

## NGS SOMATIC VARIANT ANALYSIS REPORT

Patient Details		Source Information		Sample Information	
Lab Number:	MP17-1234	Requester Ref:		Date Received:	01/02/2017
Surname:	Atient	Surgical No.:	HP1500147042 A1	Primary Tumour Site:	Rectum
Forename:	P	Sample Format:	FFPE Block	Tumour Subtype:	Adenocarcinoma
D.O.B. (D/M/Y):	17/03/1955	Consultant:	Smith	Tissue Sample Site:	Not Stated
Gender:	Female	Hospital:	Random Hospital	% Tumour (Whole):	21-50%

Variant categories assessed by requested assay MGP-1: **SNV & Small Indels** **Yes** (22 Genes) **Fusions** **No** (0 Genes) **CNVs** **No** (0 Genes)

**Yes** – Validated / certified for clinical diagnostic use, **No** – Not assessed by this assay, **RUO** – Assessed but not clinically validated, for Research Use Only.  
**SNV** – A single nucleotide variant; **Small Indel** – An Insertion, deletion or substitution of between 1 and 50 nucleotides; **Fusion** – A hybrid of two separate genes (caused by translocations, interstitial deletions, or chromosomal inversions); **CNV** – Copy Number Variation. An alteration of the number of copies of a gene within the genome. See Assay Details Section for further information.

### Detected Variants (Proportion of Total DNA >2.5%)

**KRAS** p.(Gly13Asp), c.38G>A, COSM532 (25%) **SMAD4** p.(Arg361Cys), c.1081C>T, COSM14140 (30%)  
**TP53** p.(Pro72Arg), c.215C>G, COSM250061 (65%)

### Summary Comments

**KRAS** p.(Gly13Asp), this mutation has been associated with decreased sensitivity to anti-EGFR antibodies in colorectal cancer.

No other variants with currently established therapeutic implications for NICE approved therapies were detected in this specimen. Specifically, these include **BRAF** (Codon 600), **EGFR** (Codons 719, 790, 858, 861, exon 19 deletions/insertions or exon 20 insertions), **KRAS** (Codons 12, 59, 61, 117 & 146), and **NRAS** (Codons 12, 13, 59 & 61).

### Additional Comments & Warnings

(None)

### Not Detected Variants (Proportion of Total DNA <1%)

Unless otherwise listed, unique coding mutations within the COSMIC database (version 72) were excluded from all screened genomic regions with data supporting an expected risk of a false negative result less than 1 in 1000.

For the following regions, the expected risk of a false negative result is between 1 in 100 and 1 in 1000:

(None)

For the following regions, the expected risk of a false negative result is between 1 in 10 and 1 in 100:

(None)

*NB: This above categorisation assumes that mutations are clonally represented (i.e. present in all tumour cells) and that the actual tumour cell content is not less than the estimated value above. Non 'driver' mutations may be present sub-clonally at any level and hence the false negative risk for such variants cannot be meaningfully calculated.*

### Target Regions With Insufficient Coverage

Due to little or no available sequence data, the presence or absence of certain variants contained within the target regions listed below could not be meaningfully assessed:

(None)

*NB: A significant number of genomic regions falling into this category is normally indicative of low DNA quantity and/or poor DNA quality, often as a result of very small quantities of starting tissue and/or excessive fixation or decalcification.*

## Equivocal Variants

Following automated analysis, the status of those variants listed below must be regarded as equivocal (Due to a range of poor quality metrics and/or their detection at a very low level, 1-2.5% of total DNA). Should any of these variants be of specific interest please inform us and we will review them manually as required.

