NGS with Cancer Hotspot Panel v2

Principle and Clinical Indications:

Cancer is heterogeneous disease caused by mutations in a large number of different genes. The identification of these genes and the growth, survival and immortalization pathways that they control has led to personalized therapies targeting the pathways that the mutated genes control. To take advantage of these new personalized therapies it is necessary to understand the mutational spectra of individual cancers, and in particular, the primary driver mutations that are found in the cancers. Until recently mutational analysis of cancers has been performed one gene at a time.

Next generation sequencing (NGS) technologies offer a new approach for the detection of clinically relevant mutations in human cancers, allowing detection of multiple rare mutations at low frequency, in a single test. The laboratory has developed an NGS test that targets cancer-associated genes of interest, using a commercially available primer set [AmpliSeq Cancer Hotspot Panel v2 (CHPv2), Ion Torrent/Life technologies] with sequencing carried out on an Ion Personal Genome Machine (PGM) (Ion Torrent/Life Technologies). The CHPv2 panel is used to prepare targeted gene regions for subsequent sequencing on the PGM, using as little as 10ng of FFPE tissue DNA in a single multiplex PCR reaction. The Ampliseq primer sets generate 207 amplicons covering 2855 COSMIC hotspot mutations in 50 genes.

This panel was initially chosen because it covers all of the genes and hotspot mutations that were included in our previous thoracic oncology panel (TOP panel, now retired) that had been designed for the requirements of NCI Thoracic Oncology

The Ion AmpliSeq™ Cancer Panel targets 50 genes

ABL1	*EZH2*	*JAK3*	*PTEN*
AKT1	*FBXW7*	*IDH2*	*PTPN11*
ALK	*FGFR1*	*KDR*	*RB1*
APC	*FGFR2*	*KIT*	*RET*
ATM	*FGFR3*	*KRAS*	*SMAD4*
BRAF	*FLT3*	*MET*	*SMARCB1*
CDH1	*GNA11*	*MLH1*	*SMO*
CDKN2A	*GNAS*	*MPL*	*SRC*
CSF1R	*GNAQ*	*NOTCH1*	*STK11*
CTNNB1	*HNF1A*	*NPM1*	*TP53*
EGFR	*HRAS*	*NRAS*	*VHL*
ERBB2	*IDH1*	*PDGFRA*	
ERBB4	*JAK2*	*PIK3CA*	

protocols utilizing pyrosequencing and capillary electrophoresis. The TOP panel included 80 hotspots in 7 genes. The AmpliSeq CHPv2 panel expands the panel to 11 genes, and over 300 hotspots. The assay can substitute for single gene specific EGRF mutation analysis, when only this test is indicated. It is not a substitute for ALK FISH analysis in lung adenocarcinoma. The panel also provides a multiple-gene targeting approach for detecting mutations in other cancers, including colorectal cancer, GIST, urothelial carcinoma, and melanoma.

It is important to be aware that the mutation panel is only designed to detect the targeted "hotspot" mutations. The 50 genes in this panel are not analyzed in their entirety. Variants outside of panel "hotspots" may not be detected. The limit of mutation detection (LOD) as well as other metrics for this assay are described in the assay validation section of the molecular diagnostics laboratory records.

The sequencing data generated by the PGM is analyzed with Ion Torrent Suite Software. Annotation and interpretation of all variants are performed in Ion Reporter that links to multiple databases such as RefSeq, OMIM, Oncomine, COSMIC, and dbSNP. Reported mutations are confirmed by secondary analysis with IGV Software visualization.

(A). Wet Bench Procedure:

1) DNA Sample Handling for AmpliSeq PCR

All DNA samples isolated from either fresh-frozen tissue or FFPE tissue are repurified with Zymo DNA Clean & Concentrator Kit (DCC-5, Cat# D4013 or D4014). The isolation column has a maximum binding capacity of total DNA at about 5 ug and a minimum elution volume of 6 ul. Elute DNA with nuclease-free water in appropriate volume (depending total amount DNA input).

After DNA clean-up, all samples need to be re-measured with NanoDrop and new concentrations calculated. Next dilute each DNA sample with nuclease-free water to 2ng/ul for DNA isolated from fresh-frozen tissue (or cell lines) or to 4ng/ul for DNA isolated from FFPE tissue.

