

Genomic analysis report of XYZ_STUDY_D.XYZ003_DNA *

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1 Results

1.1 Causal variant summary

- Sample ID: XYZ_STUDY_D.XYZ003_DNA
- HGVS coding variant: ENST00000380588.4:c.53G>A
- Protein variant: ENSP00000369962.4:p.Trp18Ter
- ACMG score: 2

The analysis has been concluded as follows. [Figure 1](#).

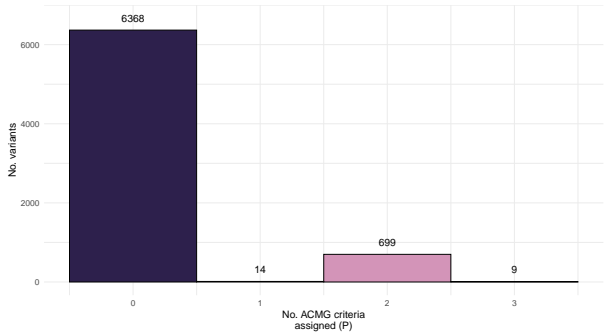


Figure 1: ACMGuru automated analysis...

1.2 Causal variant evidence interpretation

The analysis of variant interpretation performed by ACMGuru is summarised from the following evidence sources: [Figure 2](#) includes the use of gene structure and functional data including UniProt sources, protein structure data from PDB and AlphaFold. [Figure 2](#) includes the use of protein pathway construction from STRING (GO, KEGG, Reactome, etc.). [Figure 3](#) include biological protein pathway information from GO. [Figure 4](#) contains the QQ plot data from the joint cohort analysis of single variants. [Figure 4](#) contains the protein pathway identified as enriched in patients sharing the same biological mechanism as cause of disease.

* This document's source code is available from the [GitHub repository](#). All code used in this report is available on the [GitHub repository](#).

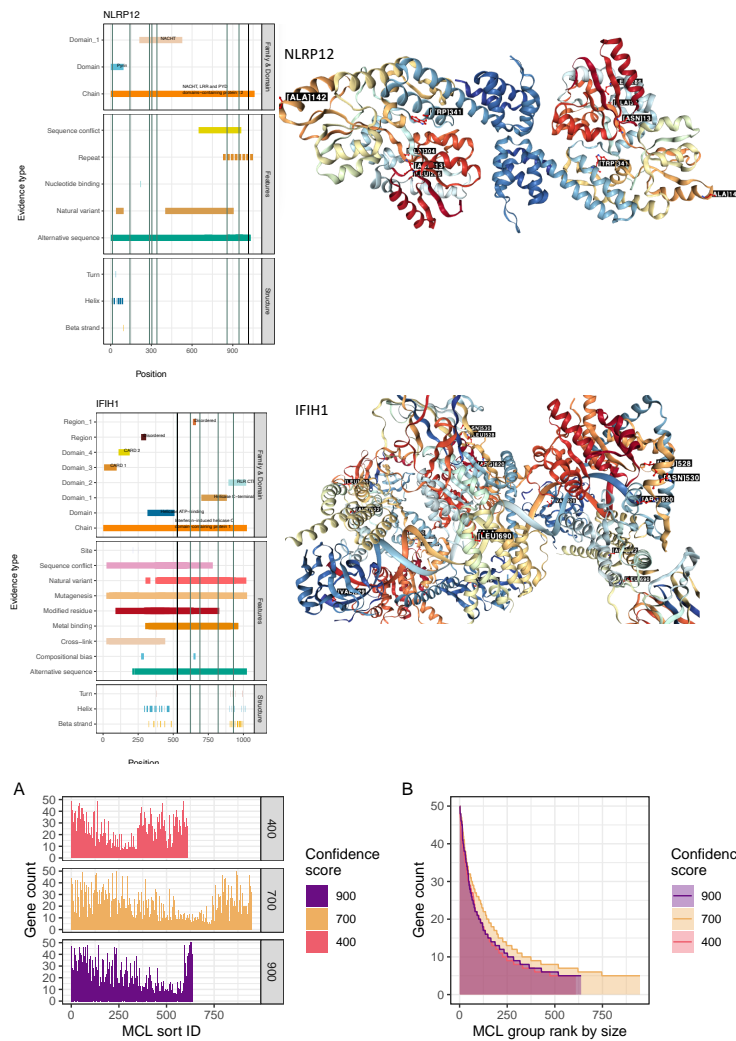


Figure 2: ACMGuru automated analysis...

2 Introduction

The two datasets (Sample ID: [XYZ_STUDY_D.XYZ003_DNA](#) and reference CH S2) of paired-end short reads were generated from the same human DNA NGS library protocol for clinical diagnosis of phenotype X. Clinical grade sequencing (ISO 15189 accredited) was used to generate whole genome sequence (WGS) data at the Swiss Multi-Omic Center (SMOC) (<http://smoc.ethz.ch>). The Illumina Novaseq6000 platform was used in combination with TruSeq DNA PCR-Free library preparation. Analysis was performed with reference to GRCh38. Handling of sensitive clinical data according to established SPHN/BioMedIT guidelines on the sciCORE platform as part of SwissPedHealth. (<https://sphn.ch/network/projects/biomedit/>) Herein, we evaluate sample performance for use in clinical diagnose.

