Sonic Genetics

Douglass Hanly Moir Pathology

ContextualGenomics

Personalised cancer care

Information for Medical Specialists

Contents

ContextualGenomics

Why choose the FIND IT® Hotspot Cancer Panel?

Every cancer is different and has a specific genetic signature. Research studies across the world are identifying genetic signatures in different solid cancers. They are also developing treatments that can target those signatures. This is leading to a revolution in cancer diagnosis and treatment.

The FIND IT Cancer Hotspot Panel can identify possible targeted treatments and enhance patient care management. It is available exclusively through Sonic Genetics.

Optimised for clinical benefit

Cancer is driven by multiple aberrations that are unique to each patient's tumour. Identifying what these aberrations are allows oncologists to select the best treatment plan for the patient. The FIND IT assay is designed to provide a clinical report to you within 7 business days upon specimen receipt. This report catalogues the genomic alterations and links them to details on treatment sensitivity and resistance, and clinical trials information.

The FIND IT Cancer Hotspot Panel has been carefully designed by the Contextual Genomics' team of cancer research scientists and physicians. Mutations have been incorporated in the panel based on their relevance to standard of care treatment, including information on treatment resistance, as well as available clinical trials that may be of potential value to the patient's treatment plan. Ninety percent of the genes on the panel are relevant to available drugs or clinical trials and 67% cover resistance mutations or positions related to new developments in the pharmaceutical industry.

Development history

The Contextual Genomics FIND IT Cancer Hotspot Panel, launched in Canada in 2016, is a multiplex, genomic assay designed for next generation sequencing. The test simultaneously evaluates the mutation status of tumour DNA at more than 120 well-characterised positions (hotspots) and 17 exons in 33 cancer-associated genes. Please check our website for the most recent version, as we review our panel regularly in response to developments in cancer research. The results of the assay identify:

- Therapeutic targets for patients
- Acquired drug resistance mutations
- Mutations with prognostic and diagnostic implications for patient care.

The report has been adapted to reflect Australian clinical practice.

Full FIND IT panel vs focused panels

The FIND IT panel is intended to be utilised in two ways: to provide the current somatic mutation testing rebated by Medicare through a focused panel (NSCLC, melanoma and colorectal tumours); and to provide a personalised genetic fingerprint of your patient's tumour via the full FIND IT panel.

Focused panels

The focused panels have been designed to reflect the Medicare items related to NSCLC, melanoma and colorectal somatic mutation testing. These panels are restricted to patients that meet these criteria. The NSCLC and melanoma focused panels can be ordered by the requesting clinician if Medicare rebate criteria are fulfilled. In addition, if processed by a Sonic Healthcare laboratory, these panels can be ordered by the reporting pathologist.
The colorectal focused panel must be requested by a specialist or consultant physician, to meet Medicare rebate criteria.

Add-on panel

The add-on panel extends any of the focused panel tests to the full FIND IT panel. This can be ordered for any patient who has already met Medicare rebate criteria. The request for the add-on panel must come from the specialist or consulting physician, accompanied by signed financial consent from the patient.

Full FIND IT panel

This test can be ordered at any time during the management of an adult patient's cancer treatment. The full FIND IT panel requires a specialist or consultant physician request. Testing will begin immediately, but the release of the results will require full payment by the patient.

Comprehensive reporting in 7 business days

FIND IT detects common, actionable mutations and reports them back to the user, allowing a turnaround time of 7 business days from sample receipt to a report. Reports feature a detailed clinical interpretation, with recommendations based on the mutation profile of the tumour. This seamless, full-service approach enables clinicians to make more informed treatment decisions and potentially offer patients the most current cancer management plan available.

Add-on panel

If a patient has already received results for any focused panel and then seeks the add-on panel, the results can be available the next business day once payment has been received. If an add-on panel is required after six months from the time the focused report panel is generated, please contact the testing laboratory.

How does FIND IT work?

