Seraseq®

SARS-CoV-2 Mutation RNA Mix

0740-0075

OVERVIEW

The Seraseq® SARS-CoV-2 Mutation RNA Mix (0740-0075) is a highly multiplexed positive reference material which contains 82 variants of interest (Table 1) blended with the Wuhan wild-type SARS-CoV-2 genomic RNA (reference sequence NC_045512) in a background of human total RNA from the well-characterized GM24385 cell line. The kit contains 3 different vials at 1%, 5%, and 20% variant allele frequency (VAF) formulated for use with Next Generation Sequencing (NGS) assays that detect variant mutations in SARS-positive patient samples. Each vial contains 25 µL fill volume at a nominal total RNA concentration of 25 ng/µL and a SARS-CoV-2 RNA concentration of 5.0E+06 copies/mL.

NGS testing was performed on each member using the Swift Normalase Amplicon SARS-CoV-2 Panel amplicon-based assay in triplicate and using the hybrid/capture-based Roche KAPA® HyperCap® SARS-CoV-2 Panel. For the amplicon-based method, approximately 30 ng of each bulk was used as input into the workflow. The resulting libraries were sequenced using the Illumina MiSeq® Reagent Kit v2 (300 cycles). NGS data analysis was performed with Swift Sarscov2 analysis docker image pulled from docker hub. This dockerized analysis pipeline was run with the run_swift_sarscov2_docker.sh script and with the sarscov2_v2_masterfile-2.txt as the -v option. Data was also aligned to a composite genome of hg19 (due to the presence of human RNA) and NC_045512.2 (SARS-CoV-2) using bwa mem. The aligned data was analyzed using 2 variant callers, GATK4 Mutect2(M2), and VarDict(VD). For the hybrid/capture-based assay, 100 ng of each bulk was used as input in the workflow and the resulting libraries were sequenced on an Illumina NextSeq® 2000 using a 300 cycle P2 kit. Data was also aligned to a composite genome of hg19 and NC_045512.2 (SARS-CoV-2) using bwa mem and analyzed using the Mutect2 and VarDict variant callers. snpEff was used for annotation.

It should be noted that the variant callers that were used can combine variants that are in close proximity and can detect some variants differently. In particular, a nearby set of 5 mutations was condensed into 3 variants by Mutect2 and 2 variants by VarDict due to differences in local realignment and merging.

Design:

```
AGGCAGCAGTAGGGGAACTTCTCCT NC_045512.2:28877,AG>TC
|||||||  ||   ||| ||||||| NC_045512.2:28881,GGG>AAC
AGGCAGCTCTAAACGAATTTCTCCT NC_045512.2:28887,C>T
```

Mutect2 realignment:

```
AGGCAGCAGTAGGGGAAC----TTCTCCT NC_045512.2:28877,AG>TC (p.203)
|||||||  ||    |||    ||||||| NC_045512.2:28880,AGGGG>A (p.R203fs)
AGGCAGCTCTA----AACGAATTTCTCCT NC_045512.2:28887,C>CGAAT (p.S206fs)
```

VarDict realignment and merging:

```
AGGCAGCAGTAGGGGAACTTCTCCT NC_045512.2:28877,AGTAGGG>TCTAAAC (p.RG203KR)
|||||||       ||| ||||||| NC_045512.2:28887,C>T (p.T205I)
AGGCAGCTCTAAACGAATTTCTCCT
```

For Research Use Only. Not for use in diagnostic procedures. Data are offered for informational purposes.
LGC Seracare does not claim that others can duplicate test results exactly.

Table 1: List of 82 SARS-CoV-2 mutations in the Seraseq SARS-CoV-2 Mutation RNA Mix product.

Amino Acid Change	Nucleotide Change	Gene	Viral isolate	Annotated Detection
T85I	1059C>T	Nsp2	B.1.351	p.T85I
S370L	3828C>T	Nsp3	P1	p.S370L
K837N	5230G>T	Nsp3	B.1.351	p.K837N
A890D	5388C>A	Nsp3	B.1.1.7	p.A890D
K977Q	5648A>C	Nsp3	P1	p.K977Q
I1683T	7767T>C	NSP3	B.1.258	p.I1683T
K90R	10323A>G	Nsp5	B.1.351	p.K90R
S106-108 del	11288-11296del	Nsp6	B.1.525	p.S106_F108del
I65V A185S	12878A>G 13993G>T	NSP9 NSP12	B.1.429 B.1.160	p.I65V p.A185S
P323L	14408C>T	Nsp12	B.1.351	p.P323L
V776L	15766G>T	NSP12	B.1.160	p.V776L
K218R	16889A>G	NSP13	B.1.160	p.K218R
D260Y	17014G>T	NSP13	B.1.429	p.D260Y
E261D	17019G>T	NSP13	B.1.160	p.E261D
E341D	17259G>T	Nsp13	global	p.E341D
S13I	21600G>T	Spike	B.1.429	p.S13I
L18F	21614C>T	Spike	P1; B.1.351	p.L18F
T20N	21621C>A	Spike	P1	p.T20N
P26S	21638C>T	Spike	P1	p.P26S
Q52R	21717A>G	Spike	B.1.525 & B1.1.207	p.Q52R
H69del V70del	21765-21770del	Spike	B.1.1.7	p.H69_V70del p.H69_V70del
D80A	21801A>C	Spike	B.1.351	p.D80A
D138Y	21974G>T	Spike	P1	p.D138Y
G142V	21987G>T	Spike	B.1.1.7	p.G142V
Y145del	21993-21995del	Spike	B.1.1.7	p.Y145del

