2025 External Quality Assessment for Next Generation Sequencing (Germline SNVs and indels)

Post-Appeals FINAL Summary Report

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Dear Colleague, 30/04/2026

This external quality assessment (EQA), 2025 Next Generation Sequencing (NGS) (Germline SNVs and indels) is provided as a collaboration between two EQA providers, EMQN and GenQA. The Summary report includes assessment data from all EQA participants. Your EQA provider is responsible for this EQA, and all correspondence related to it should be directed to either EMQN or GenQA at the relevant address.

The assessment is now complete and your individual laboratory scores have been agreed. Please go to your EQA provider's website to download your Individual Laboratory Report (ILR), Data Quality Report and Variant Call Analysis Report.

EQA DESIGN AND PURPOSE

This EQA aims to assess data quality and accuracy of NGS analysis for germline SNVs (single nucleotide variant) and indels (insertions/deletions <50 base pairs (bp)).

The EQA has been designed to be **platform and gene-target-independent**. Participating laboratories were provided with a single genomic DNA sample (with full testing consent) to process and analyse the resulting data using their normal NGS procedure(s). Participants should use their 'in-house' testing strategy which could include analysis of a single gene and/or whole exome or genome. In addition, participants were encouraged to submit up to three different sets of results/data.

Data collection, quality control (QC), storage and analysis to EMQN / GenQA-defined standards and requirements was subcontracted to a commercial company, Euformatics (http://euformatics.com/). Working with Euformatics has enabled the EQA providers to assess data quality and provide direct comparisons of different methodologies.

Participants submitted a list of identified variants as VCF (Variant Call Format) file(s) and a BED (Browser Extensible Data) file. In addition, submission of raw and processed sequence data (e.g. BAM/CRAM, FASTQ) was encouraged. All submitted data was assessed and quality metrics for each submission were calculated and analysed, as well as variant detection. The cumulative data from all participants was used to provide averages for reference.

The EQA is designed and overseen by members of the NGS EQA Specialist Advisory Group (SAG) co-ordinated by both organisations (Table 1).

Table 1: *SAG members*

Name	Role	EQA Affiliation
Dr Joo Wook Ahn	Chair	None
Dr Jonathan Coxhead	Member	None
Dr Bauke Ylstra	Member	None
Dr Paul Westwood	Member	None
Dr Chris Boustred	Member	None
Dr Erika Souche	Member	None
Dr Kevin Balbi	Member	None
Dr Joseph Halstead	Member	None
Dr Helena Ahlfors	Member	None
Becky Treacy	Deputy Director	GenQA
Dr Dave Cregeen	Deputy Director	GenQA
Prof Sandi Deans	Director	GenQA
Dr Simon Patton	CEO	EMQN
Dr Weronika Gutowska-Ding	Scheme Organiser	EMQN

If you would like to become involved in this advisory committee, please contact the EMQN (office@emqn.org) or GenQA (info@genqa.org) offices for further information.

PERFORMANCE CRITERIA

Performance Criteria have been applied to the results of this EQA[1].

The marking schema includes:

- NGS variant concordance using the F-score (see Appendix D for the definition of F-score) for only single nucleotide polymorphisms (SNVs) which are located within high-confidence (HC) regions of the genome.
- The performance outcome for this EQA is **Satisfactory** OR **Poor**. EMQN and GenQA staff will ensure consistency of scoring between and within the EQA rounds.

Poor performance is defined as follows:

- *Those participants with one or more submission(s) with an F-score below 90% for SNVs within high-confidence regions of the genome.*

Please note:

- 'High confidence' regions are defined as exons ±30 bp, exclusive of union of all segmental duplications, low-mappability regions, high/low GC regions, tandem repeats, and difficult XY regions as published by NIST in Genome In A Bottle – Genome Stratifications v.3.6 (https://ftp-trace.ncbi.nlm.nih.gov/ReferenceSamples/giab/release/genome-stratifications/v3.6/).
- The F-score of indels (<50bp) is excluded from the current Performance Criteria.

APPEALS

Following the original publication of the results, 14/376 registered participants (3.7%, a decrease from 5.9% in 2024) appealed against the scores they received.

Out of the 14 appeals, one was identified as a request for further information only, two were upheld as requests for assessment of data that was originally omitted for technical reasons and three were requests for reassessment due to an incorrect genome built selected upon submission.

The remaining eight were rejected as request for assessment of new files (BED or VCF) or request for removal from assessment files that were originally not highlighted as 'educational'.

Table below summarises the appeal types:

Appeal type	Count	Appeal decision	Further comments
Not an appeal	1	N/A	N/A
Request for removal of submission from assessment	3	Rejected	We cannot remove the submission from assessment/ mark is as 'educational' AFTER the performance have been assessed.

[1] EMQN see https://www.emqn.org/participating-in-eqa/laboratory-performance-criteria/, GenQA: https://genqa.org/performance-monitoring).

Appeal type	Count	Appeal decision	Further comments
Incorrect BED file uploaded	2	Rejected	As indicated in the pre-appeals scheme summary report (page 4), results will not be reevaluated in cases when the incorrect file was originally submitted
Incorrect VCF file uploaded	3	Rejected	As indicated in the pre-appeals scheme summary report (page 4), results will not be reevaluated in cases when the incorrect file was originally submitted
Missing assessment	2	Upheld	Missing file reassessed.
Incorrect reference genome selected	3	Upheld	Reference genome updated. Please note that in future EQAs, we will no longer be able to accommodate re-analysis due to incorrect genome build selection.

As we have highlighted in the pre-appeals summary report, we are unable to reevaluate your results in cases when the incorrect file was originally submitted (e.g. incorrect BED file), therefore all appeals requesting such analysis have been rejected.

All appeals were considered anonymously by the technical experts and the outcome can be found via your website EQA provider's account as a part of your Individual Laboratory Report (ILR).

REPORTING REQUIREMENTS

This was a technical EQA scheme and participants were not expected to submit a clinical report. Participants were expected to submit:

- A **technical survey** describing the sequencing approach, bioinformatics pipeline, and internally defined quality thresholds (submitted online to the EQA Euformatics website).
- **VCF file** of detected variants (SNVs and small indels) mapped to **hg38/GRCh38** or **hg19/GRCh37**- this list should be compiled after QC and region of interest (ROI) filtering.
    - **Variant Calling Format (VCF) version 4.x files should be submitted (VCF standard format defined at https://samtools.github.io/hts-specs/).** Additional requirements on top of the basic VCF format requirements:
    - ONLY variants with the filter set to '.' or to 'PASS' in the 7th column will be assessed. Any other text in the 7th column will exclude the variant from assessment.
    - **Any variants filtered out will not be assessed.**
- **BED file** defining (fully matching) the genomic co-ordinates of the ROI analysed. An optional second BED file can be uploaded to further limit the regions where variant calls should be assessed (the "clinical target"). BED files should have a minimum of: **Chr, Start, End**. A fourth name field is optional. Any overlapping regions will be merged. Please see the BED standard format defined at Ensembl https://www.ensembl.org/info/website/upload/bed.html for format specifications. Illumina manifest files need to be converted to the BED format.

