

Committee for Quality Assurance

Members elected:
Prof. Dr. rer. nat. Jürgen Kunz (chairman)
Dipl.-Biol. Susanne Anders
PD Dr. rer. nat. Barbara Fritz
PD Dr. rer. nat./med. habil. Thomas Liehr
Dr. rer. nat. Anja Weise

RT managers:
Dr. rer. nat. Sebastian Eck
Dr. rer. nat. Eveline Fiedler
Prof. Dr. med. Claudia Haferlach
Sarah Matos Meder, M. Sc.
Prof. Dr. med. Harald Rieder

Ring Trial Next-Generation Sequencing 2018, Final report

Dear colleagues,

Thank you for participating in the interlaboratory test Next-generation sequencing. In total 18 laboratories/institutions have completed the test. In this test the task was to determine all true-positive variants given provided reads in .fastq format and the definition of target regions in the .bed format. The provided data set is a part of a whole-exome sequencing run. The enrichment was performed using the Agilent Human All Exon v6 kit QXT reagent kit. The DNA was enzymatically fragmented and sequenced on an Illumina NextSeq 500 instrument with 2x151bp paired-end reads. The suspected indication of the sample was premature ovarian failure (POF), the target region comprised the following genes: *BMP15*, *DIAPH2*, *ERRCC6*, *ESR1*, *FIGLA*, *FOXL2*, *FSHR*, *GDF9*, *INHA*, *LHCGR*, *MCM9*, *NOBOX*, *NR5A1*, *PSMC3IP*, *SOHLH1*, *SOHLH2*, *STAG3*, *SYCE1*.

In total there were 40 genuine variants in the sample, 38 single nucleotide substitutions and 2 insertions of length 6bp and 10bp, respectively. To successfully complete the interlaboratory test it was necessary for the participants to detect at least 90% of true positive variants. During evaluation the respective detection limits, for instance minimal coverage or maximum indel length, as stated by the particular laboratories was taken into account. Except for one laboratory all participants could successfully complete the task (Figure 1, p. 3).

During evaluation a few notable issues arose, which will be briefly elaborated on:

- Some participants used a non-standard variant nomenclature in their .vcf files. In particular SNVs in close proximity to each other (affecting the same codon) were combined to a single variant. Furthermore there were additional nucleotides reported in case

23. November 2018

Coordination Office Quality Assurance
c/o Die Tastatur – Susanne Brandt &
Rainer Göbbels GbR
Feldstr. 30
52249 Eschweiler
Germany

Tel. +49 2403 83 80 54
Fax. +49 2403 83 80 56

brandt@bvdh.de
www.bvdh-ringversuche.de

RT Managers NGS

Dr. rer. nat. Sebastian Eck
Zentrum für Humangenetik und
Laboratoriumsdiagnostik (MVZ)
Dr. Klein, Dr. Rost und Kollegen
Lochhamer Str. 29
82152 Martinsried, Germany

Tel.: +49 89 89 55 78-0
sebastian.eck@medizinische-genetik.de

Dr. med. Caroline Göhringer
bio.logis Zentrum für Humangenetik
Altenhöferallee 3
60438 Frankfurt am Main, Germany

Tel.: +49 69 53 08 43 70
caroline.goehringer@bio.logis.de

Administration Office

Liniestraße 127
10115 Berlin, Germany

Tel. +49 30 55 95 44 11
Fax +49 30 55 95 44 14

info@bvdh.de
www.bvdh.de

Bank Details

Deutsche Apotheker- und Ärztebank eG
BLZ 300 606 01
Account No. 000 35 86 936

IBAN DE48 3006 0601 0003 5869 36
BIC DAAEDED3

USt.-IdNr.: DE 238 391 914
St.Nr. 27/620/53950

VR 28407 B Amtsgericht Charlottenburg

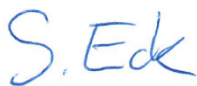
of the insertions (Figure 2, p. 3) For this test all alternative variant description were counted as correct. Starting with the next test in 2019 the task description will warrant a correct nomenclature following published standards and alternative descriptions of a variant in violation of these standards will be penalized.

- The focus of the current test was solely in the detection of true positive variants, additional variants that were reported (false positive) were not penalized. The vast majority of participants had no issues with false positives, most labs reported 1-3 additional variants (see also next paragraph). One participating laboratory reported a massive amounts of false positive variants (111). Starting with the next test in 2019 the detection of false positive variants will be part of the evaluation.
- More than 50% of participants detected an additional variant (chr7:99796146:A>C) in the data set. Re-examination of the reference data set determined that this variant resides in a region flagged as low-confidence and annotated as 3.2 MB segmental duplication. For the list of true positive variants only variants flagged as high-confidence calls in the reference data set were used, thus the said variant was omitted. The detection or not-detection of the variant had no influence on the evaluation of the current test scheme. For following test schemes, when false positive variants are also evaluated, the list of low-confidence calls of the reference data set will be made available to the reviewers so that calls from that list are not counted as false positive.

