.68% established by using HapMap samples.

10. Clinical Performance:

NYU Langone Genome PACT assay is a molecular profiling platform using next generation sequencing to detect somatic alterations (point mutations and small insertions and deletions) in tumor specimens using a 607 gene panel. The genes in the panel were selected for their role in cancer pathogenesis and tumor suppression, or for clinical or mechanistic information of relevance in the management of cancer patients. The assay reports mutations under two categories: Level 2: Cancer mutations with evidence of clinical significance, Level 3: Cancer mutations with potential clinical significance, following the FDA guidance: https://www.fda.gov/media/109050/download. The assay does not report variant of unknown significance, Benign or Likely Benign Variants. Mutations with evidence of clinical significance are represented in professional guidelines as established by consensus opinion of experts in the health care community

Clinical Evidence Curation:

NYU Langone PACT uses a clinical evidence curation resources (OncoKB and other commercial database sources reviewed by Langone) to facilitate the clinical interpretation of detected mutations. NofOne is a commercially available annotation service validated to provide state-of the art annotation of actionable variants in cancer. OncoKB is a publicly available open-source knowledge base that includes biologic, clinical and therapeutic information curated from multiple information resources including professional guidelines and recommendations, therapeutic labeling, disease specific expert and advocacy group recommendations, and medical literature. OncoKB information is publicly available through an interactive web site. Classification criteria were developed by MSKCC to communicate the level of clinical evidence available for individual mutations in the test report. The mutations are reported under two categories (i.e., cancer mutations panel with evidence of clinical significance and cancer mutations panel with potential clinical significance)

based on the pre-specified classification criteria. OncoKB undergoes periodic updates through the review of new information by a panel of experts. Upon review, the review of each mutation detected by NYU Langone Genome PACT and the assignment into an appropriate Level is performed by a health care professional upon assessment of the literature, knowledge databases and clinical information.

11. Clinical cut-off:

Not applicable

12. Expected values:

The prevalence of somatic Level 2 and Level 3 mutations in cancer has been established by large- scale clinical and research sequencing initiatives using a comprehensive genomics assays including TCGA, MSK-IMPACT, AACR GENIE and other genomic projects through which mutational tumor data for specific tumor types have been compiled. The prevalence of cancer-associated mutations across published studies can be explored via publicly available domain cBioPortal for Cancer Genomics (https://www.cbioportal.org/). The portal contains data from 273 cancer studies https://www.cbioportal.org/datasets and enables search by organ system, cancer type, by a specific gene, set of genes or mutation.

Based on the study design, tumors with known cancer driver mutations were tested. The prevalence of affected genes for cancer types corresponds to the literature, Figure 12. The Oncoprint, see Figure 13-14, shows that TP53 is the most commonly mutated gene in cancer, followed by EGFR, KRAS and BRAF corresponding to a large portion of our validation cohort being composed of Lung Adenocarcinoma, Colorectal Adenocarcinoma and Melanoma. The NYU-PACT cohort shows high % of IDH1 mutations, which corresponds to the fact that Brain tumors contribute a large group of samples in the validation cohort.

N. Instrument Name

Illumina NextSeq Sequencer (qualified by NYU).

O. System Descriptions:

1. Modes of Operation:
   The Illumina NextSeq is a high throughput sequencing system using Sequencing-By-Synthesis chemistry.

2. Software:
   FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

   Yes __ or No _____

3. Level of Concern: Moderate

4. Specimen Handling
Refer to Device Description section above.

5 Calibration and Quality Controls:
Refer to Device Description section above.

P. Other Supportive Instrument Performance Characteristics Data Not Covered in the "Performance Characteristics" Section Above:

To support the continuous implementation of process improvements to the existing gene panel, protocols with specific procedures and acceptance criteria for modifications that could be anticipated at the time of submission were provided, reviewed by FDA, and cleared as part of this marketing authorization. Future modifications by NYU for the specified types of changes below that are made in accordance with the applicable validation strategy and the pre-specified success criteria would not require a new 510(k) submission. Significant changes such as adding new genes or variant types to the panel would require a new submission with appropriate validation.

Type of change	Validation Strategy	Required result criteria
New pre-analytical protocol, kits or reagents	Sequence at least 10 specimens with known somatic Level 2 mutations. Measure sequence coverage distribution, and call somatic mutations in all samples.	Passing all QC criteria, >300X average, ensure that 95% of exons are covered to 100x or more. Concordance for known mutations should be >95%.
New library preparation protocol, kits, or reagents	Sequence at least 40 DNA specimens (tumor / normal pairs) or three pools previously sequenced by NYU Langone Gene PACT and compare VAF, variant coverage depth and quality of somatic mutations	Passing all QC criteria, >300X average, ensure that 95% of exons are covered to 100x or more. Concordance for calling somatic mutations with variant allele fraction >10% should be at least 98%. False positive rate on negative controls should be <2% and Positive Control calls should be >95%.

Type of change		Validation Strategy	Required result criteria
Changes to probes for already analytically validated genes		Re-capture existing sequence libraries from at least 3 runs (at least 40 samples) with new probes, sequence, and analyze somatic mutations in all samples.	Passing all QC criteria, >300X average, ensure that 95% of exons are covered to 100x or more. Concordance for calling somatic mutations with variant allele fraction >10% should be >98%.
New sequencing instrument or reagents using similar chemistry and technology, and the sequence depth and read length are not changed from previous platform. (Refers to new instruments for the same instrument model i.e., new serial numbers)		Re-sequence existing captured libraries from at least 3 runs and call somatic mutations in all samples.	Passing all QC criteria, >300X average, ensure that 95% of exons are covered to 100x or more. Concordance for calling somatic mutations with variant allele fraction >10% should be at least 98%. False positive rate on negative controls should be <2% and Positive Control calls should be >95%.
Bioinformatic analysis and the pipeline	Update to underlying annotation database or transcript isoforms	Reanalyze FASTQ files (raw sequencing reads) from at least 3 previous runs (at least 40 samples). Compare variants calls between the clinical analysis results and the current modified results	Confirm the changes do not change the variant call results. Confirm the annotations for the unaffected transcripts do not change. Confirm the annotations for the affected transcripts are modified as expected.
	Update to data management system and	Reanalyze FASTQ files (raw sequencing reads) from at least 3 previous runs (at least 40 samples)	Ensure that all previously called mutations are recovered and the variants in
	system database	in production mode. Compare variants calls between the clinical analysis results and the current modified results	the database of results are concordant with the variants in the pipeline output files

Type of change		Validation Strategy	Required result criteria
	Modification to an existing component of the analysis pipeline (e.g., adding a bioinformatic tool or algorithm) where the underlying analysis architecture or main parameter settings (e.g. minimal coverage/VAF threshold for SNV/indel calling) are not changed.	Reanalyze FASTQ files (raw sequencing reads) from at least 3 runs (at least 40 samples). Compare variants calls between the clinical analysis results and the current modified results	Ensure that all previously called mutations are recovered and that newly detected mutations can be explained by pipeline modifications.

Q. Proposed Labeling:

The labeling is sufficient, and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type.

R. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.

