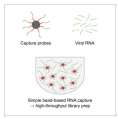

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A protocol for massively parallel diagnosis and genome sequencing of SARS-CoV-2

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Coronavirus Method Development Community


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ABSTRACT

Managing the current COVID-19 pandemic requires diagnostic testing at an unprecedented scale. However, the crisis has revealed severe deficiencies in our capacity to perform such testing. Here we build on the work of others (1-3) to develop a protocol that not only enables many thousand diagnostic tests to be run in parallel, but also provides near-whole genome sequencing data to facilitate phylogenetic analysis and contact tracing. One of the key features of our protocol is a magnetic bead-based strategy for RNA capture that may circumvent the need for SARS-CoV-2 RNA extraction, currently one of the major bottlenecks in both reagent supply and hands-on sample processing time. This approach also eliminates the requirement for per-sample reverse transcription, significantly reducing per-sample costs.

The major steps in our wet lab workflow can be summarised as follows. First, we generate a collection of bead-bound, single-stranded DNA probes tiling the entire SARS-CoV-2 genome. Multiple uniquely barcoded probe sets are prepared, each of which are used to capture viral RNA directly from patient swab samples via DNA/RNA hybridisation. Samples are combined together at this stage, enabling cDNA synthesis to be performed in a single pooled reaction. Finally, multiplex PCR is used to generate a library of overlapping amplicons ready for Illumina sequencing.

A schematic overview of the workflow is attached below, along with a more detailed figure depicting the various stages of library preparation.

References

1. https://docs.google.com/document/d/1kP2w_uTMSep2UxTCOnUhh1TMCjWvHEY0sUUpkJHPYV4/preview
2. https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w/abstract
3. https://www.biorxiv.org/content/10.1101/2020.03.20.001008v1.full.pdf

ATTACHMENTS

Workflow Overview.pdf

Library Prep Schematic.pdf

MATERIALS TEXT

Reagents

ITEM	SUPPLIER	CATALOGUE NUMBER
	General consumables, chemicals and equipment	

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UltraPure 1M Tris-HCl pH 7.5	Invitrogen	15567027
EDTA	Sigma-Aldrich	EDS-100G
NaCl	Sigma-Aldrich	S3014-500G
UltraPure DNase/RNase-free distilled water (500 mL)	Invitrogen	10977015
Ethanol, 200 proof for molecular biology (500 mL)	Sigma-Aldrich	E7023-500mL
DNA LoBind tubes, 1.5 mL (250 tubes)	Eppendorf	30108051
0.2 mL MAXYMum Recovery Thin Wall PCR Tubes	Fisher Biotec	PCR-02-L-C
20X TE buffer - RNase-free	Invitrogen	T11493
Sodium citrate	Sigma-Aldrich	C8532-100G
NaOH	Sigma-Aldrich	S8045-500G
PCR plate - Twintec LoBind DNA (Eppendorf)	Sigma-Aldrich	EP0030129512-25EA
LiCl	Sigma-Aldrich	L9650-100G
LiDS	Sigma-Aldrich	L9781-5G
DTT	Sigma-Aldrich	D9779-250MG
PCR foil	Eppendorf	30127790
	Step 1: Preparation of barcoded beads for RNA capture	
Dynabeads MyOne Streptavidin C1	Invitrogen	65001
Custom DNA oligos	IDT	-
2X B&W buffer	See Buffer list	-
1X SSC buffer	See Buffer list	-
PrimeSTAR GXL Polymerase	TaKaRa	R050A
	Step 2: Sample collection and preparation	
Universal Transport Medium (UTM)	COPAN	306C
Lysis/binding buffer	See Buffer list	-
Qubit HS RNA Assay Kit	Invitrogen	Q32855
Qubit tubes	Invitrogen	Q32856
	Step 3: RNA capture	
Washing buffer A	See Buffer list	-
	Step 4: cDNA synthesis	
SuperScript™ IV First-Strand Synthesis System	Invitrogen	18091200
Washing buffer B	See Buffer List	-
	Step 5: Library amplification	
Q5 Hot Start HiFi DNA Polymerase	NEB	M0493S
Custom DNA oligos	IDT	-
Qubit dsDNA HS Assay Kit	Invitrogen	Q33230
PrimeSTAR GXL Polymerase	TaKaRa	R050A
SPRIselect beads	Beckman Coulter	B23318

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	Step 6: Library quantitation and sequencing	
Bioanalyzer HS dsDNA	Agilent	5067-4626
Qubit dsDNA HS	Invitrogen	Q33230

Buffer list

BUFFER NAME	COMPONENTS
2X B&W buffer	10 mM Tris-HCl (pH 7.5)
	1 mM EDTA
	2 M NaCl
1X SSC buffer	0.15 M NaCl
	0.015 M sodium citrate
	Adjust pH to 7.0 with NaOH
TE buffer	10 mM Tris-HCl (pH 7.5)
	1 mM EDTA
Lysis/binding buffer	100 mM Tris-HCl (pH 7.5)
	500 mM LiCl
	10 mM EDTA, pH 8
	1% LiDS
	5 mM dithiothreitol (DTT)
Washing buffer A	10 mM Tris-HCl (pH 7.5)
	150 mM LiCl
	1 mM EDTA
	0.1% LiDS
Washing buffer B	10 mM Tris-HCl (pH 7.5)
	150mM LiCl

Individual primers [version 1]

NAME	SEQUENCE

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[Biotin]-barcoded bead tether_1	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAG CGTCAGATGTGTATAAGAGACAGGAACGTAATCGGCTCGG GTCGTCTG
[Biotin]-barcoded bead tether_2	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAG CGTCAGATGTGTATAAGAGACAGATTGATTACGGGCTCGG GTCGTCTG
[Biotin]-barcoded bead tether_3	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAG CGTCAGATGTGTATAAGAGACAGTCCGTCATCTGGCTCGGG TCGTCTG
[Biotin]-barcoded bead tether_4	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAG CGTCAGATGTGTATAAGAGACAGACTAGTTGCGGGCTCGG GTCGTCTG
[Biotin]-barcoded bead tether_5	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAG CGTCAGATGTGTATAAGAGACAGACCATATAAGGGCTCGGG TCGTCTG
[Biotin]-barcoded bead tether_6	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAG CGTCAGATGTGTATAAGAGACAGATCTCGATGAGGCTCGG GTCGTCTG
[Biotin]-barcoded bead tether_7	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAG CGTCAGATGTGTATAAGAGACAGACTTATAGTTGGCTCGGG TCGTCTG
[Biotin]-bead tether	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAG CGTC
Barcoded linker_1	CAGACGACCCGAGCCTACCATGCTTCTGTCTCTTATACACA TCTGACGCTGCCGACGA
Barcoded linker_2	CAGACGACCCGAGCCAATGGAGCCGCTGTCTCTTATACACA TCTGACGCTGCCGACGA
Barcoded linker_3	CAGACGACCCGAGCCCTAATCCGGTCTGTCTCTTATACACA TCTGACGCTGCCGACGA
Barcoded linker_4	CAGACGACCCGAGCCTGCCTAACTCCTGTCTCTTATACACA TCTGACGCTGCCGACGA
Barcoded linker_5	CAGACGACCCGAGCCAATAGTAGGCCTGTCTCTTATACACA TCTGACGCTGCCGACGA
Barcoded linker_6	CAGACGACCCGAGCCGCAACTTGGACTGTCTCTTATACACA TCTGACGCTGCCGACGA
Barcoded linker_7	CAGACGACCCGAGCCGAGATCGTCACTGTCTCTTATACACA TCTGACGCTGCCGACGA
Illumina P5 index primer_1	AATGATACGGCGACCACCGAGATCTACACTCGCTGAATCGT CGGCAGCGTC
Illumina P5 index primer_2	AATGATACGGCGACCACCGAGATCTACACCTCCAGGATCGT CGGCAGCGTC
Illumina P7 index primer_1	CAAGCAGAAGACGGCATACGAGATAACCGCGGGTGACTGG AGTTCAGACGTGTGCTCTTCCGATCT
Illumina P7 index primer_2	CAAGCAGAAGACGGCATACGAGATGGTTATAAGTGACTGGA GTTCAGACGTGTGCTCTTCCGATCT
Illumina P5 flow cell primer	AATGATACGGCGACCACCGAGATCTACAC

SARS-CoV-2 probe pool 1 [version 1]