Add 6 ul of diluted DNA sample into each AmpliSeq PCR reaction (total 12 ng DNA input for fresh-frozen tissue or 24 ng DNA input for FFPE tissue. However, as little as 5-6 ng DNA can be used if the DNA concentration is less. This amount of DNA was shown to be adequate during the validation process).

Perform 22 cycles of thermal cycling (condition was modified from the manufacture protocol for all FFPE tissue.) Zymo DCC manual and AmpliSeq PCR setup template are available in LP (N:)\LP Clinical\Molecular Diagnostics Unit\Clinical Assay Experiments & Setup\Ion PGM.

2) Library Generation:

Instruments and Materials:

PCR/UV Work Station
GeneAmp® PCR System 9700, 96-well plate
96-well plates (Life Technologies, Part #: 4306737)
MicroAmp® Clear Adhesive Film (Life Technologies, Cat. #: 4306311)
DynaMag™-96 Side (Life Technologies, Cat. #: 12331D)
ViiA 7 real-time PCR system (Life Technologies)
1.5-mL DNA LoBind Tube (Cat#: 022431021, Eppendorf)
0.2-mL MAXYMum Recovery® Thin Wall PCR Tubes, Flat Cap (do not use polystyrene tubes) (PCR-02-L-C, Axygen)

Reagents:

Ion AmpliSeq Library Kit 2.0 (Part#: 4475345)
Ion AmpliSeq Cancer Hotspot, Panel V2 (Part#: 4475346)
Ion Xpress Barcode Adapters 1-16 (Part#: 4471250)
Agencourt® AMPure® XP Kit (Beckman Coulter, Cat. #: A63880)
Ion Library TaqMan Quantitation Kit (Part#: 4468802)

Procedure:

We recommend to exactly follow the user guides from manufacture. Please use "IonAmpliSeqLibraryPrepUG_10Sept2012_MAN0006735" for more detailed protocol or "IonAmpliSeqLibraryPrep_QR_qPCR_13Sept2012-1_MAN0006775" for a

quick reference. The hardcopy of both user guides is available on the lab bench or PDF in LP (N:)\LP Clinical\Molecular Diagnostics Unit\Clinical Assay Experiments & Setup\Ion PGM\User Guides-Protocols.

3) a. Template Preparation:

(This protocol is applicable for OT2 200 Kit Only).

Instruments and Materials:

PCR/UV Work Station
BioRad iCycler, 96-well plate
Ion OneTouch™ 2 System
Guava® easyCyte™ 5 Flow Cytometer or/and Qubit® 2.0 Fluorometer (alternative use)
Microtube (Guava tube), 1.5 mL (VWR Cat. no. 16466-030)
Screw cap (VWR Cat no. 16466-082)
1.5-mL DNA LoBind Tube (Cat#: 022431021, Eppendorf)
0.2-mL MAXYMum Recovery® Thin Wall PCR Tubes, Flat Cap (do not use polystyrene tubes) (PCR-02-L-C, Axygen)

Reagents:

Ion PGM Template OT2 200 Kit (Part#: 4480974)
SYBR® Green Nucleic Acid Gel Stain, 10,000X Concentrate in DMSO (Life Technologies, Cat. no. S7563)
Annealing Buffer from Ion PGM Sequencing 200 v2 Kit (to replace 1x PBS with 0.05% Tween 20 for ISP dilutions)
Ion Sphere™ Quality Control Kit (alternative use, Part#: 4468656)

Procedure:

We recommend to exactly follow the user guides from manufacture. Please use "Ion PGM Template OT2 200 Kit User Guide_MAN0007220" for the detailed protocol" or "Ion PGM Template OT2 200 Kit Quick Reference_MAN0007221" for a quick reference. The hardcopy of both user guides is available on the lab bench or PDF in LP LP (N:)\LP Clinical\Molecular Diagnostics Unit\Clinical Assay Experiments & Setup\Ion PGM\User Guides-Protocols.

Step by step instructions are also available from the screen of the OneTouch 2 instrument.

For ISP quality assessment on Guava easyCyte Flow Cytometer use the user guide "Ion Sphere Quality Assessment using Guava easyCyte_4470082D". A hardcopy of both user guides is available on the lab bench or as a PDF in LP LP (N:)\LP Clinical\Molecular Diagnostics Unit\Clinical Assay Experiments & Setup\Ion PGM\User Guides-Protocols.

