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Submission and Reporting	
Customer	WiCell
Specimen	human ESCs
Technologist	James Johnson
Report Date	Jun 11, 2025

Sample	
Cell Line	WA01
Passage #	35
CTR #	1000G_phase1.snps.high_confidence.hg38
Collection date	Jun 6, 2025

ONCOPANEL ANALYSIS REPORT

Test Description

This report summarizes findings from a whole genome sequencing assay with a targeted analysis of 65 cancer-associated genes. Although the entire genome is sequenced, variant interpretation is restricted to this carefully selected gene panel to assess genomic alterations relevant to the quality, safety, and clinical applicability of human pluripotent stem cells (hPSCs), while providing the most actionable data and minimizing interpretation burden.

The gene panel was developed by identifying the most commonly mutated genes in cancers affecting tissues that are frequently used either as sources for iPSC derivation (e.g., fibroblasts, PBMCs, keratinocytes, among others) or as target differentiation lineages for both ESCs and iPSCs (e.g., cardiomyocytes, pancreatic β cells, neural cells, retinal cells, immune cells, etc.). Data were compiled from the COSMIC Cancer Gene Census, the Genomic Data Commons, and population-level studies of somatic mutation frequency (Mendiratta et al., 2021), ensuring coverage of the most frequently mutated cancer driver genes. In addition, genes known to acquire recurrent mutations during prolonged culture of hPSCs were incorporated based on findings from Merkle et al. (2017) and Rouhani et al. (2022). This approach ensures that the panel captures mutations most relevant to stem cell research and clinical applications.

The goal of the assay is to identify pathogenic or likely pathogenic sequence variants, whether inherited or acquired, that could compromise genomic integrity and potentially affect research or clinical outcomes. Variant interpretation is based on expert-curated databases, computational prediction tools, published literature, and professional guidelines. This report highlights clinically relevant variants and provides detailed annotations to support risk assessment, decision-making, and clinical translation.

ANALYSIS RESULTS: Pathogenic or Likely Pathogenic Variants detected

2 Pathogenic Variants

Gene	Variant	Allelic fraction
JAK2	p.V617F, c.1849G>T	-
MET	p.R970C, c.2908C>T	-

4 Likely Pathogenic Variants

Gene	Variant	Allelic fraction
DNMT3A	p.E30A, c.89A>C	-
KIT	p.V530I, c.1588G>A	-
WT1	p.R385Q, c.1154G>A	-
SETD2	p.T2421A, c.7261A>G	-

Report content

3 Variants of Uncertain Significance

Gene	Variant	Allelic fraction
CREBBP	p.A698G, c.2093C>G	-
EGFR	p.R962H, c.2885G>A	-
NOTCH1	p.R621H, c.1862G>A	-

4 Likely Benign Variants

Gene	Variant	Allelic fraction
BCORL1	p.K1053R, c.3158A>G	-
BRAF	p.I711L, c.2131A>C	-
RB1	p.T377S, c.1129A>T	-
TP53	c.-1556G>A	-

2 Benign Variants

Gene	Variant	Allelic fraction
APC	p.R99W, c.295C>T	-
FGFR4	p.G388R, c.1162G>A	-

VARIANT DETAILS

Pathogenic Variants (2)

JAK2 V617F

Gene: *JAK2*
Exon: 14
Nucleotide:
NM_004972.4
g.5073770G>T
c.1849G>T
Amino Acid: p.V617F
Assessment: Pathogenic

Biomarker summary: *JAK2*-V617F (NM_004972) is an activating mutation.

Clinical relevance: *JAK2* encodes the Janus kinase 2 (Jak2) protein, a tyrosine kinase that regulates signals triggered by cytokines and growth factors [55]. Activation of the Jak/Stat pathway may predict sensitivity to Jak inhibitors, including ruxolitinib, momelotinib, fedratinib, and pacritinib, which have been approved for treatment of primary myelofibrosis, post-polycythemia vera myelofibrosis, and post-essential thrombocythemia myelofibrosis independently of mutations. Additionally, ruxolitinib has been approved for the treatment of polycythemia vera. Hsp90 inhibitors are also being investigated in preclinical studies to target components of the Jak/Stat pathway [70, 94, 145, 87, 30, 147, 107].