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NAME	SEQUENCE
ncov_cap_1_RIGHT	CAGCACATCTAGGTTTCGTCCGCAGACGACCCGAGCC
ncov_cap_3_RIGHT	CCTCATGGTCATGTTATGGTTGAGCCAGACGACCCGAGCC
ncov_cap_5_RIGHT	GTTACCCGTGAACTCATGCGTGCAGACGACCCGAGCC
ncov_cap_7_RIGHT	GCAGACACCTTTTGAAATTAAATTGGCCAGACGACCCGAGCC
ncov_cap_9_RIGHT	GGTGAAACTTCATGGCAGACGGCAGACGACCCGAGCC
ncov_cap_11_RIGHT	GTAAGGGTGGTCGCACTATTGCCAGACGACCCGAGCC
ncov_cap_13_RIGHT	CTGCTTCCACAAGTGCTTTTGTCAGACGACCCGAGCC
ncov_cap_15_RIGHT	CGTGTTTTACAGAAGGCCGCTACAGACGACCCGAGCC
ncov_cap_17_RIGHT	AGAGACGGTTGGGAAATTGTTAAATTTCAGACGACCCGAGCC
ncov_cap_19_RIGHT	AGAAGAAACTGGCCTACTCATGCCAGACGACCCGAGCC
ncov_cap_21_RIGHT	TGGTAACAAACAATACCTTCACACTCACAGACGACCCGAGCC
ncov_cap_23_RIGHT	ACCACTGGGCATTGATTTAGATGACAGACGACCCGAGCC
ncov_cap_25_RIGHT	AGAGCAAGAAGAAGATTGGTTAGATGACAGACGACCCGAGCC
ncov_cap_27_RIGHT	GTGGTTGTTAATGCAGCCAATGTCAGACGACCCGAGCC
ncov_cap_29_RIGHT	CAGCACGAAGTTCTACTTGCACCAGACGACCCGAGCC
ncov_cap_31_RIGHT	ACCTTCAGTTGAACAGAGAAAACAAGCAGACGACCCGAGCC
ncov_cap_33_RIGHT	AAAGGCTGGTGGCACTACTGAACAGACGACCCGAGCC
ncov_cap_35_RIGHT	CAGAAGAAACACGCAAATTAATGCCCAGACGACCCGAGCC
ncov_cap_37_RIGHT	AAGTGCCAGCTACAGTTTCTGTCAGACGACCCGAGCC
ncov_cap_39_RIGHT	GTTATCACCTTTGACAATCTTAAGACACTCAGACGACCCGAGCC
ncov_cap_41_RIGHT	TGATCCTAGTTTTCTGGGTAGGTACACAGACGACCCGAGCC
ncov_cap_43_RIGHT	GAAGCTGCTAACTTTTGTGCACTCAGACGACCCGAGCC
ncov_cap_45_RIGHT	CGTGTGGTAAACAAGCTACAAAATATCTCAGACGACCCGAGCC
ncov_cap_47_RIGHT	CAAAGGTCCTATTACGGATGTTTTCTACCAGACGACCCGAGCC
ncov_cap_49_RIGHT	ACATTTTTCCCTGACTTAAATGGTGATGCAGACGACCCGAGCC
ncov_cap_51_RIGHT	TGAAGAAGTAGTGGAAAATCCTACCATCAGACGACCCGAGCC
ncov_cap_53_RIGHT	TGCTGTTAATAGTGTCCCTTGGGCAGACGACCCGAGCC
ncov_cap_55_RIGHT	CTCAACCGCTGCTTTAGGTGTTCAGACGACCCGAGCC
ncov_cap_57_RIGHT	TGGTTTTTGGCATATATTCTTTTCACTAGGCAGACGACCCGAGCC
ncov_cap_59_RIGHT	TCAACTTGTATGATGTGTTACAAACGTAACAGACGACCCGAGCC
ncov_cap_61_RIGHT	ACAGTGAAGAATGGTTCCATCCATCAGACGACCCGAGCC
ncov_cap_63_RIGHT	TGTTACTAGATCAGGCATTAGTGTCTGCAGACGACCCGAGCC
ncov_cap_65_RIGHT	AGGGTTTGTTGATTCAGATGTAGAAACCAGACGACCCGAGCC
ncov_cap_67_RIGHT	ACGAAAACAAATACGTAGTGCTGCCAGACGACCCGAGCC
ncov_cap_69_RIGHT	TGTCATGTCTAAACATACTGACTTTTCAAGCAGACGACCCGAGCC
ncov_cap_71_RIGHT	ATTACGCACAACTAATGGTGACTTTTCAGACGACCCGAGCC
ncov_cap_73_RIGHT	ACGTTATGTGCTCATGGATGGCCAGACGACCCGAGCC

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ncov_cap_75_RIGHT	GGTGCTTTGGACATATCAGCATCTCAGACGACCCGAGCC
ncov_cap_77_RIGHT	AGCACATATTCAGTGGATGGTTATGTTCAGACGACCCGAGCC
ncov_cap_79_RIGHT	TCTAAAGTTGCGTAGTGATGTGCTACAGACGACCCGAGCC
ncov_cap_81_RIGHT	CCCATCTGGTAAAGTTGAGGGTTGCAGACGACCCGAGCC
ncov_cap_83_RIGHT	GCTTAAGGTTGATACAGCCAATCCTCAGACGACCCGAGCC
ncov_cap_85_RIGHT	TCTTTTTGTTACATGCACCATATGGAATTCAGACGACCCGAGCC
ncov_cap_87_RIGHT	CCTCTAACACAAGACCATGTTGACACAGACGACCCGAGCC
ncov_cap_89_RIGHT	CACACCACTGGTTGTTACTCACACAGACGACCCGAGCC
ncov_cap_91_RIGHT	TGCTAGTTGGGTGATGCGTATTCAGACGACCCGAGCC
ncov_cap_93_RIGHT	TCTCTGTTACTTCTAACTACTCAGGTGTACAGACGACCCGAGCC
ncov_cap_95_RIGHT	ACTGACTCTTGGTGTTTATGATTACTTAGTCAGACGACCCGAGCC
ncov_cap_97_RIGHT	GGGCTCAATGTGTCCAGTTACACAGACGACCCGAGCC
ncov_cap_99_RIGHT	TGCTAATGGTGATTCTGAAGTTGTTCTCAGACGACCCGAGCC
ncov_cap_101_RIGHT	GCAAGAGATGGTTGTGTTCCCTCAGACGACCCGAGCC
ncov_cap_103_RIGHT	GCATGGCCTCTTATTGTAACAGCCAGACGACCCGAGCC
ncov_cap_105_RIGHT	ATGGAACTGGTACTATCTATACAGAACTGCAGACGACCCGAGCC
ncov_cap_107_RIGHT	ACACACACTGGTACTGGTCAGGCAGACGACCCGAGCC
ncov_cap_109_RIGHT	AAACACAGTCTGTACCGTCTGCCAGACGACCCGAGCC
ncov_cap_111_RIGHT	AGGACGAAGATGACAATTTAATTGATTCTTCAGACGACCCGAGCC
ncov_cap_113_RIGHT	GCATTTTGATGAAGGTAATTGTGACACACAGACGACCCGAGCC
ncov_cap_115_RIGHT	GGTATGATTTCGGTGATTTCATACAAACCCAGACGACCCGAGCC
ncov_cap_117_RIGHT	TGTTAACTGTTTGGATGACAGATGCCAGACGACCCGAGCC
ncov_cap_119_RIGHT	TGTATGCTGCTGACCCTGCTATCAGACGACCCGAGCC
ncov_cap_121_RIGHT	CCAACAATGTGTGATATCAGACAACTACTCAGACGACCCGAGCC
ncov_cap_123_RIGHT	CGTAATGTCATCCCTACTATAACTCAAATGCAGACGACCCGAGCC
ncov_cap_125_RIGHT	TTATGGCCTCACTTGTTCTTGCTCAGACGACCCGAGCC
ncov_cap_127_RIGHT	AATGCACTTTTATCTACTGATGGTAACAAACAGACGACCCGAGCC
ncov_cap_129_RIGHT	GGCTAGCATAAAGAACTTTAAGTCAGTTCAGACGACCCGAGCC
ncov_cap_131_RIGHT	GGTACACTTATGATTGAACGGTTCGCAGACGACCCGAGCC
ncov_cap_133_RIGHT	ACACACCGCATACAGTCTTACAGCAGACGACCCGAGCC
ncov_cap_135_RIGHT	TGTGCTAATGGACAAGTTTTTGGTTCAGACGACCCGAGCC
ncov_cap_137_RIGHT	TGCTGTCTGACAGAGAATTACATCTTCAGACGACCCGAGCC
ncov_cap_139_RIGHT	GCCATTAAGTGCACCTACACTAGTCAGACGACCCGAGCC
ncov_cap_141_RIGHT	CGCTGTTGATGCACTATGTGAGACAGACGACCCGAGCC
ncov_cap_143_RIGHT	CAATGCCAGATTACGTGCTAAGCCAGACGACCCGAGCC
ncov_cap_145_RIGHT	AGGGTGTTATCACGCATGATGTCAGACGACCCGAGCC
ncov_cap_147_RIGHT	CAGCTCACTCTTGTAATGTAAACAGATTCAGACGACCCGAGCC

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ncov_cap_149_RIGHT	TCAAAACTGAAGGTTTATGTGTTGACACAGACGACCCGAGCC
ncov_cap_151_RIGHT	TACCTTTACAGCTAGGTTTTTCTACAGGCAGACGACCCGAGCC
ncov_cap_153_RIGHT	GGCTTTGAGTTGACATCTATGAAGTATTTTCAGACGACCCGAGCC
ncov_cap_155_RIGHT	AGCTAGTTGTGATGCAATCATGACTCAGACGACCCGAGCC
ncov_cap_157_RIGHT	GTAGAATGGAAGTTCTATGATGCACAGCAGACGACCCGAGCC
ncov_cap_159_RIGHT	CATTCCACACACCAGCTTTTGATAACAGACGACCCGAGCC
ncov_cap_161_RIGHT	AGCTTGTGGGTTTACAAACAATTTGACAGACGACCCGAGCC
ncov_cap_163_RIGHT	AATGTAGCATTTGAGCTTTGGGCCAGACGACCCGAGCC
ncov_cap_165_RIGHT	AGAAATGCCCGTAATGGTGTTCTCAGACGACCCGAGCC
ncov_cap_167_RIGHT	AGCTATGGATGAATTCATTGAACGGTACAGACGACCCGAGCC
ncov_cap_169_RIGHT	CCCAAGATTTATCTGTAGTTTCTAAGGTTGCAGACGACCCGAGCC
ncov_cap_171_RIGHT	GTCGCAAAATATACTCAACTGTGTCACAGACGACCCGAGCC
ncov_cap_173_RIGHT	GATCTCATTATTAGTGATATGTACGACCCTCAGACGACCCGAGCC
ncov_cap_175_RIGHT	AATTGGATGTAATTATCTTGGCAAACCACAGACGACCCGAGCC
ncov_cap_177_RIGHT	TGATGTTCTTGTTAACAACTAAACGAACACAGACGACCCGAGCC
ncov_cap_179_RIGHT	TGGGACCAATGGTACTAAGAGGTTCAGACGACCCGAGCC
ncov_cap_181_RIGHT	CAAAAGTTGGATGGAAAGTGAGTTCACAGACGACCCGAGCC
ncov_cap_183_RIGHT	ACTAGGTTTCAAACTTTACTTGCTTTACACAGACGACCCGAGCC
ncov_cap_185_RIGHT	AGAGTCCAACCAACAGAATCTATTGTCAGACGACCCGAGCC
ncov_cap_187_RIGHT	GCAGATTCATTTGTAATTAGAGGTGATGACAGACGACCCGAGCC
ncov_cap_189_RIGHT	ATCTATCAGGCCGGTAGCACACCAGACGACCCGAGCC
ncov_cap_191_RIGHT	ACAAAAAGTTTCTGCCTTTCCAACACAGACGACCCGAGCC
ncov_cap_193_RIGHT	CAAACACGTGCAGGCTGTTTAACAGACGACCCGAGCC
ncov_cap_195_RIGHT	TCTACCAGTGTCTATGACCAAGACACAGACGACCCGAGCC
ncov_cap_197_RIGHT	CCATCAAAACCAAGCAAGAGGTCACAGACGACCCGAGCC
ncov_cap_199_RIGHT	TCTGGTTGGACCTTTGGTGCAGCAGACGACCCGAGCC
ncov_cap_201_RIGHT	ACGCTTGTTAAACAACTTAGCTCCCAGACGACCCGAGCC
ncov_cap_203_RIGHT	TGGAAAGGGCTATCATCTTATGTCCTCAGACGACCCGAGCC
ncov_cap_205_RIGHT	GTCTGGTAACTGTGATGTTGTAATAGGACAGACGACCCGAGCC
ncov_cap_207_RIGHT	CTCATCGATCTCCAAGAACTTGGACAGACGACCCGAGCC
ncov_cap_209_RIGHT	GGAACTGTAACTTTGAAGCAAGGTGCAGACGACCCGAGCC
ncov_cap_211_RIGHT	AACAGTTTACTCACACCTTTTGCTCCAGACGACCCGAGCC
ncov_cap_213_RIGHT	TCATTACTTCAGGTGATGGCACAACAGACGACCCGAGCC
ncov_cap_215_RIGHT	CCAATTTATGATGAACCGACGACGCAGACGACCCGAGCC
ncov_cap_217_RIGHT	AAAACCTTCTTTTTACGTTTACTCTCGTCAGACGACCCGAGCC
ncov_cap_219_RIGHT	TGTCTTCTACAATTTGCCTATGCCACAGACGACCCGAGCC
ncov_cap_221_RIGHT	CTTCTCAACGTGCCACTCCATGCAGACGACCCGAGCC
ncov_cap_223_RIGHT	GTCGCTACAGGATTGGCAACTATCAGACGACCCGAGCC
ncov_cap_225_RIGHT	TGAAGAGCAACCAATGGAGATTGACAGACGACCCGAGCC