The assay is performed on DNA extracted from formalin-fixed paraffin embedded (FFPE) cancer tissue. The results of the assay are presented in a comprehensive report which includes:

- Detailed listing of mutations identified and those which are absent that are of particular significance to the patient's case (not all absent mutations). Hotspot variants are categorised into clinical significance tiers.[1]
- Matching of mutations with locally approved drugs (medications registered with the TGA or included on the Pharmaceutical Benefits Scheme)
- Review of recommendations from the National Comprehensive Cancer Network and relevant medical literature
- Identification of potentially relevant Australian clinical trials.

Full FIND IT panel and associated treatment implications

Gene	Mutation	Clinical presentation			
Medicare rebates are available for the NSCLC, melanoma and colorectal focused panels, subject to eligibility criteria being met. Alternatively, please refer to the Medicare Benefits Schedule. A list of all up-to-date test criteria for Pathology Services is available at www.mbsonline.gov.au.		NSCLC focused	Melanoma focused	Colorectal focused	Any solid tumour
AKT1	E17				✔
ALK^	T1151, L1152, C1156, F1174, L1196, L1198, G1202, D1203, S1206, G1269, R1275				✔
AR	V716, S741, W742, H875, F877, T878				✔
BRAF	Q201, G466, F468, G469, Y472, D594, G596, L597, V600, K601	✔	✔	✔	✔
CTNNB1	D32, S33, G34, S37, T41, S45				✔
DDR2	L239, I638, S768				✔
EGFR	Exons: 18, 19, 20, 21	✔			✔
ERBB2	G309, S310, L755, Exon: 20	✔			✔
ESR1	K303, S463, V534, P535, L536, Y537, D538				✔
FGFR1	N546, K656				✔
FGFR2	S252, P253, N549, K659				✔
GNA11	Q209				✔
GNAQ	Q209				✔
GNAS	R201				✔
HRAS	G12, G13, Q61				✔
IDH1	R132				✔
IDH2	R140, R172				✔
JAK1	V658, S703				✔

Gene	Mutation	Clinical presentation			
Medicare rebates are available for the NSCLC, melanoma and colorectal focused panels, subject to eligibility criteria being met. Alternatively, please refer to the Medicare Benefits Schedule. A list of all up-to-date test criteria for Pathology Services is available at www.mbsonline.gov.au.		NSCLC focused	Melanoma focused	Colorectal focused	Any solid tumour
KIT	T670, D816, D820, N822, Y823, A829, Exons: 9, 11, 13		✔		✔
KRAS	G12, G13, A59, Q61, K117, A146	✔		✔	✔
MAP2K1 (MEK1)	Q56, K57, K59, D67, P387				✔
MAP2K2 (MEK2)	F57, Q60, K61, L119				✔
MET	Y1253, Exons: 13, 14+25, 14-50, 18, 14				✔
NRAS	G12, G13, A59, Q61, K117, A146		✔	✔	✔
PDGFRA	D842, L839-Y849, N659, R560-E571				✔
PIK3CA	R88, E542, E545, Q546, D549, M1043, N1044, A1046, H1047, G1049			✔	✔
PTCH1	W844, G1093				✔
PTEN	I122_M134, K254_K267, R130, R173, S170_Y188, Y225_F243				✔
RET	C634, V804, M918				✔
ROS1*	L2026, G2032				✔
SMO	D473, S533, W535				✔
STK11	Q37, P281				✔
TP53	R158, R175, R213, Y220, G245, R248, R273, R282, Exons: 4, 5, 6, 7, 8, 9				✔

Information contained within the above table v3.4 is correct at time of printing (May 2018).

^ ALK IHC is completed for all NSCLC samples in parallel with gene panel testing, to detect ALK over-expression. If over-expression is detected, subsequent ALK rearrangement FISH can then be performed. ALK testing within the FIND IT panel is to detect the listed hotspot mutations.

* ROS1 rearrangement FISH is available on request.