Disease summary: Mutations in the *JAK2* gene play a significant role in the pathogenesis of hematological malignancies, and aberrant Jak2 signaling has also been found in some types of solid tumors [72, 45, 109, 118].

Molecular function: *JAK2* V617F is a missense alteration in the pseudokinase domain of the Jak2 protein (UniProt) [113]. *JAK2* V617F is a hotspot mutation commonly reported in myeloproliferative neoplasms (MPNs), and has been found to result in constitutive Jak2 activation, promotion of downstream signaling, and the development of hematologic malignancies in mice [119, 17, 141, 93, 54].

Incidence: *JAK2* mutations have been reported in 26% (53541/204344) of all tumor samples analyzed in COSMIC (May 2023). Diseases in COSMIC with high incidence of *JAK2* mutations include Hepatocellular carcinoma (HCC) (5.7%, 110/1944), Endometrial carcinoma (3.8%, 39/1035), and Prostate carcinoma (3.3%, 135/4071) (May 2023). One study has reported a *JAK2* mutation in 1/442 carcinoma of unknown primary (CUP) cases [61]. A separate study reported loss of function *JAK2* mutations in 1% of 389 CUP cases [34].

Pathogenic Variants (2)

MET R970C

Gene: *MET*
Exon: 14
Nucleotide:
NM_000245.4
g.116771869C>T
c.2908C>T
Amino Acid: p.R970C
Assessment: Pathogenic

Biomarker summary: The functional consequences of *MET*-R988C (NM_001127500) are unknown.

Clinical relevance: Aberrant activation of Met in cancer can occur through *MET* gene mutation or amplification, or excessive/inappropriate signaling via the Met receptor's ligand, *HGF* [73]. Cabozantinib, which targets Met and other kinases, has been approved for certain indications [7, 24, 46, 14]. Crizotinib, a kinase inhibitor that has been approved for treatment of *ALK*- and *ROS1*-rearranged non-small cell lung cancer, also targets Met [120, 89, 121]. In addition, the kinase inhibitors capmatinib and tepotinib have been approved by the FDA, EMA and PMDA, for the treatment of adult patients with metastatic non-small cell lung cancer and a *MET* exon 14 skipping mutation [101, 150]. Telisotuzumab vedotin-tllv has been FDA-approved for the treatment of adult patients with locally advanced or metastatic, nonsquamous non–small cell lung cancer harboring high Met protein overexpression who have received a prior systemic therapy; high Met expression is defined as strong immunohistochemistry (IHC 3+) in at least 50% of tumor cells as determined by an FDA-approved test [10]. However, as this *MET* alteration has been characterized as both benign and oncogenic, and has been reported both as a germline mutation and present in the normal population, the relevance of any therapeutic approaches is unknown.

Disease summary: Met protein activation or overexpression promotes angiogenesis, resistance to apoptosis, proliferation, and invasion of cancer cells [1, 57, 36, 130].

Molecular function: *MET* R988 in NM_001127500 corresponds to R970 in NM_000245 (IGV). *MET* R988C is a missense alteration within the juxtamembrane region of Met [138]. *MET* R988C has been reported as a germline SNP deposited in dbSNP as rs34589476, with an allele frequency of 0.3% (796/279954) in the global population; homozygosity has been reported in four individuals (gnomAD browser, Mar 2025). Early studies characterized R988C as an activating mutation based on increased tyrosine phosphorylation of downstream proteins, enhanced reactive oxygen species production, and increased colony and focus formation in transformation assays [81, 82, 53]. However, more recent analyses of R988C activity in similar assays do not support a tumorigenic effect of this mutation [43, 139, 135].

Incidence: *MET* mutations have been reported in 3.0% (3089/101912) of all tumor samples analyzed in COSMIC (May 2023). Diseases in COSMIC with high incidence of *MET* mutations include Prostate carcinoma (6.3%, 264/4204), Endometrial carcinoma (6.0%, 70/1174), and Melanoma (5.8%, 255/4384) (May 2023).

Likely Pathogenic Variants (4)

DNMT3A E30A

Gene: *DNMT3A*
Exon: 3
Nucleotide:
NM_022552.5
g.25300227T>G
c.89A>C
Amino Acid: p.E30A
Assessment: Likely Pathogenic

Biomarker summary: *DNMT3A*-E30A (NM_022552) is predicted to have no effect on the protein function.