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ncov_cap_227_RIGHT	TGTTCATCAGACAAGAGGAAGTTCACAGACGACCCGAGCC
ncov_cap_229_RIGHT	TTTTCTTAGGAATCATCACAACTGTAGCCAGACGACCCGAGCC
ncov_cap_231_RIGHT	TGCCAGGAACCTAAATTGGGTAGCAGACGACCCGAGCC
ncov_cap_233_RIGHT	GGCCCCAAGGTTTACCCAATAATCAGACGACCCGAGCC
ncov_cap_235_RIGHT	TGGACTTCCCTATGGTGCTAACAACAGACGACCCGAGCC
ncov_cap_237_RIGHT	TTCAACTCCAGGCAGCAGTAGGCAGACGACCCGAGCC
ncov_cap_239_RIGHT	GGTCCAGAACAAACCCAAGGAACAGACGACCCGAGCC
ncov_cap_241_RIGHT	CGCATACAAAACATTCCCACCAACCAGACGACCCGAGCC
ncov_cap_243_RIGHT	CGTTTTCGCTTTTCCGTTTACGACAGACGACCCGAGCC
ncov_cap_245_RIGHT	TGGAAGAGCCCTAATGTGTAAAATTAATTTCAGACGACCCGAGCC
RNAse_P_bead_rev	GTGGAGACAGCCGCTCACAGACGACCCGAGCC

SARS-CoV-2 probe pool 2 [version 1]

NAME	SEQUENCE
ncov_cap_2_RIGHT	TCTTAAAGATGGCACTTGTGGCTTCAGACGACCCGAGCC
ncov_cap_4_RIGHT	AGGAGCTGGTGGCCATAGTTACCAGACGACCCGAGCC
ncov_cap_6_RIGHT	GCTTCATGCACTTTGTCCGAACCAGACGACCCGAGCC
ncov_cap_8_RIGHT	TGATGGCTTTATGGGTAGAATTCGACAGACGACCCGAGCC
ncov_cap_10_RIGHT	GCTGTTGTTAAAATTTATTGTCCAGCATCAGACGACCCGAGCC
ncov_cap_12_RIGHT	TGGAGAAGGTTCCGAAGGTCTTCAGACGACCCGAGCC
ncov_cap_14_RIGHT	GGAATATTGGTGAACAGAAATCAATACTGACAGACGACCCGAGCC
ncov_cap_16_RIGHT	AGGTGGTGTTGTTCAGTTGACTCAGACGACCCGAGCC
ncov_cap_18_RIGHT	TTTGGCTTTGTGTGCTGACTCTCAGACGACCCGAGCC
ncov_cap_20_RIGHT	TTAGAACAACCTACTAGTGAAGCTGTTCAGACGACCCGAGCC
ncov_cap_22_RIGHT	GCCTATACAGTTGAACTCGGTACAGCAGACGACCCGAGCC
ncov_cap_24_RIGHT	TGTGAAGAAGAAGAGTTTGAGCCACAGACGACCCGAGCC
ncov_cap_26_RIGHT	GGAACTTACACCAGTTGTTCAGACTCAGACGACCCGAGCC
ncov_cap_28_RIGHT	CTTAAAGTGGGTGGTAGTTGTGTTTTCAGACGACCCGAGCC
ncov_cap_30_RIGHT	CACAAATGTCTACTTAGCTGTCTTTGATCAGACGACCCGAGCC
ncov_cap_32_RIGHT	CAATCTTCATCCAGATTCTGCCACTCAGACGACCCGAGCC
ncov_cap_34_RIGHT	CAGTGCTTAAAAAGTGTAAAAGTGCCCAGACGACCCGAGCC
ncov_cap_36_RIGHT	GTAAAACAACTGTAGCGTCACTTATCACAGACGACCCGAGCC
ncov_cap_38_RIGHT	GTTCCTATAAAGATTGGTCCTATTCTGGACAGACGACCCGAGCC
ncov_cap_40_RIGHT	ACTTATTTGGATGGAGCTGATGTTACTAACAGACGACCCGAGCC
ncov_cap_42_RIGHT	CTTCTATTAAATGGGCAGATAACAACTGTCAGACGACCCGAGCC
ncov_cap_44_RIGHT	AAGAGTCTTGAACGTGGTGTGTCAGACGACCCGAGCC
ncov_cap_46_RIGHT	TGGTACATTTACTTGTGCTAGTGAGTACAGACGACCCGAGCC
ncov_cap_48_RIGHT	AACCAACCATATCCAAACGCAAGCAGACGACCCGAGCC
ncov_cap_50_RIGHT	CCTGGTGTATACGTTGTCTTTGGACAGACGACCCGAGCC
ncov_cap_52_RIGHT	CACACAGATCTAATGGCTGCTTATGTCAGACGACCCGAGCC

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ncov_cap_54_RIGHT	GCAAAGAATACTGTTAAGAGTGTCGGTACAGACGACCCGAGCC
ncov_cap_56_RIGHT	GTAGTGTTTGTCTTAGTGGTTTAGATTCTTCAGACGACCCGAGCC
ncov_cap_58_RIGHT	ATGGCCCCGATTTCAGCTATGGCAGACGACCCGAGCC
ncov_cap_60_RIGHT	CTGTGCTGGTAGTACATTTATTAGTGATGCAGACGACCCGAGCC
ncov_cap_62_RIGHT	AGCTAATAACACTAAAGGTTCATTGCCTACAGACGACCCGAGCC
ncov_cap_64_RIGHT	ACTCAAAACACTAGTTGCAACTGCCAGACGACCCGAGCC
ncov_cap_66_RIGHT	GCTTGTATTGACTGTAGTGCGCCAGACGACCCGAGCC
ncov_cap_68_RIGHT	AGCACTTAAGGGTGGTAAAATTGTTAATCAGACGACCCGAGCC
ncov_cap_70_RIGHT	GCTGATTTTGACACATGGTTTAGCCCAGACGACCCGAGCC
ncov_cap_72_RIGHT	GTGTTTTGGCTGCTGAATGTACACAGACGACCCGAGCC
ncov_cap_74_RIGHT	GCTGGTGTTTGTGTATCTACTAGTGGCAGACGACCCGAGCC
ncov_cap_76_RIGHT	GAGCTTTTGGTGAATACAGTCATGTAGCAGACGACCCGAGCC
ncov_cap_78_RIGHT	TCCACAAAGCATTTCTATTGGTTCTTTCAGACGACCCGAGCC
ncov_cap_80_RIGHT	GCTTGTTGTCATCTCGCAAAGGCAGACGACCCGAGCC
ncov_cap_82_RIGHT	TGAAGACATGCTTAACCCTAATTATGAAGACAGACGACCCGAGCC
ncov_cap_84_RIGHT	TGCTATGAGGCCCAATTTCACTCAGACGACCCGAGCC
ncov_cap_86_RIGHT	TTTTAGCTTGGTTGTACGCTGCCAGACGACCCGAGCC
ncov_cap_88_RIGHT	CCATATTGGGTAGTGCTTTATTAGAAGATGCAGACGACCCGAGCC
ncov_cap_90_RIGHT	TGTCTGCTTTTGCAATGATGTTTGTCAGACGACCCGAGCC
ncov_cap_92_RIGHT	ACTGTGTATGATGATGGTGCTAGGCAGACGACCCGAGCC
ncov_cap_94_RIGHT	TGCCCTATTTTCTTCATAACTGGTAATACACAGACGACCCGAGCC
ncov_cap_96_RIGHT	GGCAAACCTTGTATCAAAGTAGCCCAGACGACCCGAGCC
ncov_cap_98_RIGHT	GCTTTGTGAAGAAATGCTGGACACAGACGACCCGAGCC
ncov_cap_100_RIGHT	TGACCCAAATGTATAAACAGGCTAGACAGACGACCCGAGCC
ncov_cap_102_RIGHT	AAATACGTGTGATGGTACAACATTTACTTCAGACGACCCGAGCC
ncov_cap_104_RIGHT	ACAAACTGCTTGCACTGATGACACAGACGACCCGAGCC
ncov_cap_106_RIGHT	CTTGGTAGTTTAGCTGCCACAGTCAGACGACCCGAGCC
ncov_cap_108_RIGHT	TTTGGTGGTGCATCGTGTTGTCCAGACGACCCGAGCC
ncov_cap_110_RIGHT	GTATACAGGGCTTTTGACATCTACAATGCAGACGACCCGAGCC
ncov_cap_112_RIGHT	AATAGACGGTGACATGGTACCACCAGACGACCCGAGCC
ncov_cap_114_RIGHT	CGCCAACTTAGGTGAACGTGTACAGACGACCCGAGCC
ncov_cap_116_RIGHT	TGTTGACACTGACTTAACAAAGCCTTCAGACGACCCGAGCC
ncov_cap_118_RIGHT	GTGAGAAAAATATTTGTTGATGGTGTTCCCAGACGACCCGAGCC
ncov_cap_120_RIGHT	GCTTTTCAAACTGTCAAACCCGGCAGACGACCCGAGCC
ncov_cap_122_RIGHT	CCTAGACAAATCAGCTGGTTTTCCACAGACGACCCGAGCC
ncov_cap_124_RIGHT	TTGGAACAAGCAAATTCTATGGTGGCAGACGACCCGAGCC
ncov_cap_126_RIGHT	TCACTATATGTTAAACCAGGTGGAACCCAGACGACCCGAGCC
ncov_cap_128_RIGHT	AGACTTTGTGAATGAGTTTTACGCATCAGACGACCCGAGCC