3 Details for technical writing report

Note: This section contains the main information required for a technical report. The remainder of this document contains additional information which is *not* required for further reports, and includes duplicate references to files listed here. Please include:

- **Introduction:** all details from sec. 2.

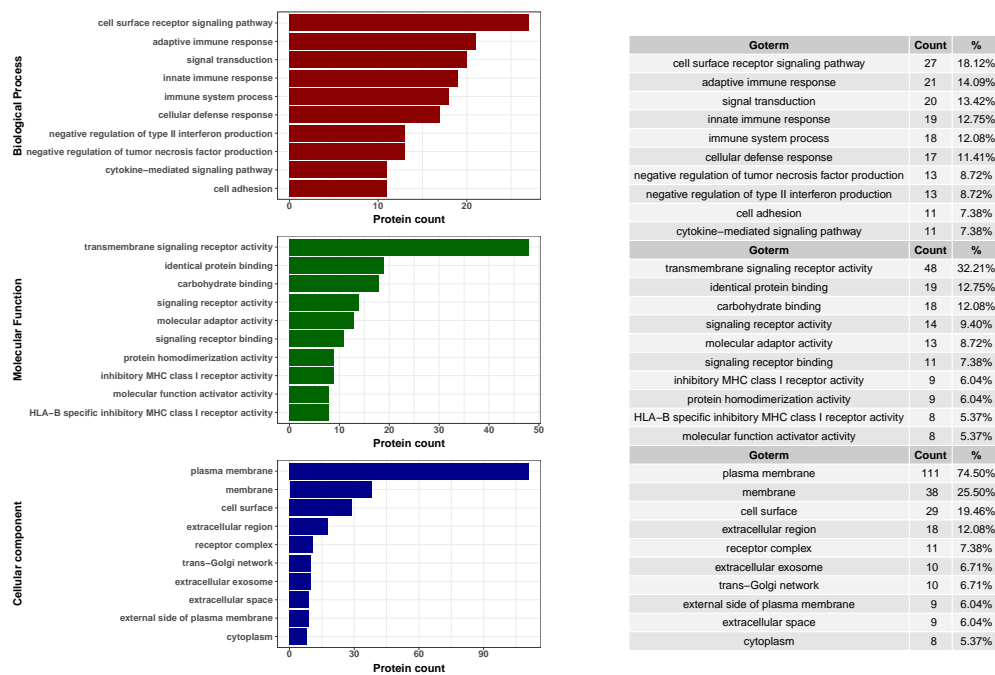


Figure 3: ACMGuru automated analysis...

- **Data source:** all details from sec. 4.1.
- **Protocol details:** a hyperlink to the protocol source: [\[link\]](#).
- **Result:** Result summary sec. 5.1.
- **Figures:** 5, 11, and 14.
- **Conclusion:** all details from sec 7.

4 Methods

4.1 Data source

- **Description:** SMOC performed library preparation for two (2) samples (sample SPH00123 and reference CH) using [TruSeq DNA PCR-Free](#). The performance of each was assessed with several bioinformatic methods including read quality control and performance when aligning to reference genome [\[code available here\]](#).
- **Data source:** raw sequence data was received from [\[link group and contact address\]](#).
- **Date:** 2022-02-14
- **Link to tickets where submission was logged:** [\[link\]](#)
- **Data integrity:** [\[link metadata md5sum\]](#).

4.2 Configuration

Local env: macOS v11.6 [software](#), Remote env: Red Hat Enterprise Linux Server 7.6 (Maipo) [software](#), compiler intel: (19.0.5 and 19.1.1), [software](#), R: R v4.1.0 Camp Pontanezen [software](#), R libraries: versions unlisted, fastqc: v0.11.7 [software](#), samtools: v1.10 [software](#), bwa: v0.7.17 [software](#), picard: v2.20.8 [software](#). qualimap v2.2.1 [qualimap](#).

4.3 Analysis code

All code available at [link: GitHub/kit_assess](#).

Protocol order:

- src/md5sum.ch

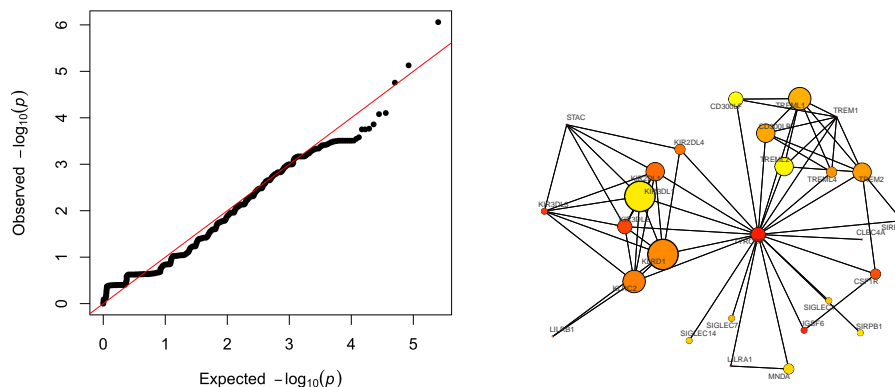


Figure 4: ACMGuru automated analysis...