Table 1 continued:

Amino Acid Change	Nucleotide Change	Gene	Viral isolate	Annotated Detection
W152C	22018G>T	Spike	B.1.429	p.W152C
R190S	22132G>T	Spike	P1	p.R190S
D215G	A22206A>G	Spike	B.1.351	p.D215G
L242del	22286-22294del	Spike	B.1.351	p.L242_L244del
A243del				p.L242_L244del
L244del				p.L242_L244del
K417N	22813G>T	Spike	B.1.351	p.K417N
N439K	22879C>A	Spike	B.1.222, 13.1.258, AV.1	p.N439K
L452R	22917T>G	Spike	B.1.429	p.L452R(M2), p.LY452RF(VD)
Y453F	22920A>T	Spike	B.1.1.7	p.Y453F(M2), p.LY452RF(VD)
E484K	23012G>A	Spike	B.1.351; P1	p.E484K
N501Y	23063A>T	Spike	B.1.1.7; B.1.351; P1	p.N501Y
A570D	23271C>A	Spike	B.1.1.7	p.A570D
D614G	23403A>G	Spike	B.1.1.7; B.1.351; P1	p.D614G
H655Y	23525C>T	Spike	P1	p.H655Y
Q677H	23593G>C	Spike	B.1.525 & B1.1.207	p.Q677H
P681H	23604C>A	Spike	B.1.1.7	p.P681H
I692V	23636A>G	Spike	Cluster 5 DK	p.I692V
A701V	23664C>T	Spike	B.1.351	p.A701V
T716I	23709C>T	Spike	B.1.1.7	p.T716I
F888L	24224T>C	Spike	B.1.525 & B1.1.207	p.F888L
L938F	24374C>T	Spike	B.1.429	p.L938F
S982A	24506T>G	Spike	B.1.1.7	p.S982A
T1027I or Y	24642C>T	Spike	P1	p.T1027I
D1118H	24914G>C	Spike	B.1.1.7	p.D1118H
S1147L	25002C>T	Spike	Cluster 5	p.S1147L
V1176F	25088G>T	Spike	P1	p.V1176F

Table 1 continued:

Amino Acid Change	Nucleotide Change	Gene	Viral isolate	Annotated Detection
K1191N	25135G>T	Spike	B.1.429	p.K1191N
M1229I	25249G>T	Spike	Cluster 5 DK	p.M1229I
Q57H	25563G>T	ORF3a	B.1.351	p.Q57H
S171L	25904C>T	ORF3a	B.1.351	p.S171L
S253P	26149T>C	ORF3a	P1	p.S253P
L21F	26305C>T	E	B.1.525	p.L21F
P71L	26456C>T	E	B.1.351	p.P71L
I82T	26767T>C	M	B.1.525	p.I82T
K162N	27008G>T	M	B.1.351	p.K162N
V93F	27670G>T	ORF7a	B.1.351, B.1.160	p.V93F
Q27*	27972C>T	ORF8	B.1.1.7	p.Q27*
R52I	28048G>T	ORF8	B.1.1.7	p.R52I
Y73C	28111A>G	ORF8	B.1.1.7	p.Y73C
E92K	28167G>A	ORF8	P1	p.E92K
Silent	28263insAACA	intergenic/non CDS	P1	28262,G>GAACA
D3L	28280_2delinsCTA	N	B.1.1.7	p.D3L
A12G	28308C>G	N	B.1.525	p.A12G
P80R	28512C>G	N	P1	p.P80R
Silent	28877A>T	N	P1	p.203(M2), p.RG203KR(VD)
Silent	28878G>C	N	P1	p.203(M2), p.RG203KR(VD)
G204R	28881GGG>AAC	N	P1 & B.1.1.7	p.R203fs(M2), p.RG203KR (VD)
R203K				p.R203fs(M2), p.RG203KR (VD)
T205I	28887C>T	N	B.1.351	p.S206fs (M2), p.T205I (VD)
M234I	28975G>C	N	multiple	p.M234I(M2), p.MS234IF (VD)
S235F	28975C>T	N	B.1.1.7	p.S235F(M2), p.MS234IF (VD)
Q289H	29140G>T	N	B.1.243	p.Q289H
Silent	29200C>T	N	Unclear	p.P309P
A376T	29399G>A	N	B.1.160	p.A376T

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Figure 1. Observed VAFs for the mutations in the data from the hybrid/capture- (HC) and amplicon-based (AMP) assays are shown. Mutect2 VAFs are in blue while VarDict VAFs are in magenta. The data for the amplicon-based panel has 3 points per mutation to reflect the 3 replicates. Mutations that were not called are set to a VAF of 0.1%.