Citation: Leigh Monahan, Kay Anantanawat, Joyce To, Aaron Darling (04/11/2020). A protocol for massively parallel diagnosis and genome sequencing of SARS-CoV-2. https://dx.doi.org/10.17504/protocols.io.betrjem6

ncov_cap_130_RIGHT	CTCTCAACATACAATGCTAGTTAAACAGGCAGACGACCCGAGCC
ncov_cap_132_RIGHT	AAGCTACATGATGAGTTAACAGGACACAGACGACCCGAGCC
ncov_cap_134_RIGHT	TCTTGTCTGTTAATCCGTATGTTTGCCAGACGACCCGAGCC
ncov_cap_136_RIGHT	TTTAGCTAACACCTGTACTGAAAGACTCAGACGACCCGAGCC
ncov_cap_138_RIGHT	CAAATAGGAGAGTACACCTTTGAAAAAGGCAGACGACCCGAGCC
ncov_cap_140_RIGHT	AAGGTTGGTATGCAAAAGTATTCTACACCAGACGACCCGAGCC
ncov_cap_142_RIGHT	GTACTGTAAATGCATTGCCTGAGACCAGACGACCCGAGCC
ncov_cap_144_RIGHT	TAGGTCCAGACATGTTCCTCGGCAGACGACCCGAGCC
ncov_cap_146_RIGHT	GCCTCAAAGATTTTGGGACTACCACAGACGACCCGAGCC
ncov_cap_148_RIGHT	CGTAGGAATGTGGCAACTTTACAAGCAGACGACCCGAGCC
ncov_cap_150_RIGHT	CCCGCGAAGAAGCTATAAGACACAGACGACCCGAGCC
ncov_cap_152_RIGHT	CCTCATACCACTTATGTACAAAGGACTCAGACGACCCGAGCC
ncov_cap_154_RIGHT	GCATCATTCTATTGGATTTGATTACGTCTCAGACGACCCGAGCC
ncov_cap_156_RIGHT	CTTGTAGAAAGGTTCAACACATGGTTCAGACGACCCGAGCC
ncov_cap_158_RIGHT	TGCAATGTCGATAGATATCCTGCTAACAGACGACCCGAGCC
ncov_cap_160_RIGHT	CCACTAAAGTCTGCTACGTGTATAACACAGACGACCCGAGCC
ncov_cap_162_RIGHT	TGATGGACAACAGGGTGAAGTACCAGACGACCCGAGCC
ncov_cap_164_RIGHT	CTCCAGCACATATATCTACTATTGGTGTCAGACGACCCGAGCC
ncov_cap_166_RIGHT	AGAAGCCGTAAAAACACAGTTCAATTCAGACGACCCGAGCC
ncov_cap_168_RIGHT	TGGTTTACATCTACTGATTGGACTAGCCAGACGACCCGAGCC
ncov_cap_170_RIGHT	AATCTAGTCAAGCGTGGCAACCCAGACGACCCGAGCC
ncov_cap_172_RIGHT	TTGCACCAGGTACAGCTGTTTTCAGACGACCCGAGCC
ncov_cap_174_RIGHT	TGGAGGTTCCGTGGCTATAAAGATCAGACGACCCGAGCC
ncov_cap_176_RIGHT	CAAATCCAATTCAGTTGTCTTCCTATTCTCAGACGACCCGAGCC
ncov_cap_178_RIGHT	CAGTGTGTTAATCTTACAACCAGAACTCCAGACGACCCGAGCC
ncov_cap_180_RIGHT	TTCGAAGACCCAGTCCCTACTTCAGACGACCCGAGCC
ncov_cap_182_RIGHT	GCCTATTAATTTAGTGCGTGATCTCCCAGACGACCCGAGCC
ncov_cap_184_RIGHT	TGAAAATGGAACCATTACAGATGCTGCAGACGACCCGAGCC
ncov_cap_186_RIGHT	ACTGTGTTGCTGATTATTCTGTCCTCAGACGACCCGAGCC
ncov_cap_188_RIGHT	CGTTATAGCTTGGAATTCTAACAATCTTGACAGACGACCCGAGCC
ncov_cap_190_RIGHT	TTCTTTTGAACTTCTACATGCACCAGCAGACGACCCGAGCC
ncov_cap_192_RIGHT	AACAAATACTTCTAACCAGGTTGCTGCAGACGACCCGAGCC
ncov_cap_194_RIGHT	ACGTAGTGTAGCTAGTCAATCCATCACAGACGACCCGAGCC
ncov_cap_196_RIGHT	ACTGGAATAGCTGTTGAACAAGACACAGACGACCCGAGCC
ncov_cap_198_RIGHT	ATTGCTGCTAGAGACCTCATTTGTCAGACGACCCGAGCC
ncov_cap_200_RIGHT	GAACCAAAAATTGATTGCCAACCAATCAGACGACCCGAGCC
ncov_cap_202_RIGHT	AAGTTTGCAGACATATGTGACTCAACCAGACGACCCGAGCC
ncov_cap_204_RIGHT	TGGAAAAGCACACTTTCCTCGTCAGACGACCCGAGCC
ncov_cap_206_RIGHT	TTGATTTAGGTGACATCTCTGGCACAGACGACCCGAGCC
ncov_cap_208_RIGHT	GCTGTATGACCAGTTGCTGTAGTCAGACGACCCGAGCC

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ncov_cap_210_RIGHT	CTTATTGTTGGCGTTGCACTTCTTCAGACGACCCGAGCC
ncov_cap_212_RIGHT	GCCGTTCCAAAAACCCATTACTTCAGACGACCCGAGCC
ncov_cap_214_RIGHT	TGTATTACACAGTTACTTCACTTCAGACTCAGACGACCCGAGCC
ncov_cap_216_RIGHT	TTCTTGCTTTCGTGGTATTCTTGCCAGACGACCCGAGCC
ncov_cap_218_RIGHT	CATGGCAGATTCCAACGGTACTCAGACGACCCGAGCC
ncov_cap_220_RIGHT	GGATCACCGGTGGAATTGCTATCAGACGACCCGAGCC
ncov_cap_222_RIGHT	ACGCTGTGACATCAAGGACCTGCAGACGACCCGAGCC
ncov_cap_224_RIGHT	GTTGACTTTCAGGTTACTATAGCAGAGACAGACGACCCGAGCC
ncov_cap_226_RIGHT	ATTCACCATTTCATCCTCTAGCTGATCAGACGACCCGAGCC
ncov_cap_228_RIGHT	ATTTGTGCTTTTTAGCCTTTCTGCTACAGACGACCCGAGCC
ncov_cap_230_RIGHT	CGTGTCCTATTCACTTCTATTCTAAATGGCAGACGACCCGAGCC
ncov_cap_232_RIGHT	CCCAAAATCAGCGAAATGCACCCAGACGACCCGAGCC
ncov_cap_234_RIGHT	GCAGTCCAGATGACCAAATTGGCCAGACGACCCGAGCC
ncov_cap_236_RIGHT	CCTCAAGGAACAACATTGCCAAACAGACGACCCGAGCC
ncov_cap_238_RIGHT	ACAACAACAAGGCCAAACTGTCCAGACGACCCGAGCC
ncov_cap_240_RIGHT	ATTGGCATGGAAGTCACACCTTCAGACGACCCGAGCC
ncov_cap_242_RIGHT	TTCTTCCTGCTGCAGATTTGGACAGACGACCCGAGCC
ncov_cap_244_RIGHT	AGTGTGTAACATTAGGGAGGACTTGCAGACGACCCGAGCC
RNAse_P_bead_rev	GTGGAGACAGCCGCTCACAGACGACCCGAGCC

SARS-CoV-2 primer pool 1 [version 1]

NAME	SEQUENCE
ncov_cap_1_LEFT	ACGTGTGCTCTTCCGATCTTCTCTTGTAGATCTGTTCTCTAAACGAAC
ncov_cap_3_LEFT	ACGTGTGCTCTTCCGATCTCTGTTTTACAGGTTCGCGACGT
ncov_cap_5_LEFT	ACGTGTGCTCTTCCGATCTCCTCATGTGGGCGAAATACCAG
ncov_cap_7_LEFT	ACGTGTGCTCTTCCGATCTATGGCTACCCTCTTGAGTGCAT
ncov_cap_9_LEFT	ACGTGTGCTCTTCCGATCTGGGGAATGTCCAAATTTTGTATTTCCC
ncov_cap_11_LEFT	ACGTGTGCTCTTCCGATCTCCACTTGCGAATTTTGTGGCAC
ncov_cap_13_LEFT	ACGTGTGCTCTTCCGATCTCTATTGGGTTCCACGTGCTAGC
ncov_cap_15_LEFT	ACGTGTGCTCTTCCGATCTTCAAACAAATTGTTGAATCCTGTGGT
ncov_cap_17_LEFT	ACGTGTGCTCTTCCGATCTTGTTCACATCTGATTTGGCTACTAACA
ncov_cap_19_LEFT	ACGTGTGCTCTTCCGATCTCCTGTGCAAAGGAAATTAAGGAGAGT
ncov_cap_21_LEFT	ACGTGTGCTCTTCCGATCTTCCCACAGAAGTGTTAACAGAGGA
ncov_cap_23_LEFT	ACGTGTGCTCTTCCGATCTAGTGCAAGGTTACAAGAGTGTGA
ncov_cap_25_LEFT	ACGTGTGCTCTTCCGATCTGGCTTCACATATGTATTGTTCTTTCTACC
ncov_cap_27_LEFT	ACGTGTGCTCTTCCGATCTAGGACAATCAGACAACTACTATTCAAACA
ncov_cap_29_LEFT	ACGTGTGCTCTTCCGATCTGGCTACTAACAATGCCATGCAAG