Both coding and non-coding regions were assessed. SNV/indel submissions were only assessed for SNVs and indels (≤50bp) even if they may contain copy number variants (CNVs) >50bp). At present we cannot accommodate pipelines which look for low level mosaicism.

RESULTS SUMMARY

Four documents[2] have been generated for EACH laboratory. These reports are accessible from your EQA provider's website account. The documents are as follows:

- **EQA Summary report** (this document) summarising all the results.
- An individual **Data quality report** containing selected quality metrics from the submitted FASTQ, BAM, and VCF files, benchmarked against the distribution of the same metric from other laboratories.
- An Individual **Variant consensus analysis report** containing a comparison of the variants reported by your laboratory against a list of consensus variants. Variants are classified as: concordant with the consensus ("Agree"); not concordant with the consensus ("Disagree"), not reported by your laboratory ("Missing"); reported by your laboratory but not present in the consensus ("Extra"); not subject to assessment for the reason stated e.g. uncertain consensus ("Not Assessed").

  Please note: this report includes the F-score which was used in assessing your laboratory's performance.
- **Individual Laboratory Report (ILR)** summarising the overall Performance status of your laboratory for this EQA.
- Performance assessment is carried out for data sets specified by participants. Participants can exclude data sets from performance assessment (for example, for pipelines in development) however at least one submission must be provided for performance assessment. The ILR records which data sets were subject to performance assessment.

[2] One report per submitted data set, i.e. if three data sets have been submitted, all of the results were collated into single document with separate headings/tabs.

- Participants are assessed on the region(s) of the genome that they have submitted for analysis and do not receive information on variants outside these regions.

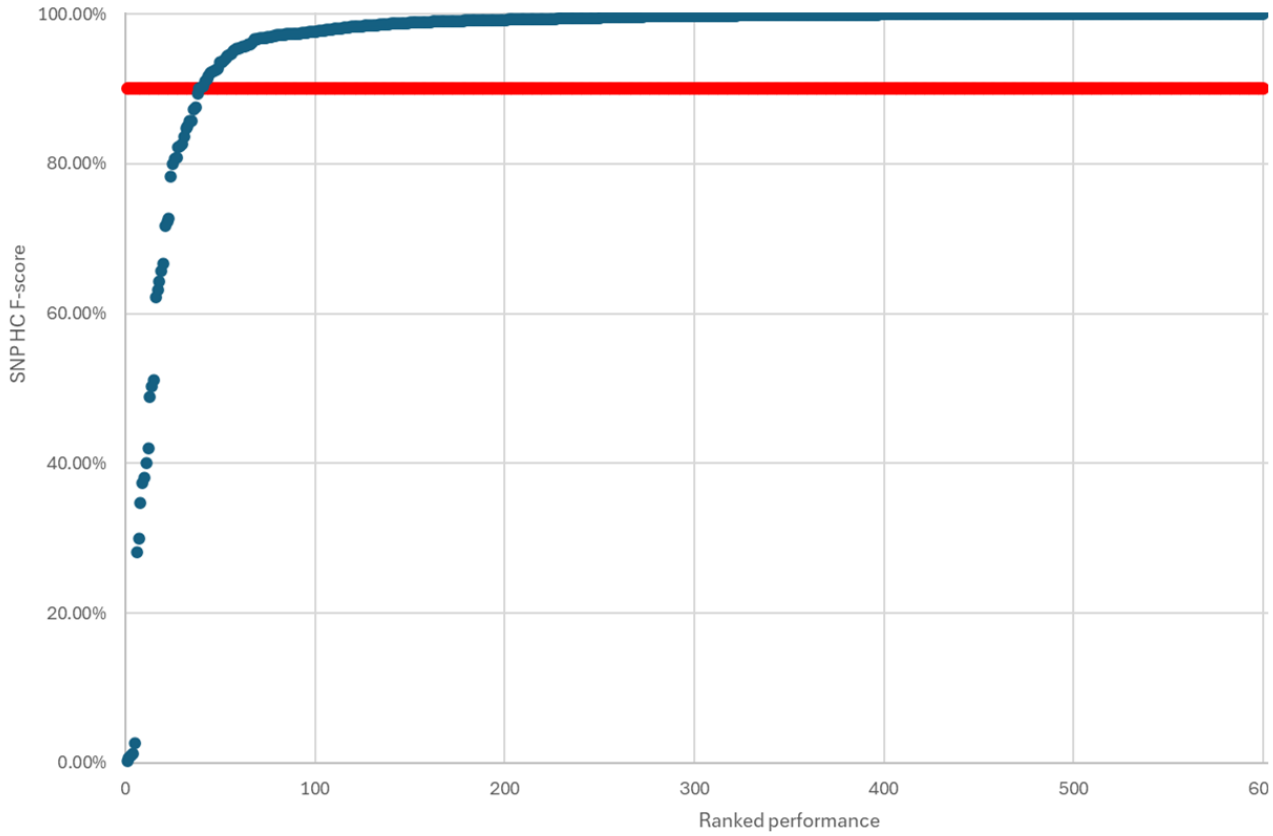
2025 EQA PERFORMANCE

This year, 376 laboratories successfully submitted 616 different datasets (an increase from 356 laboratories submitting 585 datasets in 2024). Participants asked for 76/616 submissions to be excluded from the assessment (12.3%, compared with 9.2% in 2024).

510/540 participants (94.5%) submitted data that had an F-Score above 90% for SNVs in 'high-confidence' regions. It is also worth noting that out of the 540 analysed results, 440/540 (81.5%) achieved the F-score of above 99% for SNVs within the high-confidence regions of the genome. This is an improvement from 77.6% in 2024 and 74.6% in 2023.

The improvement in the number of poor performing laboratories is encouraging; Only 5.5% of laboratories received a 'Poor Performance' (PP) status as indicated on their Individual Laboratory Report (ILR). This is lower than last year's 8% of PP. 3/540 (0.56%) of results could not be assessed as they did not contain SNVs in the high confidence region.

Figure 1: *F-score results achieved by the participants of the 2025 NGS (germline SNVs and Indels) EQA. The submissions below the red line (i.e., a score below 90%) are assigned Poor Performance.*



Submission Issues

Most issues identified in the submitted files pertained to VCF (Variant Call Format) files. According to the EBI VCF validator (https://github.com/EBIvariation/vcf-validator), only 39% of the submitted VCF files were considered valid according to VCF specification (compared with 33% in 2024). However, most validation errors do not impact the variant concordance analysis.

Some submissions contained records, where a non-existent allele was referenced from the genotype (GT) field. Such positions were excluded from the analysis.

Some submissions contained records where an unspecified allele, such as <NON_REF>, was referenced from the genotype (GT) field. While technically not an error, these genotype calls cannot be evaluated, and the affected positions were excluded from the analysis.