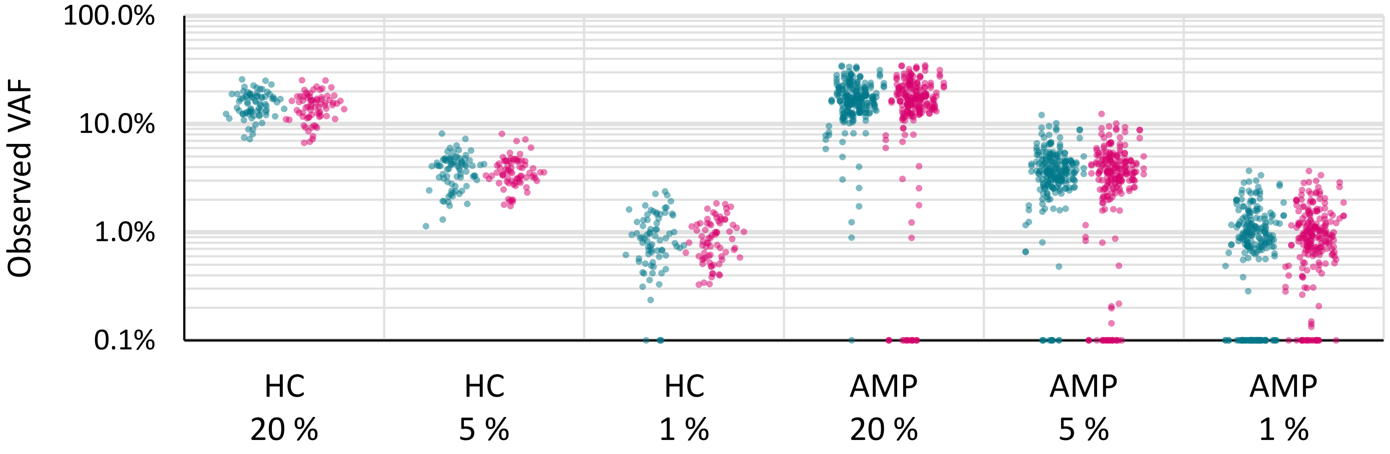


Figure 2. Observed VAFs of called mutations in the 20% data from the hybrid/capture-based assay (HC) and a representative replicate from the amplicon-based assay (AMP) are shown in orange at their locations within the SARS-CoV-2 genome. Expected mutations are highlighted in purple.

