

Supplemental data

Supplemental table 1: Sample details of 188 samples (4 duplicates)			
Run 1	Sample plate position	Cluster	Ct-value
Run 1	A01	Patient ward H	17
Run 1	A02	Patient June	34
Run 1	A03	Patient ward H	26
Run 1	A04	Patient July	25
Run 1	A05	Employee may	32
Run 1	A06	Patient ward E	23
Run 1	A07	Public health service	31
Run 1	A08	Public health service	28
Run 1	A09	Public health service	34
Run 1	A10	Public health service	21
Run 1	A11	Public health service	22
Run 1	A12	Public health service	32
Run 1	B01	Public health service	31
Run 1	B02	Public health service	27
Run 1	B03	Public health service	33
Run 1	B04	Public health service	23
Run 1	B05	Public health service	30
Run 1	B06	Public health service	30
Run 1	B07	Public health service	34
Run 1	B08	Public health service	24
Run 1	B09	Public health service	25
Run 1	B10	Public health service	34
Run 1	B11	Public health service	25
Run 1	B12	Patient may	33
Run 1	C01	Public health service	30
Run 1	C02	Public health service	34
Run 1	C03	Public health service	26
Run 1	C04	Public health service	32
Run 1	C05	Public health service	33
Run 1	C06	Public health service	16
Run 1	C07	Public health service	22
Run 1	C08	Public health service	25
Run 1	C09	Public health service	26
Run 1	C10	Public health service	23
Run 1	C11	Public health service	22
Run 1	C12	Public health service	30
Run 1	D01	Public health service	30
Run 1	D02	Public health service	26
Run 1	D03	Public health service	29
Run 1	D04	Public health service	22
Run 1	D05	Public health service	30
Run 1	D06	Public health service	24
Run 1	D07	Public health service	19
Run 1	D08	Public health service	27
Run 1	D09	Public health service	33
Run 1	D10	Public health service	23
Run 1	D11	Public health service	30
Run 1	D12	Public health service	27

Run 1	E01	Public health service	34
Run 1	E02	Public health service	27
Run 1	E03	Public health service	27
Run 1	E04	Public health service	34
Run 1	E05	Public health service	25
Run 1	E06	Public health service	24
Run 1	E07	Public health service	26
Run 1	E08	Public health service	27
Run 1	E09	Public health service	19
Run 1	E10	Public health service	24
Run 1	E11	Public health service	27
Run 1	E12	Public health service	22
Run 1	F01	Public health service	18
Run 1	F02	Public health service	26
Run 1	F03	Public health service	26
Run 1	F04	Public health service	24
Run 1	F05	Public health service	31
Run 1	F06	Public health service	29
Run 1	F07	Public health service	23
Run 1	F08	Public health service	20
Run 1	F09	Patient April	36
Run 1	F10	Patient April	36
Run 1	F11	Patient April	36
Run 1	F12	Patient April	36
Run 1	G01	Patient May	35
Run 1	G02	Patient May	41
Run 1	G03	Employee May	42
Run 1	G04	Patient May	36
Run 1	G05	Patient June	36
Run 1	G06	Public health service	36
Run 1	G07	Employee May	35
Run 1	G08	Patient July	39
Run 1	G09	Patient August	39
Run 1	G10	Patient August	37
Run 1	G11	Public health service	35
Run 1	G12	Public health service	35
Run 1	H01	Public health service	37
Run 1	H02	Patient may	37
Run 1	H03	Employee September	16
Run 1	H04	Employee September	28
Run 1	H05	Employee	neg
Run 1	H06	Employee	neg
Run 1	H07	Employee	neg
Run 1	H08	Employee	neg
Run 1	H09	Employee	neg
Run 1	H10	Employee	neg
Run 1	H11	Employee	neg
Run 1	H12	Employee	neg
Run 2	A01	Employee September	16
Run 2	A02	Patient ward H	17
Run 2	A03	Employee September	17
Run 2	A04	Patient ward H	18