Variant calling and genes analysed

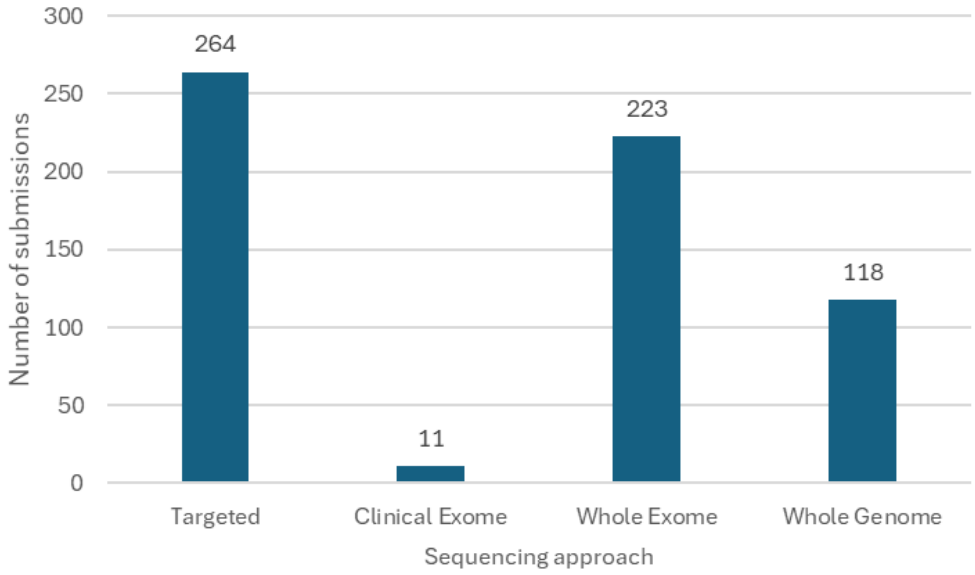
Up to three separate submissions were permitted from participants with 171/376 laboratories (45.5%) submitting more than one dataset (comparable with last year's 44.9%).

330/616 (53.6%) laboratories validate their results using an orthogonal method (Sanger sequencing or MLPA). This signifies a further decrease in laboratories seeking validation of their NGS results from 56.8% in 2024 and 66.3% in 2023.

Most submissions were targeted sequencing panels (264/616, 42.9%) followed by whole exome sequencing (223/616, 36.2%) (Figure 2).

We encourage laboratories who submitted panels to submit multiple datasets covering different genomic regions, to ensure assessment of a broader region of variants.

Figure 2: *Sequencing approaches for submissions to the 2025 NGS germline SNVs and indels EQA.*



Results mapped either to hg19/GRCh37 or hg38/GRCh38 were permitted; This year **for the first time** the number of submissions mapped to GRCh38 (328/616 (53.2%) exceeded the number of submissions mapped to GRCh37 (a steady increase from 42.9% in 2024, 33.7% and in 2023) (Figure 3).

Figure 3: *Reference genome used for submissions to the 2025 NGS germline SNVs and indels EQA.*

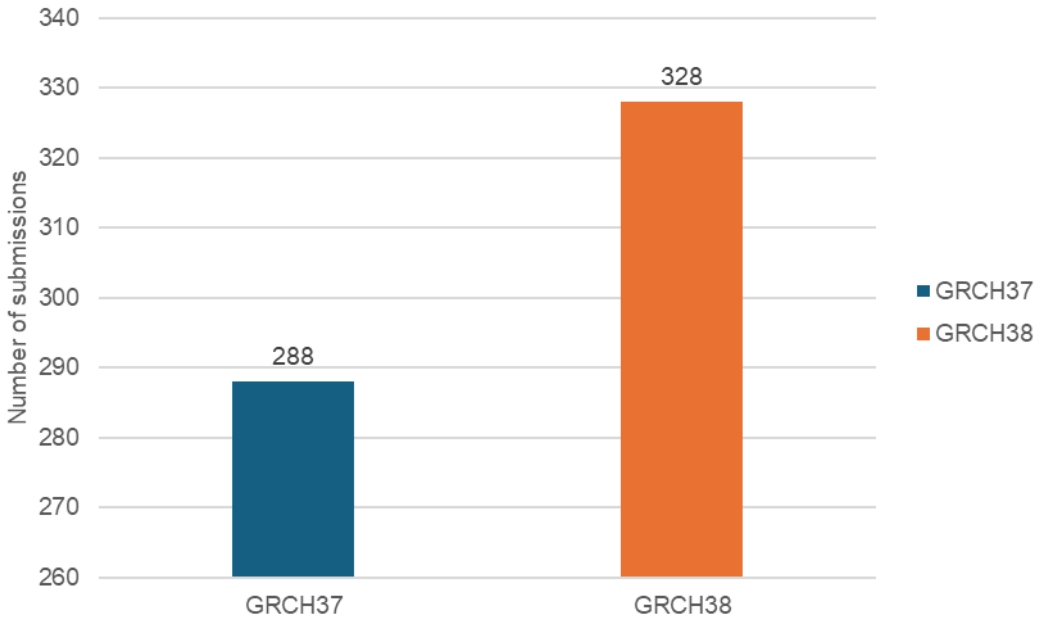
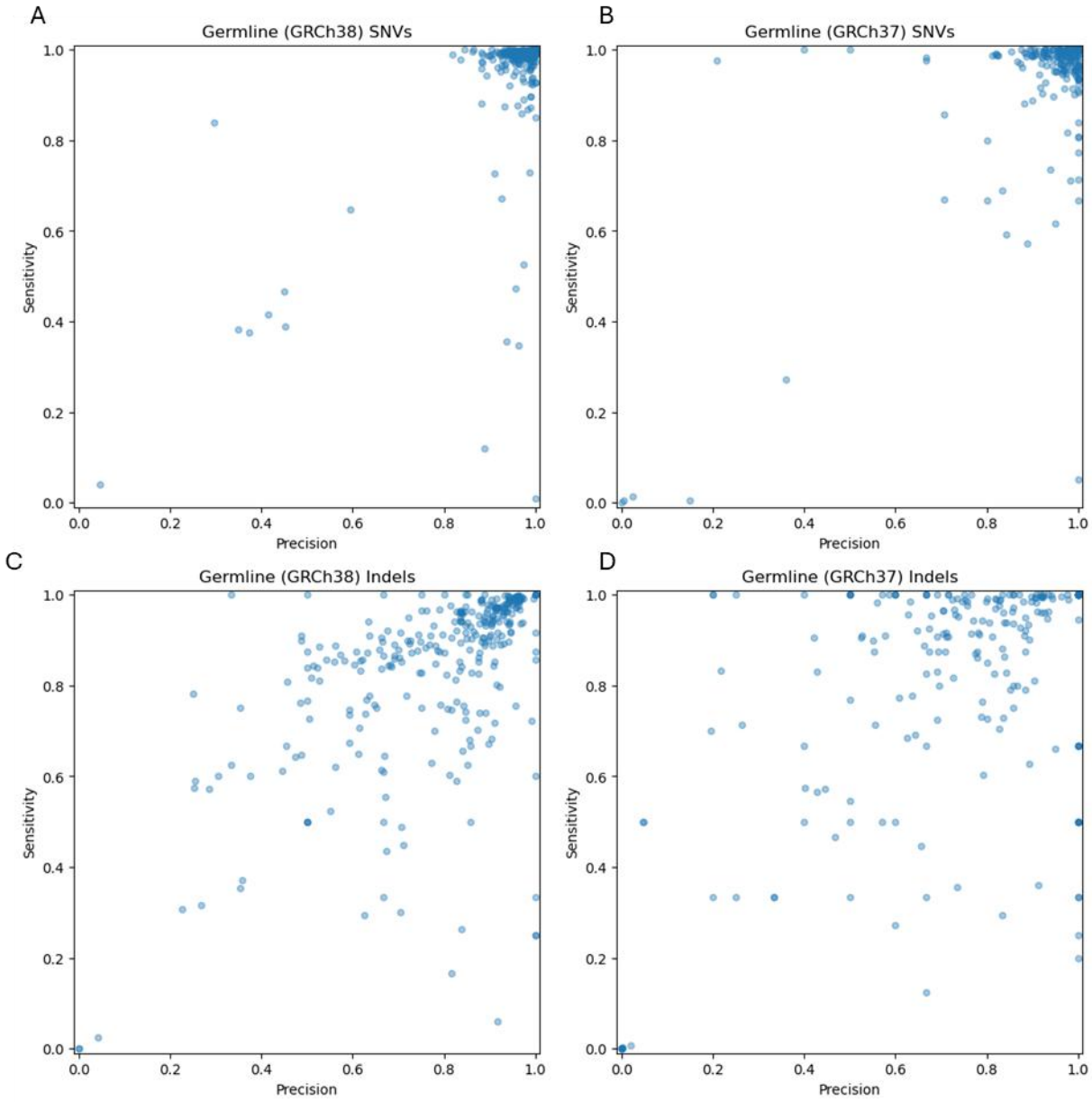