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ncov_cap_31_LEFT	ACGTGTGCTCTTCCGATCTTAAGAGTTTGTGTAGATACTGTTCGCA
ncov_cap_33_LEFT	ACGTGTGCTCTTCCGATCTTCTGGAAGAAACTAAGTTCCTCACAG
ncov_cap_35_LEFT	ACGTGTGCTCTTCCGATCTTTATATAACCACTTACCCGGGTCAGG
ncov_cap_37_LEFT	ACGTGTGCTCTTCCGATCTACAAGAGGGTGTGGTTGATTATGG
ncov_cap_39_LEFT	ACGTGTGCTCTTCCGATCTACTTCTTCTTCTAAAACACCTGAAGAACA
ncov_cap_41_LEFT	ACGTGTGCTCTTCCGATCTCGCAAGTTGTGGACATGTCAATG
ncov_cap_43_LEFT	ACGTGTGCTCTTCCGATCTTGTCAGCATTAAATCACACTAAAAAGTGG
ncov_cap_45_LEFT	ACGTGTGCTCTTCCGATCTAATGAGTTACTTGTTTCAACATGCCA
ncov_cap_47_LEFT	ACGTGTGCTCTTCCGATCTACCTTTTGTTATGATGTCAGCACCA
ncov_cap_49_LEFT	ACGTGTGCTCTTCCGATCTAGAAAGACAATTCTTATTTCACAGAGCAA
ncov_cap_51_LEFT	ACGTGTGCTCTTCCGATCTTGTTTGGCATGTTAACAATGCAACT
ncov_cap_53_LEFT	ACGTGTGCTCTTCCGATCTACCGAAGTTGTAGGAGACATTATACTTAA
ncov_cap_55_LEFT	ACGTGTGCTCTTCCGATCTAATTCTAGAATTAAAGCATCTATGCCGAC
ncov_cap_57_LEFT	ACGTGTGCTCTTCCGATCTTTGAACTCTACTAATGTCACTATTGCAACC
ncov_cap_59_LEFT	ACGTGTGCTCTTCCGATCTGCAATTGTTTTTCAGCTATTTTGCAGT
ncov_cap_61_LEFT	ACGTGTGCTCTTCCGATCTTGTCTATGCTAATGGAGGTAAAGGC
ncov_cap_63_LEFT	ACGTGTGCTCTTCCGATCTAAGACTTATGAAAGACATTCTCTCTCTCA
ncov_cap_65_LEFT	ACGTGTGCTCTTCCGATCTTAGTGCGGAAGTTGCAGTTAAAATG
ncov_cap_67_LEFT	ACGTGTGCTCTTCCGATCTGCGATAGTTGTAATAACTATATGCTCACC
ncov_cap_69_LEFT	ACGTGTGCTCTTCCGATCTACTTACCTTTTAAGTTGACATGTGCAA
ncov_cap_71_LEFT	ACGTGTGCTCTTCCGATCTTCACTCGTGACATAGCATCTACAGA
ncov_cap_73_LEFT	ACGTGTGCTCTTCCGATCTAACATCTGTTACACACCATCAAAACTT
ncov_cap_75_LEFT	ACGTGTGCTCTTCCGATCTTTCTGAGTACTGTAGGCACGGC
ncov_cap_77_LEFT	ACGTGTGCTCTTCCGATCTATGAGGTTTAGAAGAGCTTTTGGTGA
ncov_cap_79_LEFT	ACGTGTGCTCTTCCGATCTTGTTCACACCTTTAGTACCTTTCTGG
ncov_cap_81_LEFT	ACGTGTGCTCTTCCGATCTAGTACAAGTATTTTAGTGGAGCAATGGA
ncov_cap_83_LEFT	ACGTGTGCTCTTCCGATCTAACGGTCTTTGGCTTGATGACG
ncov_cap_85_LEFT	ACGTGTGCTCTTCCGATCTTAAGTTTGTTCGCATTCAACCAGGA

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ncov_cap_87_LEFT	ACGTGTGCTCTTCCGATCTTGGACCTTTTGTTGACAGGCAA
ncov_cap_89_LEFT	ACGTGTGCTCTTCCGATCTGGAATTGCCGTTTTAGATATGTGTGC
ncov_cap_91_LEFT	ACGTGTGCTCTTCCGATCTGTCCAGAGTACTCAATGGTCTTTGT
ncov_cap_93_LEFT	ACGTGTGCTCTTCCGATCTACTGTGTTATGTATGCATCAGCTGT
ncov_cap_95_LEFT	ACGTGTGCTCTTCCGATCTACAACTGTCATGTTTTTGGCCAG
ncov_cap_97_LEFT	ACGTGTGCTCTTCCGATCTACTACTCCCACCCAAGAATAGCA
ncov_cap_99_LEFT	ACGTGTGCTCTTCCGATCTTGGTTTCACTACTTTCTGTTTTGCTT
ncov_cap_101_LEFT	ACGTGTGCTCTTCCGATCTAATTTGACCGTGATGCAGCCAT
ncov_cap_103_LEFT	ACGTGTGCTCTTCCGATCTACCTCTTACAACAGCAGCCAAAC
ncov_cap_105_LEFT	ACGTGTGCTCTTCCGATCTGCTTAGTCCTGTTGCACTACGAC
ncov_cap_107_LEFT	ACGTGTGCTCTTCCGATCTGGTATGGTACTTGGTAGTTTAGCTGC
ncov_cap_109_LEFT	ACGTGTGCTCTTCCGATCTTCAGGCAATAACAGTTACACCGG
ncov_cap_111_LEFT	ACGTGTGCTCTTCCGATCTAGTCAGCTGATGCACAATCGTT
ncov_cap_113_LEFT	ACGTGTGCTCTTCCGATCTACACTTTCTCTAACTACCAACATGAAGA
ncov_cap_115_LEFT	ACGTGTGCTCTTCCGATCTAAGGACTGGTATGATTTTGTAGAAAACCC
ncov_cap_117_LEFT	ACGTGTGCTCTTCCGATCTTGTTAATGCCTATATTAACCTTGACCAGG
ncov_cap_119_LEFT	ACGTGTGCTCTTCCGATCTTGTTTTATTCTCTACAGTGTTCCCACC
ncov_cap_121_LEFT	ACGTGTGCTCTTCCGATCTTGTTGCTTTTCAAACTGTCAAACCC
ncov_cap_123_LEFT	ACGTGTGCTCTTCCGATCTGATAAGTACTTTGATTGTTACGATGGTGG
ncov_cap_125_LEFT	ACGTGTGCTCTTCCGATCTTCAAAAATTATTGAAATCAATAGCCGCCA
ncov_cap_127_LEFT	ACGTGTGCTCTTCCGATCTTGTAGCTTGTCACACCGTTTCT
ncov_cap_129_LEFT	ACGTGTGCTCTTCCGATCTTGTCCGCAATTTACAACACAGACT
ncov_cap_131_LEFT	ACGTGTGCTCTTCCGATCTATGTCTGAAGCAAAATGTTGGACTG
ncov_cap_133_LEFT	ACGTGTGCTCTTCCGATCTATGCTTACCCACTTACTAAACATCCT
ncov_cap_135_LEFT	ACGTGTGCTCTTCCGATCTGTTGTAAATGCTGTTACGACCATGT
ncov_cap_137_LEFT	ACGTGTGCTCTTCCGATCTAGCGATAATGTTACTGACTTTAATGCAA
ncov_cap_139_LEFT	ACGTGTGCTCTTCCGATCTCCACTTAACCGAAATTATGTCTTTACTGG
ncov_cap_141_LEFT	ACGTGTGCTCTTCCGATCTACCCAACACTCAATATCTCAGATGAG

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ncov_cap_143_LEFT	ACGTGTGCTCTTCCGATCTAATGTAGTAGAATTATACCTGC ACGTGC
ncov_cap_145_LEFT	ACGTGTGCTCTTCCGATCTACCAGAATATTTCAATTCAGTG TGTAGAC
ncov_cap_147_LEFT	ACGTGTGCTCTTCCGATCTGGTAAGAGAATTCCTTACACGT AACCC
ncov_cap_149_LEFT	ACGTGTGCTCTTCCGATCTCTGATAGAGACCTTTATGACAA GTTGCA
ncov_cap_151_LEFT	ACGTGTGCTCTTCCGATCTAGGACATGACCTATAGAAGACT CATCT
ncov_cap_153_LEFT	ACGTGTGCTCTTCCGATCTTTTTCCAGAGTTAGTGCTAAAC CACC
ncov_cap_155_LEFT	ACGTGTGCTCTTCCGATCTTAGACGTGCCACATGCTTTTCC
ncov_cap_157_LEFT	ACGTGTGCTCTTCCGATCTTGTTAAGCGTGTTGACTGGACT
ncov_cap_159_LEFT	ACGTGTGCTCTTCCGATCTCTATTCTTATGCCACACATTCT GACAAA
ncov_cap_161_LEFT	ACGTGTGCTCTTCCGATCTTCTGACAGTCCATGTGAGTCTC A
ncov_cap_163_LEFT	ACGTGTGCTCTTCCGATCTTGGAACACTTTTACAAGACTTC AGAGT
ncov_cap_165_LEFT	ACGTGTGCTCTTCCGATCTGGGTGTGGACATTGCTGCTAAT
ncov_cap_167_LEFT	ACGTGTGCTCTTCCGATCTAACAAGCTAGTCTTAATGGAGT CACA
ncov_cap_169_LEFT	ACGTGTGCTCTTCCGATCTTCTACTGATTGGACTAGCTAAA CGTTT
ncov_cap_171_LEFT	ACGTGTGCTCTTCCGATCTTGGTGTAAAGATGGCCATGTAG AA
ncov_cap_173_LEFT	ACGTGTGCTCTTCCGATCTCATTTTGGTGCTGGTTCTGATA AAGG
ncov_cap_175_LEFT	ACGTGTGCTCTTCCGATCTTTCACTTACATTTGTGGGTTTA TACAACA
ncov_cap_177_LEFT	ACGTGTGCTCTTCCGATCTACAAATCCAATTCAGTTGTCTT CCTATTC
ncov_cap_179_LEFT	ACGTGTGCTCTTCCGATCTAGTCAGTGTGTTAATCTTACAA CCAGA
ncov_cap_181_LEFT	ACGTGTGCTCTTCCGATCTTTTGCTTCCACTGAGAAGTCTA ACA
ncov_cap_183_LEFT	ACGTGTGCTCTTCCGATCTACCTTGAAGGAAAACAGGGTAA TTTCA
ncov_cap_185_LEFT	ACGTGTGCTCTTCCGATCTTGCTGCAGCTTATTATGTGGGT
ncov_cap_187_LEFT	ACGTGTGCTCTTCCGATCTAGTTTTTAACGCCACCAGATTT GC
ncov_cap_189_LEFT	ACGTGTGCTCTTCCGATCTGGCAAACTGGAAAGATTGCTGA T
ncov_cap_191_LEFT	ACGTGTGCTCTTCCGATCTACCCACTAATGGTGTTGGTTAC C
ncov_cap_193_LEFT	ACGTGTGCTCTTCCGATCTTCTTGACATTACACCATGTTCT TTTGG
ncov_cap_195_LEFT	ACGTGTGCTCTTCCGATCTAGGTATATGCGCTAGTTATCAG ACTCA
ncov_cap_197_LEFT	ACGTGTGCTCTTCCGATCTCAATCTTTTGTTGCAATATGGC AGTTT
ncov_cap_199_LEFT	ACGTGTGCTCTTCCGATCTAAGTGACACTTGCAGATGCTGG