Run 2	A05	Employee September	18
Run 2	A06	Employee September	18
Run 2	A07	Employee September	18
Run 2	A08	External outbreak link	19
Run 2	A09	Employee September	19
Run 2	A10	Employee September	19
Run 2	A11	Patient ward S	20
Run 2	A12	External outbreak link	20
Run 2	B01	Employee September	20
Run 2	B02	Employee September	20
Run 2	B03	Negative	neg
Run 2	B04	Employee September	20
Run 2	B05	Employee September	20
Run 2	B06	ONT samples	21
Run 2	B07	Patient ward E	21
Run 2	B08	Department C	21
Run 2	B09	Patient ward S	21
Run 2	B10	Employee September	21
Run 2	B11	Employee September	21
Run 2	B12	Employee September	21
Run 2	C01	Employee September	21
Run 2	C02	Employee September	21
Run 2	C03	ONT samples	22
Run 2	C04	ONT samples	22
Run 2	C05	ONT samples	22
Run 2	C06	Department C	22
Run 2	C07	Department C	22
Run 2	C08	Patient ward S	22
Run 2	C09	Department C	22
Run 2	C10	Employee September	22
Run 2	C11	Employee September	22
Run 2	C12	Employee September	22
Run 2	D01	Employee September	22
Run 2	D02	Employee September	22
Run 2	D03	Employee September	22
Run 2	D04	Employee September	22
Run 2	D05	Employee September	22
Run 2	D06	Employee September	22
Run 2	D07	ONT samples	23
Run 2	D08	ONT samples	23
Run 2	D09	Laboratory R	23
Run 2	D10	Laboratory R	23
Run 2	D11	External outbreak link	23
Run 2	D12	Laboratory R	23
Run 2	E01	Patient ward S	23
Run 2	E02	Employee September	23
Run 2	E03	Employee September	23
Run 2	E04	Patient ward S	24
Run 2	E05	External outbreak link	24
Run 2	E06	Employee September	24
Run 2	E07	Employee September	24
Run 2	E08	Employee September	24

Run 2	E09	Employee September	24
Run 2	E10	ONT samples	25
Run 2	E11	ONT samples	25
Run 2	E12	External outbreak link	25
Run 2	F01	Employee September	25
Run 2	F02	Employee September	25
Run 2	F03	Employee September	25
Run 2	F04	ONT samples	26
Run 2	F05	ONT samples	26
Run 2	F06	ONT samples	26
Run 2	F07	Laboratory R	26
Run 2	F08	Laboratory R	26
Run 2	F09	Laboratory R	26
Run 2	F10	Employee September	26
Run 2	F11	Employee September	26
Run 2	F12	Employee September	26
Run 2	G01	Patient ward E	26
Run 2	G02	ONT samples	27
Run 2	G03	Department C	27
Run 2	G04	Patient ward S	27
Run 2	G05	ONT samples	28
Run 2	G06	Laboratory R	28
Run 2	G07	ONT samples	29
Run 2	G08	ONT samples	29
Run 2	G09	ONT samples	29
Run 2	G10	Laboratory R	29
Run 2	G11	Laboratory R	29
Run 2	G12	ONT samples	30
Run 2	H01	ONT samples	30
Run 2	H02	Employee September	30
Run 2	H03	Employee September	30
Run 2	H04	External outbreak link	31
Run 2	H05	ONT samples	32
Run 2	H06	Employee	neg
Run 2	H07	Employee	neg
Run 2	H08	Employee	neg
Run 2	H09	Employee	neg
Run 2	H10	Employee	neg
Run 2	H11	Employee	neg
Run 2	H12	Patient ward H	26

Supplementary table 2: cDNA synthesis	
	μl
25mM MgCl ₂	5.50
2.5 mM dNTP mix	5.00
10x RT Buffer	2.50
RNase Inhibitor (20U/μL)	0.50
Multiscribe RT (50 U/μl)	0.62
Random hexamer (50 μM)	1.25
	15,37 μl mix + 10 μl RNA
RT reaction thermocycler: 10 min – 25°C / 30 min – 37°C / 5 min – 95°C / ∞ – 4°C	

Supplementary table 3: VirSEAK settings	
Settings	
Profile	SARS-CoV-2
Randomly sheared reads	no
Genome mapping	no
Single / double direction analysis	
Both direction minimum absolute coverage	3
Mutations	
Min % coverage	90% per dir.
Min % coverage homozygosity	95%
Homopolymer	on
Quality Score	
Score threshold	30
Ignore reads thresh.	40%
Score coverage warning	off
Expert Settings	
Genome Set	Haploid
Max mismatches	20%