3) b. Template Preparation:
*(This protocol is applicable for **Ion Chef** robotic instrument only).*

Instruments and Materials:

PCR/UV Work Station
1.5-mL DNA LoBind Tube (Cat#: 022431021, Eppendorf)
Ion Chef Robotic Instrument (Cat#: 4484177, Thermal Fisher)
Ion 316 V2 BC Chip Kit (Cat#: 4488145, Thermal Fisher)
Ion 318 V2 BC Chip Kit (Cat#: 4488146, Thermal Fisher)

Reagents:

Ion PGM IC 200 Kit (Cat#: 4484080, Thermal Fisher)

Procedure:

We recommend to follow the user guides from manufacture. Please use file *MANUAL_Ion_PGM_IC_200_Kit_QR*. The hardcopy of the user guide is available in PDF located on NCI cloud, LP (N:)\LP Clinical\Molecular Diagnostics Unit\Clinical Assay Experiments & Setup\Ion PGM\User Guides-Protocols.

Determine the concentration of the libraries using Thermal Fisher Ion Library TaqMan Quantitation Kit. Dilute all libraries to a concentration of 40 uM with PCR Molecular Grade Water. Total libraries pooled volume for Ion Chef must be 70 ul per sample tube regardless of number of libraries used.

Step by step instructions are also available from the screen of the Ion Chef instrument. Create run plan using Ion Torrent Server Browser before select the run parameters on Ion Chef using the touch screen interface and load the library samples, reagents, consumables, and chips onto the deck. Follow user guide instructions for unloading used reagents and loaded chips, as well as special cleaning protocol for Ion Chef Instrument.

4) PGM Sequencing:

Instruments and Materials:

BioRad iCycler, 96-well plate
Ion Personal Genome Machine® (PGM™) System (Part#: 4462917)
Torrent Server (Part#: 4462918)
Milli-Q Academic 18 MΩ water Purification system
Tank of compressed nitrogen (grade 4.5, 99.995%
or better) available at NIH Clinical Center Material Management Department, NSN: 6830-00-973-7285, 204CF)
1.5-mL DNA LoBind Tube (Cat#: 022431021, Eppendorf)
0.2-mL MAXYMum Recovery® Thin Wall PCR Tubes, Flat Cap (do not use polystyrene tubes) (PCR-02-L-C, Axygen)

Reagents:

Ion 318™ Chip Kit v2 (8-pack) (Part#: 4484355)
Ion 316™ Chip Kit v2 (8-pack) (Part#: 4483324)
ION PGM SEQUENCING 200 KIT V2 (Part#: 4482006)
NaOH (10 M) molecular biology grade (Cat#: 72068-100ML, Sigma)

Procedure:

In Torrent Browser, click the Templates tab. From Favorites, click Plan Run from *Ion AmpliSeq Cancer Panel 200v2* template for OT2 Kit. Use Plan Run from *Ion AmpliSeq Chef_CHP2* template for Ion Chef Kit. Enter run plan name (required) using format: date_SG#_CHPV2, and enter sample name with DNA # (required) under the corresponding barcode, as well as sample tube bar code number, then hit Plan Run to save.

For use with OT2 Kit:

We recommend to exactly follow the user guides from manufacture. Please use *Ion PGM Sequencing 200 Kit v2 QR 15Nov2012_MAN0007360*. The hardcopy of user guide is available as a PDF in LP (N:)\LP Clinical\Molecular Diagnostics Unit\Clinical Assay Experiments & Setup\Ion PGM\User Guides-Protocols.

For use with Ion Chef Kit:

We recommend to exactly follow the user guides from manufacture. Please use file *MANUAL_Ion_PGM_IC_200_Kit_QR*. The hardcopy of the user guide is available in PDF located on NCI cloud, LP (N:)\LP Clinical\Molecular Diagnostics Unit\Clinical Assay Experiments & Setup\Ion PGM\User Guides-Protocols.

Step by step instructions are also available from the screen of the PGM instrument.

5) Quality Control:

(1) DNA samples: All DNA samples need to be cleaned up with Zymo column to remove potential AmpliSeq PCR inhibitors and minimum concentration is required to be ≥1 ng/ul to continue the NGS assay. Otherwise, the specimen will be rejected.

(2) Libraries: The AmpliSeq libraries are quantified using Ion Library TaqMan Quantitation Kit with a standard curve of serial dilution at 6.8 pM, 0.68 pM, 0.068 pM E. coli DH10B Ion Control Library. The sample library has to be ≥ 0.068pM. Otherwise, QC is failed and the library will be re-generated with more DNA input.