Clinical relevance: The role of Dnmt3a in cancer is uncertain, as some reports describe increased expression and contribution to tumor growth, while other work proposes a role for Dnmt3a as a tumor suppressor [157, 137, 20, 25, 32, 64]. In addition, *DNMT3A* mutations often result in altered DNA methylation patterns as opposed to globally increased or decreased methylation [52, 96, 88]. There are currently no approved therapies that directly target *DNMT3A* mutations in cancer. However, in this case of likely germline polymorphism, the relevance of any therapeutic approaches is unknown.

Disease summary: The role of *DNMT3A* in cancer is unclear, with both an oncogenic and tumor suppressor role reported [157, 137, 20, 25, 32, 64]. In addition, *DNMT3A* mutations often result in altered DNA methylation patterns as opposed to globally increased or decreased methylation [52, 96, 88].

Molecular function: *DNMT3A* E30A is a missense alteration located prior to several characterized domains of the Dnmt3a protein (UniProt). *DNMT3A* E30A has been reported as a germline SNP deposited in dbSNP as rs143730975, with an allele frequency of 0.3% (945/276978) in the global population, and an allele frequency of higher than 1% in the following subpopulation (European Finnish: 2%); homozygosity has been reported in six individuals (gnomAD browser, Jan 2024). *DNMT3A* E30A has been assigned a clinical significance of benign/likely benign based on seven submissions with a two-star review status last evaluated on 2021/11/23 (ClinVar, Jan 2024). In addition, *DNMT3A* E30A has been reported to result in methyltransferase activity and protein stability similar to wild-type protein [50].

Incidence: *DNMT3A* mutations have been reported in 6.1% (5309/86886) of all tumor samples analyzed in COSMIC (May 2023). Diseases in COSMIC with high incidence of *DNMT3A* mutations include Endometrial carcinoma (7.0%, 55/789), Lymphoma (6.2%, 264/4245), and Hepatocellular carcinoma (HCC) (5.1%, 90 /1771) (May 2023).

Likely Pathogenic Variants (4)

KIT V530I

Gene: *KIT*
Exon: 10
Nucleotide:
NM_000222.3
g.54727265G>A
c.1588G>A
Amino Acid: p.V530I
Assessment: Likely Pathogenic

Biomarker summary: *KIT*-V530I (NM_000222) is an activating mutation.

Clinical relevance: Activating mutations in the Kit tyrosine kinase receptor lead to activation of downstream PI3K/Akt and Ras/MAPK signaling pathways, and may predict sensitivity to small molecule tyrosine kinase inhibitors [78, 22, 111, 126]. Several kinase inhibitors targeting Kit, including imatinib, sunitinib, and regorafenib, have been approved for certain indications; others are under investigation in clinical trials [16, 56, 40, 4, 116]. The alteration reported here, *KIT* V530I, has been shown to be sensitive to imatinib [11, 71].

Disease summary: Kit is considered to be a proto-oncogene and activating mutations of the *KIT* gene can lead to tumorigenesis [26].

Molecular function: *KIT* V530I is a missense alteration in the transmembrane domain of the Kit protein (UniProt) [71]. *KIT* V530I has been reported as a germline SNP deposited in dbSNP as rs72550822, with an allele frequency of 0.06% (168/282646) in the global population, including 0.1% (37/24962) of the African /African American and 0.1% (106/128988) of the non-Finnish European subpopulations; homozygosity has been reported in one individual (gnomAD browser, Jan 2024). This alteration has been reported to result in constitutive activation of Kit and to confer sensitivity to imatinib; a case study of a patient with extraabdominal aggressive fibromatosis harboring the V530I mutation showed a very good partial response to imatinib treatment at 34 months and subsequent complete remission of the tumor [71]. A preclinical study also reported that a cell line harboring *KIT* V530I had enhanced sensitivity to imatinib, as compared with a wild-type *KIT* cell line [11].

Incidence: *KIT* mutations have been reported in 9.3% (10845/117145) of all tumor samples analyzed in COSMIC (May 2023). Diseases in COSMIC with high incidence of *KIT* mutations include Hepatocellular carcinoma (HCC) (8.3%, 166/1994), Melanoma (7.2%, 653/9031), and Endometrial carcinoma (5.8%, 63/1084) (May 2023).