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ncov_cap_201_LEFT	ACGTGTGCTCTTCCGATCTTGGCTTATAGGTTTAATGGTATTGGAGT
ncov_cap_203_LEFT	ACGTGTGCTCTTCCGATCTACAAAGTTGAGGCTGAAGTGCA
ncov_cap_205_LEFT	ACGTGTGCTCTTCCGATCTACTTATGTCCCTGCACAAGAAAAGA
ncov_cap_207_LEFT	ACGTGTGCTCTTCCGATCTTGATCCTTTGCAACCTGAATTAGACT
ncov_cap_209_LEFT	ACGTGTGCTCTTCCGATCTTGGTGACAATTATGCTTTGCTGTATG
ncov_cap_211_LEFT	ACGTGTGCTCTTCCGATCTGTTCGCGCTACTGCAACGATAC
ncov_cap_213_LEFT	ACGTGTGCTCTTCCGATCTATGCTTTAGTCTACTTCTTGCAGAGT
ncov_cap_215_LEFT	ACGTGTGCTCTTCCGATCTACACAGTTACTTCACTTCAGACTATTACC
ncov_cap_217_LEFT	ACGTGTGCTCTTCCGATCTACAAGCTGATGAGTACGAACTTATGT
ncov_cap_219_LEFT	ACGTGTGCTCTTCCGATCTTCTAGAGTTCCTGATCTTCTGGTCT
ncov_cap_221_LEFT	ACGTGTGCTCTTCCGATCTGGCCAGTAACTTTAGCTTGTTTTGT
ncov_cap_223_LEFT	ACGTGTGCTCTTCCGATCTGTAATCGGAGCTGTGATCCTTCG
ncov_cap_225_LEFT	ACGTGTGCTCTTCCGATCTTGCTTGTACAGTAAGTGACAACAGA
ncov_cap_227_LEFT	ACGTGTGCTCTTCCGATCTACAACAGTACTTTTAAAAGAACCTTGCT
ncov_cap_229_LEFT	ACGTGTGCTCTTCCGATCTTGCTTCACACTCAAAAGAAAGACAGA
ncov_cap_231_LEFT	ACGTGTGCTCTTCCGATCTTGTAGTTGATGACCCGTGTCCT
ncov_cap_233_LEFT	ACGTGTGCTCTTCCGATCTAGAGTATCATGACGTTCGTGTTGT
ncov_cap_235_LEFT	ACGTGTGCTCTTCCGATCTAGGAAGACCTTAAATTCCCTCGAGG
ncov_cap_237_LEFT	ACGTGTGCTCTTCCGATCTTGAGGGAGCCTTGAATACACCA
ncov_cap_239_LEFT	ACGTGTGCTCTTCCGATCTCTCTTGCTTTGCTGCTGCTTGA
ncov_cap_241_LEFT	ACGTGTGCTCTTCCGATCTCATTGGCCGCAAATTGCACAAT
ncov_cap_243_LEFT	ACGTGTGCTCTTCCGATCTAGGCTGATGAAACTCAAGCCTT
ncov_cap_245_LEFT	ACGTGTGCTCTTCCGATCTTGAATTCTCGTAACTACATAGCACAAGT
RNAse_P_LEFT	ACGTGTGCTCTTCCGATCTGATTTGGACCTGCGAGCG

SARS-CoV-2 primer pool 2 [version 1]

NAME	SEQUENCE
ncov_cap_2_LEFT	ACGTGTGCTCTTCCGATCTTCAGCACATCTAGGTTTCGTCC
ncov_cap_4_LEFT	ACGTGTGCTCTTCCGATCTCATCAAACGTTCGGATGCTCGA
ncov_cap_6_LEFT	ACGTGTGCTCTTCCGATCTTGGCACTGATCCTTATGAAGATTTTCA
ncov_cap_8_LEFT	ACGTGTGCTCTTCCGATCTGCATGAAATTGCTTGGTACACGG
ncov_cap_10_LEFT	ACGTGTGCTCTTCCGATCTGCGTCACCAAATGAATGCAACC
ncov_cap_12_LEFT	ACGTGTGCTCTTCCGATCTACCTGAGCATAGTCTTGCCGAA

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ncov_cap_14_LEFT	ACGTGTGCTCTTCCGATCTGTGACTTTAAACTTAATGAAGAGATCGCC
ncov_cap_16_LEFT	ACGTGTGCTCTTCCGATCTTGTACGATCAATTTTCTCCCGCA
ncov_cap_18_LEFT	ACGTGTGCTCTTCCGATCTAAACCCGTCCTTGATTGGCTTG
ncov_cap_20_LEFT	ACGTGTGCTCTTCCGATCTTTTGTCACGCACTCAAAGGGAT
ncov_cap_22_LEFT	ACGTGTGCTCTTCCGATCTGCCCTTGCACCTAATATGATGGT
ncov_cap_24_LEFT	ACGTGTGCTCTTCCGATCTTGCTGTCATAAAAACTTTGCAACCA
ncov_cap_26_LEFT	ACGTGTGCTCTTCCGATCTGGTAAACCTTTGGAATTTGGTGCC
ncov_cap_28_LEFT	ACGTGTGCTCTTCCGATCTACATTAAAAATGCAGACATTGTGGAAGA
ncov_cap_30_LEFT	ACGTGTGCTCTTCCGATCTTGTCTTCATGTTGTCGGCCCAA
ncov_cap_32_LEFT	ACGTGTGCTCTTCCGATCTAAGATCGCTGAGATTCCTAAAGAGG
ncov_cap_34_LEFT	ACGTGTGCTCTTCCGATCTGTGGGTGATGTTGTTCAAGAGGG
ncov_cap_36_LEFT	ACGTGTGCTCTTCCGATCTTGGAACTGTTTCTTGGAATTTGCG
ncov_cap_38_LEFT	ACGTGTGCTCTTCCGATCTTGTAACACATGGCTTAAATTTGGAAGAA
ncov_cap_40_LEFT	ACGTGTGCTCTTCCGATCTTCCTACCACATTCCACCTAGATGG
ncov_cap_42_LEFT	ACGTGTGCTCTTCCGATCTAACCTCATAATTCACATGAAGGTAAAACA
ncov_cap_44_LEFT	ACGTGTGCTCTTCCGATCTAGAGTTGAAGTTTAATCCACCTGCT
ncov_cap_46_LEFT	ACGTGTGCTCTTCCGATCTCCTTAAGGGTGTAGAAGCTGTTATGT
ncov_cap_48_LEFT	ACGTGTGCTCTTCCGATCTCCTCAGAATACAAAGGTCCTATTACGG
ncov_cap_50_LEFT	ACGTGTGCTCTTCCGATCTAGAAACCTGCTTCAAGAGAGCTT
ncov_cap_52_LEFT	ACGTGTGCTCTTCCGATCTGGGAATGGATAATCTTGCCTGCG
ncov_cap_54_LEFT	ACGTGTGCTCTTCCGATCTTGCTAAGCCTTTTCTTAACAAAGTTGT
ncov_cap_56_LEFT	ACGTGTGCTCTTCCGATCTTGGTTTTTACTATTAAGTGTTTGCCTAGGT
ncov_cap_58_LEFT	ACGTGTGCTCTTCCGATCTAATGGGATTTAACTGCTTTTGGCTT
ncov_cap_60_LEFT	ACGTGTGCTCTTCCGATCTGCATGTTGTAGACGGTTGTAATTCA
ncov_cap_62_LEFT	ACGTGTGCTCTTCCGATCTGACTTGTCACTACAGTTTAAAAGACCAA
ncov_cap_64_LEFT	ACGTGTGCTCTTCCGATCTAGAATCATCTGCAAAATCAGCGTCT
ncov_cap_66_LEFT	ACGTGTGCTCTTCCGATCTCTACTTTTATTTCAGCAGCTCGGC
ncov_cap_68_LEFT	ACGTGTGCTCTTCCGATCTGCGCAGGTAGCAAAAAGTCACA
ncov_cap_70_LEFT	ACGTGTGCTCTTCCGATCTGCAGTTAATTAAAGTTACACTTGTGTTCC