- src/read_count.sh
- fastQC: manual run all fastq and save to ./processed/fastqc
- fastqcr: Assess fastqc repots further
- src/target_info.sh
- src/1.trim.sh
- src/2.align.sh
- src/3.sort.sh
- src/mapping.sh

5 Results

Data for this report can be found on link: [GitHub/kit_assess](https://github.com/kit_assess). Analysis results are separated into QC of fastq data (sec. 5.2), QC after alignment to reference genome GRCh37 (sec. 5.3), and exploration of target coding regions (sec. 5.4).

5.1 Result summary

Based on [1] QC of fastq data, [2] QC after alignment to reference genome GRCh37, and [3] exploration of target coding regions, AH S1 performed better than CH S2 for use in clinical diagnosis of solid tumor. The read depth and uniform coverage provided for AH S1 outperformed CH S2 and is beneficial in tumor sequencing for calling germline variants, somatic variants, CNV or structural changes.

5.2 Fastq data

To assess the quality of fastq data, [FastQC](#) was used. Full html reports for each file are linked:

- [AH_S1_L001_R1_fastqc](#)
- [AH_S1_L001_R2_fastqc](#)
- [CH_S2_L001_R1_fastqc](#)
- [CH_S2_L001_R2_fastqc](#)

The results of fastQC were also assessed by use of [fastqcr](#). The full html report is linked:

- [Report assessment of fastQC](#)

1. Total sequences or the number of reads for each samples: 1000000
2. Per base sequence quality: **All samples performed sufficiently**. Summarised in Figure 5
 - Median value (red line): **AH good quality** (qual >28), **CH good quality** (qual >28).
 - Inter-quartile range (25-75%) (yellow box): **AH good quality** (qual >28), **CH medium to good quality** (qual >20).
 - Upper and lower 10% and 90% whiskers points: **AH medium to good quality** (qual >28), **CH poor to good quality** (qual >14).
 - Mean quality (blue line): **AH good quality** (qual >28), **CH medium to good quality** (qual >20).
3. Per tile sequence quality: **All samples performed sufficiently**. No warning.
4. Per sequence quality score: **All samples performed sufficiently**, summarised in Figure 6.
5. Per base sequence content. **All samples flagged** with warning indicating a difference greater than 10% in any position. However, this is potentially due targeted capturing, Figure 7.
6. Per sequence GC content. **All samples failed** based on modal GC content as calculated from the observed data and used to build a reference distribution. The sum of the deviations from the normal distribution represents more than 30% of the reads. However, the sharp peaks (as seen on AH [Top]) are most likely due to enriched duplicate sequences from targeted capturing and do not necessarily indicate poor quality, Figure 8.
7. Sequence Length Distribution: **AH reads were all 150**, while **CH reads were 35-151**.
8. Sequence Duplication Levels: Percentage of duplicate reads were **AH 96.01%-96.55%** and **CH 65.44%-67.13%**. Figure 9
9. Adapter Content: Figure 10, **AH Illumina Universal adaptor and CH Adaptor not detected**.

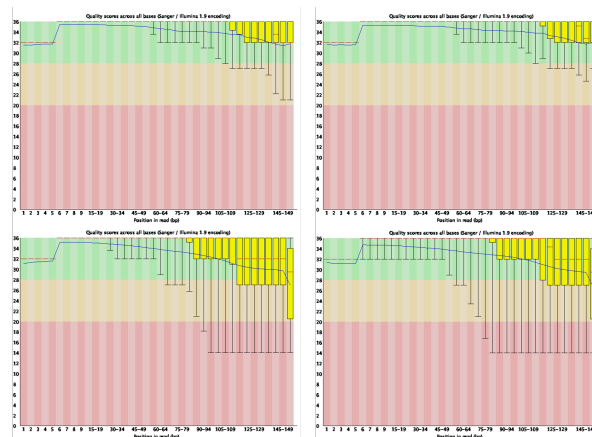


Figure 5: Per base sequence quality score: [Top] AH [Bottom] CH. AH outperformed CH for both reads. Central red line shows the median value. Inter-quartile range 25-75% (yellow box). Upper and lower whiskers represent the 10% and 90% points. Mean quality (blue line).