Appendix 1: List of all genes/transcripts on NYU Langone Gene PACT Panel

Gene	Transcript
ABL1	NM_005157
ABL2	NM_005158
ACTG1	NM_001614
ACVR1	NM_001105
ACVR2A	NM_001278579
ADAMTS20	NM_025003
AFF1	NM_005935
AFF3	NM_001025108
AKAP9	NM_005751
AKT1	NM_001014431
AKT2	NM_001626
AKT3	NM_005465
ALK	NM_004304
AMER1	NM_152424
ANKRD24	NM_133475
ANKRD26	NM_014915
APC	NM_000038
AR	NM_000044
ARAF	NM_001654
ARFGAP3	NM_001142293
ARFRP1	NM_001267548
ARID1A	NM_006015
ARID2	NM_152641
ARNT	NM_001668
ASH1L	NM_018489
ASPSCR1	NM_024083
ASXL1	NM_015338
ATF1	NM_005171
ATM	NM_000051
ATR	NM_001184
ATRX	NM_000489
AURKA	NM_003600
AURKB	NM_004217
AURKC	NM_003160
AUTS2	NM_015570
AXL	NM_001699
B2M	NM_004048
BAI3	NM_001704
BAP1	NM_004656
BARD1	NM_000465
BCL10	NM_003921
BCL11A	NM_022893
BCL11B	NM_138576

Gene	Transcript
BCL2	NM_000633
BCL2L1	NM_138578
BCL2L2	NM_004050
BCL3	NM_005178
BCL6	NM_001706
BCL9	NM_004326
BCOR	NM_001123383
BCORL1	NM_021946
BCR	NM_004327
BIRC2	NM_001166
BIRC3	NM_001165
BIRC5	NM_001168
BLM	NM_000057
BLNK	NM_013314
BMPR1A	NM_004329
BOD1L1	NM_148894
BRAF	NM_004333
BRCA1	NM_007294
BRCA2	NM_000059
BRD3	NM_007371
BRIP1	NM_032043
BTK	NM_000061
BUB1B	NM_001211
C11orf30	NM_020193
CACNA1E	NM_001205294
CALR	NM_004343
CARD11	NM_032415
CASC5	NM_144508
CASP8	NM_001228
CBFB	NM_001755
CBL	NM_005188
CBLB	NM_170662
CBLC	NM_012116
CCND1	NM_053056
CCND2	NM_001759
CCND3	NM_001760
CCNE1	NM_001238
CD74	NM_001025158
CD79A	NM_001783
CD79B	NM_000626
CDC25C	NM_001790
CDC73	NM_024529
CDH1	NM_004360
CDH11	NM_001797

Gene	Transcript
CDH2	NM_001271
CDH20	NM_031891
CDH23	NM_001171932
CDH5	NM_001795
CDK12	NM_016507
CDK4	NM_000075
CDK6	NM_001259
CDK8	NM_001260
CDKN1B	NM_004064
CDKN2A	NM_000077
CDKN2B	NM_004936
CDKN2C	NM_001262
CEBPA	NM_004364
CHD2	NM_001271
CHD4	NM_001273
CHD5	NM_015557
CHD7	NM_017780
CHEK1	NM_001114122
CHEK2	NM_007194
CHIC2	NM_012110
CIC	NM_015125
CKS1B	NM_001826
CMPK1	NM_016308
COL1A1	NM_000088
CRBN	NM_016302
CREB1	NM_134442
CREB3L2	NM_194071
CREBBP	NM_004380
CRKL	NM_005207
CRLF2	NM_022148
CRTC1	NM_015321
CSF1R	NM_005211
CSF3R	NM_156039
CSMD3	NM_198123
CSNK2B	NM_001320
CTCF	NM_006565
CTDNEP1	NM_001143775
CTNNA1	NM_001903
CTNNB1	NM_001904
CUL3	NM_003590
CUX1	NM_181552
CXCR4	NM_001008540
CYLD	NM_015247
CYP2C19	NM_000769

Gene	Transcript
CYP2D6	NM_001025161
DAXX	NM_001141969
DCC	NM_005215
DCK	NM_000788
DDB2	NM_000107
DDIT3	NM_004083
DDR2	NM_001014796
DDX3X	NM_001193416
DDX41	NM_016222
DEK	NM_003472
DHX15	NM_001358
DICER1	NM_177438
DIS3	NM_014953
DNAH9	NM_001372
DNMT3A	NM_022552
DOT1L	NM_032482
DPYD	NM_000110
DROSHA	NM_001100412
DST	NM_001723
EED	NM_003797
EGFR	NM_005228
EGR1	NM_001964
EML4	NM_019063
EP300	NM_001429
EP400	NM_015409
EPCAM	NM_002354
EPHA3	NM_005233
EPHA5	NM_004439
EPHA7	NM_004440
EPHB1	NM_004441
EPHB4	NM_004444
EPHB6	NM_004445
ERBB2	NM_004448
ERBB3	NM_001982
ERBB4	NM_005235
ERCC1	NM_202001
ERCC2	NM_000400
ERCC3	NM_000122
ERCC4	NM_005236
ERCC5	NM_000123
ERG	NM_004449
ESR1	NM_001122742
ETNK1	NM_018638
ETS1	NM_005238

Gene	Transcript
ETV1	NM_004956
ETV4	NM_001079675
ETV5	NM_004454
ETV6	NM_001987
EWSR1	NM_005243
EXT1	NM_000127
EXT2	NM_207122
EZH1	NM_001991
EZH2	NM_004456
EZR	NM_003379
FAM175A	NM_139076
FAM46C	NM_017709
FAM5C	NM_199051
FANCA	NM_000135
FANCC	NM_000136
FANCD2	NM_033084
FANCE	NM_021922
FANCF	NM_022725
FANCG	NM_004629
FANCL	NM_018062
FAS	NM_000043
FBXW7	NM_033632
FGF10	NM_004465
FGF14	NM_175929
FGF19	NM_005117
FGF23	NM_020638
FGF3	NM_005247
FGF4	NM_002007
FGF6	NM_020996
FGFR1	NM_023110
FGFR2	NM_000141
FGFR3	NM_000142
FGFR4	NM_022963
FH	NM_000143
FIP1L1	NM_001134937
FLCN	NM_144997
FLI1	NM_002017
FLT1	NM_002019
FLT3	NM_004119
FLT4	NM_002020
FN1	NM_212476
FOXA1	NM_004496
FOXL2	NM_023067
FOXO1	NM_002015

Gene	Transcript
FOXO3	NM_201559
FOXP1	NM_032682
FOXP4	NM_001012426
FUBP1	NM_003902
FUS	NM_004960
FZR1	NM_001136197
G6PD	NM_000402
GABRA6	NM_000811
GATA1	NM_002049
GATA2	NM_032638
GATA3	NM_001002295
GDNF	NM_001278098
GID4	NM_024052
GNA11	NM_002067
GNA13	NM_006572
GNAI3	NM_006496
GNAQ	NM_002072
GNAS	NM_000516
GPR124	NM_032777
GPS2	NM_004489
GRIN2A	NM_000833
GRM8	NM_001127323
GSK3B	NM_002093
GUCY1A2	NM_001256424
H3F3A	NM_002107
H3F3B	NM_005324
HCAR1	NM_032554
HGF	NM_000601
HIF1A	NM_001530
HIST1H1B	NM_005322
HIST1H1C	NM_005319
HIST1H1D	NM_005320
HIST1H1E	NM_005321
HIST1H1E	NM_005321
HIST1H2AC	NM_003512
HIST1H3B	NM_003537
HIST2H3C	NM_021059
HLF	NM_002126
HMGA2	NM_003483
HNF1A	NM_000545
HNRNPK	NM_002140
HOOK3	NM_032410
HOXB13	NM_006361
HRAS	NM_005343