FIND IT panel genes associated with tumour types

Cancer	Associated genes
Basal cell carcinoma	PTCH1, SMO
Bladder cancer	HRAS, PIK3CA
Breast cancer	AKT1, BRAF, DDR2, ERBB2 (HER2), ESR1, PIK3CA, PTEN
Colorectal cancer	AKT1, BRAF, CTNNB1, KRAS, MET, NRAS, PIK3CA, PTEN
Endometrial cancer	DDR2, PIK3CA, PTEN
GIST	BRAF, KIT, PDGFRA
Glioma	BRAF, EGFR, FGFR1, IDH1, IDH2, PIK3CA, PTEN, SMO
Head and neck cancer	BRAF, DDR2, PIK3CA
Lung cancer	AKT1, ALK, BRAF, DDR2, EGFR, ERBB2, KRAS, MAP2K1, MET, NRAS, PIK3CA, PTEN, ROS1, STK11
Melanoma	BRAF, CTNNB1, GNA11, GNAQ, KIT, MAP2K1, NRAS
Neuroblastoma	ALK
Ovarian cancer	BRAF, KRAS, PIK3CA, PTEN
Pancreatic cancer	CTNNB1, KRAS
Prostate cancer	AR, DDR2, KRAS, PTEN
Thymic cancer	KIT
Thyroid cancer	BRAF, CTNNB1, HRAS, KRAS, NRAS, RET

Please note: The genes included in each panel are under continual review and subject to change. Refer to the Sonic Genetics website for current information.

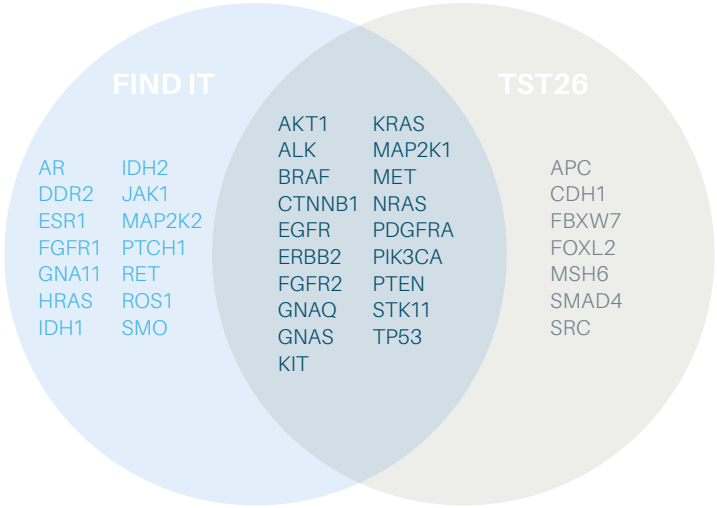
Accurate and robust genome analytics

Contextual Genomics has developed a robust, accurate and high-throughput bioinformatics system for the identification of mutations from targeted NGS data using the FIND IT multiplex PCR panel. The central component of this system is the proprietary bioinformatics and genome analytics pipeline. The pipeline has been built on state-of-the-art computational algorithms and has been rigorously validated using commercially available cell-line controls and more than 200 tumour samples with available orthogonal data. In a Canada-wide study the pipeline powered the generation of more than 1,000 reports from banked specimens and real-time patient samples used in a clinical setting. In that study, the pipeline demonstrated high accuracy with sensitivity and specificity greater than 99.9%.

Comparison with other platforms

A comparison of FIND IT to the Illumina TruSight Tumour 26 test was performed by Sonic Genetics in Australia, using a cohort of more than 70 samples. This showed >99% concordance in regions common to both panels.

FIND IT vs. Illumina TruSight Tumour 26: Targets



Reproducibility and repeatability

Experiments to investigate reproducibility and repeatability have shown correlations of allele frequency at >0.99 and >0.98 within and between experiments respectively, for different types of mutations (SNVs and insertions-deletions). Furthermore, >99% reproducibility has been shown for the FIND IT assay, including comparisons between sequencing runs, laboratory technologists, MiSeq® instruments and dates.

Why use FIND IT for your patient?

Improved therapeutic precision and increased treatment options

Unlike technologies that have limited detection potential or whole gene sequencing strategies that often yield variants of unknown significance, the FIND IT test targets known and clinically actionable alterations, designed to reduce ambiguity and potentially improve clinical management options. We limit the range of genes tested, to maximise the reporting of usable information that will assist doctors to make more precise decisions about therapeutics.