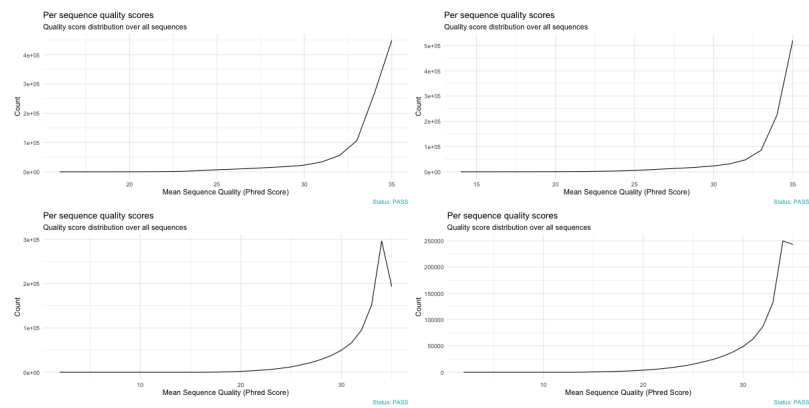


Figure 6: Per sequence quality score: [Top] AH [Bottom] CH. All samples performed sufficiently. AH outperformed CH for both reads. Most frequently observed mean quality was above 30 for all samples; less than 0.2% error rate.

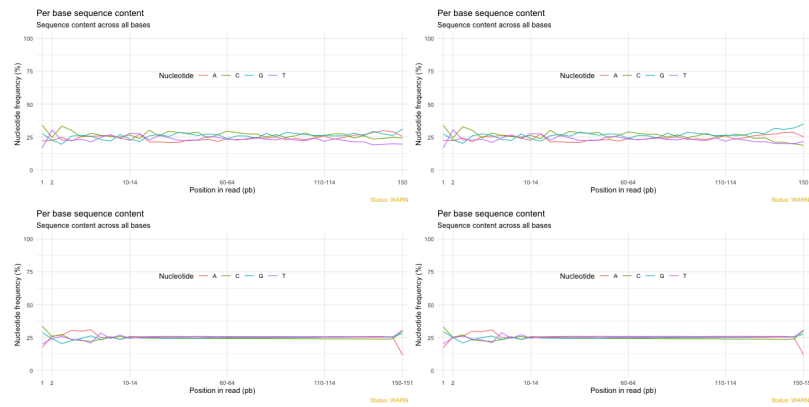


Figure 7: Per base sequence content: [Top] AH [Bottom] CH. *Note:* The frequencies observed for CH are unexpected; additional background information is required for their interpretation.

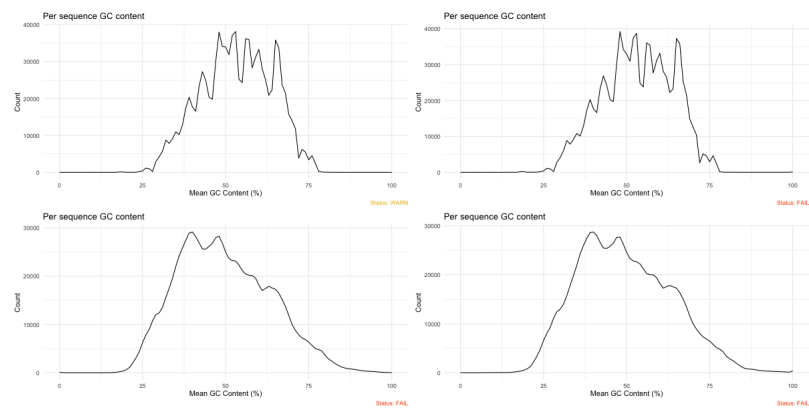


Figure 8: Per sequence GC content: [Top] AH [Bottom] CH. A normal distribution is expected for genomic DNA. However, targeted sequencing may produce difference distributions as seen here.

