Center for Genomic Interpretation

ELEVATEGENETICS BRILLIANT

Advanced *in silico* proficiency testing. For Research Use Only. Not for clinical use.

Test Provider	Test Provider Contact
Laboratory Name	Laboratory Contact
Test Evaluated: Test Name CGP v. 2.1	Email
Date: 6 Mar 2022	Phone Number

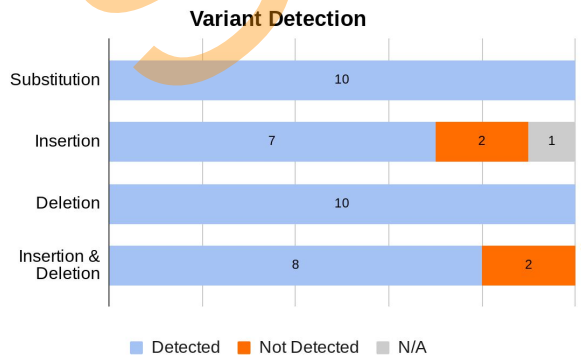
Report Summary

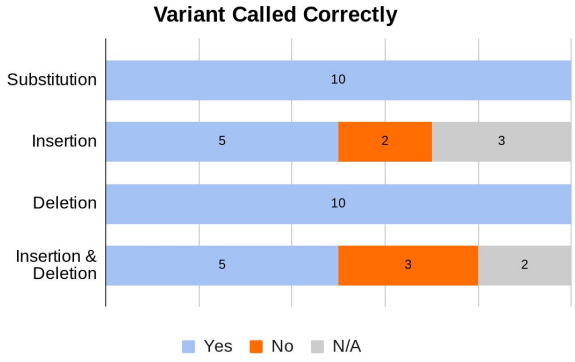
The test reported 75% (30/40) of variants present in ELEVATEGENETICS BRILLIANT 2021/2022 *in silico* proficiency samples for Comprehensive Genomic Profiling. Nine variants are not included in the final reports or have been miscalled. One variant is outside of the claimed scope of the test and is therefore not considered an analytical false negative. One analytical false positive variant is reported. Variant classification for the test leans more sensitive (90%) than specific (10%). A total of six proficiency samples are included for analysis of appropriate drug therapy recommendations. Three out of six proficiency samples are directed to the most appropriate therapy based on the variants present in the *in silico* proficiency samples.

Test Report Accuracy and Clarity Ratings

AVERAGE: 2.5 out of 5 stars ★★½

Proficiency Test 1 ★★★	Proficiency Test 2 ★★	Proficiency Test 3 ★★★
Proficiency Test 4 ★★	Proficiency Test 5 ★★★	Proficiency Test 6 ★★





*Undetected variants were not scored for naming/classification

Table Summary of Analytical True Positive Results

Variant Type	Substitution	Insertion	Deletion	Insertion & Deletion
Number of Variants	**10**	**10**	**10**	**10**
Variants Detected	10	7	10	8
Variant Called Correctly	10	5	10	5

Table Summary of Analytical False Positive Results

Variant Type	Substitution	Insertion	Deletion	Insertion & Deletion
Number of Variants	1	0	0	0

Table Summary of Variant Classification Results

Variant Type	Correct	Incorrect	Sensitive	Specific
Number of Variants	5	0	30	10

Report Methodology

In silico variants were designed to test and characterize variant detection capability and variant calling capability. The variants were also designed to allow for characterization of the test's variant classification approach. NIST cell line NA12878 was sequenced by the test provider and FASTQ files were delivered to CGI. Variants were inserted into the FASTQ files *in silico* and returned to the test provider for variant calling and final report generation. The variants are listed in this report using the GRCh38 (hg38) genome assembly coordinates.

Analytical True Positive Results

Variant Detection: Variants were classified as detected if the test's variant calling pipeline output (final, filtered VCF or other equivalent text file) reported a variant present in the proficiency testing samples. Detection of an incorrect but comparable variant at the same location where a variant was present in the proficiency testing sample was also considered a variant detection. Comparable variants included differences in naming conventions such as left/right alignment, and truncated or expanded insertion and deletion calls. Absence of any variant call at any location where a variant was present in the proficiency testing samples was considered a missed variant detection, except when a variant was not covered or reportable by the test (e.g. gene not included in the panel for somatic and/or germline variants). The latter variants are denoted with "N/A."