Gene	Transcript
HSP90AA1	NM_001017963
HSP90AB1	NM_007355
ICK	NM_016513
ID3	NM_002167
IDH1	NM_005896
IDH2	NM_002168
IGF1R	NM_000875
IGF2	NM_001291861
IGF2R	NM_000876
IKBKB	NM_001556
IKBKE	NM_014002
IKZF1	NM_006060
IL2	NM_000586
IL21R	NM_021798
IL3	NM_000588
IL6ST	NM_001190981
IL7R	NM_002185
ING4	NM_001127586
INHBA	NM_002192
INPP4B	NM_003866
IRF4	NM_002460
IRF8	NM_002163
IRS2	NM_003749
ITGA10	NM_003637
ITGA9	NM_002207
ITGB2	NM_001127491
ITGB3	NM_000212
JAK1	NM_002227
JAK2	NM_004972
JAK3	NM_000215
JARID2	NM_004973
JUN	NM_002228
KAT6A	NM_006766
KAT6B	NM_012330
KDM5A	NM_001042603
KDM5C	NM_004187
KDM6A	NM_021140
KDR	NM_002253
KEAP1	NM_012289
KEL	NM_000420
KIT	NM_000222
KLF6	NM_001160125
KLHL6	NM_130446
KMT2A	NM_005933

Gene	Transcript
KMT2B	NM_014727
KMT2C	NM_170606
KMT2D	NM_003482
KRAS	NM_004985
LAMP1	NM_005561
LCK	NM_005356
LIFR	NM_002310
LPHN3	NM_015236
LPP	NM_005578
LRP1B	NM_018557
LTF	NM_001199149
LTK	NM_002344
LUC7L2	NM_016019
LZTR1	NM_006767
MAF	NM_005360
MAFB	NM_005461
MAGEA1	NM_004988
MAGI1	NM_004742
MALT1	NM_173844
MAML2	NM_032427
MAP2K1	NM_002755
MAP2K2	NM_030662
MAP2K4	NM_003010
MAP3K1	NM_005921
MAP3K7	NM_003188
MAPK1	NM_002745
MAPK8	NM_001323302
MARK1	NM_018650
MARK4	NM_031417
MBD1	NM_001204139
MCL1	NM_021960
MDM2	NM_002392
MDM4	NM_002393
MECOM	NM_001105077
MED12	NM_005120
MEF2B	NM_001145785
MEN1	NM_130799
MET	NM_001127500
MITF	NM_000248
MKL1	NM_020831
MLF1	NM_001130156
MLH1	NM_000249
MLH3	NM_001040108
MLL	NM_005933

Gene	Transcript
MLLT1	NM_005934
MLLT10	NM_004641
MLLT3	NM_004529
MLLT4	NM_001040000
MMP2	NM_001127891
MN1	NM_002430
MNX1	NM_005515
MPL	NM_005373
MRE11A	NM_005590
MSH2	NM_000251
MSH3	NM_002439
MSH6	NM_000179
MTOR	NM_004958
MTR	NM_000254
MTRR	NM_024010
MUC1	NM_001044392
MUTYH	NM_012222
MYB	NM_005375
MYBL1	NM_001080416
MYC	NM_002467
MYCL	NM_001033082
MYCN	NM_005378
MYD88	NM_002468
MYH11	NM_001040114
MYH9	NM_002473
NBN	NM_002485
NCOA1	NM_003743
NCOA2	NM_006540
NCOA4	NM_005437
NCOR2	NM_006312
NF1	NM_001042492
NF2	NM_000268
NFE2L2	NM_006164
NFKB1	NM_001165412
NFKB2	NM_002502
NFKBIA	NM_020529
NIN	NM_182946
NKX2-1	NM_003317
NLRP1	NM_001033053
NOTCH1	NM_017617
NOTCH2	NM_024408
NOTCH4	NM_004557
NPM1	NM_002520
NRAS	NM_002524

Gene	Transcript
NSD1	NM_022455
NTRK1	NM_002529
NTRK2	NM_006180
NTRK3	NM_002530
NUMA1	NM_006185
NUP214	NM_005085
NUP93	NM_014669
NUP98	NM_016320
PAK3	NM_001128166
PALB2	NM_024675
PARP1	NM_001618
PAX3	NM_181459
PAX5	NM_016734
PAX7	NM_002584
PAX8	NM_003466
PBRM1	NM_018313
PBX1	NM_001204963
PCDHAC2	NM_018899
PDE4DIP	NM_014644
PDGFB	NM_002608
PDGFRA	NM_006206
PDGFRB	NM_002609
PDK1	NM_002610
PER1	NM_002616
PGAP3	NM_033419
PHF6	NM_032458
PHLPP2	NM_015020
PHOX2B	NM_003924
PIK3C2B	NM_002646
PIK3C3	NM_002647
PIK3CA	NM_006218
PIK3CB	NM_006219
PIK3CD	NM_005026
PIK3CG	NM_001282427
PIK3R1	NM_181523
PIK3R2	NM_005027
PIM1	NM_002648
PKD1L2	NM_001076780
PKHD1	NM_170724
PLAG1	NM_002655
PLCG1	NM_002660
PLCG2	NM_002661
PLEKHG5	NM_020631
PML	NM_033238

Gene	Transcript
PMS1	NM_000534
PMS2	NM_000535
POLD1	NM_002691
POLE	NM_006231
POT1	NM_015450
POU5F1	NM_203289
PPARG	NM_015869
PPM1D	NM_003620
PPP2R1A	NM_014225
PPP6C	NM_002721
PRCC	NM_005973
PRDM1	NM_001198
PRDM16	NM_022114
PREX2	NM_024870
PRKAR1A	NM_212471
PRKDC	NM_006904
PRPF8	NM_006445
PSIP1	NM_021144
PTCH1	NM_000264
PTEN	NM_000314
PTGS2	NM_000963
PTPN11	NM_002834
PTPRD	NM_002839
PTPRT	NM_133170
RAC1	NM_006908
RAD21	NM_006265
RAD50	NM_005732
RAD51	NM_002875
RAD51C	NM_058216
RAD51D	NM_002878
RAF1	NM_002880
RALGDS	NM_006266
RARA	NM_000964
RB1	NM_000321
RBBP6	NM_006910
RBM15	NM_022768
RECQL4	NM_004260
REL	NM_002908
RET	NM_020975
RHOH	NM_004310
RICTOR	NM_152756
RNASEL	NM_021133
RNF2	NM_007212
RNF213	NM_020954

Gene	Transcript
RNF43	NM_017763
ROS1	NM_002944
RPL22	NM_000983
RPN1	NM_002950
RPS14	NM_001025070
RPS6KA2	NM_021135
RPTOR	NM_020761
RRM1	NM_001033
RUNX1	NM_001754
RUNX1T1	NM_175635
SAMD9	NM_017654
SBDS	NM_016038
SDHA	NM_004168
SDHB	NM_003000
SDHC	NM_003001
SDHD	NM_003002
SETBP1	NM_015559
SETD2	NM_014159
SETD5	NM_001080517
SEPT9	NM_001113496
SF3B1	NM_012433
SGK1	NM_005627
SH2B3	NM_005475
SH2D1A	NM_002351
SLC29A1	NM_001304465
SMAD2	NM_005901
SMAD4	NM_005359
SMARCA4	NM_001128844
SMARCB1	NM_003073
SMARCE1	NM_003079
SMC1A	NM_006306
SMC3	NM_005445
SMO	NM_005631
SMUG1	NM_001243791
SNX31	NM_152628
SOCS1	NM_003745
SOCS3	NM_003955
SOX10	NM_006941
SOX11	NM_003108
SOX2	NM_003106
SP140	NM_001005176
SPEN	NM_015001
SPI1	NM_003120
SPOP	NM_003563