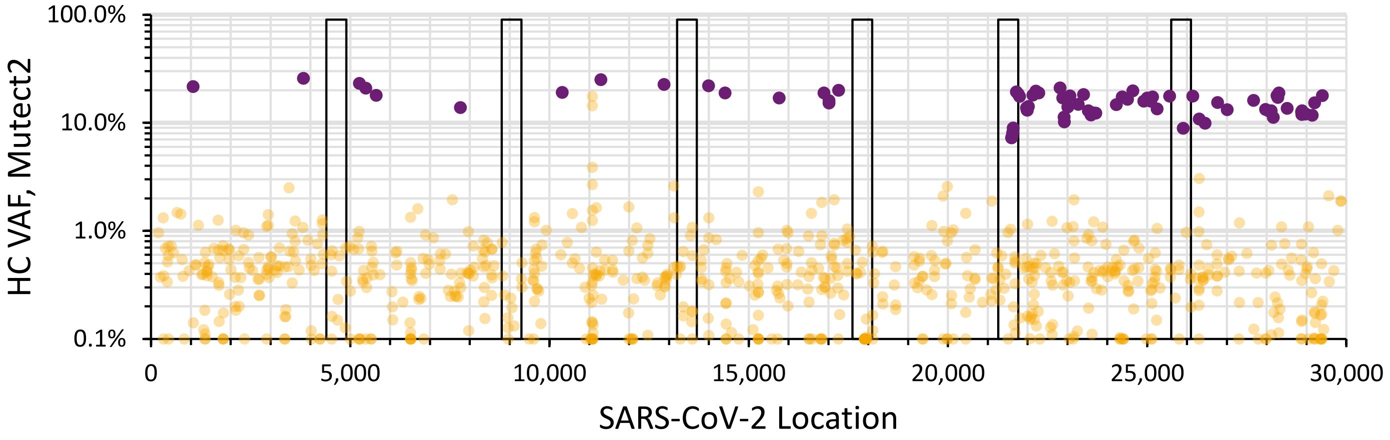
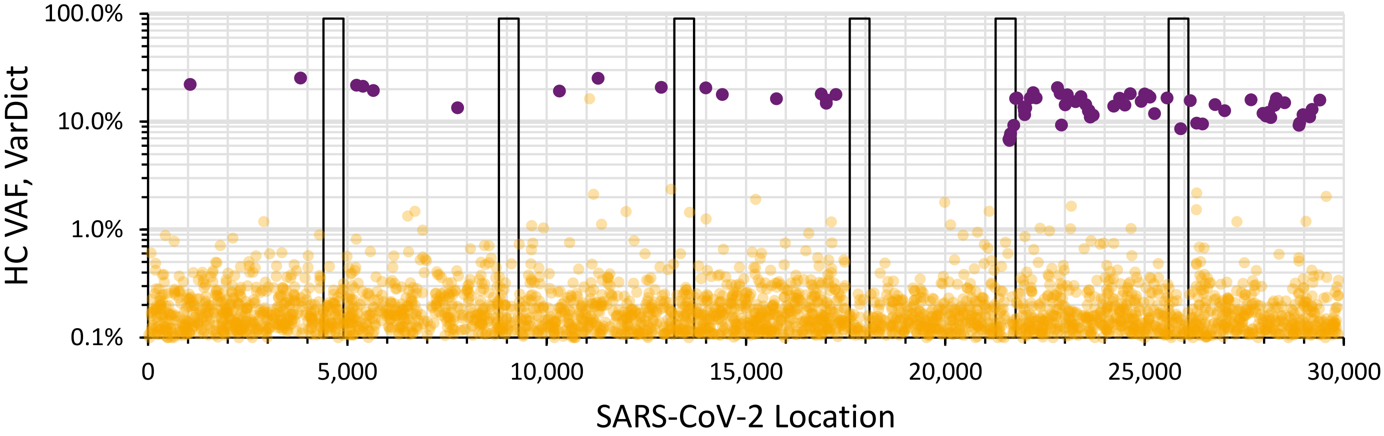
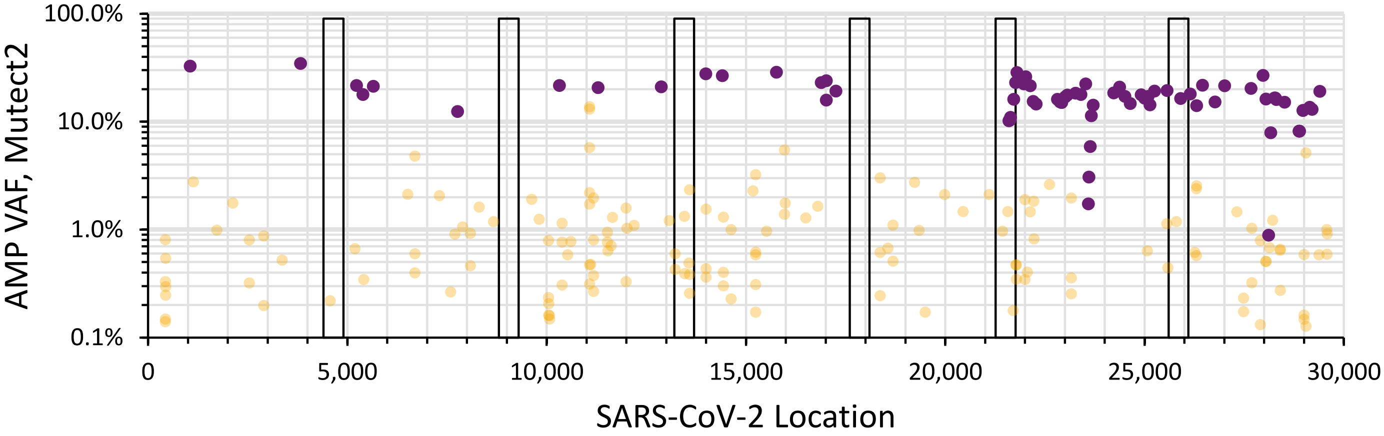