Reliable turnaround time

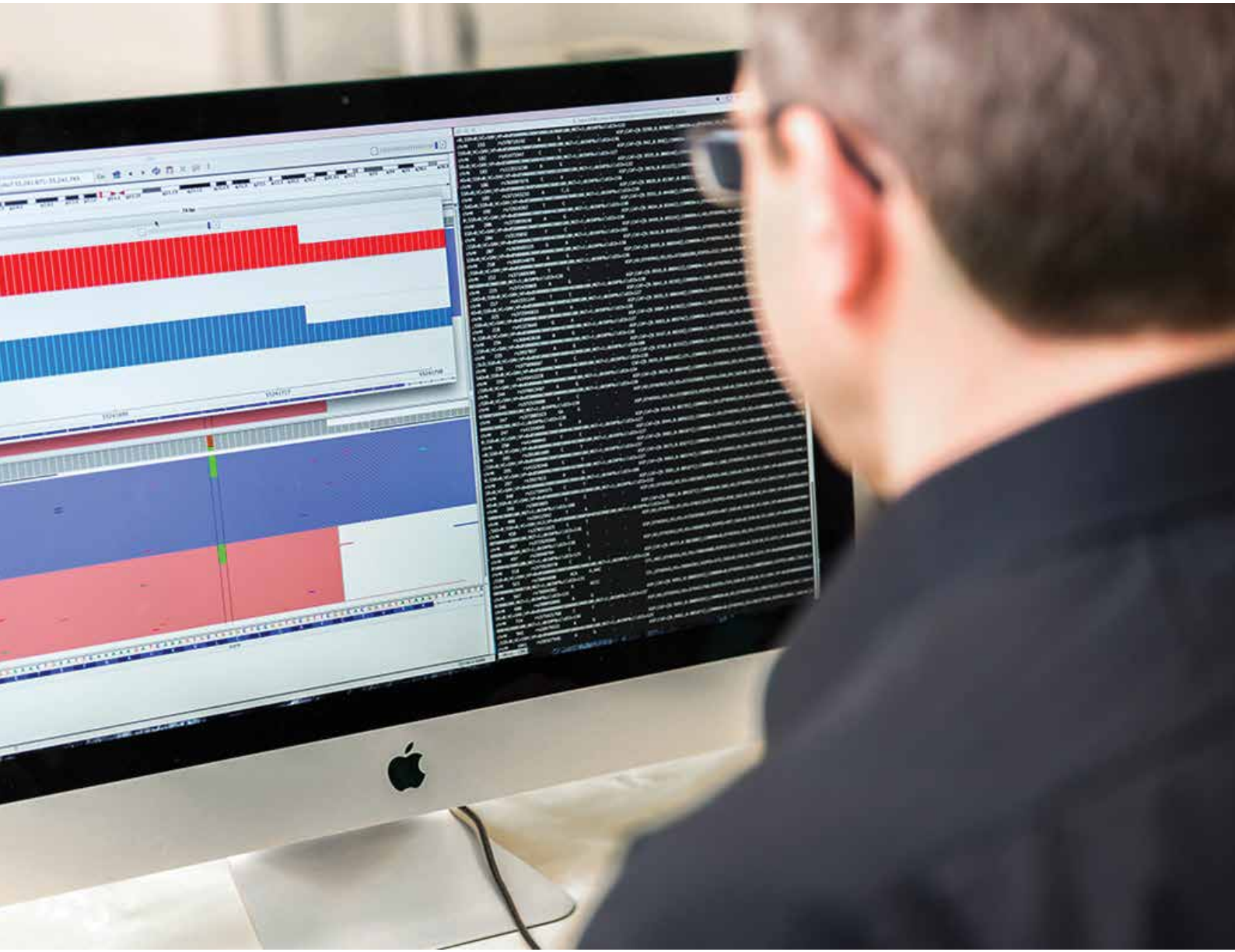
We are committed to report results in 7 business days from the time of sample receipt in the molecular testing laboratory. Since the panel tests for all hotspot mutations simultaneously, it significantly reduces time to result compared to consecutive single gene test methods.

Conserve biopsy tissue

The assay reduces the amount of biopsy tissue required by taking a single sample, extracting its DNA and testing for all hotspots.