Gene	Transcript
SPTA1	NM_003126
SRC	NM_005417
SRSF2	NM_003016
SS18L1	NM_198935
SSX1	NM_005635
SSX2	NM_003147
SSX4	NM_001243313
STAG2	NM_001282418
STAT3	NM_139276
STAT4	NM_001243835
STK11	NM_000455
STK36	NM_015690
SUFU	NM_016169
SUZ12	NM_015355
SYK	NM_001135052
SYNE1	NM_182961
SYT1	NM_001135806
TAF1	NM_138923
TAF1L	NM_153809
TAL1	NM_001287347
TBX22	NM_001109878
TCF12	NM_207036
TCF3	NM_003200
TCF7L1	NM_031283
TCF7L2	NM_030756
TCL1A	NM_021966
TERT	NM_198253
TET1	NM_030625
TET2	NM_001127208
TET3	NM_144993
TFE3	NM_001282142
TGFB1	NM_000660
TGFBR2	NM_003242
TGM7	NM_052955
THBS1	NM_003246
TIMP3	NM_000362
TLR2	NM_003264
TLR4	NM_138554
TLX1	NM_001195517
TMEM216	NM_001173990
TMPRSS2	NM_005656
TNFAIP3	NM_001270507
TNFRSF1	NM_001065
TNK2	NM_005781

Gene	Transcript
TOP1	NM_003286
TP53	NM_000546
TPM3	NM_001043351
TPR	NM_003292
TRAF3	NM_003300
TRIM24	NM_015905
TRIM33	NM_015906
TRIP11	NM_004239
TRRAP	NM_003496
TSC1	NM_000368
TSC2	NM_000548
TSHR	NM_000369
TYK2	NM_003331
U2AF1	NM_006758
UBR5	NM_015902
UGT1A1	NM_000463
UMODL1	NM_001199528
USP9X	NM_001039590
VHL	NM_000551
WAS	NM_000377
WHSC1	NM_133334
WISP3	NM_003880
WRN	NM_000553
WT1	NM_024426
XPA	NM_000380
XPC	NM_004628
XPO1	NM_003400
XRCC2	NM_005431
ZMYM2	NM_003453
ZMYM3	NM_005096
ZNF217	NM_006526
ZNF384	NM_133476
ZNF521	NM_015461
ZNF703	NM_025069
ZRSR2	NM_005089
ZSWIM4	NM_023072

Appendix 2: List of regions not reported

Gene	Blacklist
ABL2	[ABL2] chr1:179102448-179102509
AFF3	[AFF3] chr2:100627960-100628033
AFF3	[AFF3] chr2:100721131-100721178
AKT3	[AKT3] chr1:243846500-243846516
ARNT	[ARNT] chr1:150833724-150833778
ARNT	[ARNT] chr1:150833838-150833853
ATM	[ATM] chr11:108117272-108117287
BCL3	[BCL3] chr19:45251953-45252303
C5orf54	[C5orf54] chr5:159820714-159822497
CASC5	[CASC5] chr15:40902998-40903074
CASC5	[CASC5] chr15:40903677-40903709
CASP8	[CASP8] chr2:202142451-202142891
CHEK2	[CHEK2] chr22:29117507-29117619
CHEK2	[CHEK2] chr22:29126409-29126536
CSF3R	[CSF3R] chr1:36943207-36943278
DDR2	[DDR2] chr1:162681083-162681090
DNMT3A	[DNMT3A] chr2:25564696-25564771
ERCC2	[ERCC2] chr19:45862018-45862170
FANCC	[FANCC] chr9:97905807-97905825
FGF14	[FGF14] chr13:102378963-102379160
FGF14	[FGF14] chr13:102521076-102521178
FGF14	[FGF14] chr13:102527537-102527646
FGF14	[FGF14] chr13:102568804-102568995
FLT4	[FLT4] chr5:180034754-180035284
FOXL2	[FOXL2] chr3:138664435-138665564
HNF1A	[HNF1A] chr12:121426637-121426835
HNF1A	[HNF1A] chr12:121431324-121431509
HNF1A	[HNF1A] chr12:121431968-121432208
HNF1A	[HNF1A] chr12:121434345-121434969
HNF1A	[HNF1A] chr12:121435278-121435468
HNF1A	[HNF1A] chr12:121437072-121437430
HNF1A	[HNF1A] chr12:121438869-121438995
IGF2	[IGF2] chr11:2154218-2154453
IGF2	[IGF2] chr11:2156598-2156763
IGF2	[IGF2] chr11:2158423-2158531
IGF2	[IGF2] chr11:2159460-2160204
INS-IGF2	[INS-IGF2] chr11:2154218-2154453
INS-IGF2	[INS-IGF2] chr11:2156598-2156763
INS-IGF2	[INS-IGF2] chr11:2158423-2158531
INS-IGF2	[INS-IGF2] chr11:2159460-2160204
LPHN3	[LPHN3] chr4:62383034-62383236
LRP1B	[LRP1B] chr2:140992356-140992453
MEF2B	[MEF2B] chr19:19257083-19257193

Gene	Blacklist
MEF2BNB-MEF2B	[MEF2BNB-MEF2B] chr19:19257083-19257193
MET	[MET] chr7:116335812-116335853
MET	[MET] chr7:116364155-116364218
MLF1	[MLF1] chr3:158300446-158300718
MLF1	[MLF1] chr3:158306642-158306713
MLH1	[MLH1] chr3:37038648-37038791
MLLT1	[MLLT1] chr19:6279785-6279795
MLLT10	[MLLT10] chr10:21840746-21840850
MMP2	[MMP2] chr16:55515475-55515790
NLRP1	[NLRP1] chr17:5458462-5458500
NOTCH2	[NOTCH2] chr1:120572530-120572610
PAX3	[PAX3] chr2:223065894-223066161
PAX3	[PAX3] chr2:223066433-223066909
PAX3	[PAX3] chr2:223158350-223159020
PDE4DIP	[PDE4DIP] chr1:144952567-144952689
PDE4DIP	[PDE4DIP] chr1:144997084-144997111
PHLPP2	[PHLPP2] chr16:71752224-71752229
PIK3R2	[PIK3R2] chr19:18279286-18279356
PLEKHG5	[PLEKHG5] chr1:6557381-6557484
PMS2	[PMS2] chr7:6013031-6013173
POT1	[POT1] chr7:124477175-124477270
POT1	[POT1] chr7:124488595-124488713
RAD51	[RAD51] chr15:40994005-40994124
RB1	[RB1] chr13:48954190-48954220
REL	[REL] chr2:61147519-61147613
SDHD	[SDHD] chr11:111963805-111963933
SMARCA4	[SMARCA4] chr19:11144444-11144541
SP140	[SP140] chr2:231175459-231175566
SSX2	[SSX2] chrX:52727039-52727138
SSX2	[SSX2] chrX:52727837-52727981
SSX2	[SSX2] chrX:52729494-52729628
SSX2	[SSX2] chrX:52731632-52731680
SSX2	[SSX2] chrX:52733548-52733642
SSX2	[SSX2] chrX:52734179-52734292
SSX2	[SSX2] chrX:52734732-52734799
SSX4	[SSX4] chrX:48243496-48243563
SSX4	[SSX4] chrX:48244004-48244117
SSX4	[SSX4] chrX:48244795-48244889
SSX4	[SSX4] chrX:48246754-48246802
SSX4	[SSX4] chrX:48248789-48248923
SSX4	[SSX4] chrX:48251312-48251415
SSX4	[SSX4] chrX:48252199-48252224
TGFB1	[TGFB1] chr19:41836958-41837115