Figure 2 continued





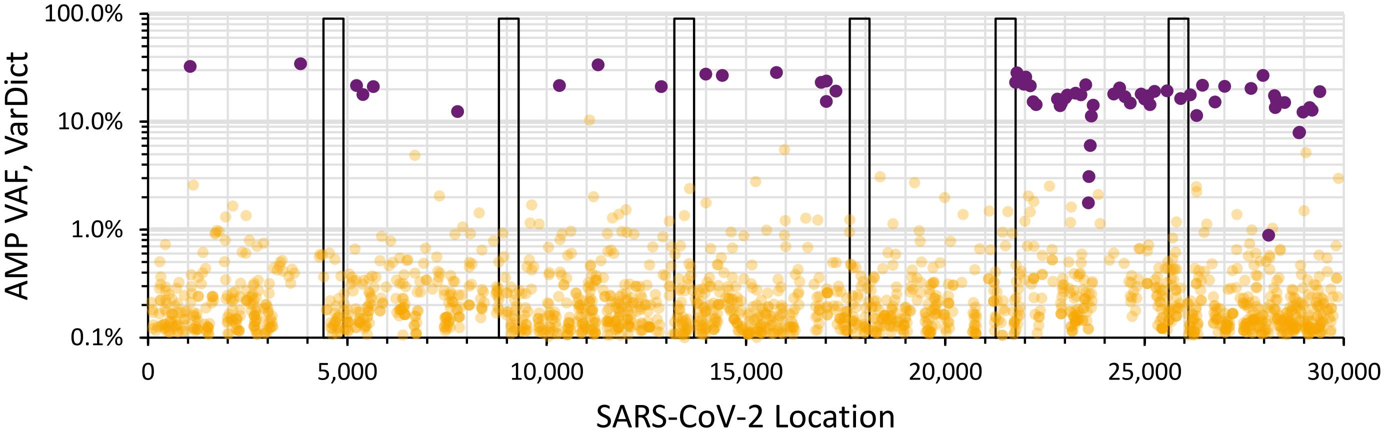


Table 2: Variant allele frequencies for the hybrid/capture-based and amplicon-based NGS method as determined by the Mutect2, VarDict, and GATK4 Mutect2(M2) variant callers. HC1, HC5, and HC20 represent the hybrid/capture-based method at 1%, 5%, and 20% VAF, respectively. A1, A5, and A20 represent the amplicon-based method at 1%, 5%, and 20% VAF, respectively. The variant callers that were used sometimes combine variants that are in close proximity.