(3) Templates: The sequencing templates (ISPs) are analyzed with a Quava easyCyte Flow Cytometer for quality assessment. To produce the highest-quality sequencing data, the percent templated ISPs in an un-enriched sample should be in the range of 10-30%. For enriched samples, the percent templated ISPs should be >80% to be considered successfully enriched. The minimum enrichment accepted is 50% templated ISPs and

30 million total ISPs to continue with loading the 316 chip. (Only applicable for OT2 instrument protocol).

(4) Sample Identifier Tracking: the Check List is required for recording DNA # and the corresponding sequencing barcode in each sequencing run (SG). The DNA # and barcode is entered into the PGM run plan and reviewed by a designee prior to starting the Ion Chef run.

(5) We recommend using the user manuals and the instructions on instrument screen for all steps in all procedures, and **REQUIRE** the use of the following check list to record completion of important key steps in ***real time***. The printout of this check list is available in the lab and must be completely filled out for each PGM run.

OT2 KIT - Ion PGM Sequencing Run Check List

I. AmpliSeq Library Preparation: Person:_____ Date:_____

Check Point	Important Item to Be Checked								Check (√)
1	PCR setup on ice; Pipet slowly and mix thoroughly Primer Pool and HiFi Master Mix								
2	Pipet PCR mixture up and down 5 times								
3	Seal the 96-well plate very well with adhesive film and spin down								
4	Record **Sample ID, Barcode, and Sequencing Groups (SG)**								
	SG_____		SG_____		SG_____		SG_____		
	DNA#	Barcode	DNA#	Barcode	DNA#	Barcode	DNA#	Barcode	
		001		005		009		013	
		002		006		010		014	
		003		007		011		015	
		004		008		012		016	
5	Bring AMPure reagent to RT & vertex thoroughly; Use freshly prepared 70% EtOH								
6	Store undiluted libraries at -20oC								

II. Template Preparation with OT2: Person:_____ Dates:_____ _____ _____ _____

Check Point	Important Item to Be Checked	SG____ Check	SG____ Check	SG____ Check	SG___ Check
7	Oil and Recovery Solution level; ensure tight Sippers				
8	Solution is fully thawed and vortex the reagent as recommended to mix				
9	If not enriched right away, store ISP in 1mL of Ion OneTouch Wash Solution 4oC				
10	Aliquot 2ul pre-IPS from Well 1 for QC (store 4oC)				
11	Check leaking, clean, empty waste				
12	Store enriched ISP at 4oC (up to 3 days)				

III. PGM sequencing: Person:_____ Dates:_____ _____ _____ _____

Check Point	Important Item to Be Checked	SG___ & SG___ Check (√)	SG___ & SG___ Check (√)
13	Create a planned run		
14	N2 gas regulator >500 psi		
15	Clean with chlorine solution, weekly, and 18MΩ water		
16	Rinse W1, W2, W3 bottle 3X		
17	Add 70 ul freshly prepared **100mM NaOH** in W2		
18	Add 350 ul **100mM NaOH** in W1, W3 solution in W3		
19	Change gloves before handling dNTP		
20	Mark new chip with SG #		
21	Clean PGM with 18 MΩ water before shut down		

22	Turn off N2 gas		

ION CHEF - Ion PGM Sequencing Run Check List

I. AmpliSeq Library Preparation: Person:_____

Date:_____

Check Point	Important Item to Be Checked								Check (√)
	Library Kit Lot_____Exp._____ Primer Pool Lot_____Exp._____								
1	PCR setup on ice; Pipet slowly and mix thoroughly Primer Pool and HiFi Master Mix								
2	Pipet PCR mixture up and down 5 times								
3	Seal the 96-well plate very well with adhesive film and spin down								
4	Record **Sample ID, Barcode, and Sequencing Groups (SG)**								BC Kit Lot No. _____ exp. Date _____
	SG_____		SG_____		SG_____		SG_____		
	DNA#	Barcode	DNA#	Barcode	DNA#	Barcode	DNA#	Barcode	
		001		005		009		013	
		002		006		010		014	
		003		007		011		015	
		004		008		012		016	
5	Bring AMPure reagent to RT & vertex thoroughly; Use freshly prepared 70% EtOH								
6	Store undiluted libraries at -20oC								