Gene	Blacklist
TGFB1	[TGFB1] chr19:41848076-41848152
TGFB1	[TGFB1] chr19:41854201-41854360
TMEM216	[TMEM216] chr11:61165733-61165748
TNK2	[TNK2] chr3:195599148-195599341
TNK2	[TNK2] chr3:195605125-195605218
TNK2	[TNK2] chr3:195622118-195622432
WISP3	[WISP3] chr6:112381217-112381278
XPA	[XPA] chr9:100444483-100444712

Appendix 3: List of Variants of by Tumor Type

Gene	Hotspot mutations	Tumor Type
ALK	K1062, D1091, C1156, M1166, I1171, F1174, L1196, A1234, F1245, I1250, R1275, Y1278	Non-Small Cell Lung Cancer
BRAF	G464, G466, G469, Y472, N581, D594, F595, G596, L597, A598_T599, V600, V600_K601, K601, V60010, K6010, G4694, N5810, G4660	Melanoma
EGFR	R108, A289, G598, R677, E709, G719, K745_E749, K745_E746, E746_A750, E746_S752, E746_T751, E746_E749, E746_T751, L747_P753, L747_A750, L747_T751, L747_S752, L747_T751, L747_E749, L747, T751, S752_I759, D761, S768, V769_D770, D770_N771, H773_V774, R776, T790, L833, H835, T847, P848, T854, L858, L861, G863, L8587, A2898, R252, R222	Non-Small Cell Lung Cancer
FGFR3	R248, S249, G370, S371, Y373, G380, A391, K650, G697, S2492, Y3730	Bladder Cancer
FLT3	D835, I836, D8358	Myeloid sarcoma
IDH1	G70, V71, R132, V178, R13239, P33	Glioma, Cholangiocarcinoma
IDH2	R140, R172, V294, R1402, R1721	Glioma, Cholangiocarcinoma
KIT	D52, D419, Y503_F504, K509, M541, K550_K558, P551_V555 , P551_E554, P551_M552, Y553_K558, E554_K558, Q556_V560, W557_K558, W557, W557_V559, W557_E561, W557_V559, K558_E562, K558, K558_V560, V559, V559_V560, V559_E561, V560, E56 Y570_L576, D572, L576, D579, K642, V654, T670, S715, D816, K818, D820, N822, Y823, V 25, D8160	Gastrointestinal Stromal Tumor, Thymic tumors, Melanoma
PIK3CA	R38, E81, R88, R93, G106, R108, K111, G118, V344, N345, C37 8, E418, C420, E453, P539, E542, E545, Q546, E547, S553, K567, H701, E726, C901, G1007, Y1021, T1025, M1043, N1044, D1045, A1046, H1047, G1049, T1052, A1066, N1068, E54534, H104715, E54217, Q5467, R887, N3453, C4209, G1187, E7265, E4535, K1113, R932, R382, R1080, E39	Breast Cancer
TSC2	N1515	CNS Cancer, Renal Cell Carcinoma
MET	D1010H, D1010N, D1010Y, Exon 14 Deletion, Exon 14 splice mutation, Y1003C, Y1003F, Y1003N	Non-Small Cell Lung Cancer
PDGFRA	V561, S566_E571, N659, D842, I843_D846, D1071N	Gastrointestinal Stromal Tumor
AKT1	E17, Q124, G171, E170	Breast Cancer

Gene	Hotspot mutations	Tumor Type
HRAS	G12, G13, Q61, E62, Q614, G136, G122	Head and Neck Squamous Cell Carcinoma
KRAS	G10_A11, G12, G13, V14, L19, Q22, T58, A59, Q61, K117, A146, G1242, G133, Q619, A146	Histiocytosis
MAP2K1	Q56, K57, D67, P124, P1240, F53, E203	Melanoma, Non-Small Cell Lung Cancer, Low-Grade Sero
MTOR	L1460P, L2209V, L2427Q, E2014K, E2419K	Renal Cell Carcinoma, Bladder Cancer
NRAS	G12, G13, A18, G60, Q61, Q6193, G128, G138	Melanoma, Thyroid Cancer
PIK3CA	R38, E81, R88, R93, G106, R108, K111, G118, V344, N345, C37 8, E418, C420, E453, P539, E542, E545, Q546, E547, S553, K567, H701, E726, C901, G1007, Y1021, T1025, M1043, N1044, D1045, A1046, H1047, G1049, T1052, A1066, N1068, E54534, H104715, E54217, Q5467, R887, N3453, C4209, G1187, E7265, E4535, K1113, R932, R382, R1080, E39	Breast Cancer
PTCH1	P1315	Skin Cancer, Non-Melanoma, Embryonal Tumor
RAF1	S2570	Histiocytosis

Appendix 4: List of hotspot mutations (i.e., commonly somatically mutated in cancers) for genes in the NYU Langone Genome PACT panel.

Gene	Codons
ABL1	G250, Q252, Y253, E255, T315, F317, M351, F359, H396R
AKT1	E17, Q124, G171, E170
AKT2	V140
ALK	K1062, D1091, C1156, M1166, I1171, F1174, L1196, A1234, F1245, I1250, R1275, Y1278
APC	S1234, I1307, E1309, E1317, P1319, G1339, S1341, P1361, P1372, P1373, R1399, S1400, S1407, S1411, V1414, S1415, S1421, T1438, P1439, P1440, T1445, P1453, N1455, E1464, S1465, T1487, L1488, F1491, T1493, E1494, T1537, K1555, T1556, I1557, C1578
AR	T878, T8782, Q581
ARAF	S214
ARID1A	D1850, G2087
ARID2	R314, S297, R285, A1773
ASXL1	Y591, E635, G645, G646, E1102D
ASXL2	R591
ATM	D1853, R3008, R3376, E2164
ATRX	K1936, E625
BARD1	P24
BCL6	R594, R618
BCOR	N1425, N14591
BRAF	G464, G466, G469, Y472, N581, D594, F595, G596, L597, A598_T599, V600, V600_K601, K601, V60010, K6010, G4694, N5810, G4660
CARD11	R170
CBL	Y371, L380, C384, C404, R420Q
CDH1	T263
CDK4	R24
CDKN2A	S43, P48, A57, A68, D74, L78, P81, H83, D84, L97, D108, P114, H831, D1081, P1140
CEBPA	P23, H24, Q83, K304_Q305, E309_T310, Q312_K313, K313_V314, K313_V314, K313, E316_L317, E316_L317insQ
CHEK2	K373, K3732
CIC	R215
CREBBP	R1446, S1680, R14460
CRLF2	F232C
CSF1R	Y969C
CTCF	R377
CTNNB1	D32, S33, G34, I35, H36, S37, T40, T41, T42, A43, P44, S45, G48, K49, E53, K335, S376, S334, D324, T412, G349, S455, C619
DICER1	E1813
DIS3	R382, D488
DNMT1	E432
DNMT3A	G543, R635, S714, F731, R882, R8820
DOT1L	G1386
EGFR	R108, A289, G598, R677, E709, G719, K745_E749, K745_E746, E746_A750, E746_S752, E746_T751, E746_E749, E746_T751, L747_P753, L747_A750, L747_T751, L747_S752, L747_T751, L747_E749, L747, T751, S752_I759, D761, S768, V769_D770, D770_N771, H773_V774, R776, T790, L833, H835, T847, P848, T854, L858, L861, G863, L8587, A2898, R252, R222