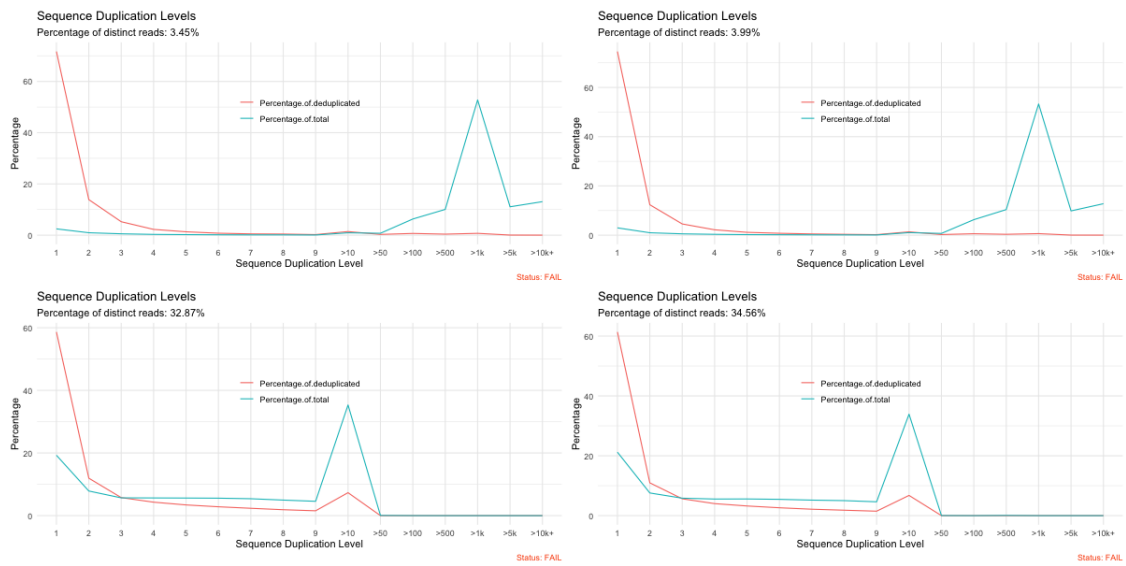


Figure 9: Sequence Duplication Levels: [Top] AH [Bottom] CH. The plot shows the proportion of the library which is made up of sequences in each of the different duplication level bins. The blue line shows the duplication level distribution for full sequence sets. The red line shows de-duplicated sequences and the proportions shown are the proportions of the de-duplicated set which come from different duplication levels in the original data. In a properly diverse library most sequences should fall into the far left of the plot in both the red and blue lines. The fail status is due to non-unique sequences making up more than 50% of the total, which is not a problem for targeted capture libraries.

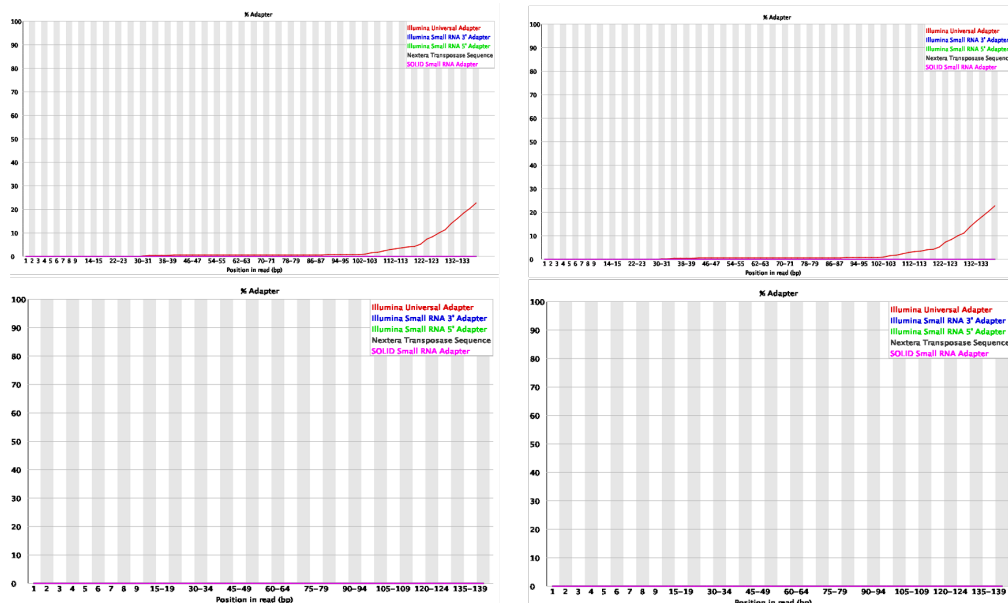


Figure 10: Adapter content plot: [Top] AH [Bottom] CH. Accumulative percentage count of the proportion of library which has seen each of the adapter sequences at each position. Adapter sequence Illumina universal was found for AH, and no adaptor sequence was identified for CH.

5.3 Alignment data

Fastq were trimmed using [TrimGalore](#). with the use of [cutadapt](#).

Reads were aligned to GRCh37 using [BWA MEM](#) and converted to bam format with [samtools](#).

The alignment data was assessed using

- [samtools](#) flagstat: get mapping summary.
- [samtools](#) depth: read depth for at all positions of the reference genome, e.g. how many reads are overlapping the genomic position.
- [qualimap](#): examines sequencing alignment data in SAM/BAM files according to the features of the mapped reads and provides an overall view of the data that helps to the detect biases in the sequencing and/or mapping of the data and eases decision-making for further analysis.

Qualimap full html report link: [sample AH](#)

Qualimap full html report link: [sample CH](#)

1. Samtools flagstat mapping summary shows alignment performance with GRCh37 for sorted reads, Table 1.
2. Mapping quality histogram indicate that AH performed better than CH, Figure 11.
3. Genome coverage histogram shows that AH produced a normal distribution of coverage depths while CH had an enrichment for some genomic regions, Figure 12.
4. The duplication rate histograms are shown in Figure 13.
5. Genome coverage across GRCh37 shows a uniform distribution of reads for AH [Top], while CH [Bottom] has high depth in some regions with lower coverage in others, Figure 14.

