NGS SOMATIC MUTATION ANALYSIS REPORT

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PLEASE READ THE ACCOMPANYING ASSAY GUIDELINES ON THE FINAL REPORT PAGE FIRST

SAMPLE DETAILS

Lab No:	MA14-1234	**Date received:**	01/02/2014	**Primary Tumour Site:**	Colon
Surname:	Smith	**Surgical No.**	AA1-01234	**Tumour %: Full Section:**	21-50%
Forename:	John	**Sample Type:**	FFPE Block	**% in micro-dissected area:**	N/A
DOB (D/M/Y):	01/01/2000	**Tissue Type:**	Colon	**Consultant:**	A. N. Other
Gender:	Male	**Tumour Type:**	Adeno	**Source:**	UCLH
Gene panel:	MGP-1 (see accompanying notes)	**NGS Run(s):**		MGP1-30	

DETECTED VARIANTS (Frequency>2.5%)

VARIANTS DETECTED IN BRAF, EGFR, KRAS or NRAS:

(None)

ALL OTHER DETECTED VARIANTS:

MAP2K1 p.Arg49Leu, c.146G>T, COSM1235482 (2.93%)
SMAD4 p.Ala532Val, c.1595C>T, COSM1150752 (5.85%)
TP53 p.Arg306*, c.916C>T, COSM99947 (19.39%)
TP53 p.Pro72Arg, c.215C>G, COSM250061 (64.33%)
PIK3CA p.Ala533Val, c.1598C>T, COSM29314 (3.45%)
TP53 p.Arg290His, c.869G>A, COSM44017 (6.18%)
TP53 p.Gly105Asp, c.314G>A, COSM45997 (9.20%)

NOT DETECTED VARIANTS (Frequency<1%)

Unless otherwise listed, unique coding variants within the COSMIC database (version 68) were excluded from all screened genomic regions with data supporting an expected risk of a false negative result lower than 1 in 1000 (assuming a 5% minimum tumour content).

For the following regions, the expected risk of a false negative result is between 1 in 100 and 1 in 1000 (assuming a 5% minimum tumour content):

(None)

For the following regions, the expected risk of a false negative result is between 1 in 10 and 1 in 100 (assuming a 5% minimum tumour content):

(None)

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:

PTEN Exon 6 codons 165-184

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.

UNASSIGNABLE VARIANTS-1 (Frequency 1-2.5%)

BRAF:
p.Phe610Leu, c.1828T>C, COSM1448587
CTNNB1:
p.Gln26Arg, c.77A>G, COSM1670090
FBXW7:
p.His379Arg, c.1136A>G, COSM48375
p.His580Tyr, c.1738C>T, COSM1566962
FGFR2:
p.Pro164Leu, c.491C>T, COSM537801
SMAD4:
p.Arg416Ser, c.1248A>C, COSM14162
TP53:
p.Ser95Phe, c.284C>T, COSM44673
p.Val172Ala, c.515T>C, COSM44996

UNASSIGNABLE VARIANTS-2 (Analysis inconclusive)

TP53:

p.Ala74Val, c.221C>T, COSM43671	p.Ala74fs*49, c.220delG, COSM46350
p.Ala79_Ala88del10, c.235_264del30, COSM44559	p.Ala86fs*59, c.252_262del11, COSM46122
p.Gly105fs*18, c.312delG, COSM45801	p.Gly105fs*18, c.313delG, COSM45833
p.Leu93Met, c.277C>A, COSM43812	p.Leu93Val, c.277C>G, COSM1564163
p.Leu93fs*30, c.274delC, COSM45290	p.Leu93fs*30, c.277delC, COSM96581
p.Pro72fs*76, c.210_211delTC, COSM111573	p.Pro85His, c.254C>A, COSM1386905
p.Pro85Leu, c.254C>T, COSM45837	p.Pro85fs*59, c.253_266del14, COSM46349
p.Pro85fs*63, c.253_254delCC, COSM45724	p.Pro89Leu, c.266C>T, COSM44677
p.Pro89Ser, c.265C>T, COSM43688	p.Pro92Leu, c.275C>T, COSM45972
p.Ser90Thr, c.268T>A, COSM1735384	p.Trp91fs*57, c.273_274delGC, COSM46010
p.Val73Leu, c.217G>C, COSM45288	p.Val73Leu, c.217G>T, COSM46408
p.Val73Met, c.217G>A, COSM43787	p.Val73fs*50, c.211delC, COSM46307
p.Val73fs*50, c.214delC, COSM44256	p.Val73fs*50, c.216delC, COSM18569

COMMENTS

Report Signed by/Date:

_____ ___/___/_____

Clinical Scientist ☐
Pathologist ☐
BMS (Senior) ☐

Report checked by/Date:

_____ ___/___/_____

BMS ☐
BMS (Trainee) ☐
Mol. Biol. PhD ☐

How do we look for mutations?

Selected regions from a multi-gene panel (*Life Technologies Colon & Lung Panel V2*: see below) are amplified using a highly multiplex Polymerase Chain Reaction approach. These are labelled using 'DNA barcodes' unique to each specimen and then collectively sequenced on a Life Technologies PGM instrument using Ion PGM™ Sequencing 200 Kit v2 chemistry and a 318v2 chip. Data is analysed using Torrent suite v4.0.2 and VariantCaller v4.0 (r76860). An in-house developed script is then used to group 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 follows. (Format: Gene Name (Reference Sequence), Exon, Codons.)

