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Technical information Document

# Document History

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## Version History

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| Version | Date | Description |
| 1.0.main | 05/10/2016 | This Technical information Document will accompany Whole Genome Analysis: Preliminary Analysis document |
| 1.1.main | 11/11/2016 | This Technical information Document will accompany Whole Genome Analysis: Preliminary and Supplementary Analysis documents |
| 1.2.main | 16/01/2017 | This Technical information Document will accompany Whole Genome Analysis: Preliminary and Supplementary Analysis documents |
| 1.3.main | 05/03/2017 | This Technical information Document will accompany Whole Genome Analysis: Preliminary and Supplementary Analysis documents. Section on germline variant calling was added and explanation of report fields was extended. |
| 1.4.main | 30/03/2017 |  |
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## Reviewers

This document must be reviewed by the following:

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| Augusto Rendon | Director of Bioinformatics | Dec 2016 | 1.1.main |
| Clare Turnbull | Clinical Lead for Cancer Data | 18/01/2017 | 1.2.main |
| Joanne Mason | Director of Sequencing | 05/10/2016 | 1.0.main |

Technical Information Document

Main program

# Sequencing and alignment

Samples were prepared using an Illumina TruSeq DNA Nano, TruSeq DNA PCR-Free or FFPE library preparation kit and then sequenced on a HiSeq X generating 150 bp paired-end reads. Germline samples were sequenced to produce at least 85 Gb of sequences with sequencing quality of at least 30. For tumour samples at least 212.5 Gb were required. Alignments for the germline sample must cover at least 95% of genome at 15x or above with well mapped reads (mapping quality > 10) after discarding duplicates.

# Variant detection

## Small variants

Illumina’s North Star pipeline (version 2.6.53.23) was used for primary WGS analysis. Read alignment against human reference genome GRCh38-ALT+Decoy+EBV was performed with ISAAC (version iSAAC-03.16.02.19); small variant calling together with tumour-normal subtraction was performed using Strelka (version 2.4.7).

Strelka filters out the following germline variant calls:

* All calls with a sample depth three times higher than the chromosomal mean
* Site genotype conflicts with proximal indel call. This is typically a heterozygous SNV call made inside of a heterozygous deletion
* Locus read evidence displays unbalanced phasing patterns
* Genotype call from variant caller not consistent with chromosome ploidy
* The fraction of basecalls filtered out at a site > 0.4
* Locus quality score < 14 for for het or hom SNP
* Locus quality score < 6 for het, hom or het-alt indels
* Locus quality score < 30 for other small variant types or quality score is not calculated

Strelka filters out the following somatic variant calls:

* All calls with a normal sample depth three times higher than the chromosomal mean
* All calls where the site in the normal sample is not a homozygous reference
* Somatic SNV calls with empirically fitted VQSR score < 2.75 (recalibrated quality score expressing the phred scaled probability of the somatic call being a false positive observation)
* Somatic indels where fraction of basecalls filtered out in a window extending 50 bases to either side of the indel’s call position is > 0.3
* Somatic indels with quality score < 30 (joint probability of the somatic variant and a homo ref normal genotype)

Variants are not removed on the basis of low read count/frequency in the current version of the analysis pipeline. This is to allow for the detection of low level variants but may be reviewed in subsequent versions of the pipeline.

Variants were not filtered out on the basis of being common in the general population. Small indels intersecting with reference homopolymers of at least 8 nucleotides in length have been highlighted on the analysis with an (H): such variants arise commonly, especially in the context of deficits in base-excision repair, but overall have a higher likelihood of being false positive artefacts of sequencing or calling. Small indels in regions with high levels of sequencing noise have been highlighted (N) if at least 10% of the basecalls in a window extending 50 bases to either side of the variant have been filtered out due to the poor quality. These indels have a higher likelihood of being false positive artefacts of misalignment.

## Structural variants

Structural variants (SVs) and long indel (>50bp) calling was performed with Manta (version 0.28.0) which combines paired and split-read evidence for SV discovery and scoring. Copy number variants (CNVs) were called with Canvas (version 1.3.1) that employs coverage and minor allele frequencies to assign copy number. These tools filter out the following variant calls:

* Manta-called SVs with a normal sample depth near one or both variant break-ends three times higher than the chromosomal mean
* Manta-called SVs with somatic quality score < 30
* Manta-called somatic deletions and duplications with length > 10kb
* Manta-called somatic small variant (<1kb) where fraction of reads with MAPQ0 around either break-end > 0.4
* Canvas-called somatic CNVs with length < 10kb
* Canvas-called somatic CNVs with quality score < 10

# Variant annotation

SNVs and small indels were normalized (left aligned, trimmed, MNVs decomposed), uploaded to Open-CGA and annotated by Cellbase against ENSEMBL (version 82/GRCh38), COSMIC (version v78/GRCh38) and ClinVar (December 2016 release) databases. CellBase takes advantage of the data integrated in its database to implement a rich and high-performance variant annotator (with 99.9991% concordance with Ensembl VEP Consequence Types across 1000 genomes phase 3 variants). Only variants annotated with the following consequence types in canonical transcripts (see List of canonical transcripts v1.4) are reported:

|  |  |  |  |
| --- | --- | --- | --- |
| SO term | Consequence type |  |  |
| SO:0001893 | transcript ablation | | |
| SO:0001574 | splice\_acceptor\_variant | | |
| SO:0001575 | splice\_donor\_variant | | |
| SO:0001587 | stop\_gained | | |
| SO:0001589 | frameshift\_variant | | |
| SO:0001578 | stop\_lost | | |
| SO:0002012 | start\_lost | | |
| SO:0001889 | transcript\_amplification | | |
| SO:0001821 | inframe\_insertion | | |
| SO:0001822 | inframe\_deletion | | |
| SO:0001650 | Inframe\_variant | | |
| SO:0001583 | missense\_variant | | |
| SO:0001630 | splice\_region\_variant | | |