Figure 4 shows the sensitivity and precision for submissions using GRCh38 and GRCh37, respectively, separated into indels and SNVs.

Figure 4: *Sensitivity and precision of variant calling A) Germline GRCh38 SNVs, B) Germline GRCh37 SNVs, C) Germline GRCh38 indels and D) Germline GRCh37 indels.*



Technical data analysis

Laboratory practice

Most submissions (87.3%) use one of the Illumina NGS platforms with NovaSeq 6000 and NextSeq 500 remaining the most popular sequencing platforms, accounting for 127/616 (20.6%, decreased from 27.8% of all submissions in 2024) and 113/616 (18.3%, decreased from 19.9% in 2024) submissions, respectively. This year seven laboratories opted to submit long read sequencing results, four more than in previous years.

For the detailed breakdown of platforms used please see Table 2.

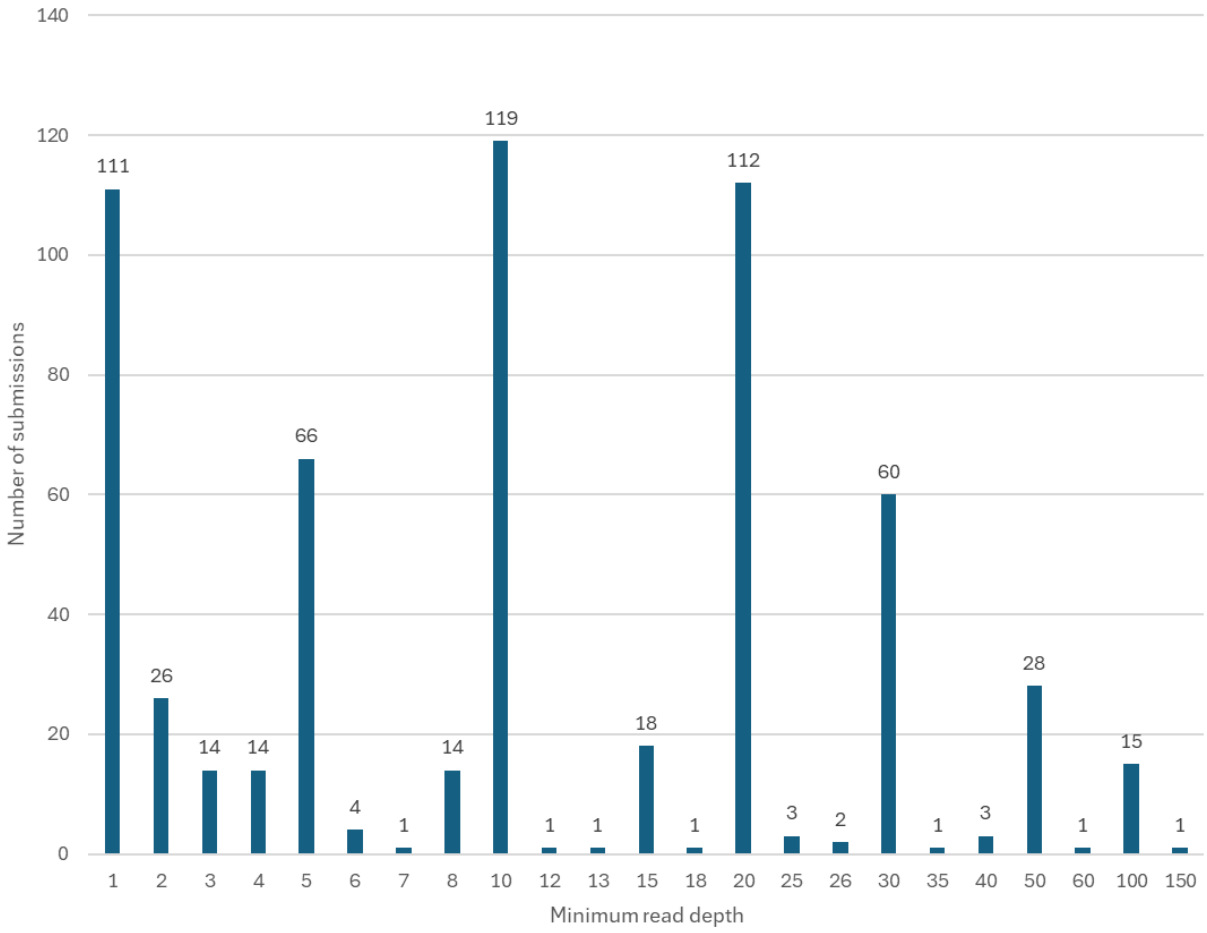
Table 2. *Sequencing platforms used by the participants.*

Illumina	**538**
NovaSeq 6000	127
NextSeq 500	113
NovaSeq X Plus	97
NextSeq 2000	81
MiSeq	71
NovaSeq X	24
NextSeq 1000	16
MiniSeq	8
iSEQ 100	1
Life Technologies	**31**
Ion S5	31
MGI	**22**
DNBSEQ-T7	12
DNBSEQ-G400	6
DNBSEQ-G50	4
Element Biosciences	**15**
AVITI	15
Oxford Nanopore	**5**
PromethION	5
Other	**3**
Other	3
Pacific Biosciences	**2**
PacBio Revio	2

Most participants opt to use commercially available capture kits in library preparation (85.9%, an increase from 79.3% in 2024). Appendix E summarises the different commercial kits used by participants in the NGS (Germline SNVs and Indels) EQA which accounts for over 95% of submissions.

Figure 6 shows the distribution of minimum reportable coverage used to call a variant. For 58.6% of the submissions (361/616; an increase from 45.5% in 2024), participants stated that they would report a diagnostic test result if it met their minimum coverage threshold.