Variant Called Correctly: To distinguish between how variants are called in a VCF and ultimately reported based on subsequent downstream analysis, any detected variants were also characterized for correct reporting on the proficiency testing sample report. Variants were considered to be called correctly if at least one nomenclature format of the variant reported in the final report matched the variant present in the proficiency testing samples. These could be either either g dot, c dot or p dot formats. Comparable reported variant calls that do not accurately name the variant were not counted as a correct variant call.

Analytical False Positive Results

Any variants reported by the test that were not present in the proficiency testing samples were considered analytical false positive results. Detection of a comparable variant (see explanation in "Variant Detection") at the same location where a variant was present in the proficiency testing sample was not considered an analytical false positive result. Variants present in NA12878 prior to *in silico* mutagenesis are not considered false positives.

Variant Classification

The variants tested represent both simple and complex classification challenges. While some variants had clear indications for classification based on status as an FDA approved companion diagnostic (CDx), other complex classifications were graded more broadly based on the presentation of the variant and justification given on the final report.

CDx variants were given a rating of Correct or Incorrect if the final report included the corresponding treatment based on the treatment CDx Intended Use and the drug label for Indications and Usage.

Non-CDx variants were characterized as either sensitive or specific. Sensitive classifications indicate the variant classification procedures for the test enrich for true positives but likely also enrich for false positives. Specific classifications indicate that the variant classification procedures for the test minimize false positives, potentially resulting in less true positives captured by the test.

Star Rating for Test Report Accuracy and Clarity

A maximum of 5 stars is possible for each proficiency sample final report from the laboratory. A star is earned for each of the following accuracy and clarity features of the final reports:

1. All current CDx targets that match the sample's tumor type are represented in the panel (of those that are detectable by Next-Generation Sequencing).
2. All variants present in the *in silico* sample that are deemed reportable by the technical specifications of the test are called correctly on the report.
3. All true positive CDx targets are reported as CDx (or equivalent).
4. No false positive CDx targets (or equivalent) are reported.
5. The variety of drug treatment options are clearly and accurately ranked to alert clinicians to treatments that are supported by greater evidence/regulatory approvals for the tumor type.

Some variant omissions or classification errors may impact more than one of the above criteria. In these cases, star deductions are additive.

Table of Proficiency Variant Detection and Calling Results

Sample	Gene	Variant	Tumor Variant Allele Frequency (%)	Variant Type	Variant Detected	Variant Called Correctly (not yet classified)
1	1	c.402_403insAG p.Asp134Pro Chr#:g.##	52	Somatic Insertion	Yes	Yes
	2	c.679_680insCTA p.Phe233Leu Chr#:g.##	14	Somatic Insertion	No	N/A
	3	c.A>T p.Asp135Tyr Chr#:g.##	36	Somatic Substitution	Yes	Yes
	4	c.1236_1237del p.Pro453Val Chr#:g.##	77	Somatic Deletion	Yes	Yes
2	5	c.3516_3521delinsTCCA p.Thr459Gln Chr#:g.##	89	Somatic Insertion & Deletion	No	N/A
	6	c.689_690del p.Ser267Ile Chr#:g.##	3	Germline Deletion	Yes	Yes
	7	c.G>A p.Gly14Val Chr#:g.##	5	Somatic Substitution	Yes	Yes
	8	c.127_128del p.Met34Trp Chr#:g.##	7	Germline Deletion	Yes	Yes
	9	c.T>G p.Phe157His Chr#:g.##	21.4	Somatic Substitution	Yes	Yes
	10	c.624_625del p.Asp208Leu Chr#:g.##	36	Somatic Deletion	Yes	Yes
	11	c.4672_4675delinsATTA p.Glu806Lys Chr#:g.##	18	Somatic Insertion & Deletion	Yes	No
	12	c.T>G p.Pro68Ala Chr#:g.##	25	Somatic Substitution	Yes	Yes
	13	c.C>A p.Gly59Cys Chr#:g.##	77	Somatic Substitution	Yes	Yes