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ncov_cap_72_LEFT	ACGTGTGCTCTTCCGATCTTGCTGCAGTCATAACAAGAGAAGT
ncov_cap_74_LEFT	ACGTGTGCTCTTCCGATCTACCAATGTACTAGAAGGTTCTGTTGC
ncov_cap_76_LEFT	ACGTGTGCTCTTCCGATCTGATCTTTACCAGGAGTTTTCTGTGGT
ncov_cap_78_LEFT	ACGTGTGCTCTTCCGATCTACTGTACTCTGTTTAACACCAGTTTACT
ncov_cap_80_LEFT	ACGTGTGCTCTTCCGATCTCCTTTAGTACTTTTGAAGAAGCTGCG
ncov_cap_82_LEFT	ACGTGTGCTCTTCCGATCTCCACAAACCTCTATCACCTCAGC
ncov_cap_84_LEFT	ACGTGTGCTCTTCCGATCTGGCTGGTAATGTTCAACTCAGGG
ncov_cap_86_LEFT	ACGTGTGCTCTTCCGATCTTGGTTCATGTGGTAGTGTTGGTT
ncov_cap_88_LEFT	ACGTGTGCTCTTCCGATCTACCACAACTCTTAATGACTTTAACCTTGT
ncov_cap_90_LEFT	ACGTGTGCTCTTCCGATCTACAATGCTCAGGTGTTACTTTCCA
ncov_cap_92_LEFT	ACGTGTGCTCTTCCGATCTTTACCTTCTCTTGCCACTGTAGCT
ncov_cap_94_LEFT	ACGTGTGCTCTTCCGATCTACACTTATGAATGTCTTGACACTCGT
ncov_cap_96_LEFT	ACGTGTGCTCTTCCGATCTTGTACTTGTTACTTTGGCCTCTTTTGT
ncov_cap_98_LEFT	ACGTGTGCTCTTCCGATCTAGTCTTACTCTCAGTTTTGCAACAACT
ncov_cap_100_LEFT	ACGTGTGCTCTTCCGATCTGTTCCCTTCCATCATATGCAGCT
ncov_cap_102_LEFT	ACGTGTGCTCTTCCGATCTGGGCAAAAGTTACTAGTGCTATGCA
ncov_cap_104_LEFT	ACGTGTGCTCTTCCGATCTCAGGTTGTAGATGCAGATAGTAAAATTGT
ncov_cap_106_LEFT	ACGTGTGCTCTTCCGATCTTGCACTGTTATCCGATTTACAGGA
ncov_cap_108_LEFT	ACGTGTGCTCTTCCGATCTAAGTGCCTGCCAATTCAACTGT
ncov_cap_110_LEFT	ACGTGTGCTCTTCCGATCTAAAAACACAGTCTGTACCGTCTGC
ncov_cap_112_LEFT	ACGTGTGCTCTTCCGATCTAATTCCTAAAAACTAATTGTTGTCGCTTCC
ncov_cap_114_LEFT	ACGTGTGCTCTTCCGATCTTCAACGTCTTACTAAATACACAATGGCA
ncov_cap_116_LEFT	ACGTGTGCTCTTCCGATCTCGAAATGCTGGTATTGTTGGTGT
ncov_cap_118_LEFT	ACGTGTGCTCTTCCGATCTTGACTTCACGGAAGAGAGGTTAAAAC
ncov_cap_120_LEFT	ACGTGTGCTCTTCCGATCTCCACTTCAGAGAGCTAGGTGTTG
ncov_cap_122_LEFT	ACGTGTGCTCTTCCGATCTCACTTCTTCTTTGCTCAGGATGGT
ncov_cap_124_LEFT	ACGTGTGCTCTTCCGATCTCGCATATACAAAACGTAATGTCATCCC

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ncov_cap_126_LEFT	ACGTGTGCTCTTCCGATCTATGGGTTGGGATTATCCTAAATGTGA
ncov_cap_128_LEFT	ACGTGTGCTCTTCCGATCTTCAGGAGATGCCACAACTGCTT
ncov_cap_130_LEFT	ACGTGTGCTCTTCCGATCTTACTCTCTGACGATGCTGTTGTG
ncov_cap_132_LEFT	ACGTGTGCTCTTCCGATCTAGATCCATCAAGAATCCTAGGGGC
ncov_cap_134_LEFT	ACGTGTGCTCTTCCGATCTGGAACCTGAGTTTTATGAGGCTATGT
ncov_cap_136_LEFT	ACGTGTGCTCTTCCGATCTACTCAACTTTACTTAGGAGGTATGAGCT
ncov_cap_138_LEFT	ACGTGTGCTCTTCCGATCTACGCTCAAAGCTACTGAGGAGA
ncov_cap_140_LEFT	ACGTGTGCTCTTCCGATCTACCGAGGTACAACAACTTACAAATTAAAT
ncov_cap_142_LEFT	ACGTGTGCTCTTCCGATCTCTCTACTACCCTTCTGCTCGCA
ncov_cap_144_LEFT	ACGTGTGCTCTTCCGATCTCTTTGATGAAATTTCAATGGCCACAAA
ncov_cap_146_LEFT	ACGTGTGCTCTTCCGATCTGCACATAAAGACAAATCAGCTCAATGC
ncov_cap_148_LEFT	ACGTGTGCTCTTCCGATCTGTTGATTCATCACAGGGCTCAGA
ncov_cap_150_LEFT	ACGTGTGCTCTTCCGATCTTGGGTTACATCCTACACAGGCA
ncov_cap_152_LEFT	ACGTGTGCTCTTCCGATCTGTCGAGGGGTGTCATGCTACTA
ncov_cap_154_LEFT	ACGTGTGCTCTTCCGATCTAGTGACACACTTAAAAATCTCTCTGACA
ncov_cap_156_LEFT	ACGTGTGCTCTTCCGATCTCAGGTAACCTACAAAGCAACCATGA
ncov_cap_158_LEFT	ACGTGTGCTCTTCCGATCTCCCAGTTCTTCACGACATTGGT
ncov_cap_160_LEFT	ACGTGTGCTCTTCCGATCTAACTTGCCTGGTTGTGATGGTG
ncov_cap_162_LEFT	ACGTGTGCTCTTCCGATCTTGTAGACATCATGCTAATGAGTACAGAT
ncov_cap_164_LEFT	ACGTGTGCTCTTCCGATCTCACAAAAGTTGATGGTGTTGATGTAGA
ncov_cap_166_LEFT	ACGTGTGCTCTTCCGATCTACTGAAACGATTTGTGCACCACT
ncov_cap_168_LEFT	ACGTGTGCTCTTCCGATCTCCAACAATTACCTGAAACTTACTTTACTCA
ncov_cap_170_LEFT	ACGTGTGCTCTTCCGATCTGGTTCATCTAAGTGTGTGTGTTCTGT
ncov_cap_172_LEFT	ACGTGTGCTCTTCCGATCTAGAATGCTATTAGAAAAGTGTGACCTTCA
ncov_cap_174_LEFT	ACGTGTGCTCTTCCGATCTTGATGCAGATTCAACTTTGATTGGTG
ncov_cap_176_LEFT	ACGTGTGCTCTTCCGATCTGCTGATCTTTATAAGCTCATGGGACA
ncov_cap_178_LEFT	ACGTGTGCTCTTCCGATCTAGGGGTACTGCTGTTATGTCTTTAAA
ncov_cap_180_LEFT	ACGTGTGCTCTTCCGATCTAGGACTTGTTCTTACCTTTCTTTTTCCA
ncov_cap_182_LEFT	ACGTGTGCTCTTCCGATCTGTTGGATGGAAAGTGAGTTCAGAGT
ncov_cap_184_LEFT	ACGTGTGCTCTTCCGATCTTCGGCTTTAGAACCATTGGTAGATT

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ncov_cap_186_LEFT	ACGTGTGCTCTTCCGATCTAGTGTACGTTGAAATCCTTCACTGT
ncov_cap_188_LEFT	ACGTGTGCTCTTCCGATCTACTTTTAAGTGTTATGGAGTGTCTCCT
ncov_cap_190_LEFT	ACGTGTGCTCTTCCGATCTAGATTGTTTAGGAAGTCTAATCTCAAACCT
ncov_cap_192_LEFT	ACGTGTGCTCTTCCGATCTATGGTTTAACAGGCACAGGTGT
ncov_cap_194_LEFT	ACGTGTGCTCTTCCGATCTATCAACTTACTCCTACTTGGCGTG
ncov_cap_196_LEFT	ACGTGTGCTCTTCCGATCTAACTCTATTGCCATACCCACAAATTTT
ncov_cap_198_LEFT	ACGTGTGCTCTTCCGATCTAGTCAAACAAATTTACAAAACACCACCA
ncov_cap_200_LEFT	ACGTGTGCTCTTCCGATCTTTGCCACCTTTGCTCACAGATG
ncov_cap_202_LEFT	ACGTGTGCTCTTCCGATCTGCACTTGGAAAACTTCAAGATGTGG
ncov_cap_204_LEFT	ACGTGTGCTCTTCCGATCTTGCTGCTACTAAAATGTCAGAGTGT
ncov_cap_206_LEFT	ACGTGTGCTCTTCCGATCTTGTAACACAAAGGAATTTTTATGAACCACA
ncov_cap_208_LEFT	ACGTGTGCTCTTCCGATCTGTAAACATTCAAAAAGAAATTGACCGCC
ncov_cap_210_LEFT	ACGTGTGCTCTTCCGATCTGCTCAAAGGAGTCAAATTACATTACACA
ncov_cap_212_LEFT	ACGTGTGCTCTTCCGATCTCCAAGGGTGTTCACTTTGTTTGC
ncov_cap_214_LEFT	ACGTGTGCTCTTCCGATCTGCCAACTATTTTCTTTGCTGGCA
ncov_cap_216_LEFT	ACGTGTGCTCTTCCGATCTTTCACACAATCGACGGTTCATCC
ncov_cap_218_LEFT	ACGTGTGCTCTTCCGATCTCCTTACTGCGCTTCGATTGTGT
ncov_cap_220_LEFT	ACGTGTGCTCTTCCGATCTAAGCTCCTTGAACAATGGAACCT
ncov_cap_222_LEFT	ACGTGTGCTCTTCCGATCTTTCTTTCAGACTGTTTGCGCGT
ncov_cap_224_LEFT	ACGTGTGCTCTTCCGATCTAACGCTTTCTTATTACAAATTGGGAGC
ncov_cap_226_LEFT	ACGTGTGCTCTTCCGATCTTCTCAATTAGATGAAGAGCAACCAATGG
ncov_cap_228_LEFT	ACGTGTGCTCTTCCGATCTCGTCTATCAGTTACGTGCCAGAT
ncov_cap_230_LEFT	ACGTGTGCTCTTCCGATCTATTATCTTTTGGTTCTCACTTGAACTGC
ncov_cap_232_LEFT	ACGTGTGCTCTTCCGATCTACATCGATATCGGTAATTATACAGTTTCCT
ncov_cap_234_LEFT	ACGTGTGCTCTTCCGATCTACCCTCAGATTCAACTGGCAGT
ncov_cap_236_LEFT	ACGTGTGCTCTTCCGATCTAATGAAAGATCTCAGTCCAAGATGGT
ncov_cap_238_LEFT	ACGTGTGCTCTTCCGATCTGCAGTCAAGCCTCTTCTCGTTC
ncov_cap_240_LEFT	ACGTGTGCTCTTCCGATCTGCAAAAACGTACTGCCACTAAAGC
ncov_cap_242_LEFT	ACGTGTGCTCTTCCGATCTACACAGGTGCCATCAAATTGGA
ncov_cap_244_LEFT	ACGTGTGCTCTTCCGATCTTGCTGACTCAACTCAGGCCTAA
RNAse_P_LEFT	ACGTGTGCTCTTCCGATCTGATTTGGACCTGCGAGCG

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Preparation of barcoded beads for RNA capture

1

The first stage in our workflow is the preparation of bead-bound DNA probes that will facilitate subsequent viral RNA capture, cDNA synthesis and multiplex PCR to generate ~200 nt amplicons covering the entire SARS-CoV-2 genome. We use two distinct sets of probes for this, requiring two independent bead preparations for each sample to be processed. Ultimately, this enables library preparation to be performed in two parallel reactions, designed such that neighbouring amplicons do not overlap within the same library PCR. This strategy protects against preferential amplification of short overlap products, and has been successfully developed and employed elsewhere (Quick *et al.* [2017] *Nat Protoc.* 12: 1261; https://www.protocols.io/view/ncov-2019-sequencing-protocol-bbmuik6w/abstract). Throughout the protocol we use the generic terms “pool 1” and “pool 2” to refer to our two capture probe sets, as well as the corresponding primer sets needed for library amplification.