Figure 6: *Distribution of minimum sequencing coverage for reporting variants applied by different participants for submissions. Coverage is an average number of reads that align to known reference bases (also known as read depth).*



Bioinformatics analysis

In-house bioinformatics was used by participants for 38.5% (237/616) of submissions (a decrease from 55.1% in 2024), with 3.9% (24/616) outsourcing the analysis (a large decrease from 9% in 2023). 24.2% (149/616) of participants used the platform provided (an increase from 12.6% in 2024) and 33.4% (206/616) commercial pipelines (an increase from 18.5% in 2024), respectively.

Not all submissions specified the aligner or variant caller used, but from those that did the number of submissions using Burrows-Wheeler Aligners (BWA) was 79.8% (further decrease from 85% in 2024). Following this, over 51% of participants reported using the GATK Haplotype Caller.

CONFIDENTIALITY

Details of the confidentiality policies of each provider can be found:

- EMQN: https://www.emqn.org/participating-in-eqa/terms-conditions/ - In section 4.6 Performance evaluation.
- GenQA: https://genqa.org/confidentiality.php

SUBCONTRACTED ACTIVITIES

Your EQA provider does not subcontract activities such as EQA planning, evaluation of performance or the authorization of reports. However, some activities are subcontracted, for example the preparation of materials may be performed by suitably accredited providers.

FINAL COMMENTS

The EQA service is designed to educate and support the improvement of standards across the field. We hope you found participating in this EQA both valuable and beneficial. Please note that a separate EQA is available for the NGS detection of germline copy-number variants (CNVs) (ongoing for 2025).

We would like to thank all participants for their hard work, prompt submission of results, and co-operation during this exercise.

Registration for the 2026 EQA is now open on the GenQA and EMQN websites, and we look forward to your participation in the upcoming round.

Kind regards,

Dr Simon Patton
CEO
EMQN

Professor Sandi Deans
Director
GenQA

REFERENCES

1 ISO15189:2022(en), Medical laboratories — Requirements for quality and competence. https://www.iso.org/obp/ui/#iso:std:iso:15189:ed-4:v1:en4.

2. Deans ZC, *et al*. Recommendations for reporting results of diagnostic genomic testing. Eur J Hum Genet. 2022 Apr 1. doi: 10.1038/s41431-022-01091-0. Epub ahead of print. PMID: 35361922.

AUTHORISATION/APPROVAL

This document has been authorised/approved on behalf of EMQN by: Dr Simon Patton on 30/04/2026