Quantitation Kit Lot No._____ **Exp.** _____

II. Ion Chef Emulsion PCR/Enrichment/Chip Loading: Person:_____

Dates:_____

Check Point	Important Item to Be Checked	SG____ Check	SG____ Check	SG____ Check	SG____ Check
7	Thaw frozen reagents at room temperature for 1 hr Lot#_____ Exp. Date_____				
8	Create IC Template Sequencing Run on server				
9	Turn on Ion Chef Instrument and perform QC Check				
10	Chip version_____ Chip Lot No._____	BC_____	BC____	BC____	BC____
11	Prepare sample pools and titrate sample concentrations. Tube No_____/_____				
12	Run IC/Unload Chip and perform inverted 5s spin/Clean up and run UV instrument clean				

Sequencing Reagent Lot No._____ **Exp.**_____ **Buffer Lot No.**_____**Exp.**_____

III. PGM sequencing: Person:_____ Dates:_____ _____ _____ _____

Check Point	Important Item to Be Checked	SG____ & SG____ Check (√)	SG____ & SG___ Check (√)
13	Create a planned run		
14	N2 gas regulator >500 psi		

15	Clean with chlorine solution, weekly, and 18MΩ water		
16	Rinse W1, W2, W3 bottle 3X		
17	Add 70 ul freshly prepared **100mM NaOH** in W2		
18	Add 350 ul **100mM NaOH** in W1, W3 solution in W3		
19	Change gloves before handling dNTP		
20	Mark new chip with SG #		
21	Clean PGM with 18 MΩ water before shut down		
22	Turn off N2 gas		

(B) Bioinformatics Pipeline (by Staff Scientist)

1) Steps to analyze the CHP2 clinical test:

i. Default run coverageAnalysis v5.0 and variantCaller v5.0 from run plan.
ii. After the analysis is complete, check run report, coverageAnalysis result for QC. Print Coverage Analysis and attach it to the test record.
iii. Screen the variant call table and check each variant called in IGV if necessary. Save the variant table in Excel format in LP\Clinical Assay Results\Ion PGM Variant Reports and attach it to a printout to the test record.
iv. From Select plugins to run tab, select IonReporterUploader v5.0 and use Upload Options of .vcf only to launch IRU.
v. In Ion Reporter, start analysis work flow "NCI LP CHP2-50 Cancer Gene Mutation Panel" to annotate the variants.
vi. Annotate the coding sequence, codon, and AA changes by using saved filter "CHP2-clin2" to filter in all functional impact variants including: missense, nonframe shift detection, nonframe shift block substitution, nonsense, stoploss, frame shift insertion, frame shift deletion, frame shift block substitution, unknown, nonframe shift insertion.

vii. Review the filtered list and the check sequence read quality and evaluate for possible artifacts with IGV for EACH variant. Select and save the variants that pass quality check for predicted functional classification of the variant (see below for algorithm used).

viii. A PDF report is generated and saved in LP\Clinical Assay Results\Ion PGM Variant Reports and attached a printout to the test record.

2) Quality Metrics for Sequencing Data and Quality Control

Through more than 100 validation and pre-clinical runs, we have defined the following key parameters for optimal performance of NGS with Ion CHPv2 using 316 chip or 316 chip v2.

Table 1. Summary of sequence quality, total reads, read length, and coverage depth by sample

Assay	Sample Type	# of Seq Samples	Summary	>=Q20 Seq Bases per sample (M)	Total Reads	Mean Read Length (bp)	Read Depth Cov.	100x Cov. (%)
Clinical Validation	FFPE DNA	34	Median	47.6	569,559	104	2,161	100.0
			Min	12.7	167,871	76	664	98.6
			Max	102.0	1,315,176	109	5,052	100.0
Analytical Sensitivity Specificity	Fresh Cell Line DNA	30	Median	57.6	677,315	102	2,676	100.0
			Min	30.6	434,397	82	613	100.0
			Max	81.1	860,299	108	3,366	100.0
No Template Control	H_2O	1		0.0	0	0	0	0
	H_2O	1		0.5	12,958	43	10	3.23

Table 2. Additional metrics to assess sequencing quality for targeted bases

Quality Interpre-tation	Mapped Reads	On Target (%)	Mapped Reads on Target	Target Base Coverage at 100x (%)	Target Base Coverage at 500x (%)	Target Base Coverage Average (X)	Target Base Uniformity(%)
High	>300,000	80	>240,000	100	>80	>1000	>95
OK	150,000-300,000	60-80	100,000-240,000	95-99	60-80	500-1000	90-85
Caution* / fail on some targets	<150,000	<60	<100,000	<95	<60	<500	<85

*caution depending upon percent tumor in specimen and amplicon target to report.

