DNA sequencing (Art.nr 246)

External quality assessment for sequencing of human genomic DNA. Both ability to identify the sequence and report according to HGVS nomenclature are assessed. Two samples (amplicons) and two primer pairs for a total of 4 sequence reactions are distributed to the participants. The samples are to be sequenced according to standard procedures for patient samples.

Frequency: 1/year

Dispatched	2022-11-09
Closing date	2022-12-09

Scheme coordinator Sara Ekvall
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Test material

Label	DNA sequencing 2022:01/A, 2022:01/B and 2022:01/C. **2022:01/A:** Three tubes – PCR-A (sample, yellow tube), Primer A-forward (green tube) and Primer A-reverse (red tube). **2022:01/B:**Three tubes – PCR-B (sample, blue tube), Primer B-forward (white tube) and Primer B-reverse (purple tube). **2022:01/C:** Four digital files – F/R_ab1_sample C_246_2022-01.ab1 and F/R_seq_sample C_246_2022-01.seq)
Description	2022:01/A and 2022:01/B are 20 µL purified PCR-products. The four primer vials contain 20 µL sequencing primer each, with a concentration of 3.0 pmol/µL. 2022:01/C is a digital sample available for download at Equalis Online. The sample is available in two different formats (.ab1 and .seq), with each format consisting of one forward sequence and one reverse sequence. In total, four different files.
Storage	2022:01/A and 2022:01/B: Upon arrival, in refrigerator +2 to +8°C.
Infectious diseases	2022:01/A and 2022:01/B: Not tested for infectious diseases. For safety reasons, the test material should always be handled using the same precautions as an unknown patient sample.

Extra
In this round, in addition to the two PCR-products, we are distributing a digital sample (2022:01/C) for interpretation in your ordinary software program as a test for future development of the EQA scheme.

Instruction for analysis

You are asked to perform sequencing reactions for two PCR products, PCR-A and PCR-B, in both forward (sense) and reverse (antisense) direction. Please use Primer A-forward and Primer A-reverse for sequencing PCR-A, and Primer B-forward and Primer B-reverse for sequencing PCR-B.

In addition, you are asked to download the digital sample (2022:01/C) from Equalis Online (www.equalis.se), import the preferred files (.ab1 or .seq) into your ordinary software program and perform a regular interpretation of the sequence.

Furthermore, you are asked to identify and report any variants found in the coding/translated DNA sequences (named as coding DNA, e.g. c.76A>T, **and** as protein, e.g. p.Lys26Asn) according to HGVS standard nomenclature (http://www.hgvs.org/). Please use reference sequence NM_ 001002236.2 for sample A, NM_ 000519.4 for sample B and NM_000410.3 for sample C.

Registration of results

Please fill in the electronic reply forms, available for download from Equalis Online (www.equalis.se) and send them by e-mail to sara.ekvall@equalis.se.
Registered results may be changed until the closing date.

Reports

A summary of the results is sent to the participants within two months from the closing date.

Round dispatched	2022-11-09
Closing date	2022-12-09
Scheme coordinator	Sara Ekvall
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For the subcontractor	Caroline Adelfalk, Lund

Summary of results

Sample 2022:01/A

This PCR product consisted of a 519 bp long fragment including exon 5 of the human *SERPINA1* gene. It contained one coding variant, a heterozygous substitution of one nucleotide, see table 1.

Table 1. Expected result for sample 2022:01/A.

#	Variant
1	NM_001002236.2:c.710T>C, heterozygous NP_001002236.1:p.(Val237Ala)

Below is the sequence of the PCR product of sample A; primers (green, excluding sequence tags), exon 5 (capital letters) and the coding variant (red). Only variants detected in the protein coding DNA sequence were asked to be identified and reported.