Accreditation

Our testing laboratory, Sullivan Nicolaides Pathology, is fully accredited by Australian regulatory authorities for this test assay



Manufacturer's specifications

Key metrics and requirements	
Genes covered	33 genes
Exons covered	17 full exons
Hotspots covered	123 hotspots
Mutations types	SNVs, insertions and deletions (up to 24bp)
Turnaround time	Designed to provide a clinical report within 7 days
Sample types	FFPE blocks and unstained slides (15 x4µm sections)
Sample multiplexing	Up to 21 samples per experiment
Sequencing depth	Average 6,500x and at least 500x
Assay sensitivity	>99%
Assay PPV	100%
Assay reproducibility	>99%
Assay cut-off	≥5% allele frequency
Sample requirements	Sample formats are FFPE blocks or unstained slides (4µm sections) with a minimum of 10% tumour cellularity

The FIND IT report and interpretation of results

The FIND IT Cancer Hotspot Panel can:

Detect mutations that have prognostic and diagnostic value.

Identify potential therapeutics or eligibility for a clinical trial.

Find acquired resistance mutations to drugs and assist in the selection of alternative therapies.

The panel targets a defined set of genes and hotspots, meaning that it will not detect genetic alterations outside of those target areas. As well, the test will not detect mutations below the test's detection limit of 5% variant allelic frequency. In some cases, a negative result is actionable and can help direct patient care. In a small proportion of cases, the test will find a mutation associated with a hereditary cancer. The assay cannot distinguish between somatic (no risk to relatives) and hereditary (risk to relatives) mutations. Discussion with or referral to a family cancer clinic or clinical genetics service may be considered if a mutation is detected in a gene known to be associated with a hereditary cancer syndrome. This is particularly important to consider if the patient has a family history, and/or other factors, such as age of onset, consistent with a hereditary cancer syndrome.

How much does the FIND IT test cost?

The full panel is available on request. Currently, it is not covered by a Medicare rebate and will be privately billed to the patient. Focused panels are available for the relevant Medicare rebate fee, avoiding any out-of-pocket charges for your patient. An add-on panel can be requested to extend a focused panel to a full panel for the subsidised price listed below.

Panel required and clinical indication		Genes analysed	Cost
Full FIND IT panel*		33 genes	$595
Focused panels†	NSCLC† Melanoma† Colorectal†	4 genes (BRAF, EGFR, ERBB2, KRAS) 3 genes (BRAF, KIT, NRAS) 4 genes (BRAF, KRAS, NRAS, PIK3CA)	MBS rebatable (no gap) MBS rebatable (no gap) MBS rebatable (no gap)
Add-on panel		Extend a focused panel to the full 33 genes	$395

Prices correct at time of printing.
*Partial rebate may be available, subject to Medicare criteria being met for the NSCLC, Melanoma or Colorectal panels listed.
†Medicare rebates available, subject to Medicare criteria being met.

The NSCLC, melanoma and colorectal cancer panels are fully rebated when MBS criteria are fulfilled. The table below details the criteria which must be fulfilled for a patient to be entitled to a rebate.

*Please note: For patient samples held by histopathology laboratories that are not part of the Sonic Healthcare network, a sample retrieval and processing fee may be applied and invoiced to the patient by the laboratory holding the sample block and delay testing.

Medicare criteria (as of May 2018)

Indication	Item #	MBS rebate requirements
NSCLC	73337	A test of tumour tissue from a patient diagnosed with non-small cell lung cancer, shown to have non-squamous histology or histology not otherwise specified, requested by, or on behalf of, a specialist or consultant physician, to determine if the requirements relating to epidermal growth factor receptor (EGFR) gene status for access to erlotinib or gefitinib under the Pharmaceutical Benefits Scheme (PBS) are fulfilled.
Melanoma	73336	A test of tumour tissue from a patient with unresectable stage III or stage IV metastatic cutaneous melanoma, requested by, or on behalf of, a specialist or consultant physician, to determine if the requirements relating to BRAF V600 mutation status for access to dabrafenib or vemurafenib under the Pharmaceutical Benefits Scheme (PBS) are fulfilled.
Colorectal	73338	A test of tumour tissue from a patient with metastatic colorectal cancer (stage IV), requested by a specialist or consultant physician, to determine if the requirements relating to rat sarcoma oncogene (RAS) gene mutation status for access to cetuximab or panitumumab under the Pharmaceutical Benefits Scheme (PBS) are fulfilled, if: (a) the test is conducted for all clinically relevant mutations on KRAS exons 2, 3 and 4 and NRAS exons 2, 3 and 4; or (b) a RAS mutation is found.