AKT1 (NM_005163.2) Exon 3 Codons 17-52; **ALK** (NM_004304.3) Exon 22 Codons 1151-1171, Exon 23 Codons 1173-1216, Exon 25 Codons 1252-1279; **BRAF** (NM_004333.4) Exon 11 Codons 439-472, Exon 15 Codons 583-611; **CTNNB1** (NM_001904.3) Exon 3 Codons 9-48; **DDR2** (NM_006182.2) Exon 5 Codons 63-65, Exon 5 Codons 92-135, Exon 8 Codons 226-265, Exon 12 Codons 440-484, Exon 13 Codons 502-537, Exon 14 Codons 577-607, Exon 15 Codons 621-668, Exon 17 Codons 762-790; **EGFR** (NM_005228.3) Exon 12 Codons 473-499, Exon 18 Codons 693-726, Exon 19 Codons 729-761, Exon 20 Codons 762-800, Exon 21 Codons 854-875; **ERBB2** (NM_004448.2) Exon 19 Codons 753-769, Exon 20 Codons 770-797, Exon 21 Codons 839-882; **ERBB4** (NM_005235.2) Exon 3 Codons 135-140, Exon 4 Codons 167-185, Exon 6 Codons 226-247, Exon 7 Codons 254-290, Exon 8 Codons 296-323, Exon 9 Codons 334-368, Exon 15 Codons 578-622, Exon 23 Codons 917-947; **FBXW7** (NM_0033632.2) Exon 5 Codons 262-287, Exon 8 Codons 371-403, Exon 9 Codons 434-472, Exon 10 Codons 478-508, Exon 11 Codons 566-597; **FGFR1** (NM_023110.2) Exon 4 Codons 121-150, Exon 7 Codons 250-275; **FGFR2** (NM_000141.4) Exon 7 Codons 251-278, Exon 7 Codons 295-313, Exon 9 Codons 363-399, Exon 12 Codons 542-557; **FGFR3** (NM_000142.4) Exon 7 Codons 248-277, Exon 9 Codons 365-401, Exon 14 Codons 631-653, Exon 16 Codons 689-719, Exon 18 Codons 772-807; **KRAS** (NM_004985.3) Exon 2 Codons 5-37, Exon 3 Codons 38-66, Exon 4 Codons 114-150; **MAP2K1** (NM_002755.3) Exon 2 Codons 43-83; **MET** (NM_001127500.1) Exon 2 Codons 159-188, Exon 2 Codons 339-378, Exon 14 Codons 982-1014, Exon 16 Codons 1106-1131, Exon 19 Codons 1244-1274; **NOTCH1** (NM_017617.3) Exon 26 Codons 1566-1602, Exon 27 Codons 1674-1680; **NRAS** (NM_002524.3) Exon 2 Codons 3-31, Exon 3 Codons 41-69, Exon 4 Codons 112-150; **PIK3CA** (NM_006218.2) Exon 10 Codons 522-550, Exon 14 Codons 676-720, Exon 21 Codons 1017-1051, Exon 21 Codons 1063-1069; **PTEN** (NM_000314.4) Exon 1 Codons 1-25, Exon 3 Codons 56-69, Exon 6 Codons 165-184, Exon 7 Codons 213-218, Exon 7 Codons 230-267, Exon 8 Codons 280-302, Exon 8 Codons 312-342; **SMAD4** (NM_005359.5) Exon 3 Codons 98-136, Exon 5 Codons 165-202, Exon 6 Codons 241-263, Exon 8 Codons 307-318, Exon 9 Codons 326-365, Exon 10 Codons 384-426, Exon 11 Codons 444-473, Exon 12 Codons 494-533; **STK11** (NM_000455.4) Exon 1 Codons 22-64, Exon 4&5 Codons 191-207, Exon 6 Codons 254-286, Exon 8 Codons 317-360; **TP53** (NM_000546.5) Exon 2 Codons 1-20, Exon 4 Codons 67-114, Exon 5 Codons 126-139, Exon 5 Codons 151-186, Exon 6 Codons 188-224, Exon 7 Codons 226-257, Exon 8 Codons 262-306, Exon 10 Codons 332-366

What mutations do we look for?

A total of 7884 mutations in the COSMIC (Catalogue Of Somatic Mutations In Cancer http://cancer.sanger.ac.uk/) database (version 68) are encompassed by the genomic regions described above. The removal of silent mutations (those not causing a change in the protein sequence), intronic variants and duplicate entries (present due to alternate transcripts for certain genes) leaves 4214 unique coding variants. 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 within target regions with insufficient coverage.

TARGET REGIONS WITH INSUFFICIENT COVERAGE: As a result of amplification and/or sequencing failure, the presence or absence of variants contained within the listed target regions could not be meaningfully assessed.

UNASSIGNABLE VARIANTS-1: Variants with an observed frequency of 1-2.5% are by definition unassignable as they do not fall into either of the above categories. Some specimens display high numbers of variants in this category 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.

UNASSIGNABLE VARIANTS-2: 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.

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 details laboratory test findings only, no interpretive comments 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