cttccaaaccttcactcacccctggtgatgcccaccttcccctctctccagGCAAATGGGAGAGACCCTTTGAAGTCAA
GGACACCGAGGAAGAGGACTTCCACGTGGACCAGG(T/C)GACCACCGTGAAGGTGCCTATGATGAAGCGTTTAGGCAT
GTTTAACATCCAGCACTGTAAGAAGCTGTCCAGCTGGGTGCTGCTGATGAAATACCTGGGCAATGCCACCGCCATCTTC
TTCCTGCCTGATGAGGGGAAACTACAGCACCTGGAAAATGAACTCACCCACGATATCATCACCAAGTTCCTGGAAAATG
AAGACAGAAGgtgattccccaacctgagggtgaccaagaagctgcccacacctcttagccatgttgggactgaggccca
tcaggactggccagagggctgaggagggtgaaccccacatccctgggtcactgctactctgtataaacttggcttccag
aatgaggccaccactgagttcaggcagcgccatccatgctccatgagga

Sample 2022:01/B

This PCR product consisted of a 407 bp long fragment including exon 2 of the human *HBD* gene. It contained one coding variant, a heterozygous deletion of one nucleotide, see table 2.

Table 2. Expected result for sample 2022:01/B.

#	Variant
1	NM_000519.4:c.179del, heterozygous NP_000510.1:p.(Lys60Argfs*2) or short NP_000510.1:p.(Lys60fs) (Note that either * or Ter can be used)

Below is the sequence of the PCR product of sample B; primers (green, excluding sequence tags), exon 2 (capital letters) and the coding variant (red). Only variants detected in the protein coding DNA sequence were asked to be reported.

caaggaggcaaatggaaactgggcatgtgtagacagagaagactcttgggtttctgataggcactgactctctgtccct
tgggctgttttcctaccctcagATTACTGGTGGTCTACCCTTGGACCCAGAGGTTCTTTGAGTCCTTTGGGGATCTGTC
CTCTCCTGATGCTGTTATGGGCAACCCTA(A/)GGTGAAGGCTCATGGCAAGAAGGTGCTAGGTGCCTTTAGTGATGG
CCTGGCTCACCTGGACAACCTCAAGGGCACTTTTTCTCAGCTGAGTGAGCTGCACTGTGACAAGCTGCACGTGGATCCT
GAGAACTTCAGGgtgagtccaggagatgcttcacttttctctttttactttctaatcttacattttggttcttttacct
acctgctcttctccca

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Sample 2022:01/C

This digital sample, available in both ab1- and seq-format, originated from a PCR product consisting of a 582 bp long fragment including exon 4 of the human *HFE* gene. It contained two coding variants, both heterozygous substitutions of one nucleotide, see table 3.

Table 3. Expected result for sample 2022:01/C.

#	Variant
1	NM_000410.3:c.845G>A, heterozygous NP_000401.1:p.(Cys282Tyr)
2	NM_000410.3:c.848A>C, heterozygous NP_000401.1:p.(Gln283Pro)

Below is the sequence of the PCR product of sample C; primers (green, excluding sequence tags), exon 4 (capital letters) and the two coding variants (red). Only variants detected in the protein coding DNA sequence were asked to be reported.

catgtgaggagaacaagctgatctgactgctctccaagtgacactgtgttagagtccaatcttaggacacaaaatggtg
tctctcctgtagcttgtttttttctgaaaagggtatttccttcctccaacctatagaaggaagtgaaagttccagtctt
cctggcaagggtaaacagatcccctctcctcatccttcctctttcctgtcaagTGCCTCCTTTGGTGAAGGTGACACAT
CATGTGACCTCTTCAGTGACCACTCTACGGTGTCGGGCCTTGAACTACTACCCCCAGAACATCACCATGAAGTGGCTGA
AGGATAAGCAGCCAATGGATGCCAAGGAGTTCGAACCTAAAGACGTATTGCCCAATGGGGATGGGACCTACCAGGGCTG
GATAACCTTGGCTGTACCCCCTGGGGAAGAGCAGAGATATACGT(G/A)CC(A/C)GGTGGAGCACCCAGGCCTGGATC
AGCCCCTCATTGTGATCTGGGgtatgtgactgatgagagccaggagctgagaaaatctattgggggttgagaggagtgcc
ctgaggaggtaattatggcagtgagatgaggatctgc

Comments

All nine participating laboratories submitted results in this round.