Arrange FIND IT testing with Sonic Genetics

1. Complete a FIND IT Test Request Form available on the Sonic Genetics website, www.sonicgenetics. com.au/FIND IT. To enable interpretation of genomic findings and drug therapy recommendations, please include:
   - The patient's clinical history and diagnosis
   - Previous molecular test results including gene fusion FISH results (if available)
   - A copy of the histology report
   - Details of the laboratory holding the original tissue sample, block number and laboratory reference

   Please fax this request to 1800 952 202 or email to oncology@sonicgenetics.com.au

2. Prepayment is required from the patient for the add-on or full FIND IT panel. The laboratory will contact the patient for prepayment by credit card over the phone if criteria for Medicare or other reimbursement is not met.

3. Once we have received your request and payment, we will contact the laboratory to obtain the tissue sample for analysis. Please be aware, and ensure the patient is informed, that if the sample is held by a laboratory outside of the Sonic Healthcare network, there may be a delay in receiving the sample and the holding laboratory may charge the patient for processing, postage and administration.

4. Once the tissue sample is received, we will perform the analysis and report results within 7 business days.

   Note: If the sample yields insufficient DNA for analysis, the laboratory may attempt to use other technology to obtain a result, which may cause delays. Results provided in these cases will not necessarily comprise all genes of the focused or full panel.

When to order this test

- This test is designed to identify treatment opportunities for newly diagnosed tumours and to detect developed resistance to current or previous therapies.
- The FIND IT panel is not a diagnostic tool.
- The FIND IT panel is not designed to determine the susceptibility of an individual or individual's family's risk of cancer.
- The FIND IT panel is not designed to test mutations found in paediatric cancers. However, it can be ordered for paediatric patients if an oncologist determines that it may have clinical utility.

Methodology

The FIND IT assay includes targeted sequence analysis of hotspot mutations/coding exons of the requested genes and transcripts. FFPE slides and/or tissue blocks are required for the FIND IT assay. Samples undergo pathology evaluation, and tumour cell enrichment through macro-dissection is performed, if appropriate. Genomic DNA is extracted and targets of interest amplified using a highly multiplexed PCR assay. Sequence reads that pass defined quality threshold metrics are aligned to the reference sequence (Genome Build hg19) and variants are identified and annotated using a validated, custom-built bioinformatics pipeline. Hotspot variants are categorised into clinical significance tiers, and reported if they are tier I or II (strong or potential clinical significance).[1]

Limitations

The FIND IT assay evaluates single nucleotide changes, insertions and deletions at the targeted hotspots and exons. Variants with ≥5% allele ratio will be reported. The ability to detect a particular variant in a given specimen will depend upon the allele proportion of the variant in the extracted DNA combined with the lower limit of detection of the assay. FIND IT does not differentiate between germline and somatic mutations, and rare germline variants may interfere with the assay.

This assay does not detect copy number variation, including amplification, nor does it detect gene fusions, such as those leading to ALK gene rearrangements. These can be tested for using a different methodology. In addition, the assay does not assess for mutations in BRCA or other genes associated with homologous recombination and DNA damage repair which could be associated with response to PARP inhibitors.

References

1. Li MM et al, Standards and Guidelines for the Interpretation and Reporting of Sequence Variants in Cancer: A Joint Consensus Recommendation of the Association for Molecular Pathology, American Society of Clinical Oncology, and College of American Pathologists. *J Mol Diagn* 2017; 19(1): 4-23

DOUGLASS
HANLY MOIR
PATHOLOGY

For further information please refer to our website,
www.sonicgenetics.com.au or call us on 1800 010 447

14 Giffnock Avenue, Macquarie Park, NSW 2113, Australia
T 1800 010 447 | **E** info@sonicgenetics.com.au
www.dhm.com.au

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