3) **Classification of Sequence Variants**

The classification is based on two sets of evidence that are incorporated into Ion Reporter 5.0: (1) variant frequency in population: MAF (1000 genomes global minor allele frequency) and GMAF (5000 exomes global minor allele frequency). UCSC Genome Browser defined MAF≥1% as Common SNPs. (2) protein functional damage prediction algorithms: SIFT(http://sift.jcvi.org) and PolyPhen (http://genetics.bwh.harvard.edu/pph2).

Classification	**MAF/ GMAF**	**UCSC Common SNPs**	**1º PolyPhen -2 Score**	**2º SIFT Score**	**Stand-alone**
Deleterious			0.85-1.0	0.0-0.05	Truncating variant - nonsense, frameshift, canonical +/-1, 2 splice sites, initiation codon.
Suspected Deleterious			0.15-0.85		
Suspected Benign	≥0 and <0.01				
Benign	≥0.01	Yes	0.0-0.15	0.05-1.0	
Unknown	NA	NA	NA	NA	NA

(C) Interpretation of Sequence Variants and Molecular Report [by <u>Molecular Pathologist(s)</u>]

After the bioinformatics pipeline is completed, the staff scientist prepares the report using Ion Reporter 4.0 following the parameters described

above. The report is given to the molecular pathologist(s) for clinical reporting.

The variants are to be reported as three levels:
(1) Mutation of known significance: pathogenic/likely pathogenic.
(2) Mutation of uncertain significance
(3) Benign/likely benign

To assist in the interpretation of variants reported from Ion Reporter 4.0, the following database/web sites are suggested for use:

1) NCBI dbSNP (http://www.ncbi.nlm.nih.gov/snp): Provides detailed information on all known SNVs and related information. Allows you to confirm the variant classification from Ion Reporter, decide whether a variant has been previously reported as germline, or as a somatic variant, links out to ClinVar and other useful databases.

2) NCBI ClinVar (http://www.ncbi.nlm.nih.gov/clinvar): ClinVar currently includes clinical annotations for variants identified through the following methods: (1) clinical testing - clinical significance reported as part of the genetic testing process in CLIA certified or ISO 1589 accredited laboratories; (2) research - general term for variations identified in humans as part of a research project; (3) literature only - reports of the variant effect on phenotype as extracted from the literature without modification of authors' statements. Used by third parties, not the authors of the paper being cited.

3) COSMIC (http://cancer.sanger.ac.uk/cancergenome/projects/cosmic): Valuable resource in which all cancer associated SNVs are recorded and searchable by a COSMIC ID designator. Note that the inclusion of an SNV in this database is not predicated on its functional effect, and may be nonfunctional or a known polymorphism. Contains useful information on the frequency of each variant allele and/or variant locus by cancer type. Both the initial variant caller report and the final Ion Reporter report provide Cosmic IDs if available.

4) mycancergenome.org lists the cancer mutations with all national and international trials registered within NCI PDQ (physician data query) and clinicaltrials.gov. If you wish to add clinical trial information, it can be found here. Any SNV listed in this database can be assumed to be deleterious.

5) Both Polyphen 2 (http://genetics.bwh.harvard.edu/pph2/) and Sift (http://sift.jcvi.org/) are user friendly databases that can be accessed by the molecular pathologist for additional information concerning the effect of missense mutations on protein function. These databases take into account phylogenetic conservation, the degree of conservation of amino acid residues in sequence alignments derived from closely related sequences, and the location of the mutation to predict the effect of a particular mutation. Although a functional score based on these databases is generated in the Ion Reporter bioinformatics pipeline, a more detailed explanation of the score can be generated and reviewed directly through the database.

All reports will include the full list of genes screened for genetic variants. However, this mutation panel is only designed to detect the targeted "hotspot" mutations, and a limited amount of surrounding nucleotides. The 50 genes in this panel are not analyzed in their entirety. Therefore, variants outside of panel "hotspots" may not be detected. The complete panel of hotspots is available on request from the Molecular Diagnostics Laboratory, Laboratory of Pathology, NCI.

Once the report is generated and signed by the staff molecular pathologist, a preliminary secured email is sent to requesting physician. The report is given to the section secretary who finalizes the report in the SoftPath reporting system, and e-mails the official finalized copy to all physicians on the distribution list of the report.

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