Gene	Codons
EP300	D1399, D13990, C1164
EPHB1	R170
ERBB2	S310, L755, D769, A775_G776, G776, V777, V842, S3108, L7553, E930, R678
ERBB3	V1043, D297, M91
ERBB4	R711
ERCC2	D312
ESR1	Y537
ETV1	R187
ETV6	R369
EZH2	Y646, R690
FBXW7	G423, R465, R479, R505, S582, R689, R4652, R5054, R4792
FGFR2	S252, P253, C382, N549, N550, K659
FGFR3	R248, S249, G370, S371, Y373, G380, A391, K650, G697, S2492, Y3730
FGFR4	V550
FLT3	D835, I836, D8358
FOXL2	C134W
FUBP1	R430
GATA1	M1, S30, V74I
GATA2	G320, L321, L359, R362Q
GNA11	R183, Q209, R256
GNAQ	R183, Q209
GNAS	R201, Q227, R8448
GRIN2A	R1067
HIST1H3	E74
HNF1A	W206, P291, G292
HRAS	G12, G13, Q61, E62, Q614, G136, G122
IDH1	G70, V71, R132, V178, R13239, P33
IDH2	R140, R172, V294, R1402, R1721
IL7R	K395
IRS2	G1057
JAK1	R873
JAK2	F537_K539, H538_K539, K539, I540_E543, R541_E543, N542_E543, E543_D544, V617 R683
JAK3	A572, A573, R657Q
KDR	S1100, E759
KEAP1	R470
KIT	D52, D419, Y503_F504, K509, M541, K550_K558, P551_V555, P551_E554, P551_M552, Y553_K558, E554_K558, Q556_V560, W557_K558, W557, W557_V559, W557_E561, W557_V559, K558_E562, K558, K558_V560, V559, V559_V560, V559_E561, V560, E56 Y570_L576, D572, L576, D579, K642, V654, T670, S715, D816, K818, D820, N822, Y823, V 25, D8160
KMT2C	V656
KRAS	G10_A11, G12, G13, V14, L19, Q22, T58, A59, Q61, K117, A146, G1242, G133, Q619, A146
LATS2	A3243, G3630
MAP2K1	Q56, K57, D67, P124, P1240, F53, E203
MAP2K4	R134
MAP3K1	S1330, S939

Gene	Codons
MAPK1	E322
MAX	R600
MED12	L36, Q43, G44, L1224, L12240
MEF2B	D83V
MET	T1010, Y1248, Y1253, M1268, K1360
MLL3	K2797
MPL	S505, W515, W515R
MSH6	F1088, T1219I
MTOR	S22152, F1888
MYC	T58
MYCN	P44
MYD88	S219, S243, L265P
NF1	L844
NFE2L2	D29, L30, G31, R34, E79, T80, G81, E82, E794, D294, R342
NOTCH1	L1574, L1575, V1578, L1585, L1586, F1592, L1593, L1594, R1598, R1599, L1600, L1601, L1678, L1679, Q2460, P2514, A1944
NOTCH2	E385, N463
NPM1	W288, W290
NRAS	G12, G13, A18, G60, Q61, Q6193, G128, G138
NTRK1	T264
PAK7	E144
PARP1	I562
PAX5	P80R
PDGFRA	V561, S566_E571, N659, D842, I843_D846, D1071N
PIK3C2G	S670
PIK3CA	R38, E81, R88, R93, G106, R108, K111, G118, V344, N345, C378, E418, C420, E453, P539, E542, E545, Q546, E547, S553, K567, H701, E726, C901, G1007, Y1021, T1025, M1043, N1044, D1045, A1046, H1047, G1049, T1052, A1066, N1068, E54534, H104715, E54217, Q5467, R887, N3453, C4209, G1187, E7265, E4535, K1113, R932, R382, R1080, E39
PIK3R1	G376, D560, N564, K567
POLE	P2864, V4111
PPP2R1A	P179, R182, R183, S256, W257, R258, R1832
PREX2	G233C
PTCH1	P1315
PTEN	K6, P38, L42, H61, Y68, Y76, Y88, H93, I101, C105, L112, H123, A126, G129, R130, C136, A151, Y155, R159, K164, G165, S170, R173, N184, E242, P246, P248, C250, K267, V290, L318, T319, T321, N323, F347, R1309, R1730, K128
PTPN11	G60, D61, E69, A72, T73, E76, S502, G503, Q510
PTPRD	S431, P666
RAC1	P295
RAF1	S2570
RET	E632_T636, E632_L633, C634, M918T
RHOA	E40, Y42
RICTOR	S1101
RIT1	M90
RUNX1	L56, R107, D198, R201, R204, R162, R205
SDHA	S4560, A466, R465

Gene	Codons
SF3B1	E622, R625, H662, K666, K700, K7002
SMAD4	A118, D351, R361, G386, R3619, D537, P356
SMARCA	T910, G1232
SMARCB	R377, A382, P383
SMO	W535L
SPOP	F133, F1338, W131, F102
SRSF2	P95, P95_R102, P107H
STAG2	R370
STK11	D194, P281, F354L
TET2	C25, C262, Q764, F868, R1261, H1380, V1718L
TNFAIP3	L324
TP53	E11, D49, P82, T102, G105, Y107, R110, L111, F113, K120, T125, Y126, Y126_K132, S127, P128, L130, N131, K132, M133, F134, C135, A138, K139, T140, C141, P142, V143, Q144, L145, V147, S149, P151, P152, P153, G154, T155, R156, V157, R158, A159, M160, A161, I162, Y163, K164, S166, H168, M169, T170, E171, V172, V173, R174, R175, C176, P177, P177_C182, H178, H179, E180, R181, C182, D184, D186, G187, P190, P191, Q192, H193, L194, I195, R196, V197, E198, G199, N200, R202, V203, Y205, D208, R209, T211, F212, R21 , , S215, V216, V217, V218, Y220, E224, G226, S227, D228, C229, T230, I232, Y234, N235, Y2 6, M237, C238, N239, S240, S241, C242, M243, G244, G245, M246, N247, R248, R249, P250 I251, L252, T253, I254, I255, L257, E258, D259, G262, L265, G266, R267, F270, E271, V272, R273, V274, C275, A276, C277, P278, G279, R280, D281, R282, R283, T284, E285, E286, E2 7, N288, R290, K291, K292, E294, P300, P301, S303, K320, G334, R337, R27328, R24892, R17538, R2820, G2451, Y2202, H1938, H1797, R1583, C1763, P2783, Y1633, R2800, G2660, I1950, S2419, R2499, V1577, C2386, E2856, R3375, G2445, V1733, P1512, C2752, K1321, Y2050, V2720, C1359, D2818, E2718, V2168, M2378, Y2347, E2867, L1946, A1596, R2675, S1275, C2425, Y2364, C1414, F2704, A1613, V2743, S2153, R2132, H2142, R1101, N2390, T1550, P1520, P2500, G1050, L1300, Q136, F109
TP6	R379
TSC	N1515
TSH	M453, I486, L512, I568, D619, A623, L629, I630, T632, D633, D633E
U2A	S34, Q157, S347
VHL	V62, S65, S72, V74, F76, N78, S80, P81, L85, P86, L89, N90, S111, G114, H115, L118, D121, L128, V130, G144, F148, I151, L153, V155, L158, E160, C162, V166, R167, L169, L184
WT1	V303, R312, A314, R394, D396, R462