Grading has been made according to table 4. When evaluating the results, points have been given irrespective of the use of brackets and parentheses. No limit for passing or not passing the round is present. The overview of results in table 5–8 display the grading of each parameter for each sample and participating laboratory. Table 9 presents the analysis set up reported by each participating laboratory. Each laboratory's own lab number is displayed in the file "Your_lab_no".

Table 4. Grading of reported results.

Total per variant	6 points
Variant detected	1 point
Correct coding DNA position	1 point
Correct nucleotide change	1 point
Correct zygosity	1 point
Correct protein position	1 point
Correct amino acids (both if changed)	1 point

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Sample 2022:01/A

For sample A, all nine participants identified the heterozygous substitution. However, one participant also reported two additional heterozygous variants, c.995A>T and c.1259G>A, which are incorrect. See table 5 and 8 for more information about the results for sample A.

Table 5. Sample 2022:01/A – one variant, max 6 points.

Lab	Variant detected	Described as coded DNA	Zygosity	Described as protein	Points	Total	Comments
1	Yes	c.710T>C	HT	p.(Val237Ala)	4+2=6	**6**	-
2	Yes	c.**893**T>C	HT	p.Val237Ala	3+2=5	**5**	Incorrect DNA position. Two additional incorrect variants reported (c.995A>T and c.1259G>A).
4	Yes	c.710T>C	HT	p.(Val237Ala)	4+2=6	**6**	-
10	Yes	c.710T>C	HT	p.(Val237Ala)	4+2=6	**6**	-
12	Yes	c.710T>C	HT	p.(Val237Ala)	4+2=6	**6**	-
13	Yes	c.710T>C	HT	p.Val237Ala	4+2=6	**6**	-
14	Yes	c.710T>C	HT	p.(Val237Ala)	4+2=6	**6**	-
15	Yes	c.710T>C	HT	p.Val237Ala	4+2=6	**6**	-
16	Yes	c.710T>C	HT	p.Val237Ala	4+2=6	**6**	-

Sample 2022:01/B

For sample B, 8 out of 9 participants identified the heterozygous deletion. However, one participant also reported one additional heterozygous variant, c.221_222dup, which is incorrect. One laboratory reported that it was not possible to sequence the sample and suspected a contamination. See table 6 and 8 for more information about the results for sample B.

Table 6. Sample 2022:01/B – one variant, max 6 points.

Lab	Variant detected	Described as coded DNA	Zygosity	Described as protein	Points	Total	Comments
1	Yes	c.179del**A**	HT	p.(Lys60Argfs*2)	4+2=6	**6**	The recommendation is to not describe the deleted sequence.
2	-	-	-	-	0+0=0	**0**	Not possible to sequence, suspected contamination?
4	Yes	c.179del	HT	p.(Lys60Argfs*2)	4+2=6	**6**	-
10	Yes	c.179del	HT	p.(Lys60Argfs*2)	4+2=6	**6**	-
12	Yes	c.179del	HT	p.(Lys60ArgfsTer2)	4+2=6	**6**	-
13	Yes	c.179del	HT	p.Lys60fs	4+2=6	**6**	One additional incorrect variant reported (c.221_222dup).
14	Yes	c.179del	HT	p.(Lys60Argfs*2)	4+2=6	**6**	-
15	Yes	c.**178**del	HT	p.Lys60fs	3+2=5	**5**	Incorrect DNA position.
16	Yes	c.179del	HT	p.Lys60Argfs*2	4+2=6	**6**	-

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Sample 2022:01/C

In this round, in addition to the two PCR products, a digital sample (2022:01/C) was distributed for interpretation in the participants' ordinary software.

All nine participants reported that it was possible to import the sequencing files, of which seven used the ab1-format and two did not report the format used. Two participants stated that they had to use an additional software for interpretation besides their ordinary one, the remaining seven used their ordinary software for interpretation of the digital sample.

Five participants reported that they preferred the option "Only digital sequencing files to evaluate" in the EQA scheme in the future, whereas the remaining four preferred "Both PCR products and digital sequencing files". The option "Only PCR products to sequence by yourself" was not preferred by anyone. We will evaluate this outcome for future development of the EQA scheme.

Regarding the results, all nine participants identified the two heterozygous substitutions. See table 7 and 8 for more information about the results for sample C.