<b>AKT1</b> p.(Phe35Leu), c.103T>C, COSM48226	p.(Glu49Lys), c.145G>A, COSM36918
<b>FBXW7</b> p.(Gly391Asp), c.1172G>A, COSM1485737 p.(Arg473fs*25), c.1417del, COSM24620	p.(Arg473fs*4), c.1417_1418insA, COSM22968
<b>FGFR3</b> p.(Arg248_Ser249del), c.742_747del, COSM86030 p.(Lys403fs*93), c.1199_1200insC, COSM3337723 p.(Lys715Met), c.2144A>T, COSM732992	p.(Glu368Lys), c.1102G>A, COSM4748566 p.(Lys403fs*93), c.1200_1201insC, COSM5516105 p.(Ser779fs*28), c.2330del, COSM4684605
<b>MAP2K1</b> p.(Gly61_Asp65del), c.182_196del, COSM5702510	
<b>NOTCH1</b> p.(Leu1574Pro), c.4721T>C, COSM12772 p.(Leu1574fs*36), c.4720_4721insC, COSM5751263 p.(Val1577_Val1578del), c.4721_4726del, COSM5751216	p.(Leu1574Gln), c.4721T>A, COSM25835 p.(Val1577Met), c.4729G>A, COSM308595 p.(Val1578del), c.4732_4734del, COSM13047
<b>PTEN</b> p.(Leu57fs*42), c.170del, COSM5835 p.(Cys218*), c.653_654delinsAA, COSM4745580 p.(Asp268fs*30), c.794_795insA, COSM86078 p.(Thr321fs*23), c.963del, COSM5823	p.(Cys218fs*1), c.652_653insA, COSM1173640 p.(Lys267fs*9), c.795del, COSM30622 p.(Ser302fs*8), c.902_906del, COSM921145 p.(Asn323fs*2), c.962_963insA, COSM23626
<b>SMAD4</b> p.(Pro356fs*21), c.1067_1068del, COSM4386692	
<b>STK11</b> p.(Asp53fs*11), c.153del, COSM27282 p.(Pro203Leu), c.608C>T, COSM3692365	p.(Asp53fs*11), c.157del, COSM48969
<b>TP53</b> p.(Pro67fs*56), c.199del, COSM46345 p.(Glu68*), c.202G>T, COSM11513 p.(Glu68Lys), c.202G>A, COSM5681716 p.(Pro85fs*59), c.253_266del, COSM46349 p.(Gly105fs*18), c.312del, COSM45801 p.(Gly105fs*18), c.314del, COSM5646708 p.(Leu114*), c.341T>A, COSM46344 p.(Thr150_Pro153del), c.448_459del, COSM43627 p.(Thr150Arg), c.449C>G, COSM45139 p.(Thr150_Pro151del), c.448_453del, COSM46364 p.(Pro151Leu), c.452_453delinsTT, COSM43586 p.(Pro151fs*27), c.452_459del, COSM5174080 p.(Pro152Thr), c.454C>A, COSM44561 p.(Pro152fs*27), c.455_459del, COSM507584 p.(Pro153Thr), c.457C>A, COSM45660 p.(Gly154fs*27), c.456_457insC, COSM26863 p.(Gly154Ser), c.460G>A, COSM43692 p.(Gly154fs*16), c.456del, COSM44958 p.(Cys176Tyr), c.527G>A, COSM10687 p.(Cys176fs*71), c.526del, COSM44759 p.(Asp186fs*61), c.556del, COSM45838 p.(Pro223fs*1), c.666_667del, COSM111639 p.(Pro223fs*4), c.667_668insGAGCCGC, COSM1318439 p.(Pro223Arg), c.668C>G, COSM45255 p.(Glu258Gln), c.772G>C, COSM10751 p.(Glu258*), c.772G>T, COSM43568 p.(Gly262His), c.784_785delinsCA, COSM44761 p.(Gly262del), c.784_786del, COSM45437 p.(Gly262fs*2), c.784_806del, COSM46343 p.(Asn263His), c.787A>C, COSM45781 p.(Ser269Arg), c.807C>A, COSM44331 p.(Phe270Leu), c.808T>C, COSM44262 p.(Glu271Gly), c.812A>G, COSM43879 p.(Cys277Arg), c.829T>C, COSM45871 p.(Lys351Glu), c.1051A>G, COSM107991 p.(Lys351fs*19), c.1050del, COSM45375	p.(Pro67fs*81), c.200_201del, COSM4949638 p.(Glu68Gln), c.202G>C, COSM44917 p.(Glu68fs*81), c.200_201insC, COSM5752813 p.(Ser90fs*33), c.267del, COSM1268330 p.(Gly105fs*18), c.313del, COSM45833 p.(Gly112fs*9), c.334_340del, COSM45797 p.(Leu130Pro), c.389T>C, COSM45481 p.(Thr150Ile), c.449C>T, COSM44038 p.(Thr150Lys), c.449C>A, COSM45563 p.(Thr150fs*16), c.447_459del, COSM5752222 p.(Pro151His), c.452_453delinsAT, COSM46297 p.(Pro152Ser), c.454C>T, COSM43582 p.(Pro152Ala), c.454C>G, COSM44788 p.(Pro153Ser), c.457C>T, COSM43675 p.(Pro153Ala), c.457C>G, COSM45821 p.(Gly154fs*16), c.459del, COSM404850 p.(Gly154Cys), c.460G>T, COSM44715 p.(Cys176Phe), c.527G>T, COSM10645 p.(Cys176Ser), c.527G>C, COSM44645 p.(Cys176Ser), c.525_526delinsTA, COSM46255 p.(Gly199Arg), c.595G>A, COSM43749 p.(Pro223Leu), c.668C>T, COSM12193 p.(Pro223His), c.668C>A, COSM44456 p.(Leu257fs*6), c.770_771del, COSM43575 p.(Glu258Lys), c.772G>A, COSM10988 p.(Gly262_Ser269del), c.784_807del, COSM43998 p.(Gly262_Phe270del), c.784_810del, COSM44878 p.(Gly262fs*83), c.785del, COSM45527 p.(Asn263Asp), c.787A>G, COSM45752 p.(Ser269fs*76), c.806del, COSM44010 p.(Phe270Ile), c.808T>A, COSM43809 p.(Phe270Val), c.808T>G, COSM44956 p.(Glu271Val), c.812A>T, COSM44469 p.(Leu348_Gly361delinsTrp), c.1043_1081del, COSM5944166 p.(Lys351*), c.1051A>T, COSM1522202 p.(Ser362fs*8), c.1083del, COSM13159