Appendix 5: Table of all variants with corresponding VAF and Depth detected during the Dilution experiments in Figures 6 and 7

Mutation ID	Dilution	DNA input (ng)	Depth	Freq.
AKT1 p.E17K	1	250	396	0.088
APC p.R1450*	1	250	1071	0.073
APC p.T1556fs*3	1	250	969	0.071
ATM p.C353fs*5	1	250	475	0.084
BRAF p.V600E	1	250	434	0.032
CTNNB1 p.T41A	1	250	613	0.06
EGFR p.D770_N771insG	1	250	757	0.036
EGFR p.E746_A750delELREA	1	250	471	0.013
EGFR p.L858R	1	250	705	0.054
EGFR p.T790M	1	250	781	0.038
ERBB2 p.A775_G776insYVMA	1	250	503	0.068
FGFR3 p.S249C	1	250	865	0.034
FLT3 p.D835Y	1	250	870	0.037
GNA11 p.Q209L	1	250	831	0.108
GNAQ p.Q209P	1	250	608	0.054
GNAS p.R201C	1	250	914	0.046
JAK2 p.V617F	1	250	446	0.074
KIT p.D816V	1	250	646	0.06
KRAS p.G12D	1	250	373	0.051
MPL p.W515L	1	250	513	0.101
NPM1 p.W288fs*12	1	250	232	0.043
NRAS p.Q61R	1	250	241	0.079
PDGFRA p.D842V	1	250	929	0.095
PDGFRA p.S566fs*6	1	250	673	0.045
PIK3CA p.E545K	1	250	666	0.084
PIK3CA p.H1047R	1	250	751	0.039
PIK3CA p.N1068fs*4	1	250	619	0.031
PTEN p.K267fs*9	1	250	489	0.049
PTEN p.P248fs*5	1	250	556	0.058
RET p.M918T	1	250	576	0.04
SMAD4 p.A466fs*28	1	250	546	0.097
TP53 p.C242fs*5	1	250	491	0.059
TP53 p.R175H	1	250	783	0.046
TP53 p.R248Q	1	250	504	0.063
TP53 p.R273H	1	250	869	0.07
AKT1 p.E17K	1	100	487	0.084
APC p.R1450*	1	100	1190	0.087
APC p.T1556fs*3	1	100	1252	0.081
ATM p.C353fs*5	1	100	676	0.056
BRAF p.V600E	1	100	663	0.023
CTNNB1 p.T41A	1	100	739	0.062
EGFR p.D770_N771insG	1	100	820	0.021

Mutation ID	Dilution	DNA input (ng)	Depth	Freq.
EGFR p.E746_A750delELREA	1	100	621	0.026
EGFR p.L858R	1	100	929	0.05
EGFR p.T790M	1	100	835	0.035
ERBB2 p.A775_G776insYVMA	1	100	682	0.04
FGFR3 p.S249C	1	100	999	0.027
FLT3 p.D835Y	1	100	1061	0.027
GNA11 p.Q209L	1	100	1161	0.098
GNAQ p.Q209P	1	100	642	0.058
GNAS p.R201C	1	100	976	0.042
JAK2 p.V617F	1	100	622	0.059
KIT p.D816V	1	100	725	0.08
KRAS p.G12D	1	100	481	0.054
MPL p.W515L	1	100	703	0.08
NPM1 p.W288fs*12	1	100	377	0.016
NRAS p.Q61R	1	100	329	0.058
PDGFRA p.D842V	1	100	962	0.088
PDGFRA p.S566fs*6	1	100	993	0.046
PIK3CA p.E545K	1	100	831	0.103
PIK3CA p.H1047R	1	100	914	0.037
PIK3CA p.N1068fs*4	1	100	768	0.021
PTEN p.K267fs*9	1	100	708	0.041
PTEN p.P248fs*5	1	100	796	0.041
RET p.M918T	1	100	772	0.035
SMAD4 p.A466fs*28	1	100	634	0.063
TP53 p.C242fs*5	1	100	497	0.06
TP53 p.R175H	1	100	900	0.067
TP53 p.R248Q	1	100	501	0.058
TP53 p.R273H	1	100	912	0.053
AKT1 p.E17K	1	50	408	0.069
APC p.R1450*	1	50	1017	0.073
APC p.T1556fs*3	1	50	1001	0.081
ATM p.C353fs*5	1	50	559	0.089
CTNNB1 p.T41A	1	50	600	0.043
EGFR p.D770_N771insG	1	50	712	0.027
EGFR p.E746_A750delELREA	1	50	594	0.025
EGFR p.L858R	1	50	848	0.05
EGFR p.T790M	1	50	769	0.038
ERBB2 p.A775_G776insYVMA	1	50	641	0.041
FGFR3 p.S249C	1	50	957	0.038
FLT3 p.D835Y	1	50	872	0.025
GNA11 p.Q209L	1	50	1025	0.1
GNAQ p.Q209P	1	50	518	0.064
GNAS p.R201C	1	50	887	0.063
JAK2 p.V617F	1	50	453	0.055

Mutation ID	Dilution	DNA input (ng)	Depth	Freq.
KIT p.D816V	1	50	629	0.07
KRAS p.G12D	1	50	393	0.048
MPL p.W515L	1	50	700	0.089
NPM1 p.W288fs*12	1	50	326	0.031
NRAS p.Q61R	1	50	243	0.074
PDGFRA p.D842V	1	50	915	0.102
PDGFRA p.S566fs*6	1	50	857	0.036
PIK3CA p.E545K	1	50	669	0.075
PIK3CA p.H1047R	1	50	743	0.027
PIK3CA p.N1068fs*4	1	50	650	0.032
PTEN p.K267fs*9	1	50	490	0.014
PTEN p.P248fs*5	1	50	609	0.044
RET p.M918T	1	50	682	0.026
SMAD4 p.A466fs*28	1	50	547	0.071
TP53 p.C242fs*5	1	50	472	0.032
TP53 p.R175H	1	50	802	0.056
TP53 p.R248Q	1	50	475	0.034
TP53 p.R273H	1	50	939	0.037
AKT1 p.E17K	0.5	250	393	0.038
APC p.R1450*	0.5	250	832	0.049
APC p.T1556fs*3	0.5	250	912	0.046
ATM p.C353fs*5	0.5	250	587	0.061
BRAF p.V600E	0.5	250	433	0.021
CTNNB1 p.T41A	0.5	250	598	0.032
EGFR p.D770_N771insG	0.5	250	751	0.012
EGFR p.E746_A750delELREA	0.5	250	515	0.021
EGFR p.L858R	0.5	250	698	0.029
ERBB2 p.A775_G776insYVMA	0.5	250	490	0.022
FGFR3 p.S249C	0.5	250	788	0.013
FLT3 p.D835Y	0.5	250	940	0.022
GNA11 p.Q209L	0.5	250	787	0.07
GNAQ p.Q209P	0.5	250	560	0.041
GNAS p.R201C	0.5	250	767	0.026
GNAS p.R201C	0.5	250	816	0.026
JAK2 p.V617F	0.5	250	505	0.034
KIT p.D816V	0.5	250	612	0.034
KRAS p.G12D	0.5	250	369	0.03
MPL p.W515L	0.5	250	539	0.05
NPM1 p.W288fs*12	0.5	250	289	0.031
NRAS p.Q61R	0.5	250	223	0.036
PDGFRA p.D842V	0.5	250	865	0.051
PDGFRA p.D842V	0.5	250	837	0.051
PDGFRA p.S566fs*6	0.5	250	871	0.021
PIK3CA p.E545K	0.5	250	623	0.051