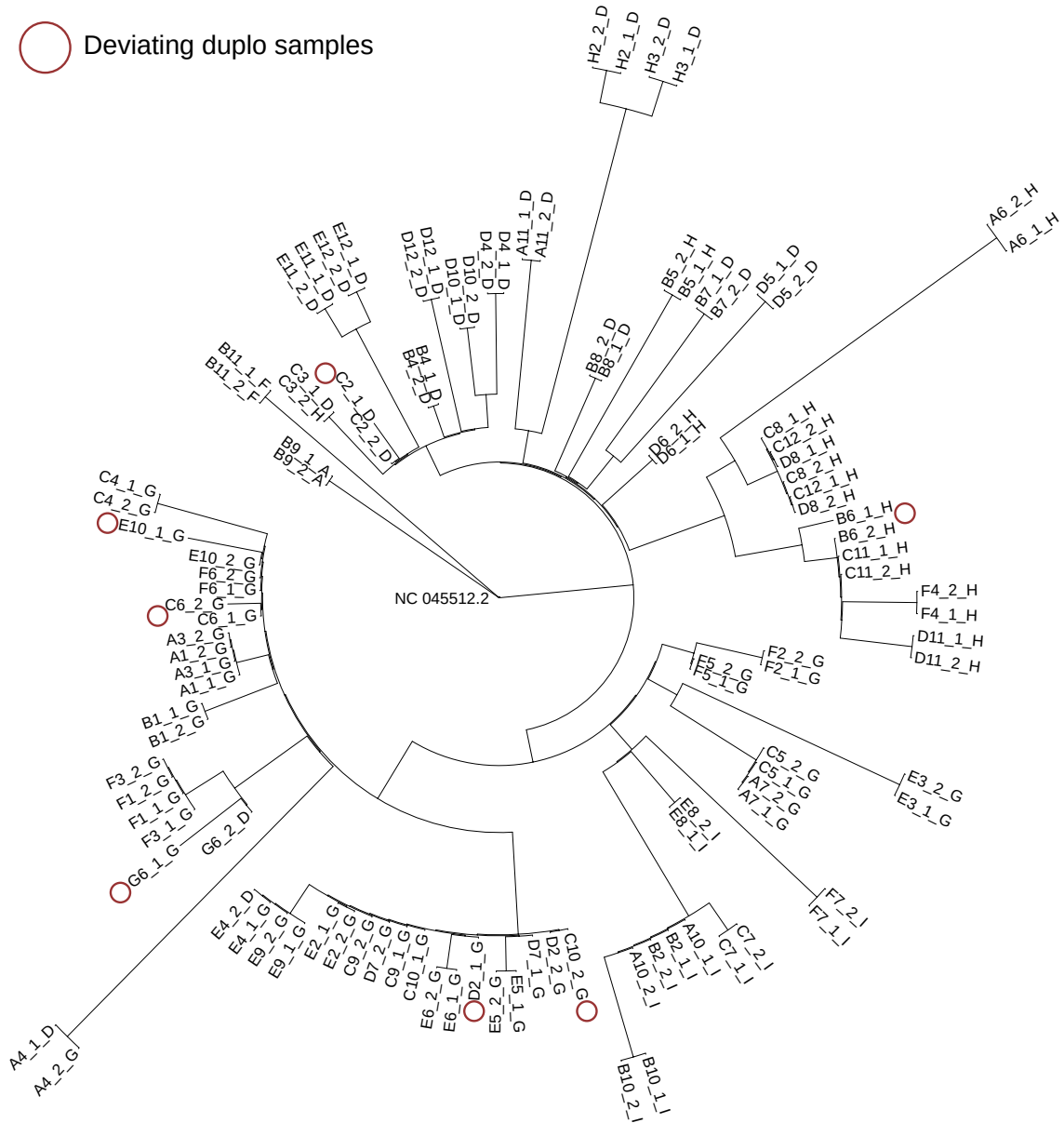
Supplementary table 4: Run statistics and details			
	Run1	Run1_new	Run2
Protocol	Default	Ct Library pooling strategy	Ct Library pooling strategy
Total Reads	19209544	18564880	18094162
PF Reads	18152046	17436566	17333104
% Reads Identified (PF)	89.18	93.92	88.79
Forward Read (% >= Q30)	97.15	96.41	97.43
Reverse Read (% >= Q30)	96.3	95.02	97.27
Total number of samples	96	96	96
Negative samples	8 (8%)	8 (8%)	7 (7%)
VirSEAK Consensus	57 (65%)*	59 (67%)*	89 (100%)*
Failed samples	31 (34%)*	29 (33%)*	0 (0%)*
Average amplicon depth	877.8	657	555.7
Average Genome Coverage	92.75%	88.38%	96.69%

** % is calculated in relation to total number of samples excluding the negative samples since output is not expected for these samples.*

Supplemental figures

Tree scale: 0.0001

○ Deviating duplo samples



Supplement Figure 1. Validation of RC-PCR by performing a technical duplo of 57 sample of which we performed RC-PCR on the same cDNA. Sample pairs that deviate on the phylogene-
nary are indicated by a red circle.