### Report Signed by / Date:

Dr F. Irst  
Clinical Scientist  
12/02/2017



### Report Checked by / Date:

Dr S. Econd  
Mol. Biol. PhD  
12/02/2017



**How do we look for mutations?**

Selected regions from a commercially available multi-gene DNA panel (*Life Technologies Oncomine Solid Tumour CE-IVD*: see below) are amplified using a highly multiplex Polymerase Chain Reaction approach. These are labelled using 'DNA barcodes' unique to each specimen and then sequenced collectively on a Life Technologies PGM instrument using Ion Torrent Hi-Q™ View chemistry and a 318v2 chip. Data is analysed using Torrent suite v5.0.5 and VariantCaller v5.0.4.0. An in-house developed script is then used to group the Variant Caller output into the reported categories and construct variant descriptors according to Human Genome Variation Society recommended nomenclature (<http://www.hgvs.org/>) and COSMIC reference number.

**Where do we look for mutations (screened regions)?**

**Loci included in this assay gene panel are as follow: (Format: Gene Name (Reference Sequence), Exon Codon-Range.)**

**AKT1** (NM\_001014432.1) **4** 17-52; **ALK** (NM\_004304.3) **22** 1151-1171, **23** 1173-1215, **25** 1251-1278; **BRAF** (NM\_004333.4) **11** 439-472, **15** 582-609; **CTNNB1** (NM\_001904.3) **3** 9-48; **DDR2** (NM\_001014796.1) **5** 63-64, **5** 92-135, **8** 226-265, **12** 440-483, **13** 503-537, **14** 577-607, **15** 621-668, **17** 762-790; **EGFR** (NM\_005228.3) **12** 472-499, **18** 693-726, **19** 729-761, **20** 762-800, **21** 854-875; **ERBB2** (NM\_004448.2) **19** 753-769, **20** 770-797, **21** 839-882; **ERBB4** (NM\_005235.2) **3** 135-140, **4** 167-185, **6** 226-247, **7** 254-290, **8** 296-323, **9** 334-368, **15** 578-622, **23** 917-947; **FBXW7** (NM\_0033632.3) **5** 261-287, **8** 377-402, **9** 434-472, **10** 478-508, **11** 567-597; **FGFR1** (NM\_023110.2) **4** 121-149, **7** 250-275; **FGFR2** (NM\_000141.4) **7** 295-313, **7** 251-278, **9** 363-399, **12** 542-557; **FGFR3** (NM\_000142.4) **7** 248-277, **9** 367-402, **14** 631-653, **16** 689-719, **18** 772-807; **KRAS** (NM\_004985.4) **2** 5-37, **3** 38-66, **4** 114-150; **MAP2K1** (NM\_002755.3) **2** 43-83; **MET** (NM\_001127500.1) **2** 159-188, **2** 339-378, **14** 982-1014, **16** 1106-1131, **19** 1244-1274; **NOTCH1** (NM\_017617.3) **26** 1566-1602, **27** 1674-1680; **NRAS** (NM\_002524.3) **2** 3-31, **3** 41-69, **4** 112-150; **PIK3CA** (NM\_006218.2) **10** 522-550, **14** 676-720, **21** 1017-1051, **21** 1063-1069; **PTEN** (NM\_000314.4) **1** 1-25, **3** 56-69, **6** 165-184, **7** 213-218, **7** 230-267, **8** 280-302, **8** 312-342; **SMAD4** (NM\_005359.5) **3** 98-136, **5** 165-202, **6** 241-262, **8** 307-318, **9** 326-365, **10** 384-426, **11** 444-473, **12** 494-533; **STK11** (NM\_000455.4) **1** 22-64, **4-5** 192-207, **6** 254-286, **8** 317-361; **TP53** (NM\_000546.5) **2** 1-20, **4** 67-114, **5** 150-186, **5** 126-138, **6** 188-221, **7** 225-257, **8** 262-306, **10** 332-366.