Mutation ID	Dilution	DNA input (ng)	Depth	Freq.
PIK3CA p.H1047R	0.5	250	713	0.027
PIK3CA p.N1068fs*4	0.5	250	573	0.021
PTEN p.K267fs*9	0.5	250	460	0.026
PTEN p.P248fs*5	0.5	250	639	0.028
RET p.M918T	0.5	250	544	0.029
SMAD4 p.A466fs*28	0.5	250	438	0.066
TP53 p.C242fs*5	0.5	250	380	0.026
TP53 p.R175H	0.5	250	757	0.038
TP53 p.R273H	0.5	250	766	0.039
AKT1 p.E17K	0.5	100	436	0.023
APC p.R1450*	0.5	100	940	0.03
APC p.T1556fs*3	0.5	100	1038	0.049
ATM p.C353fs*5	0.5	100	563	0.034
BRAF p.V600E	0.5	100	501	0.024
CTNNB1 p.T41A	0.5	100	628	0.022
EGFR p.D770_N771insG	0.5	100	670	0.01
EGFR p.E746_A750delELREA	0.5	100	560	0.02
EGFR p.L858R	0.5	100	806	0.038
ERBB2 p.A775_G776insYVMA	0.5	100	512	0.029
FLT3 p.D835Y	0.5	100	962	0.014
GNA11 p.Q209L	0.5	100	862	0.059
GNAQ p.Q209P	0.5	100	626	0.037
JAK2 p.V617F	0.5	100	521	0.046
KIT p.D816V	0.5	100	681	0.047
KRAS p.G12D	0.5	100	380	0.029
MPL p.W515L	0.5	100	568	0.055
NPM1 p.W288fs*12	0.5	100	280	0.021
NRAS p.Q61R	0.5	100	266	0.026
PDGFRA p.D842V	0.5	100	862	0.038
PDGFRA p.S566fs*6	0.5	100	801	0.017
PIK3CA p.E545K	0.5	100	669	0.039
PIK3CA p.H1047R	0.5	100	799	0.015
PIK3CA p.N1068fs*4	0.5	100	675	0.018
PTEN p.K267fs*9	0.5	100	582	0.012
PTEN p.P248fs*5	0.5	100	636	0.016
SMAD4 p.A466fs*28	0.5	100	468	0.041
TP53 p.R175H	0.5	100	726	0.047
TP53 p.R273H	0.5	100	807	0.048
AKT1 p.E17K	0.5	50	462	0.052
APC p.R1450*	0.5	50	896	0.052
APC p.T1556fs*3	0.5	50	1027	0.036
ATM p.C353fs*5	0.5	50	508	0.031
CTNNB1 p.T41A	0.5	50	690	0.025
EGFR p.L858R	0.5	50	835	0.032

Mutation ID	Dilution	DNA input (ng)	Depth	Freq.
ERBB2 p.A775_G776insYVMA	0.5	50	531	0.028
FGFR3 p.S249C	0.5	50	856	0.016
FLT3 p.D835Y	0.5	50	889	0.02
GNA11 p.Q209L	0.5	50	876	0.061
GNAQ p.Q209P	0.5	50	565	0.018
GNAS p.R201C	0.5	50	785	0.032
JAK2 p.V617F	0.5	50	506	0.03
KIT p.D816V	0.5	50	642	0.036
KRAS p.G12D	0.5	50	390	0.033
MPL p.W515L	0.5	50	565	0.058
NPM1 p.W288fs*12	0.5	50	276	0.014
NRAS p.Q61R	0.5	50	245	0.037
PDGFRA p.D842V	0.5	50	856	0.042
PDGFRA p.S566fs*6	0.5	50	848	0.02
PIK3CA p.E545K	0.5	50	657	0.043
PIK3CA p.H1047R	0.5	50	729	0.014
PIK3CA p.N1068fs*4	0.5	50	631	0.013
PTEN p.K267fs*9	0.5	50	552	0.029
PTEN p.P248fs*5	0.5	50	617	0.021
SMAD4 p.A466fs*28	0.5	50	565	0.034
TP53 p.C242fs*5	0.5	50	495	0.018
TP53 p.R175H	0.5	50	872	0.042
TP53 p.R273H	0.5	50	810	0.033
APC p.R1450*	0.25	250	830	0.033
APC p.T1556fs*3	0.25	250	969	0.021
ATM p.C353fs*5	0.25	250	337	0.018
EGFR p.E746_A750delELREA	0.25	250	461	0.011
EGFR p.L858R	0.25	250	789	0.018
ERBB2 p.A775_G776insYVMA	0.25	250	556	0.018
FGFR3 p.S249C	0.25	250	869	0.013
GNA11 p.Q209L	0.25	250	886	0.035
JAK2 p.V617F	0.25	250	354	0.028
KIT p.D816V	0.25	250	361	0.033
MPL p.W515L	0.25	250	484	0.029
PDGFRA p.D842V	0.25	250	628	0.032
PDGFRA p.S566fs*6	0.25	250	612	0.013
PIK3CA p.E545K	0.25	250	417	0.034
PIK3CA p.H1047R	0.25	250	644	0.019
PIK3CA p.N1068fs*4	0.25	250	412	0.012
PTEN p.K267fs*9	0.25	250	444	0.02
PTEN p.P248fs*5	0.25	250	520	0.023
SMAD4 p.A466fs*28	0.25	250	403	0.037
TP53 p.C242fs*5	0.25	250	416	0.019
AKT1 p.E17K	0.25	100	484	0.033

Mutation ID	Dilution	DNA input (ng)	Depth	Freq.
APC p.R1450*	0.25	100	1133	0.026
APC p.T1556fs*3	0.25	100	1152	0.013
ATM p.C353fs*5	0.25	100	435	0.023
EGFR p.L858R	0.25	100	880	0.017
FLT3 p.D835Y	0.25	100	1028	0.014
GNA11 p.Q209L	0.25	100	1009	0.033
GNAQ p.Q209P	0.25	100	409	0.017
KIT p.D816V	0.25	100	533	0.024
MPL p.W515L	0.25	100	605	0.031
PDGFRA p.D842V	0.25	100	908	0.02
PIK3CA p.E545K	0.25	100	430	0.03
PTEN p.K267fs*9	0.25	100	461	0.017
PTEN p.P248fs*5	0.25	100	521	0.023
SMAD4 p.A466fs*28	0.25	100	466	0.028
TP53 p.C242fs*5	0.25	100	460	0.015
TP53 p.R175H	0.25	100	815	0.028
TP53 p.R273H	0.25	100	906	0.025
APC p.R1450*	0.25	50	860	0.021
APC p.T1556fs*3	0.25	50	861	0.016
ATM p.C353fs*5	0.25	50	421	0.019
ERBB2 p.A775_G776insYVMA	0.25	50	495	0.012
GNA11 p.Q209L	0.25	50	776	0.032
MPL p.W515L	0.25	50	495	0.026
NPM1 p.W288fs*12	0.25	50	266	0.011
PDGFRA p.D842V	0.25	50	739	0.032
PIK3CA p.E545K	0.25	50	517	0.021
PIK3CA p.N1068fs*4	0.25	50	577	0.01
PTEN p.K267fs*9	0.25	50	477	0.013
PTEN p.P248fs*5	0.25	50	555	0.013
SMAD4 p.A466fs*28	0.25	50	426	0.031
TP53 p.C242fs*5	0.25	50	371	0.013