Amino Acid Change	Mutect2	Vardict	Mutect2	Vardict	HC1_M2	HC5_M2	HC20_M2	HC1_VD	HC5_VD	HC20_VD	A1_VD	A5_VD	A20_VD	A1_M2	A5_M2	A20_M2
T85I	1059,C>T	1059,C>T	p.T85I	p.T85I	1.5%	6.3%	21.6%	1.84%	6.94%	22.16%	2.54%	9.33%	33.04%	2.55%	9.35%	33.11%
S370L	3828,C>T	3828,C>T	p.S370L	p.S370L	0.9%	8.1%	25.8%	0.92%	8.11%	25.31%	2.11%	9.85%	30.37%	2.15%	9.80%	30.30%
K837N	5230,G>T	5230,G>T	p.K837N	p.K837N	1.9%	5.7%	23.2%	1.71%	6.03%	21.72%	1.21%	4.57%	22.72%	1.22%	4.57%	22.75%
A890D	5388,C>A	5388,C>A	p.A890D	p.A890D	0.7%	4.3%	20.9%	0.56%	3.92%	21.31%	0.73%	3.26%	17.55%	0.89%	3.21%	17.57%
K977Q	5648,A>C	5648,A>C	p.K977Q	p.K977Q		4.6%	18.0%	0.73%	4.55%	19.45%	0.93%	5.09%	21.58%	1.05%	5.08%	21.64%
I1683T	7767,T>C	7767,T>C	p.I1683T	p.I1683T	0.7%	2.2%	13.8%	0.62%	1.75%	13.42%	0.88%	2.94%	13.43%	0.91%	2.93%	13.52%
K90R	10323,A>G	10323,A>G	p.K90R	p.K90R	1.0%	6.0%	19.1%	1.20%	5.38%	19.21%	1.09%	5.63%	24.85%	1.10%	5.65%	24.90%
S106-108 del	11287,GTCTGGTTTT>G	11287,GTCTGGTTTT>G	p.S106_F108del	p.S106_F108del	1.5%	7.3%	25.0%	1.81%	7.18%	25.21%	1.85%	8.04%	33.20%	0.90%	3.87%	20.60%
I65V	12878,A>G	12878,A>G	p.I65V	p.I65V	1.6%	5.5%	22.7%	1.52%	4.72%	20.76%	1.47%	4.96%	24.48%	1.94%	5.06%	24.33%
P323L	14408,C>T	14408,C>T	p.P323L	p.P323L	1.0%	4.8%	18.9%	0.52%	4.55%	17.84%	2.99%	8.00%	29.72%	3.01%	8.00%	30.25%
E341D	17259,G>T	17259,G>T	p.E341D	p.E341D	1.1%	4.7%	20.0%	0.82%	4.93%	17.86%	1.33%	4.80%	20.88%	1.34%	4.82%	20.88%
A185S	13993,G>T	13993,G>T	p.A185S	p.A185S	0.9%	4.7%	22.0%	0.64%	4.56%	20.53%	1.93%	6.60%	27.76%	1.93%	6.63%	27.81%
V776L	15766,G>T	15766,G>T	p.V776L	p.V776L	0.7%	4.2%	17.0%	0.58%	3.56%	16.34%	2.28%	8.32%	29.16%	2.22%	8.33%	29.19%
K218R	16889,A>G	16889,A>G	p.K218R	p.K218R	1.2%	4.1%	18.9%	1.17%	4.13%	18.00%	1.44%	5.44%	20.36%	1.46%	5.84%	20.39%
D260Y	17014,G>T	17014,G>T	p.D260Y	p.D260Y	2.4%	4.8%	15.2%	1.31%	4.52%	14.79%	1.29%	4.87%	22.45%	1.37%	4.89%	22.54%
E261D	17019,G>T	17019,G>T	p.E261D	p.E261D	2.3%	5.0%	16.0%	1.47%	4.74%	15.91%	0.68%	2.65%	14.59%	0.70%	2.89%	15.34%
S13I	21600,G>T	21600,G>T	p.S13I	p.S13I	0.4%	1.8%	7.2%	0.33%	1.93%	6.85%	0.98%		12.18%	0.99%	3.02%	11.58%
L18F	21614,C>T	21614,C>T	p.L18F	p.L18F	0.4%	1.9%	7.5%	0.34%	1.79%	6.70%	0.96%	2.11%	12.16%	0.96%	3.02%	11.63%
T20N	21621,C>A	21621,C>A	p.T20N	p.T20N	0.4%	2.1%	8.1%	0.41%	1.91%	7.24%	0.96%	2.11%	12.08%	0.96%	3.02%	11.63%
P26S	21638,C>T	21638,C>T	p.P26S	p.P26S	0.5%	2.3%	8.9%	0.41%	1.99%	7.71%	0.99%	2.31%	12.70%	1.06%	3.07%	11.95%
Q52R	21717,A>G	21717,A>G	p.Q52R	p.Q52R	1.1%	3.3%	19.3%	1.00%	3.13%	9.24%	0.69%			1.00%	3.39%	17.55%
H69_V70del	21764,ATACATG>A	21764,ATACATG>A	p.H69_V70del	p.H69_V70del	1.1%	5.0%	18.4%	0.88%	4.81%	16.35%	1.41%	5.01%	23.87%	1.41%	4.98%	23.86%
D80A	21801,A>C	21801,A>C	p.D80A	p.D80A	0.5%	4.3%	17.6%	0.48%	3.93%	16.61%	1.84%	6.68%	28.61%	1.85%	6.67%	28.76%
D138Y	21974,G>T	21974,G>T	p.D138Y	p.D138Y	0.8%	4.3%	13.8%	1.01%	3.57%	13.71%	1.57%	4.10%	22.64%	1.57%	4.12%	22.69%
G142V	21987,G>T	21987,G>T	p.G142V	p.G142V	0.7%	4.1%	13.2%	0.76%	3.09%	12.61%	1.59%	4.15%	23.81%	1.65%	4.16%	24.04%
Y145del	21990,TTTA>T	21990,TTTA>T	p.Y145del	p.Y145del	0.7%	3.8%	13.1%	0.60%	2.83%	11.63%	1.59%	4.13%	23.37%	1.59%	4.16%	24.04%
W152C	22018,G>T	22018,G>T	p.W152C	p.W152C	1.0%	4.4%	14.1%	1.04%	3.55%	13.41%	1.97%	4.91%	26.97%	1.97%	4.74%	26.26%
R190S	22132,G>T	22132,G>T	p.R190S	p.R190S	1.5%	5.0%	18.0%	1.36%	4.49%	16.58%	1.19%	3.59%	19.42%		3.60%	19.46%
D215G	22206,A>G	22206,A>G	p.D215G	p.D215G	1.5%	5.5%	19.7%	1.65%	4.09%	18.61%	0.66%	2.21%	13.77%	0.83%	2.22%	13.84%
L242_L244del	22280,ACTTTACTTG>A	22280,ACTTTACTTG>A	p.L242_L244del	p.L242_L244del	1.0%	4.6%	18.8%	1.02%	3.54%	16.56%	0.84%	2.64%	13.14%	0.85%	2.68%	13.27%
K417N	22813,G>T	22813,G>T	p.K417N	p.K417N	0.8%	4.8%	21.0%	1.21%	5.26%	20.65%	0.83%	2.42%	14.46%	0.94%	2.43%	14.43%
N439K	22879,C>A	22879,C>A	p.N439K	p.N439K	1.0%	3.3%	16.9%	0.69%	3.36%	18.26%	0.86%	3.06%	15.37%	0.79%	3.06%	15.85%
L452R	22917,T>G	22917,TGTA>GGTT	p.L452R	p.LY452RF	0.7%	2.4%	11.3%	0.49%	1.98%	9.30%	0.88%	3.28%	16.11%	0.83%	3.28%	16.22%
Y453F	22920,A>T	22917,TGTA>GGTT	p.Y453F	p.LY452RF	0.7%	2.4%	10.2%	0.49%	1.98%	9.30%	0.88%	3.28%	16.11%	0.83%	3.28%	16.22%
E484K	23012,G>A	23012,G>A	p.E484K	p.E484K	1.3%	5.7%	14.0%	1.27%	4.54%	14.32%	1.83%	3.63%	16.01%	2.61%	3.53%	16.24%
N501Y	23063,A>T	23063,A>T	p.N501Y	p.N501Y		4.5%	17.7%	1.31%	3.30%	17.70%	1.76%	3.78%	17.58%	2.22%	3.73%	17.57%
A570D	23271,C>A	23271,C>A	p.A570D	p.A570D	1.5%	3.2%	14.8%	1.39%	3.30%	15.32%	1.63%	5.19%	20.19%	1.95%	5.18%	20.29%
D614G	23403,A>G	23403,A>G	p.D614G	p.D614G	1.2%	4.3%	18.3%	0.98%	4.68%	17.11%	0.91%	4.19%	18.62%	1.11%	4.25%	18.76%

Table 2 continued:

Amino Acid Change	Mutect2	Vardict	Mutect2	Vardict	HC1_M2	HC5_M2	HC20_M2	HC1_VD	HC5_VD	HC20_VD	A1_VD	A5_VD	A20_VD	A1_M2	A5_M2	A20_M2
H655Y	23525,C>T	23525,C>T	p.H655Y	p.H655Y	0.2%	4.2%	12.9%	0.74%	3.61%	14.37%	1.26%	4.60%	23.50%	1.13%	4.37%	23.67%
Q677H	23593,G>C	23593,G>C	p.Q677H	p.Q677H	0.3%	2.3%	11.8%	0.41%	3.15%	12.58%	0.21%	0.35%	2.80%		0.48%	2.77%
P681H	23604,C>A	23604,C>A	p.P681H	p.P681H	0.3%	1.9%	12.3%	0.55%	3.40%	12.73%	0.32%	0.80%	4.97%		0.80%	4.94%
I692V	23636,A>G	23636,A>G	p.I692V	p.I692V	0.6%	1.1%	12.4%	0.64%	3.33%	11.03%	0.36%	0.96%	6.98%	0.49%	0.82%	6.95%
A701V	23664,C>T	23664,C>T	p.A701V	p.A701V	0.4%	1.3%	12.3%	0.51%	3.43%	11.67%	0.58%	1.82%	11.61%	0.89%	1.80%	11.53%
T716I	23709,C>T	23709,C>T	p.T716I	p.T716I	0.6%	2.1%	12.3%	0.88%	3.76%	11.45%	0.84%	2.50%	15.15%	0.70%	2.53%	15.19%
F888L	24224,T>C	24224,T>C	p.F888L	p.F888L	0.9%	3.6%	14.7%	0.65%	3.22%	13.89%	0.90%	4.03%	16.55%	1.10%	4.09%	16.76%
L938F	24374,C>T	24374,C>T	p.L938F	p.L938F	0.8%	3.4%	17.3%	0.76%	3.33%	16.44%	2.45%	3.92%	16.34%	2.46%	3.95%	16.46%
S982A	24506,T>G	24506,T>G	p.S982A	p.S982A	0.9%	2.7%	16.5%	1.00%	2.87%	14.26%	1.12%	3.74%	16.41%	1.15%	3.77%	16.53%
T1027I or Y	24642,C>T	24642,C>T	p.T1027I	p.T1027I	2.2%	1.8%	19.7%	1.50%	2.32%	18.19%	0.81%	3.53%	15.28%	0.81%	3.63%	15.33%
D1118H	24914,G>C	24914,G>C	p.D1118H	p.D1118H	0.7%	3.0%	15.8%	1.10%	2.99%	15.42%	0.89%	4.07%	16.88%	1.22%	4.14%	16.73%
S1147L	25002,C>T	25002,C>T	p.S1147L	p.S1147L	0.9%	4.1%	17.0%	1.21%	3.88%	18.04%	0.89%	3.28%	16.18%	1.06%	3.28%	16.30%
V1176F	25088,G>T	25088,G>T	p.V1176F	p.V1176F	0.8%	3.8%	15.9%	1.20%	3.65%	17.44%	0.94%	3.30%	16.88%	0.94%	3.34%	16.90%
K1191N	25135,G>T	25135,G>T	p.K1191N	p.K1191N	0.4%	4.4%	17.3%	0.60%	4.57%	16.85%	0.75%	2.73%	14.04%	0.75%	2.75%	14.11%
M1229I	25249,G>T	25249,G>T	p.M1229I	p.M1229I	1.7%	2.7%	13.5%	1.66%	2.59%	11.86%	1.01%	4.00%	18.58%	1.09%	4.03%	18.66%
Q57H	25563,G>T	25563,G>T	p.Q57H	p.Q57H	0.8%	4.3%	17.7%	0.94%	3.51%	16.57%	1.09%	3.89%	18.04%	1.10%	3.83%	18.03%
S171L	25904,C>T	25904,C>T	p.S171L	p.S171L	0.7%	1.9%	8.9%	0.79%	2.02%	8.59%	0.77%	3.62%	16.19%	0.76%	3.64%	16.30%
S253P	26149,T>C	26149,T>C	p.S253P	p.S253P	1.4%	3.3%	17.6%	1.17%	3.89%	15.65%	0.99%	3.55%	16.91%	1.19%	3.58%	17.02%
L21F	26305,C>T	26305,C>T	p.L21F	p.L21F	1.3%	3.1%	10.9%	0.57%	2.48%	9.68%	0.56%	2.15%	9.89%	0.60%	2.61%	12.32%
P71L	26456,C>T	26456,C>T	p.P71L	p.P71L	1.1%	2.4%	9.9%	0.95%	2.67%	9.52%	1.11%	2.69%	19.67%	1.11%	4.00%	19.64%
I82T	26767,T>C	26767,T>C	p.I82T	p.I82T	1.3%	2.9%	15.4%	1.32%	3.19%	14.41%	0.78%	3.47%	14.61%	0.96%	3.46%	14.66%
K162N	27008,G>T	27008,G>T	p.K162N	p.K162N	0.5%	3.2%	13.3%	0.60%	2.83%	12.64%	1.22%	4.90%	21.53%	1.25%	4.97%	21.64%
V93F	27670,G>T	27670,G>T	p.V93F	p.V93F	0.6%	4.1%	16.2%	0.33%	4.31%	15.95%	1.05%	3.34%	18.99%	1.06%	3.34%	19.05%
Q27*	27972,C>T	27972,C>T	p.Q27*	p.Q27*		3.4%	13.3%	0.71%	2.87%	11.92%	1.91%	6.71%	27.27%	1.93%	6.70%	27.32%
R52I	28048,G>T	28048,G>T	p.R52I	p.R52I	0.9%	3.3%	12.9%	0.79%	2.93%	11.31%				1.09%	3.47%	17.62%
Y73C	28111,A>G	28111,A>G	p.Y73C	p.Y73C	0.7%	2.8%	12.9%	0.38%	2.98%	12.18%	0.14%	0.18%	1.06%			1.06%
E92K	28167,G>A	28167,G>A	p.E92K	p.E92K	1.6%	2.4%	11.2%	0.80%	3.10%	10.92%	0.44%			0.65%	1.54%	8.61%
Silent	28262,G>GAACA	28262,G>GAACA	0	0	1.8%	4.6%	17.9%	1.05%	3.78%	14.13%	1.06%	4.28%	18.13%	1.01%	4.19%	17.43%
D3L	28280,GAT>CTA	28280,GAT>CTA	p.D3L	p.D3L	1.5%	4.1%	17.1%	0.86%	3.24%	14.95%	0.75%	3.43%	13.85%	0.99%	4.19%	17.32%
A12G	28308,C>G	28308,C>G	p.A12G	p.A12G	0.9%	4.1%	18.9%	1.13%	3.32%	16.35%	0.89%	3.95%	16.21%	0.90%	4.01%	16.33%
P80R	28512,C>G	28512,C>G	p.P80R	p.P80R	1.6%	4.7%	13.6%	1.16%	4.34%	14.97%	0.76%	3.66%	15.60%	0.73%	3.67%	15.69%
RG203KR	28877,AG>TC	28877,AGTAGGG>TCTAAAC	p.203	p.RG203KR	0.5%	4.8%	12.6%	0.58%	4.57%	9.27%	0.49%	2.23%	10.09%	0.65%	2.24%	10.20%
T205I	28887,C>T	28887,C>T	p.T205I	p.T205I			0.1%	0.50%	2.76%	9.67%	0.47%	2.23%	10.13%			
MS234IF	28975,G>C	28975,GTC>CTT	p.M234I	p.MS234IF	0.6%	3.9%	12.1%	0.51%	2.95%	11.61%	0.63%	3.19%	12.67%	0.73%	3.17%	13.04%
Q289H	29140,G>T	29140,G>T	p.Q289H	p.Q289H	0.8%	3.3%	11.8%	0.72%	3.18%	11.09%	0.59%	3.05%	14.19%	0.62%	3.04%	14.28%
Silent	29200,C>T	29200,C>T	p.P309P	p.P309P		3.7%	15.3%	0.40%	3.99%	12.98%	0.62%	2.95%	13.30%	0.71%	2.96%	13.38%
A376T	29399,G>A	29399,G>A	p.A376T	p.A376T	1.7%	4.2%	17.8%	1.30%	4.18%	15.82%	0.93%	4.54%	18.70%	0.95%	4.51%	18.74%