Table 7. Sample 2022:01/C – two variants, max 12 points (6 points per variant).

Lab	Variant detected	Described as coded DNA	Zygosity	Described as protein	Points	Total	Comments
1	Yes	c.845G>A	HT	p.(Cys282Tyr)	4+2=6	**12**	-
	Yes	c.848A>C	HT	p.(Gln283Pro)	4+2=6		
2	Yes	c.845G>A	HT	p.Cys282**Ter**	4+1=5	**10**	Incorrect amino acids for both variants.
	Yes	c.848A>C	HT	p.**Glu**283**Leu**	4+1=5		
4	Yes	c.845G>A	HT	p.(Cys282Tyr)	4+2=6	**12**	-
	Yes	c.848A>C	HT	p.(Gln283Pro)	4+2=6		
10	Yes	c.845G>A	HT	p.(Cys282Tyr)	4+2=6	**12**	-
	Yes	c.848A>C	HT	p.(Gln283Pro)	4+2=6		
12	Yes	c.845G>A	HT	p.(Cys282Tyr)	4+2=6	**12**	-
	Yes	c.848A>C	HT	p.(Gln283Pro)	4+2=6		
13	Yes	c.845G>A	HT	p.Cys282Tyr	4+2=6	**12**	-
	Yes	c.848A>C	HT	p.Gln283Pro	4+2=6		
14	Yes	c.845G>A	HT	p.(Cys282Tyr)	4+2=6	**12**	-
	Yes	c.848A>C	HT	p.(Gln283Pro)	4+2=6		
15	Yes	c.845G>A	HT	p.Cys282Tyr	4+2=6	**12**	**-**
	Yes	c.848A>C	HT	p.Gln283Pro	4+2=6		
16	Yes	c.845G>A	HT	p.Cys282Tyr	4+2=6	**12**	**-**
	Yes	c.848A>C	HT	p.Gln283Pro	4+2=6		

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Table 8. Overview of the results for each parameter and sample.

Lab	2022:01/A						2022:01/B						2022:01/C												Total (max 24 points)
	Variant 1						Variant 1						Variant 1						Variant 2						
	Variant detected	Coding DNA position	Nucleotide change	Zygosity	Protein position	Amino acids	Variant detected	Coding DNA position	Nucleotide change	Zygosity	Protein position	Amino acids	Variant detected	Coding DNA position	Nucleotide change	Zygosity	Protein position	Amino acids	Variant detected	Coding DNA position	Nucleotide change	Zygosity	Protein position	Amino acids	
1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	24
2	■	■	■	■	■	■	□	□	□	□	□	□	■	■	■	■	■	■	■	■	■	■	■	■	15
4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	24
10	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	24
12	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	24
13	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	24
14	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	24
15	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	23
16	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	24

■ As expected result = 1p ■ Not as expected result = 0p □ No result = 0p

Table 9. Analysis set up used at participating laboratories.

Lab	Method	Instrument	Software program
1	Sanger sequencing	ABI 3500Dx	SeqScape v2.5 (Sequencing Analysis 5.2.0 for sample C)
2	Sanger sequencing	ABI 3730xL	SeqScape
4	Sanger sequencing	ABI 3500xL	SeqScape
10	Sanger sequencing	ABI 3500xL	SeqScape v3 (Sequencing Analysis v6 for sample C)
12	Sanger sequencing	ABI 3730	Vector NTI
13	Sanger sequencing	ABI 3730xL	Sequencher 5.0
14	Sanger sequencing	ABI 3500Dx	Sequencher 5.4.6
15	Sanger sequencing	ABI 3500	Mutation Surveyor
16	Sanger sequencing	Seq Studio	Geneious Prime, Alamut Visual Plus

Test material

2022:01/A and 2022:01/B: Two samples containing 20 µL of purified PCR product each. Four primer vials containing 20 µL of sequencing primer each, with a concentration of 3.0 pmol/µL.

2022:01/C: A digital sample consisting of four files, F/R_ab1_sample C_246_2022-01.ab1 and F/R_seq_sample C_246_2022-01.seq.

Based on previous tests and the results of this round, the samples are homogeneous, stable, and suitable for the external quality assessment scheme.

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