PLEASE NOTE:-

1. Complex indels are not annotated at the protein level
2. Indels intersecting with reference homopolymers of at least 8 nucleotides in length are denoted with an (H) after the annotation in the predicted consequence column.
3. Small indels in regions with high levels of sequencing noise where at least 10% of the basecalls in a window extending 50 bases to either side of the indel’s call have been filtered out due to the poor quality are denoted with an (N) after the annotation in the predicted consequence column.

# Germline findings

Analysis for pertinent germline findings was performed to detect pathogenic or likely pathogenic variants in genes conferring susceptibility to the relevant tumour type. For a list of the genes analysed for each tumour type, see [The Gene List for Reporting Germline Findings in Cancer Patients v1.4](https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/cancer-genome-analysis/). Pathogenic or likely pathogenic variants include (i) variants predicted to truncate the protein in genes for which the mechanism of pathogenicity is loss of function (ii) variants listed in ClinVar as pathogenic or likely pathogenic (with a rating of at least two stars). Clinical review within Genomics England was undertaken for all pathogenic or likely pathogenic variants.

# Explanation of report fields

## Sample and variant description

|  |  |  |  |
| --- | --- | --- | --- |
| Column name | Explanation |  |  |
| Tumour Sample Cross-contamination | Cross-contamination is a measure, which indicates whether the tumour DNA sample is contaminated with DNA from other individuals. Contamination is calculated at homozygote sites derived from the germline genotyping array. PASS status means that contamination is below 2%. | | |
| Reported Tumour Content | Reported tumour content as estimated in host GMC Pathology lab (Low <40%; Medium 40-60%; High >60%.). | | |
| Gene- or variant– level actionability | Therapies or clinical trials which the patient may be eligible for. Cancer type abbreviations have been used expansions can be accessed in [Cancer type abbreviations v1.4](https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/cancer-genome-analysis/) document | | |
| cDNA change | cDNA change was calculated with the [Mutalyzer API](https://www.mutalyzer.nl) | | |
| VAF | Calculated as alt/(alt + ref) where alt and ref are the number of reads passing filter (reads excluded are read pairs with a mapping quality < 40; read pairs with only a single end mapped or with an anomalous insert size) | | |
| Gene mode of action | Classification for gene mode of action (oncogene, tumour suppressor or both) was extracted from the manually curated list of Cancer Census Genes (downloaded on 26/09/2016 from <http://cancer.sanger.ac.uk/census>; see the list at [Cancer census genes v1.4](https://www.genomicsengland.co.uk/information-for-gmc-staff/cancer-programme/cancer-genome-analysis/)) | | |

## Sequencing and coverage quality metrics

All coverage metrics are calculated by including fragments (rather than reads) with minimal base quality of 30 and minimal mapping quality of 10, with duplicates removed. Quality metrics (mapped reads, chimeric DNA fragments and insert size) were calculated with samtools (version 1.1).

|  |  |  |  |
| --- | --- | --- | --- |
| Column name | Explanation |  |  |
| Mapped Reads | The percentage of reads which can be mapped to the reference sequence. A low percentage could indicate DNA degradation and/or cross-species (e.g. bacterial) contamination. Median values for good quality FF samples are 95.7% with standard deviation of 0.6%. | | |
| Chimeric DNA fragments, % | This metric indicates the proportion of chimeric DNA fragments. Random Inter-chromosomal DNA cross-linking due to DNA strand breakage can cause high proportions of chimeric DNA fragments. This can reflect problems with tissue processing or DNA extraction. For good quality FF samples the median percentage of chimeric DNA fragments is 0.3 % with standard deviation of 0.1%. | | |
| Average fragment size, bp | Short fragments could result from DNA fragmentation due to poor sample handling. Very long fragments (2 or 3 times longer than expected) could result from artefacts introduced during the PCR amplification step, which is required to obtain libraries from samples containing low levels of DNA. Median fragment size for good quality FF samples is 490 bp with standard deviation of 29 bp. | | |
| Genome-wide coverage mean | Coverage represents the median number of reads (depth) per base in the reference genome. Coverage is calculated for autosomes only. The median value for good quality FF samples is 101x with standard deviation of 8x. | | |
| Unevenness of Local Genome Coverage | This metric represents how evenly the read coverage is distributed across the genome. Unevenness is calculated as median for the root mean square deviation (RMSD) of coverage calculated in non-overlapping 100 kb windows. This metric would be 0 for a genome with absolutely uniform coverage. Median value for good quality FF samples is 16.2 with standard deviation of 1.4. | | |
| COSMIC content with low coverage | This metric represents the “discoverability” of known somatic mutations. It is calculated as the % of hypothetical somatic mutation sites (obtained from COSMIC) with coverage of <30x. Median value for this metric for good quality FF samples is 0.9% with standard deviation of 0.2%. | | |
| Total somatic  SNVs, indels and SVs | High numbers of somatic calls can signal a high rate of false positives. However caution is required when interpreting this metric as different tumour types typically have different levels of mutation burden. Additionally, tumours arising from particular mechanisms (e.g severe loss of function in DNA repair genes) may contain very high numbers of somatic mutations. | | |