WT1 R385Q

Gene: *WT1*
Exon: 7
Nucleotide:
NM_024426.6
g.32396367C>T
c.1154G>A
Amino Acid: p.R385Q
Assessment: Likely Pathogenic

Biomarker summary: The functional consequences of *WT1*-R380Q (NM_024426) are unknown.

Clinical relevance: *WT1* encodes the Wilms tumor 1 protein (Wt1). Wt1 has been reported to have both tumor suppressive and tumorigenic properties [51, 124]. There are no approved targeted therapies for *WT1* mutations or expression. Wt1 vaccines are in clinical trials for Wt1-expressing hematologic and solid tumor cancers, including renal cell carcinoma, biliary tract cancers, and non-small cell lung cancer [124, 97, 128, 127]. As the alteration reported here has not been functionally characterized, the relevance of any available therapeutic approaches is unknown.

Disease summary: The role of *WT1* and the transcription factor it encodes in cancer is complex; while in some tumor types, *WT1* is inactivated and behaves as a tumor suppressor, in other tumor types it is overexpressed and oncogenic [51, 124]. Germline mutation in *WT1* has been associated with Wilms' tumor, a pediatric renal cancer [149, 13, 112]. Please note: This gene has not been researched in the context of this specific cancer type by N-of-One.

Molecular function: *WT1* R380 in transcript NM_024426.4/ ENST00000332351 corresponds to R385 in NM_024426.6/ENST00000452863 (IGV, Ensembl genome browser 106). *WT1* R380Q is a missense alteration that occurs outside of several characterized domains in the Wt1 protein (UniProt). *WT1* R380Q has also been reported in COSMIC (Feb 2022), but it has not been functionally characterized (PubMed, Feb 2022), and its effect on protein function is unknown.

Incidence: *WT1* mutations have been reported in 3.1% (2398/76936) of all tumor samples analyzed in COSMIC (May 2023). Diseases in COSMIC with high incidence of *WT1* mutations include Prostate carcinoma (7.2%, 269/3743), Hepatocellular carcinoma (HCC) (4.3%, 77/1790), and Pancreatic carcinoma (4.0%, 71 /1793) (May 2023).

Likely Pathogenic Variants (4)

SETD2 T2421A

Gene: *SETD2*
Exon: 18
Nucleotide:
NM_014159.7
g.47037755T>C
c.7261A>G
Amino Acid: p.T2421A
Assessment: Likely Pathogenic

Biomarker summary: The functional consequences of *SETD2*-T2421A (NM_014159) are unknown.

Clinical relevance: *SETD2* encodes Setd2, a lysine-specific histone methyltransferase. Loss of function of Setd2 through mutation or loss has been reported in various tumor types, including breast and renal cancer, as well as glioma [23, 95, 69, 28, 35, 44]. Inactivating *SETD2* alterations in cancer have been associated with impaired DNA repair, microsatellite instability, and DNA hypomethylation [58, 12, 156, 76, 123]. There are currently no approved therapies that directly target *SETD2* alterations in cancer. However, preclinical studies have reported that *SETD2*-deficient cancer cell lines have increased sensitivity to Wee1 inhibitors such as adavosertib, which is currently under investigation in clinical trials [105, 86]. As the alteration reported here has not been functionally characterized, the relevance of any available therapeutic approaches is unknown.

Disease summary: *SETD2* inactivation through deletion, mutation, or protein loss has been reported in various cancer types, including breast and renal cancer, as well as glioma [23, 95, 69, 28, 35, 44]. *SETD2* is considered to be a tumor suppressor gene; *SETD2* loss and mutation in cancer have been variously reported to lead to replication stress, impaired DNA repair, microsatellite instability, and extensive DNA hypomethylation [58, 12, 156, 76, 123].

Molecular function: *SETD2* T2421A is a missense alteration located within the WW domain of the Setd2 protein [33]. *SETD2* T2421A has been assigned a clinical significance of likely benign based on two submissions with a one-star review status last evaluated on 2022/05/27 (ClinVar, Feb 2025). This alteration has been reported (COSMIC, Feb 2025), but it has not been functionally characterized (PubMed, Feb 2025), and its effect on protein function is unknown.