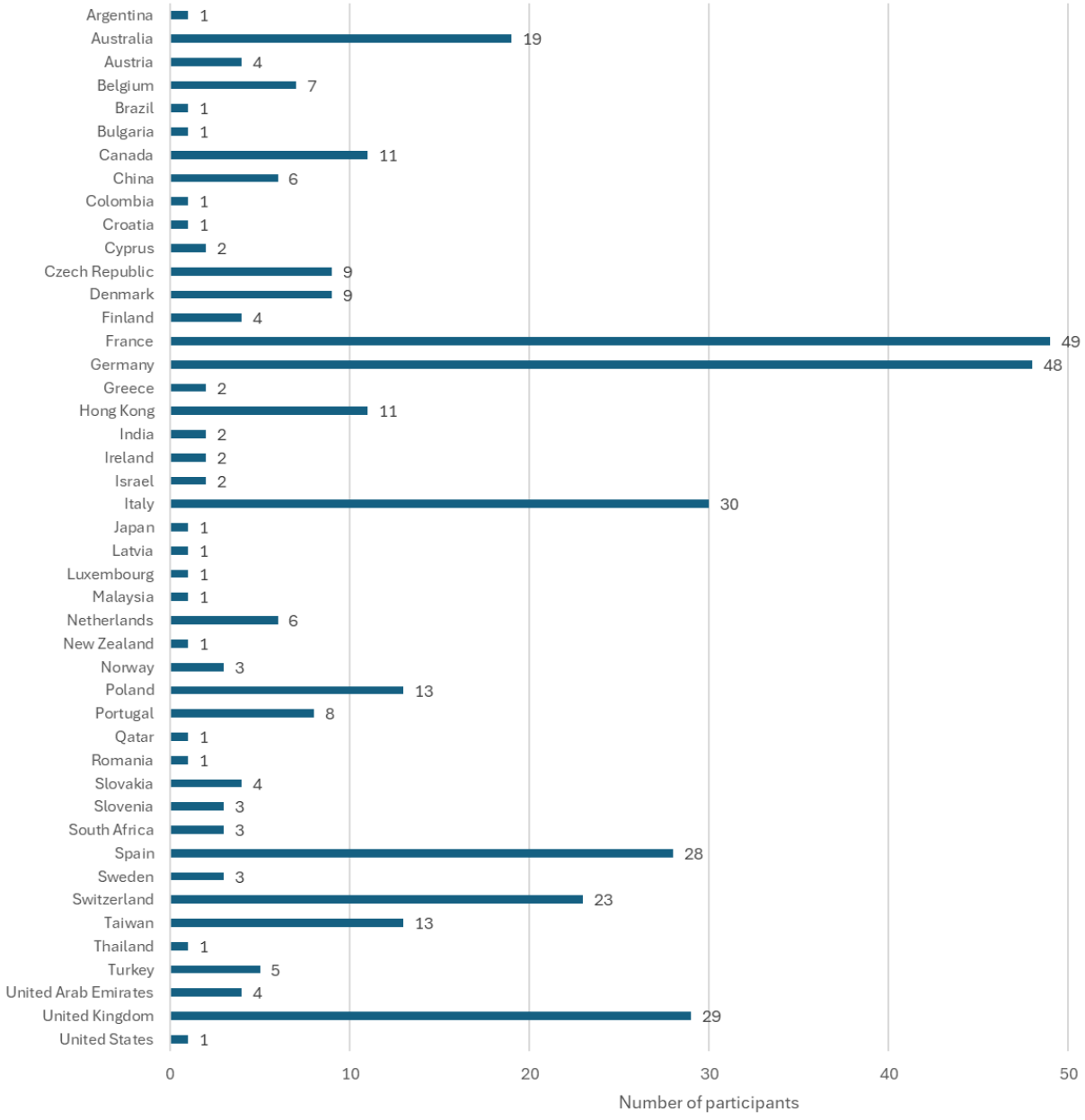
Chief Executive, EMQN CIC

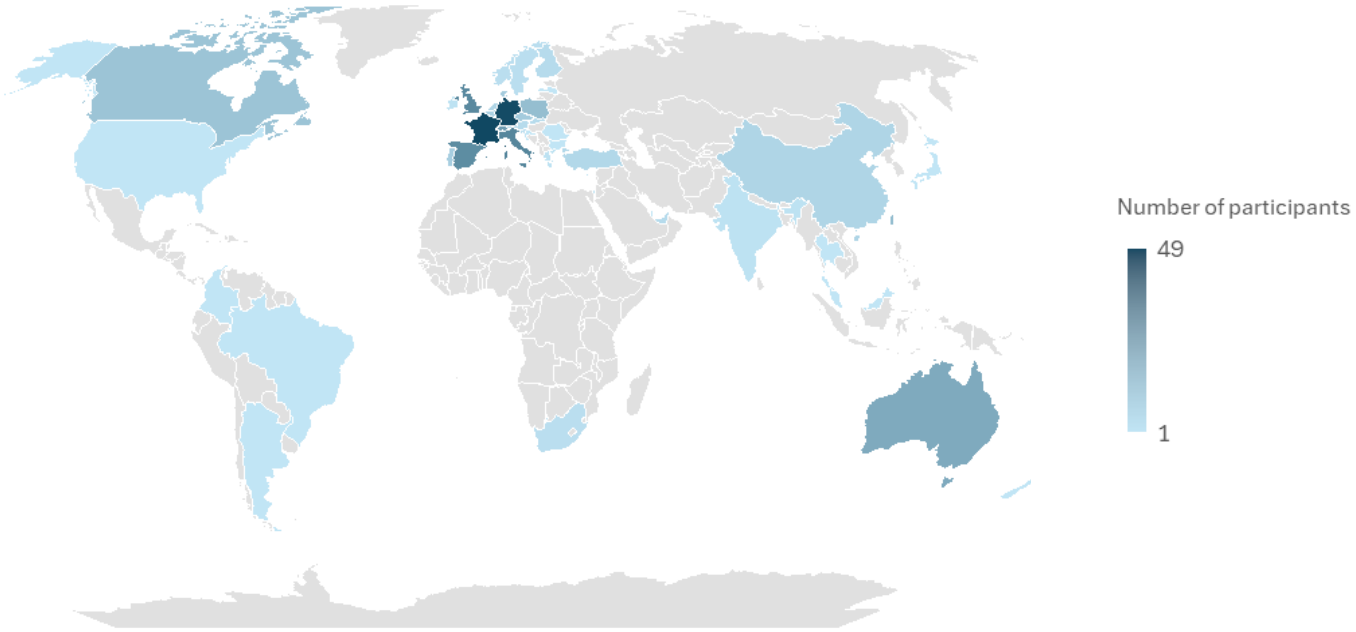
APPENDIX A – PARTICIPATION

Table 3: *Number of participating laboratories*

Number of registrations	**384**
Number of withdrawals	**8**
Number of laboratories that did not submit results	**0**
Total number participating laboratories	**376**
Number of laboratories submitting more than one set of results	**171**

Figure 7: *Participating countries*





APPENDIX B - SAMPLES PROVIDED AND GENERATING PARTICIPANT CONSENSUS

The participants received one genomic DNA sample (NA24385, https://www.coriell.org/0/Sections/Search/Sample_Detail.aspx?Ref=NA24385&Product=DNA) extracted from a single large, homogenized growth of B lymphoblastoid cell lines and purchased from Coriell Cell Repositories.

A participant consensus variant set was established separately for GRCh37 and GRCh38 submissions. For both submissions the consensus required at least five submissions to target each variant position with >2/3 agreeing on the genotype call. Where there was less than 1/3 support for a variant call, the consensus was taken to be the absence of a variant; where there was more than 2/3 support for a variant call, the consensus was taken to be presence of a variant. For positions where the agreement was between 1/3 and 2/3, the consensus was considered to be inconclusive.

The mitochondrial genome is not currently assessed as part of this EQA due to complexities with heteroplasmy and contig naming in the VCF.

This year, the EQA utilised the well-characterised GIAB reference sample HG002 (https://www.nist.gov/programs-projects/genome-bottle), which enabled a direct comparison between the participant consensus genotype and the established benchmark sequence. During analysis, 673 apparent false negatives were excluded from performance scoring, as further investigation demonstrated that at least 80% of these were in fact correctly identified variants that only a minority of participants were able to call. In many of these instances, the DeepVariant caller detected the variants whereas the DRAGEN pipeline did not. This is consistent with expectations, as DeepVariant is a machine-learning–based variant caller trained specifically on HG002, and therefore exhibits enhanced sensitivity for this sample.

- The participant consensus variant set was computed separately for SNVs and indels.
- Any duplicate submissions were excluded from the consensus. In case of multiple submissions with identical VCF files but different BED files, only the submission with the largest BED file was kept for generating the consensus.
- Submissions with no SNVs/indels called within the evaluated regions were excluded from the SNV/indel participant consensus, respectively.
- For many submissions, the genomic regions of interest indicated by the BED file accompanying the laboratory submission included genomic regions where no variant calling was performed. To counteract this and allow us to build the Germline consensus variant sets, we have excluded submissions based on variant density after normalisation.
- Participants can choose to exclude submissions from the consensus and performance assessment e.g. for new pipelines under evaluation, providing there was still at least one submission left to analyse.

The individual participant submissions were normalised and evaluated against the participant consensus following the Global Alliance for Genomics and Health's (GA4GH) guidelines (https://www.ga4gh.org/).

Unfortunately, due to the commercial nature of the DNA sample, it is not possible to provide the full consensus sequence to participants.

APPENDIX C – INDIVIDUAL LABORATORY FEEDBACK

Data quality and variant analysis are reported separately in the following documents: (a) **Data Quality Report** (PDF), and (b) **Variant Consensus Analysis Report** (Excel). These reports, plus the **Individual Laboratory Report** (ILR) are specific to YOUR laboratory and are designed to give feedback that you can use to help improve your processes. The following pages provide an explanation of the content of each report, both of which can be downloaded from your respective EQA organiser's scheme webpage.

Data quality report [3]

The Data Quality Report contains our assessment of a range of selected quality metrics from the data submitted by your laboratory for the three file types: FASTQ, BAM, and VCF. We have also provided a brief glossary describing the applied quality metrics and an explanation about why it is important to consider them for validation and ongoing QC of your NGS processes.

Variant consensus analysis report [4]

Reported variants were normalised and compared against the consensus variants computed from participant submissions (EQA Participant consensus variant set). Following the GA4GH's recommendations we have stratified the results by SNVs and Indels.

Classification:

- **Agree** – participant's variant that matched the participants' consensus (True positive)
- **Extra** – participant's variant which was not present in the participants' consensus (False positive)
- **Missing** – participant has missed a variant present in the participants' consensus genotype (False negative)
- **Disagree** – participant's variant did not match the participants' consensus (False positive, false negative)
- **Not Assessed** – participant's variant could not be assessed against the consensus (e.g. uncertain consensus)

Figure 8: *An extract from the Variant consensus analysis report. This data describes each laboratory's submitted variants (VCF file) cross-referenced against the consensus variants that should have been detected based on each laboratory's region of interest (BED file).*

	A	B	C	D	E	F	G	H	I
1	Analysis set:	2025 Germline (GRCh38)							
2	Reference genome:	GRCh38							
3									
4	Region:	All		High confidence					
5	Type:	SNV	Indel	SNV	Indel				
6	True positives:	534	32	358	6				
7	False positives:	6	34	0	1				
8	False negatives:	24	10	10	0				
9	Sensitivity:	95.70%	76.19%	97.28%	100.00%				
10	Precision:	98.89%	48.48%	100.00%	85.71%				
11	F-Score:	97.27%	59.26%	**98.62%**	92.31%				
12									
13	Variant position	Type	Gene	Submitted genotype	EQA genotype	EQA consensus ratio	Classification	Notes	Region
14	1:2228788	Indel	SKI	C/CGGGGGGG			Extra		Low confidence
15	1:47417347	SNV	FOXE3		C/C	78/82	Missing		Low confidence
16	1:47417352	SNV	FOXE3		G/G	76/80	Missing		Low confidence
17	1:74436055	Indel	FPGT-TNNI3K		C/C	63/109	Not Assessed	Uncertain consensus	Low confidence
18	1:74436055	Indel	FPGT-TNNI3K	CTT/C			Extra		Low confidence
19	1:74540343	SNV	FPGT-TNNI3K	G/G	G/G	95/99	Agree		High confidence

[3] Each laboratory's report will be called Data quality report (NGS EQA 2025) Germline

[4] Each laboratory's report will be called Variant consensus analysis report (NGS EQA 2025) Germline

APPENDIX D – GLOSSARY OF QUALITY METRICS TERMS

Variant call assessment

- **Sensitivity**

  Proportion of actual positives that are correctly identified as such. Defined as Sensitivity = TP / (TP + FN), where TP is the number of true positives and FN is the number of false negatives. Also known as recall.