Table 2 summarizes the calculated AF for each variant as determined by the Mutect2, VarDict, and GATK4 Mutect2(M2) variant callers. Table 3 summarizes the average AF and standard deviation (SD) for each member by NGS method and variant caller. The average AF for the 1% and 5% members were similar for both NGS methods for both of the variant callers. However, for the 20% member, the amplicon-based method for both variant callers gave higher % AF than the hybrid/capture-based method. The SD was similar across the NGS methods and variant callers for each member respectively.

All 82 variants in each member were detected for the hybrid/capture-based method with the VarDict variant caller. As highlighted earlier, the variant callers used in these analyses sometimes combine variants that are in close proximity. The hybrid/capture-based method with the Mutect2 variant caller detected all variants for the 20% member, 81/82 (98.7%) for the 5% member, and 77/82 (93.9%) for the 1% member.

For the amplicon-based method, neither variant caller was able to detect all of the variants. One variant was excluded from the average AF and SD calculations in Table 3 because of low or no frequency, Y73C. In Table 2, the AFs for the amplicon-based method are highlighted. The amplicon-based method with the VarDict or GATK4:Mutect2(M2) variant caller did not detect 2 to 5 variants depending on member (Table 2).

Table 3: Summary of average AF and SD for each member. M2=Mutect2, VD=VarDict, M=GATK4:Mutect2(M2).

	HC1_M2	**HC5_M2**	**HC20_M2**	**HC1_VD**	**HC5_VD**	**HC20_VD**	**A1_VD**	**A5_VD**	**A20_VD**	**A1_M2**	**A5_M2**	**A20_M2**
Avg	1.0%	3.8%	15.5%	0.9%	3.7%	14.7%	1.1%	4.0%	18.1%	1.2%	4.0%	18.0%
SD	0.5%	1.3%	4.4%	0.4%	1.2%	4.1%	0.6%	1.8%	6.3%	0.6%	1.7%	6.0%