Incidence: *SETD2* mutations have been reported in 4.7% (2967/63711) of all tumor samples analyzed in COSMIC (May 2023). Diseases in COSMIC with high incidence of *SETD2* mutations include Renal cell carcinoma (13%, 439/3406), Hepatocellular carcinoma (HCC) (9.3%, 170/1821), and Endometrial carcinoma (8.9%, 70/783) (May 2023). One study has reported *SETD2* mutations in 2.8% (13/464) of patients with carcinoma of unknown primary [148].

Variants of Uncertain Significance (3)

CREBBP A698G

Gene: *CREBBP*
Exon: 10
Nucleotide:
NM_004380.3
g.3778031G>C
c.2093C>G
Amino Acid: p.A698G
Assessment: Uncertain Significance

CREBBP encodes the CREB binding protein, CBP, a histone acetyltransferase (HAT) that acts as a transcriptional co-activator by binding to a number of transcription factors, including c-jun, c-myb, MyoD, E2F-1, YY1, and members of the steroid hormone receptor superfamily [90, 39]. CBP activates p53 signaling in response to DNA damage and may mediate beta-catenin signaling [129, 42]. Germline *CREBBP* mutations have been reported in Rubinstein-Taybi syndrome, a congenital syndrome characterized by growth retardation, developmental delay, and an increased risk of cancer [3, 117, 104]. Alterations in the *CREBBP* gene have been implicated in hematological malignancies and in the predisposition to other cancers [39, 37].

EGFR R962H

Gene: *EGFR*
Exon: 24
Nucleotide:
NM_005228.5
g.55200352G>A
c.2885G>A
Amino Acid: p.R962H
Assessment: Uncertain Significance

EGFR encodes the Epidermal growth factor receptor (Egfr), a receptor tyrosine kinase that functions as an oncogene. Egfr activates signaling pathways, such as the Ras/Raf/MAPK and PI3K pathways, and stimulates the cell to grow and divide [115]. Amplification, mutation, and overexpression of EGFR may cause excessive proliferation and tumor formation [15, 6]. *EGFR* activating mutations or amplification may predict sensitivity to Egfr-targeted therapies, including inhibitors of multiple ErbB family members, and several have received FDA approval in some tumor types [92, 110, 132]. Small in-frame deletions in exon 19 and the exon 21 missense mutation L858R account for approximately 40% of all *EGFR* alterations in non-small cell lung cancer [84, 18]. In-frame insertions within exon 20 of *EGFR* are found in over 4% of non-small cell lung cancer and associated with resistance to tyrosine kinase inhibitors [2, 91, 99, 151, 152].

Located in a critical and well-established functional domain (PKc_like) without benign variation.

Multiple lines of computational evidence support a deleterious effect on the gene or gene product [CADD = 29.5].

Variants of Uncertain Significance (3)

NOTCH1 R621H

Gene: *NOTCH1*
Exon: 11
Nucleotide:
NM_017617.5
g.136515524C>T
c.1862G>A
Amino Acid: p.R621H
Assessment: Uncertain Significance

NOTCH1 encodes Notch1, a member of the Notch family of receptors, which contain an extracellular domain with several EGF-like repeats. Upon binding of membrane bound ligands, the Notch1 intracellular domain (NICD) is cleaved and forms part of a transcription factor complex regulating genes involved in cell fate determination, proliferation, and apoptosis [68]. Depending on cellular context, *NOTCH1* can act as either a tumor suppressor or oncogene [65, 144].

Located in a critical and well-established functional domain (EGF_CA) without benign variation.

Multiple lines of computational evidence support a deleterious effect on the gene or gene product [CADD = 23.5].

Reputable source recently reports variant as benign, but the evidence is not available to the laboratory to perform an independent evaluation.