- **Precision**

  Proportion of actual positives among all reported positives. Defined as Precision = TP / (TP + FP), where TP is the number of true positives and FP is the number of false positives.

- **F-Score**

  Harmonic mean of sensitivity and precision. Defined as F-Score = 2 × Sensitivity × Precision / (Sensitivity + Precision). Also known as the F_1 score.

FASTQ file

- **Base quality (Phred quality score)**

  Base quality score (Phred quality score) is a measure of the quality of the identification of the nucleotide bases generated by automated DNA sequencing. Phred quality scores are assigned to each nucleotide base call in automated sequencer traces and are used for assessment of sequence quality, recognition and removal of low-quality sequence, and determination of accurate consensus sequences.

  - **Consideration for validation:** An acceptable raw base call quality score threshold should be established during validation. Informatics filters should be established to eliminate any reads with raw base calls lower than the established quality score. The tolerance for low base quality is higher in long-read than in short read technologies because the sequence length and accuracy at the base level is less critical for alignment.
  - **Consideration for ongoing quality control:** The quality of the base calling signal should be monitored over time and among used instruments by examining the base quality across reads for each sequencing run.

BAM file

- **Uniformity (%)**

  The percentage of bases on target covered at 0.1 x median coverage.

- **Reads on/off target (%)**

  The fraction of reads mapped to the target region as a function of the total amount of (mapped and unmapped) reads. This is a raw, or minimum value for the amount of informative reads, while the read enrichment disregards unmapped reads and provides a filtered, or maximum value for the amount of informative reads. The fraction of reads on the target region is equal to 100 minus the fraction of off target reads.

  - **Consideration for validation:** Following the on or off target fraction will give an idea about the target specificity. A high off target percentage means low specificity of the run. The off target depicts the non reliable reads and should be established during validation.
  - **Consideration for ongoing quality control:** It is important to keep a record of the fraction of reads on the target region because it provides confidence in the validity of results in the region of study and will make sure that no regions have been omitted in the test.

- **Error rate on target**

  The proportion of mismatched bases on target.

- **Insert size**

  Number of bases between paired-end reads.

VCF file

- **Ti/Tv ratio**

  This is the ratio of the number of transitions (substitutions between purines or between pyrimidines) to the number of transversions (substitutions between a purine and a pyrimidine) in the variants called in an experiment. The Ti/Tv ratio is close to 2.1 for the whole human genome and closer to 2.9 in exons, and depends among other on the GC content of the DNA fragment considered. It is therefore specific to the target area in a normal genome. Cancer genomes show aberrant evolution and can have quite different Ti/Tv ratios.

  - **Consideration for validation:** The ratio of transitions to transversions (Ti/Tv) should be established separately for each target capture protocol and compared to published values.
  - **Consideration for ongoing quality control:** The Ti/Tv ratio should be monitored with every sample to detect a change in test performance. When the Ti/Tv ratio is lower or higher than expected, this is an indication that the quality of base calls was low, and potentially contains errors.

- **Het:hom ratio (SNV)**

  Usually, the ratio of Het/Hom is expected to be 2:1. As homozygous cases would be either due to mendelian transmission due to inbreeding-like effects in a small, finite population or due to some founder effects. If that ratio is off (which can be established by comparing your results with the consensus of other participants, as shown in the boxplot), that indicates that there might be an issue with sequencing quality (e.g., the problem might be that the variant calling pipeline was not set up correctly).

APPENDIX E – CAPTURE KITS USED

Table 4. *Commercial capture kits as submitted by participants of the 2025 NGS Germline SNVs and indels EQA. Table excludes in house/custom kits. Kits that were described in a way that made it impossible to identify them were also excluded.*

Kit	Number of submissions
4Bases	
4bases ClinEx Pro	1
Agilent	
SureSelect Clinical Research Exome V4	4
SureSelect XT Custom Enrichment	5
HaloPlex Custom Kit	1
SureSelect Custom 1-499kb	4
SureSelect Custom Constitutional Panel 17Mb	1
SureSelect Focused Exome	2
SureSelect Human All Exon V6	1
SureSelect Human All Exon V7	2
Sureselect Human All Exon V8	2
SureSelect PreCap Custom tier2	1
SureSelect QXT	5
SureSelect XT HS	42
SureSelect XT Low Input Reagent Kit	8
TruRiskÂ® Vers.4.2	2
BGI	
Hereditary tumor gene testing kit	1
Universal Exome	1
Whole Genome Library Preparation Set	1
Devyser	
BRCA	1
FH	1
GeneReach Biotechnology	
SATLite DNA Prep	1
Health in Code	
Hereditary Plus OncoKitDx V2	1
IDT	
96rxn xGEN-lockdown-reagents	1
EZ UNI	2
xGen DNA library prep EZ	3
xGen DNA Lib Prep MC UNI 96 rxn	2
xGen Exome Hyb Panel v2, custom oligo pools	1
xGen Exome Research Panel v2.0	2
xGen Exome Research Panel v1.0	1

Illumina	
AmpliSeq Custom DNA Panel for Illumina	2
EBM Panel	2
Illumina DNA PCR-Free Prep	52
Illumina DNA Prep with Enrichment	27
Illumina DNA prep with exome 2.0 plus enrichment	2
Illumina DNA Prep with Exome 2.5 Enrichment	24
Illumina Nextera DNA Flex	1
JMML	1
Lipidpanel	1
Nextera Flex for Enrichment	4
Nextera Rapid Capture Custom Enrichment kit	1
Nextera XT Index Kit v2	1
NovaSeq 6000 S4 Reagent Kit (300 cycles)	1
TrueSeq DNA PCR free	16
TruSeq Nano DNA	6
TruSight Cancer	1
TruSight Custom	1
TruSight Hereditary Cancer Panel	9
TruSight Oncology 500	2
TruSight One Sequencing Panel	1
Integrated DNA Technologies	
xGen Exome Research Panel v1.0	1
MGI	
MGIEasy FS PCR-Free DNA Library Prep Set	3
Nanpore	
Ligation Sequencing Kit	1
New England Biolabs	
NEBNext Ultra II FS DNA Library Prep Kit (WGS)	1
NEBNext Ultra II FS DNA Library Prep Kit for Illumina	1
NEBNext Ultra II FS DNA PCR-free Library Prep Kit	3
Nonacus Ltd	
Cell3 Target	6
Nonacus Ltd Cell3 Target	2
Novogene Co. Ltd	
NGS DNA Library Prep Kit	4
Nukendo	
Illumina DNA Prep with Enrichment	1
ONT	
Ligation Sequencing Kit XL	1
Oxfor Nanopore Technology	
Ligation Sequencing Kit	3