**What mutations do we look for?**

All mutations in the COSMIC (Catalogue Of Somatic Mutations In Cancer <http://cancer.sanger.ac.uk/>) database (version 79), encompassed by the genomic regions described above, are filtered to remove silent mutations (those not causing a change in the protein sequence), intronic variants and duplicate entries (present due to alternate transcripts for certain genes) leaving 5100 unique coding variants (Full list available electronically upon request). The presence or absence of all of these unique coding variants is assessed and reported for each specimen tested. Other 'Non-COSMIC' mutations are identified by the assay but will not be routinely reported.

**How do we assign the presence or absence of a mutation (variant)?**

**DETECTED VARIANTS:** Given that a specimen tumour content of not less than 5% is an assay acceptance criteria, only variants with a frequency >2.5%, in not less than 100 high quality unbiased reads, will be classified as 'Detected'. These are reported in the format Gene name, protein change, cDNA change, COSMIC reference, observed variant frequency. Note that variants with a frequency of essentially 100% (i.e. allowing for background noise) are assumed to be homozygous germ line mutations and are excluded from reports.

**NOT DETECTED VARIANTS:** Potential variants with a frequency less than 1% are considered to be indistinguishable from background noise, which can arise from a number of sources both intrinsic and extrinsic to the assay. These will be classified as 'not detected'. The confidence with which these variants can be classified as 'Not detected' will increase in line with the 'quantity of data' from which their presence has been excluded. Although the principle metric in this 'quantity of data' is total sequencing read depth, it is not the only metric, nor is a single read depth value potentially meaningful (without also considering read length, direction etc.). Consequently, not detected variants (or negatives) have been grouped by target region according to the likelihood that they represent a false negative, assuming the worst case scenario of a 5% tumour content. Variants which are not detected but which have a false negative likelihood greater than 1 in 10 are included within target regions with insufficient coverage.

**EQUIVOCAL VARIANTS:** Variants with an observed frequency of 1-2.5% are by definition equivocal, as they do not fall into either the above categories. Some specimens display high numbers of such variants, and whilst often regarded as artefacts of excessive fixation (see below) or other 'chemical' processing of the specimen, the possibility that they may reflect actual physiological processes cannot be excluded. Other variants in this category are the result of low confidence calls, sufficient data is available and analysis has been performed but results are deemed inconclusive (irrespective of apparent variant frequency) due to the combination of other metrics including, but not limited to, read quality, mapping quality, and sequencing/variant strand bias.

**TARGET REGIONS WITH INSUFFICIENT COVERAGE:** Any target regions where the presence or absence of variants cannot be assessed with any meaningful level of confidence (due to insufficient data quantity and/or quality) are specifically listed.

**What are the assay limitations?**

Whilst every precaution has been taken to ensure this assay is as sensitive as possible, it has been validated with total genomic DNA inputs not less than 10ng, of which at least 2.5% must be tumour DNA (5% tumour at the cellular level). Below either of these cut off levels, variants may not be consistently identified.

C>T or G>A transitions resulting from cytosine deamination, as a consequence of formalin fixation, cannot be distinguished from genuine tumour specific mutations. Although the observed variant frequencies of such artefacts is usually low, in specimens subject to excessive fixation they may exceed the 2.5% threshold for detection. Consequently, great caution should be exercised when assessing the likely significance of low frequency detected variants, especially if they are numerous and/or in stark contrast to tumour content.

Although extensive and frequently updated, there will inevitably be a delay between the discovery of any new variants, their inclusion in the COSMIC database and the subsequent update of our list of assessed unique coding variants (based on the new COSMIC version number). Any such variants may not be identified and/or reported.

**Other important points to note!**

**Interpretation:** This report primarily details laboratory test findings. Only limited interpretive comments relating to mutations with established therapeutic implications are provided. Any identified variants of unknown or unclear significance should be discussed in an appropriate MDT or other suitable forum. In order to assist with this, full 'raw data' can be provided on request, there will however be an additional charge for this which may vary depending upon format requirements.

**Germ line mutations:** Please note that although this assay has been designed to assess somatic mutations (and hence is unsuitable for germ line screening), the possibility that any variant identified may be of germ line origin cannot be entirely excluded.