Benign variants (6)

Gene	Variant	Allelic fraction	Assessment
APC	c.295C>T p.R99W	-	Benign
BCORL1	c.3158A>G p.K1053R	-	Likely Benign
BRAF	c.2131A>C p.I711L	-	Likely Benign
FGFR4	c.1162G>A p.G388R	-	Benign
RB1	c.1129A>T p.T377S	-	Likely Benign
TP53	c.-1556G>A	-	Likely Benign

REPORT INFORMATION

Genes tested (65)

APC, ASXL1, BAP1, BCOR, BCORL1, BRAF, CALR, CDKN2A, CIC, CREBBP, CSMD3, CTNNB1, CYSLTR2, DDX3X, DNMT3A, EGFR, EIF1AX, FAS, FAT4, FBXW7, FGFR4, FLT3, GNA11, GNAQ, GNAS, GRIN2A, H3-3A, IDH1, IDH2, IKZF1, JAK2, KIT, KMT2D, KRAS, LRP1B, MAP2K1, MAP3K1, MET, MTOR, MUC16, MYD88, MYOD1, NF1, NOTCH1, NPM1, NRAS, NSD2, PAX5, PBRM1, PHF6, PIK3CA, PIM1, PTEN, PTPN11, RB1, SETD2, SF3B1, SMAD4, SMARCB1, STAT3, TET2, TP53, U2AF1, VHL, WT1

Methods and limitations

Methods

Genomic DNA was extracted from the submitted human pluripotent stem cell (hPSC) line and subjected to whole genome sequencing (WGS) at an average read depth of 100x using Illumina technology. Sequencing reads were aligned to the GRCh38 reference genome, and sequence variants (SNVs and small indels) were identified using validated secondary analysis software.

Resulting VCF files were analyzed using Qiagen Clinical Insight Interpret (QCI Interpret) for variant annotation and classification. Only variants located within a curated panel of 65 cancer-associated genes were interpreted and reported. Variant classification incorporated data from public databases (e.g., ClinVar, COSMIC) and predictive algorithms in accordance with current clinical guidelines. Variants were filtered for call quality greater than 20 and read depth greater than 10. Variants were excluded from analysis if present in greater than 1% of any population except if they have been previously established as pathogenic common variants.

Limitations

This assay reports only sequence variants within the defined 65-gene panel. Variants outside this panel are not interpreted. The assay does not detect large structural variants, copy number changes, gene fusions, mitochondrial variants, or epigenetic alterations. Low-frequency variants (below ~5% allele frequency) may not be reliably detected. Variants of uncertain significance (VUS) are reported but may lack clear functional relevance. Interpretation is based on current knowledge and may evolve as new data become available. This assay is not a comprehensive assessment of genomic integrity. Additional assays may be necessary for a complete evaluation of hPSC safety and suitability.

QIAGEN Clinical Insight (QCI™) is a variant analysis, interpretation and decision support tool for research and clinical labs analyzing human genetics data and is not intended to be used for diagnostic purposes. QCI Interpret software includes the following underlying databases, data reference sets and tools: QIAGEN Clinical Insight Interpret (9.4.2.20250513), Ingenuity Knowledge Base (N-release), CADD (1.6), NCBI Gene (2023-08-25), Allele Frequency Community (2019-09-25), EVS (ESP6500SI-V2), Refseq Gene Model (2023-10-03), JASPAR (2013-11), Ingenuity Knowledge Base Snapshot Timestamp (2025-05-30 12:37:16.159), Vista Enhancer hg18 (2012-07), Vista Enhancer hg19 (2012-07), Clinical Trials (2025-05-30), MITOMAP: A Human Mitochondrial Genome Database. http://www.mitomap.org, 2019 (2020-06-19), REVEL (1.3), PolyPhen-2 (2.2.2 (HumVar)), 1000 Genome Frequency (phase3v5b), ExAC (0.3.1), TargetScan (7.2), phyloP hg18 (GRCh37 (hg19) 2019-11, GRCh38 2019-11), phyloP hg19 (GRCh37 (hg19) 2019-11, GRCh38 2019-11), GENCODE (Release 44), CentoMD (5.3), dbVar (2021_04), OMIM (2023-10-23), gnomAD (GRCh37 (hg19) 2.1.1, GRCh38 (hg38) 4.1.0), BSIFT (2016-02-23), TCGA (2013-09-05), Clinvar (2025-04-13), DGV (2016-05-15), COSMIC (99), HGMD (2025.1), Matched Annotation from NCBI and EMBL-EBI (MANE) (1.2), OncoTree (oncotree_2021_11_02), SpliceAI (1.3), dbSNP (GRCh37 (hg19) 156, GRCh38 156), SIFT4G (2016-02-23)

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