PacBio	
PacBio SMRTbell prep kit 3.0	1
SMRTbell Template Prep Kit (WGS)	1
Paragon Genomics	
CleanPlexÂ® Comprehensive Hereditary Cancer Panel	1
Qiagen	
QiaSeq Custom Targeted DNA Pro Panel	1
QIAseq Human Breast Cancer Panel	2
QiaSeq Targeted DNA Kit	7
QIAseq xHyb Human Hybrid Capture Panel	1
RefPleks	
MEFV NGS Panel	1
Revvity	
Nextflex library preparation module	1
Roche KAPA	
DysliSeq v7	1
HemostaseV4	1
Kapa Biosystems + Roche HyperPlu	1
KAPA EvoPlus V2 Kit	8
Kapa Hyper Plus with Roche Hyper capture (custom panel)	15
Kapa HyperChoice	7
KAPA HyperExome	16
KAPA HyperExome V2 EvoPlus Kit+KAPA HyperChoice MAX	7
KAPA HyperExplore	1
Kapa Hyperplus with IDT custom panel	1
Kapa Hyperplus with Roche Hyper capture (custom panel)	4
KAPA HyperPrep kit	4
Kapa Library Preparation	1
Other Roche Kapa	2
Roche KAPA EvoPlus Kit+KAPA HyperChoice MAX 3Mb T3 probes	1
Roche KAPA EvoPlus with Roche KAPA HyperChoice MAX 3Mb T3	1
Roche KAPA EvoPlus with Roche KAPA HyperExome probes	4
Sistemas Genomicos	
HubExomePlusPanel-GeneSGKit	2
Sophia Genetics	
CES	2
Custom HCS	4
HCS1.1	2
ID Seq	1
SOPHiA Custom Solution CAUT_A	1
Sophia Custom Solution CSLA_A	1
Whole Exome Solution	3

Hereditary Cancer Solution v2.0	2
SOPHiA Custom Bundle Solution	1
Thermo Fisher Scientific	
Carrier seq ECS	1
CEN	1
Custom Panel	24
DSDv1.1	1
Dysplasie squelettique	1
Exome 2.0 + Comprehensive Exome Spike-in	30
Exome Core + RefSeq + mtDNA	2
Genetikum Panel	1
HCC_v1	1
Hieff NGS C102P2 OnePot Pro DNA Library Prep Kit for MGI	2
HousePanel	1
Human Core Exome + RefSeq	9
Human Exome 2.0 + Comprehensive Exome + mtDNA	17
Ion AmpliSeq BRCA1 and BRCA2 Panel	1
Ion AmpliSeq Exome RDY Kit	1
Ion AmpliSeqâ„¢ Kit for Chef DL8 "Custom"	3
Ion AmpliSeqâ„¢Library Kit	12
LIBRARY PREPARATION EF KIT	4
Library Preparation Kit	3
NS_157v2	2
Oncomine BRCA Expanded	1
Oncomine BRCA Research Assay	2
PCR free Genome	1
Precision Prep and Enrichment Dx Kit	5
SYW3	1
ThermoFisher Scientific Ion AmpliSeqâ„¢Library Plus Kit	3
ThermoFisher Scientific Ion torrent smMIP in house Lib prep	1
Twist Bioscience	
Twist Alliance CNTG Hereditary Oncology Panel	2
Twist Alliance VCGS exome	5
Twist Bioscience CP2	1
Twist Bioscience Superexome	1
Twist Bioscience VCP1	1
Twist Biosciences Comprehensive Exome V2 + spike-in	1
Twist Biosciences TwistCMH_v4bis	1
Twist Biosciences TwistOnco_v2	1
Twist Biosciences TwistTubulo_v3	1
Twist Comprehensive Exome	27
Twist Core Exome	2

Twist custom	3
Twist Exome 2.0	28
Twist Exome 2.5	5
Twist Library Preparation EF kit	4
Twist Precision Prep and Enrichment Dx Kit	1
Twistpanel_v2c	1
Watchmaker	
Watchmaker	5
Yeasen Biotech Co., Ltd	**2**

APPENDIX F - AMENDMENTS TO 2025 SUMMARY EQA REPORT

This EQA report was originally issued as Pre-appeals version 1 on 20/02/2026.

<table>
<tr><th>Section</th><th>Amendments made</th></tr>
<tr><td>TITLE</td><td>Removed: Pre-appeals scheme summary report

Replaced with: Post-appeals FINAL summary report</td></tr>
<tr><td>APPEALS</td><td>Removed:

The marking is subject to appeal. If you wish to appeal against any deduction, then please do so by 13th March 2026 (23:59h GMT). Laboratories can submit an appeal by opening the Appeals Submission Form from the appropriate EQA webpage and completing the online form with details of your appeal.

All appeals will be considered anonymously by the NGS SAG and the outcome of your appeal will be available via your website account. You will be notified by email when the appeals outcome and the final scores are published, and the EQA Summary Report will be amended if required and released as the Final version.

PLEASE NOTE: if you have any questions regarding our assessment of your data, please also submit those as an appeal.

We are unable to reevaluate your results in cases when the incorrect file was originally submitted (e.g. incorrect BED file).

Replaced with:

Following the original publication of the results, 14/376 registered participants (3.7%, a decrease from 5.9% in 2024) appealed against the scores they received.

Out of the 14 appeals, one was identified as a request for further information only, two were upheld as requests for assessment of data that was originally omitted for technical reasons and three were requests for reassessment due to an incorrect genome built selected upon submission.

The remaining eight were rejected as request for assessment of new files (BED or VCF) or request for removal from assessment files that were originally not highlighted as ‘educational’.

Table below summarises the appeal types:

<table>
<tr><th>Appeal type</th><th>Count</th><th>Appeal decision</th><th>Further comments</th></tr>
<tr><td>Not an appeal</td><td>1</td><td>N/A</td><td>N/A</td></tr>
<tr><td>Request for removal of submission from assessment</td><td>3</td><td>Rejected</td><td>We cannot remove the submission from assessment/ mark is as ‘educational’ AFTER the performance have been assessed.</td></tr>
</table>
</td></tr>
</table>

	Incorrect BED file uploaded	2	Rejected	As indicated in the pre-appeals scheme summary report (page 4), results will not be reevaluated in cases when the incorrect file was originally submitted
	Incorrect VCF file uploaded	3	Rejected	As indicated in the pre-appeals scheme summary report (page 4), results will not be reevaluated in cases when the incorrect file was originally submitted
	Missing assessment	2	Upheld	Missing file reassessed.
	Incorrect reference genome selected	3	Upheld	Reference genome updated. Please note that in future EQAs, we will no longer be able to accommodate re-analysis due to incorrect genome build selection.

As we have highlighted in the pre-appeals summary report, we are unable to reevaluate your results in cases when the incorrect file was originally submitted (e.g. incorrect BED file), therefore all appeals requesting such analysis have been rejected.

All appeals were considered anonymously by the technical experts and the outcome can be found via your website EQA provider's account as a part of your Individual Laboratory Report (ILR).