Table 1: Samtools flagstat mapping summary. Alignment with GRCh37, sorted reads.

CH	AH	
2011262	1999498	in total (QC-passed reads & + QC-failed reads)
15710	612	secondary
0	0	supplementary
0	0	duplicates
2006501	1997929	mapped (99.76% : N/A, 99.92% : N/A)
1995552	1998886	paired in sequencing
997776	999443	read1
997776	999443	read2
1968314	1992738	properly paired (98.64% : N/A, 99.69% : N/A)
1986886	1996840	with itself and mate mapped
3905	477	singletons (0.20% : N/A, 0.02% : N/A)
14488	1612	with mate mapped to a different chr
8748	1426	with mate mapped to a different chr (mapQ>=5)

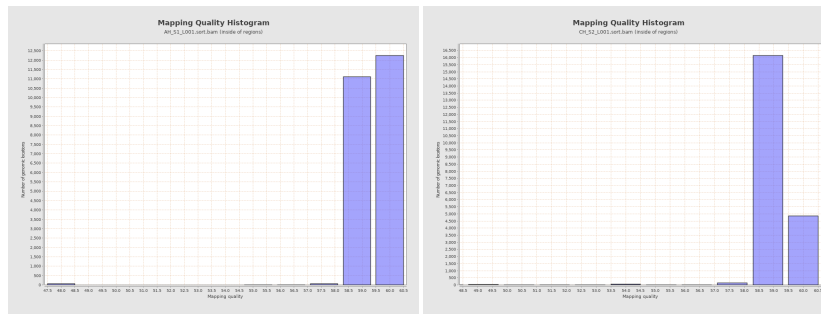


Figure 11: Mapping quality histogram (GRCh37). [Left] AH [Right] CH. Histogram of the number of genomic locations having a given mapping quality. To construct the histogram mean mapping quality is computed at each genome position with non-zero coverage and collected. According to Specification of the SAM format the range for the mapping quality is [0-255].

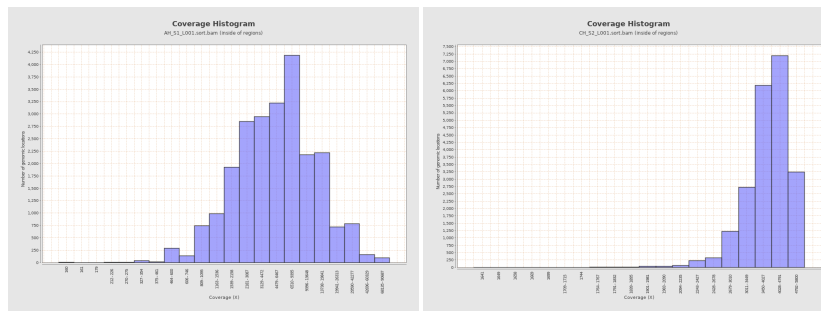


Figure 12: Genome coverage histogram (GRCh37). [Left] AH [Right] CH. Histogram of the number of genomic locations having a given coverage rate. The bins of the x-axis are scaled by aggregating some coverage values in order to produce a representative histogram also in presence of the usual NGS peaks of coverage.

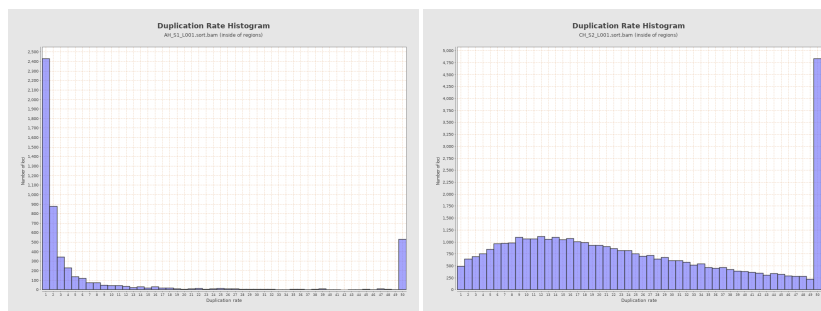


Figure 13: Duplication rate histogram (GRCh37). [Left] AH [Right] CH. This plot shows the distribution of duplicated read starts. Due to several factors (e.g. amount of starting material, sample preparation, etc) it is possible that the same fragments are sequenced several times. For some experiments where enrichment is used (e.g. ChIP-seq) this is expected at some low rate. If most of the reads share the exact same genomic positions there is very likely an associated bias.