Table of Proficiency Variant Detection and Calling Results

Sample	Gene	Variant	Tumor Variant Allele Frequency (%)	Variant Type	Variant Detected	Variant Called Correctly (not yet classified)
3	14	c.652_655delinsACCT p.Val207Pro Chr#:g.##	63	Somatic Insertion & Deletion	Yes	Yes
	15	c.36_37del p.Gly12Ala Chr#:g.##	28	Germline Deletion	Yes	Yes
	16	c.365_368insCTA p.Ala123Val Chr#:g.##	14.7	Somatic Insertion	Yes	No
4	17	c.217_220delinsTGCA p.Val34Cys Chr#:g.##	16	Somatic Insertion & Deletion	Yes	Yes
	18	c.150_152insAC p.Cys45Pro Chr#:g.##	34	Somatic Insertion	Yes	Yes
	19	c.215_220delinsACCTA p.Pro56Leu Chr#:g.##	28	Somatic Insertion & Deletion	No	N/A
	20	c.452_453del p.Leu267Ile Chr#:g.##	92	Somatic Deletion	Yes	Yes
	21	c.A>G p.Ile78Met Chr#:g.##	10	Germline Substitution	Yes	Yes
	22	c.A>T p.Met89Trp Chr#:g.##	5	Somatic Substitution	Yes	Yes
	23	c.186_188delinsACCA p.Trp91Phe Chr#:g.##	34	Somatic Insertion & Deletion	Yes	No
	24	c.264_266del p.Phe82Lys Chr#:g.##	57	Somatic Deletion	Yes	Yes
	25	c.C>T p.Lys761Arg Chr#:g.##	86	Somatic Substitution	Yes	Yes
	26	c.165_167delinsTCC p.Arg53His Chr#:g.##	22	Somatic Insertion & Deletion	Yes	Yes

Table of Proficiency Variant Detection and Calling Results

Sample	Gene	Variant	Tumor Variant Allele Frequency (%)	Variant Type	Variant Detected	Variant Called Correctly (not yet classified)
5	27	c.A>G p.Gly99Leu Chr#:g.##	55	Somatic Substitution	Yes	Yes
	28	c.136_138delinsTGG p.Ala87Ile Chr#:g.##	24	Somatic Insertion & Deletion	Yes	Yes
	29	c.982_983insACA p.Val367Met Chr#:g.##	3	Somatic Insertion	Yes	No
	30	c.679_682delinsTTTA p.Cys254Trp Chr#:g.##	36	Somatic Insertion & Deletion	Yes	Yes
6	31	c.156_157del p.Ser87Thr Chr#:g.##	76	Somatic Deletion	Yes	Yes
	32	c.231_232insAA p.Gln94Tyr Chr#:g.##	78.6	Somatic Insertion	No	N/A
	33	c.3241_3243delinsCCA p.Asn768Ser Chr#:g.##	47	Germline Insertion & Deletion	Yes	Yes
	34	c.4650_4652insGT p.Asp654Cys Chr#:g.##	33	Somatic Insertion	Yes	Yes
	35	c.961_963delinsCTG p.Glu348Ala Chr#:g.##	2	Somatic Insertion & Deletion	Yes	N/A
	36	c.4531_4533del p.Gly983Pro Chr#:g.##	45	Somatic Deletion	Yes	Yes
	37	c.921_922insCC p.His349Lys Chr#:g.##	59	Somatic Insertion	Yes	Yes
	38	c.1754_1757insCTAG p.Thr938Leu Chr#:g.##	42	Somatic Insertion	No	N/A

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6	39	c.A>T p.Asp324Gly Chr#:g.##	55	Somatic Substitution	Yes	Yes
	40	c.894_896insCCA p.Trp298Met Chr#:g.##	3	Somatic Insertion	Yes	Yes

Table of Proficiency Variant Classification Results

Sample	Gene	Variant	Tumor Variant Allele Frequency (%)	Variant Type	Variant Classification
1	1	c.402_403insAG p.Asp134Pro Chr#:g.##	52	Somatic Insertion	Sensitive
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	4	c.1236_1237del p.Pro453Val Chr#:g.##	77	Somatic Deletion	Correct
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	6	c.689_690del p.Ser267Ile Chr#:g.##	3	Germline Deletion	Sensitive
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	13	c.C>A p.Gly59Cys Chr#:g.##	77	Somatic Substitution	Correct

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	25	c.1754_1757insCTAG p.Thr938Leu Chr#:g.##	42	Somatic Insertion	N/A

Table of Proficiency Variant Classification Results

Sample	Gene	Variant	Tumor Variant Allele Frequency (%)	Variant Type	Variant Classification
6	1	c.A>T p.Asp324Gly Chr#:g.##	55	Somatic Substitution	Sensitive
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Summary of Drug Treatment Options

Tier I: Tumor and variant matches a variant-drug recommendation for a drug with an FDA Companion Diagnostic (CDx), or with a standard clinical guideline (eg. NCCN).