We have also received many suggestions and improvements, for which we are very thankful. We will try to implement all of them for the next test scheme in 2019.

We would like to thank all participants of the interlaboratory test Next-generation sequencing 2018 and look forward to your participation in 2019. We would like to express our sincere gratitude to all reviewers of this years' test scheme, Dr. Hinderhofer, Dr. Eichhorn, Dr. Martin and Dr. Schanze.

Best regards,



Dr. rer. nat. Sebastian Eck
Ring Trial manager



Dr. med. Caroline Göhringer
Deputy RT manager



Prof. Dr. Jürgen Kunz
Chairman of Committee QA

Figure 1: Results RT NGS

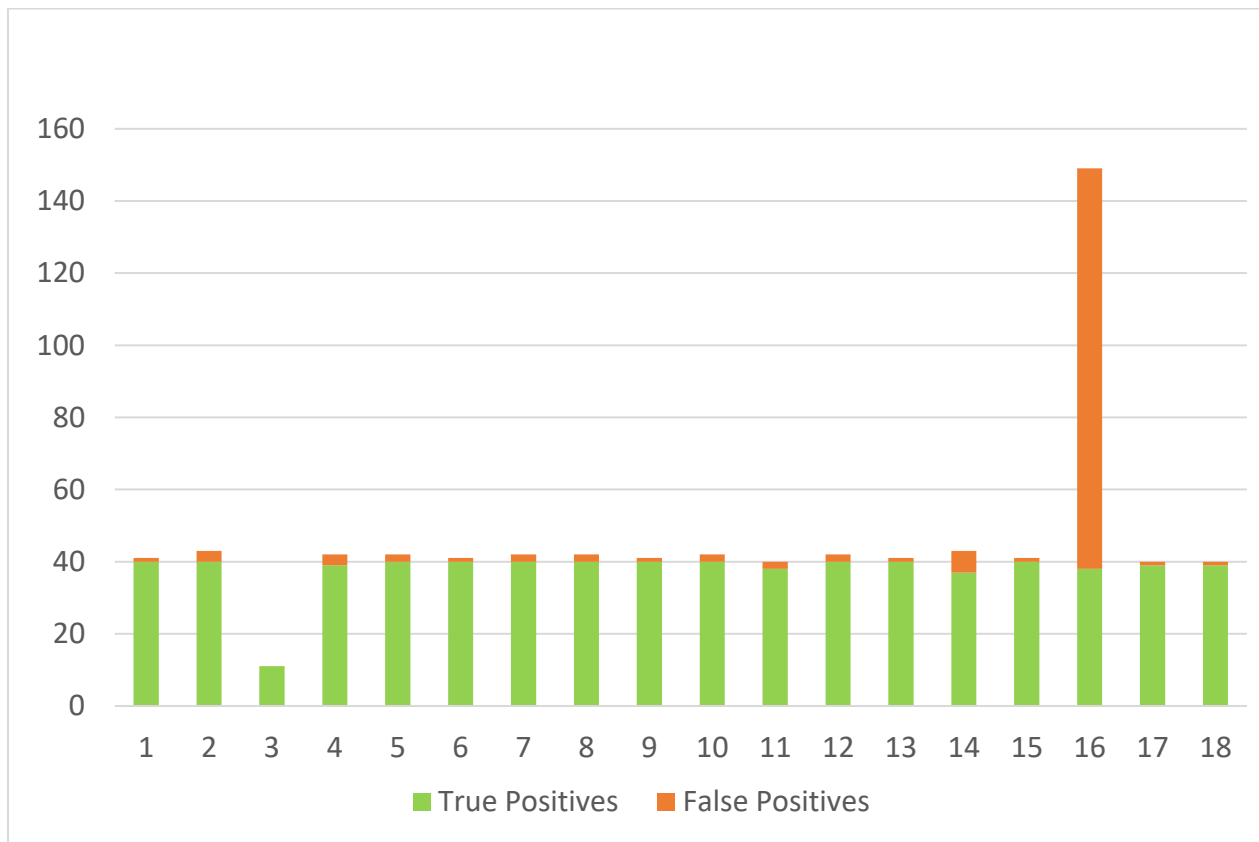


Figure 2: Variant nomenclature

#CHROM	POS	ID	REF	ALT
2	48.915.871	rs11125179	A	G
2	48.921.375	COSM3766585	T	C
2	48.925.746	COSM4001691	C	T
2	48.982.755	rs142537840	GGCTGCAGC	GGCTGCAGCTGCAGC
2	71.004.492	-	T	C
2	71.004.494	-	A	T
2	71.004.492	rs386647171	TCA	CCT
9	138.586.966	-	G	A
9	138.586.967	-	C	A
9	138.586.966	rs386739489	GC	AA
10	135.368.489	rs150216780	AGCTG	AGCTGAGACGGGCTG