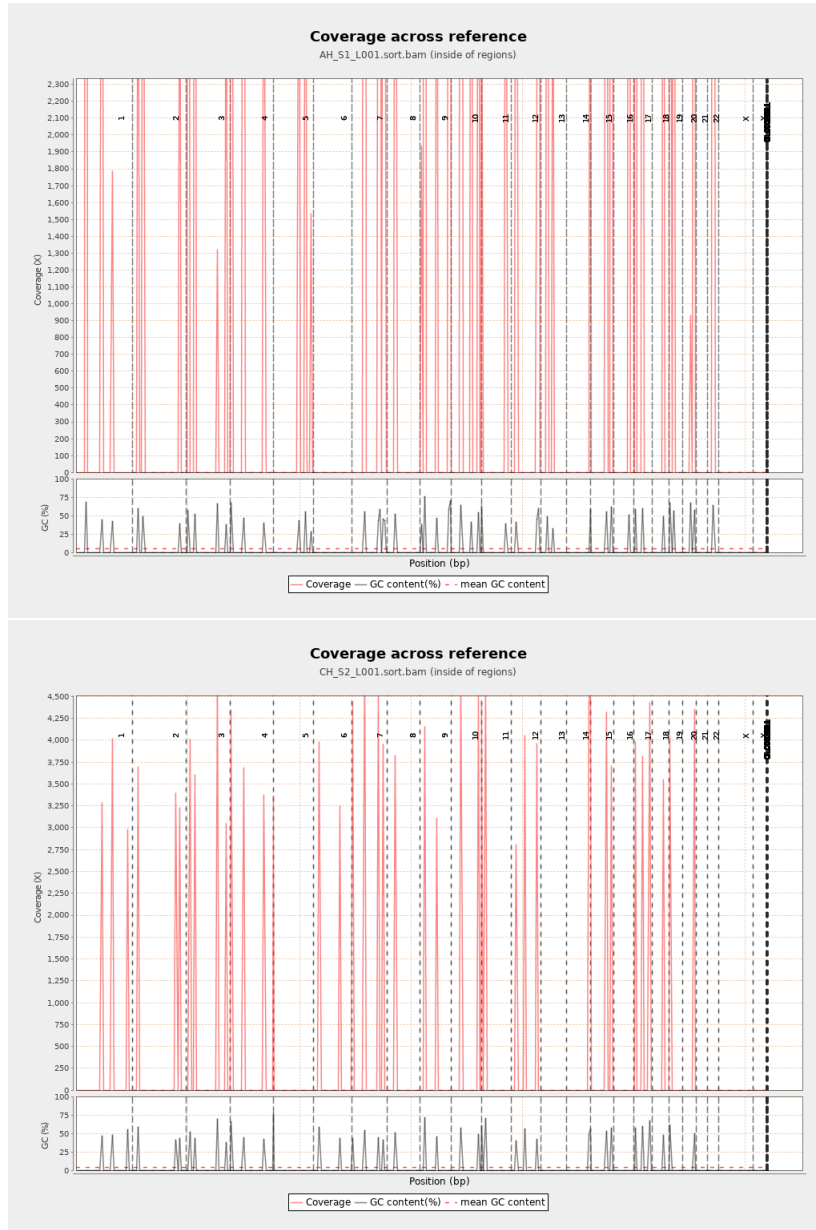


Figure 14: Genome coverage across reference (GRCh37). [Top] AH [Bottom] CH. Each plot consists of two figures. The upper figure provides the coverage distribution (red line) and coverage deviation across the reference sequence. The coverage is measured in X number of reads. The lower figure shows GC content across reference (black line) together with its average value (red dotted line).

5.4 Target coding genes

To learn about what target regions are captured in this dataset, the coordinates (hg19) were annotated using Ensembl Biomart. The data was annotated using grch37.ensembl.org/biomart dataset Ensembl Genes 105 (GRCh37.p13). Output attributes queried for these regions: Gene stable ID, Transcript stable ID, Gene name, Gene start (bp), Gene end (bp), Chromosome/scaffold name, Gene % GC content, HGNC symbol, and HGNC ID. The gene symbols for each target list are shown in Table 2:

- [Target coding genes table \(html version\).](#)

Table 2: Targeted coding genes in GRCh37 present in for each method.