Tier II: Variant matches drugs with potential clinical significance where there is limited evidence of the drug-biomarker relationship, and/or Tier I evidence of a drug-biomarker relationship, but in a different tumor type.

Proficiency Test 1: Male, age 72, colorectal cancer			
Tier I	Tier II		
Variant 1	Variant 2	Variant 3	Variant 4
Drugs of benefit (True positive)	**Drugs of potential benefit**	**Drugs of potential benefit**	**Lack of Benefit**

Proficiency Test 2: Female, age 45, ovarian cancer		
Tier I		Tier II
Variant 5	False Positive Variant	Variant 6
Drug of benefit (True positive)	**Drug not indicated for patient (False positive)**	**Lack of Benefit**

Proficiency Test 3: Male, age 68, prostate cancer			
Tier I	Tier II		
Variant 1	Variant 2	Variant 3	Variant 4
Drugs of benefit (True positive)	**Drugs of potential benefit**	**Drugs of potential benefit**	**Lack of Benefit**

Proficiency Test 4: Female, age 55, breast cancer			
Tier I	Tier II		
Variant 1	Variant 2	Variant 3	Variant 4
Drugs of benefit (True positive)	**Drugs of potential benefit**	**Drugs of potential benefit**	**Lack of Benefit**

Proficiency Test 5: Female, age 63, NSCLC			
Tier I	Tier II		
Variant 1	Variant 2	Variant 3	Variant 4
Drugs of benefit (True positive)	**Drugs of potential benefit**	**Drugs of potential benefit**	**Lack of Benefit**

Proficiency Test 6: Female, age 51, bladder cancer			
Tier I	Tier II		
Variant 1	Variant 2	Variant 3	Variant 4
Drugs of benefit (True positive)	**Drugs of potential benefit**	**Drugs of potential benefit**	**Lack of Benefit**

Calculation of Test Report Accuracy and Clarity Ratings

AVERAGE: 2.5 out of 5 stars ★★½		
Proficiency Test 1 ★★★	Proficiency Test 2 ★★	Proficiency Test 3 ★★★
Proficiency Test 4 ★★	Proficiency Test 5 ★★★	Proficiency Test 6 ★★

A star is earned for each of the following accuracy and clarity features of the final reports:

1. All current CDx targets that match the sample's tumor type are represented in the panel (of those that are detectable by Next-Generation Sequencing).
2. All variants present in the *in silico* sample that are deemed reportable by the technical specifications of the test are called correctly on the report.
3. All true positive CDx targets are reported as CDx (or equivalent).
4. No false positive CDx targets (or equivalent) are reported.
5. The variety of drug treatment options are clearly and accurately ranked to alert clinicians to treatments that are supported by greater evidence/regulatory approvals for the tumor type.

Some variant omissions or classification errors may impact more than one of the above criteria. In these cases, star deductions are additive.

		Star Category				
		1	2	3	4	5
Proficiency Sample	1	★	★	★		
	2	★		★		
	3	★		★	★	
	4	★	★			
	5	★	★	★		
	6	★		★		

Proficiency Testing Disclaimer

In silico proficiency testing has the following limitations:

- Laboratory processes such as handling of patient samples, micro/macrodissection, and nucleotide extraction from Formalin-Fixed Paraffin-Embedded (FFPE) samples and other sample types could not be assessed using *in silico* proficiency samples.
- Test performance may vary between the NIST cell line samples used for *in silico* proficiency testing and patient FFPE and/or blood samples.
- Like all proficiency testing, ELEVATEGENETICS BRILLIANT is a non exhaustive assessment of variant detection and reporting capabilities of the test. A different set of proficiency testing samples could result in varying performance of the test.

Although every ELEVATEGENETICS BRILLIANT report is checked for accuracy prior to delivery, there may be an error in interpreting the results of a test final report due to the complexity and variation in reporting practices. If the test provider disagrees with the findings of this report, CGI will support discrepancy resolution for a period of 8 weeks following report delivery if initiated by the ordering client. Amended report(s) will be provided if necessary to accurately reflect proficiency testing results.

This report is for Research Use Only.