Chr	Start	End	Gene name	% GC content	AH S1	CH S2
1	43803478	43818443	MPL	48.47	Yes	No
1	115247090	115259515	NRAS	39	Yes	Yes
1	162601163	162757190	DDR2	40.47	Yes	Yes
2	25455845	25565459	DNMT3A	53.5	Yes	No
2	29415640	30144432	ALK	43.51	Yes	Yes
2	47922669	48037240	MSH6	44.65	Yes	No
2	48016455	48132932	FBXO11	39.46	Yes	No
2	209100951	209130798	IDH1	41.27	Yes	Yes
2	212240446	213403565	ERBB4	34.69	Yes	Yes
3	10182692	10193904	VHL	47.55	Yes	No
3	37034823	37107380	MLH1	42.01	Yes	No
3	41236328	41301587	CTNNB1	39.91	Yes	Yes
3	138663066	138665982	FOXL2	64.62	Yes	Yes
3	178865902	178957881	PIK3CA	35.78	Yes	Yes
4	1795034	1810599	FGFR3	65.82	Yes	Yes
4	54243810	55161439	FIP1L1	40.97	Yes	Yes
4	55095264	55164414	PDGFRA	43.65	Yes	Yes
4	55524085	55606881	KIT	40.87	Yes	Yes
4	55919227	55958701	RP11-530I17.1	40.25	Yes	No
4	55944644	55991756	KDR	40.69	Yes	No
4	153242410	153457253	FBXW7	35.41	Yes	Yes
4	153258807	153259250	RP11-461L13.2	34.91	Yes	No
5	112043195	112181936	APC	37.63	Yes	No
5	112162910	112203279	CTC-554D6.1	39.75	Yes	No
5	149432854	149492935	CSF1R	48.1	Yes	No
5	170814120	170838141	NPM1	43.6	Yes	No
7	55086714	55324313	EGFR	45.08	Yes	Yes
7	55247443	55256627	EGFR-AS1	50.41	Yes	Yes
7	116312444	116438440	MET	39.03	Yes	Yes
7	128828713	128853386	SMO	49.17	Yes	No
7	128849616	128853386	RP11-286H14.8	57.23	Yes	No
7	140419127	140624564	BRAF	37.97	Yes	Yes
7	148504475	148581413	EZH2	39.21	Yes	No
8	38268656	38326352	FGFR1	50.55	Yes	Yes
8	38279407	38283614	RP11-350N15.4	50.78	Yes	No
9	4985033	5128183	JAK2	37.53	Yes	No
9	5077163	5084580	AL161450.1	34.29	Yes	No
9	21802635	22032985	RP11-145E5.5	40.29	Yes	Yes
9	21967751	21995300	CDKN2A	41.19	Yes	Yes
9	80331003	80646374	GNAQ	39.13	Yes	Yes
9	133589333	133763062	ABL1	44.47	Yes	No
9	135766735	135820020	TSC1	42.78	Yes	No
9	139388896	139440314	NOTCH1	63.39	Yes	No
10	43572475	43625799	RET	55.73	Yes	Yes

10	89622870	89731687	PTEN	35.77	Yes	No
10	123237848	123357972	FGFR2	45.41	Yes	Yes
11	532242	537287	HRAS	69.01	Yes	Yes
11	108093211	108239829	ATM	37.52	Yes	No
11	108179246	108338258	C11orf65	38.99	Yes	No
12	25357723	25403870	KRAS	36.37	Yes	Yes
12	112856155	112947717	PTPN11	43.16	Yes	Yes
12	121416346	121440315	HNF1A	52.2	Yes	No
13	28577411	28674729	FLT3	43.22	Yes	No
13	48877887	49056122	RB1	36.91	Yes	No
14	105235686	105262088	AKT1	64.08	Yes	Yes
15	66679155	66784650	MAP2K1	44.72	Yes	Yes
15	66782086	66784447	CTD-3185P2.1	44.37	Yes	No
15	66782473	66790151	SNAPC5	44.54	Yes	No
15	90626277	90645736	IDH2	52.54	Yes	Yes
16	68771128	68869451	CDH1	46.89	Yes	No
17	7565097	7590856	TP53	48.85	Yes	Yes
17	37844167	37886679	ERBB2	52.09	Yes	Yes
18	48494389	48584514	RP11-729L2.2	41.11	Yes	No
18	48494410	48611415	SMAD4	40.04	Yes	Yes
19	1189406	1228428	STK11	58.47	Yes	No
19	3094408	3124002	GNA11	61.66	Yes	Yes
19	3118663	3119302	AC005262.3	64.69	Yes	Yes
19	17935589	17958880	JAK3	54.93	Yes	No
20	35973088	36034453	SRC	54.1	Yes	No
20	57414773	57486247	GNAS	47.09	Yes	Yes
22	24129150	24176703	SMARCB1	50.77	Yes	No

6 Variant classification was performed using ACMGuru

<https://github.com/DylanLawless/ACMGuru>

Interpretation of variants included the use of ACMG standards and guidelines. Extensive annotation is applied during our genomics analysis. Interpretation of genetic determinants of disease is based on many evidence sources. One important source of interpretation comes from the Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richards et al., 2015), full text at [doi: 10.1038/gim.2015.30](https://doi.org/10.1038/gim.2015.30). A table of [ACMG criteria](#) is used in our filtering protocol for the classification of candidate causal variants.

7 Conclusion

[1] Based on QC of fastq data (sec. 5.2), **Sample ID: XYZ_STUDY_D.XYZ003_DNA** sequencing generated data sufficient for analysis. [2] Based on QC after alignment to reference genome GRCh37 (sec. 5.3), **Sample ID: XYZ_STUDY_D.XYZ003_DNA** was aligned suitably. However, it should be noted that non-uniform coverage favouring a subset of genes may have occurred - in some circumstances; additional information required. [3] Based on coverage for coding sequences within targeted regions (sec. 5.4), **Sample ID: XYZ_STUDY_D.XYZ003_DNA** analysis is complete. However, additional information on study design required for confirmation.