We envisage that capture beads could be prepared in bulk in advance and supplied to testing labs in 96-well or 384-well format. Each uniquely barcoded bead preparation could thus be used for multiple sample batches, provided each batch is sequenced independently. The protocol below is for a small-scale preparation of up to 10 uniquely barcoded bead sets, to be used in development and evaluation of a prototype. We also provide an alternative, lower-cost bead preparation method at the end of this page.

Wash **200 µl** of streptavidin-coated Dynabeads™ MyOne™ C1 magnetic beads as per the manufacturer’s instructions:

1.1 Resuspend the Dynabeads™ magnetic beads in the vial (vortex for > **00:00:30**).

1.2 Transfer **200 µl** of Dynabeads™ magnetic beads to a tube.

1.3 Add an equal volume of 1X B&W buffer and resuspend.

1.4 Place the tube on a magnet for **00:01:00** and discard the supernatant.

1.5 Remove the tube from the magnet and resuspend the washed magnetic beads in **200 µl** of 1X B&W buffer.

1.6 Repeat steps 1.4–1.5 twice, for a total of 3 washes.

1.7 Pellet beads once more, and resuspend in **400 µl** of 2X B&W buffer.

2 For each unique barcode, take an aliquot of the prepared beads and immobilise an oligonucleotide of the structure 5'-[biotin][spacer][partial Illumina p5 adapter][sample barcode][generic stub]-3’ onto the beads as follows:

2.1 Add **40 µl** of 5 µM oligonucleotide ([Biotin]-barcoded bead tether) to **40 µl** of washed beads.

2.2 Incubate for **00:15:00** at room temperature using gentle rotation.

2.3 Separate the biotinylated DNA coated beads with a magnet for 2–3 min.

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2.4 Wash the coated beads 2–3 times with **100 µl** 1X B&W buffer.

2.5 Resuspend in **120 µl** of nuclease-free water.

3 For each barcoded sample, generate two distinct libraries of bead-bound SARS-CoV-2 probes by annealing separate oligonucleotide pools with the general structure 3'-[generic stub complement][SARS-CoV-2 target]-5' and performing strand extension:

3.1 Mix the following components in 0.2 mL PCR tubes:

Component	Pool 1	Pool 2
Bead-bound template (previous step)	**60 µl**	**60 µl**
5X PrimeSTAR GXL buffer	**20 µl**	**20 µl**
dNTP mixture (2.5 mM each)	**8 µl**	**8 µl**
SARS-CoV-2 probe pool 1 or 2 (10 µM)	**10 µl**	**10 µl**
PrimeSTAR GXL DNA polymerase	**2 µl**	**2 µl**

3.2 Incubate the reaction as follows:

Step	Temperature	Time	Cycles
Denaturation	**98 °C**	**00:00:10**	1
Annealing	**55 °C**	**00:00:15**	1
Extension	**68 °C**	**00:02:00**	1
Hold	**4 °C**	Indefinite	1

4 For each bead sample, dissociate and remove the non-biotinylated strand as follows:

4.1 Pellet the beads on a magnet and wash once in **50 µl** 1X SSC.

4.2 Resuspend the beads in **20 µl** of freshly prepared 0.15 M NaOH and incubate at room temperature for **00:10:00** .

4.3 Pellet the beads on a magnet and discard the supernatant containing the non-biotinylated strand.

4.4 Wash the beads once with **50 µl** 0.1M NaOH, once with **50 µl** of 1X B&W buffer and once with **50 µl** TE buffer.

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4.5 Resuspend beads in **50 µl** TE buffer.

The above steps are designed to produce two libraries of single-stranded, bead-bound SARS-CoV-2 probes for each sample barcode, with the general structure 5'-[biotin][spacer][partial Illumina p5 adapter][sample barcode][generic stub][SARS-CoV-2 target]-3'. Once the method has been suitably tested and is ready for deployment at scale, we envisage that these steps could be simplified substantially by synthesising the biotinylated probe sets in full for each sample barcode and immobilising these directly onto the streptavidin-coated beads.

Sample collection and preparation

5 Collect nasopharyngeal swabs in a **1.5 ml** volume of transport medium.

We are currently testing a range of media with the aim of identifying a formulation that optimally preserves viral RNA and supports hybridisation with our bead-bound probes in the following step. These include UTM, Lysis/binding buffer and a combination of the two (see Materials). Alternatively, kit-extracted RNA or synthetic control RNA can be used.

6 Quantify **10 µl** of swab diluent using the Qubit RNA HS assay or similar.

RNA capture

7 For each sample to be tested, transfer 2 x **25 µl** of unique barcoded beads (one for each probe set) into separate 0.2 mL PCR tubes.

Alternatively, use multi-well PCR plates. For 384-well plates, sample volumes will need to be scaled down in subsequent steps.

8 Pellet the beads on a magnet and discard the supernatant.

9 Add **50 µl** of swab diluent directly to each bead pellet (i.e. 2 x **50 µl** per swab sample) and resuspend completely by pipette mixing. Alternatively, use purified or synthetic control RNA in a buffer suitable for probe hybridisation e.g. 1X SSC.

10 Place reactions in a preheated thermal cycler with a heated lid and incubate at **75 °C** for **00:02:00** , then ramp to **25 °C** at a rate of -0.1°C/s and proceed immediately to the next step.

11 Pellet the beads on a magnet and discard the supernatant.

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12 Resuspend each reaction in **20 µl** of Washing buffer A.

13 Combine all “pool 1” samples together into a fresh tube and repeat this process for “pool 2”.

For large sample batches it should only be necessary to pool a small aliquot of each reaction. The combined samples will be used as input for cDNA synthesis. We are currently testing whether there is a limit to the amount of beads that can be tolerated in the reverse transcription reaction, as well as the subsequent library amplification.

cDNA synthesis

14 Wash each pooled bead sample once with an equivalent volume of Washing buffer A and twice with the same volume of Washing buffer B, using a magnet to separate the beads from the solution between each washing step. After the final wash, leave the beads suspended in Washing buffer B at room temperature and proceed immediately to the next step.

15 Prepare a reverse transcriptase master mix by combining the following components from the SuperScript™ IV First-Strand Synthesis System:

Component	Volume
5X SSIV buffer	**20 µl**
10 mM dNTP mix (10 mM each)	**5 µl**
100 mM DTT	**5 µl**
Ribonuclease inhibitor (40 U/µL)	**5 µl**
DEPC-treated water	**60 µl**

16 Pellet beads from step 14 on a magnet and discard the supernatant. Pulse spin tubes for 5 s, return to the magnet and discard any remaining liquid to ensure all the Washing buffer B has been removed (residual buffer may inhibit the reverse transcriptase reaction).

17 Perform one final wash in **25 µl** of reverse transcriptase master mix. Again pellet beads on a magnet and discard the supernatant.

18 Resuspend each pooled bead sample in **19 µl** of reverse transcriptase master mix and add **1 µl** of SuperScript™ IV Reverse Transcriptase (200 U/µL). Mix well by pipetting and briefly centrifuge.

19 Incubate the reactions at **55 °C** for **00:10:00** .

20 Inactivate by incubating at **80 °C** for **00:10:00** .

21 Pellet beads by placing each tube on a magnet. Discard the supernatant and wash once in TE buffer.

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Library amplification

22

Here we generate amplicon libraries from each pooled cDNA sample by multiplex PCR. We also incorporate Illumina sequencing adapters and additional barcodes at this stage, producing sequence-ready samples that could potentially be combined with additional library pools for further scalability. Sequencing adapters are added over two sequential rounds of PCR, although we envisage that a single step protocol may also be feasible.

Pellet the “pool 1” and “pool 2” cDNA-coated bead samples from the previous step on a magnet, discard the supernatant and resuspend each in **28 µl** of nuclease-free water.

23 Combine the *first-round* PCR components in separate 0.2 mL PCR tubes:

Component	Pool 1	Pool 2
cDNA-coated beads (pool 1 or 2)	**26.7 µl**	**26.7 µl**
5X Q5 reaction buffer	**10 µl**	**10 µl**
10 mM dNTPs	**1 µl**	**1 µl**
Illumina P5 index primer (10 µM)	**2.5 µl**	**2.5 µl**
SARS-CoV-2 primer pool 1 or 2 (10 µM)	**9.3 µl**	**9.3 µl**
Q5 Hot Start High-Fidelity Polymerase	**0.5 µl**	**0.5 µl**

The SARS-CoV-2 primer pools contain 124 (pool 1) and 123 (pool 2) individual primer sequences. The concentration of each primer in the final reaction is 0.015 µM, as recommended in previous studies (Quick *et al.* [2017] *Nat Protoc.* 12: 1261).

24 Mix well by pipetting and briefly centrifuge.

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25 Incubate the *first-round* PCR reactions as follows:

Step	Temperature	Time	Cycles
Heat activation	98 °C	00:00:30	1
Denaturation	98 °C	00:00:15	25-35
Annealing/extension	65 °C	00:05:00	25-35
Hold	4 °C	Indefinite	1

26 For each reaction, pellet the cDNA-coated beads on a magnet and transfer the supernatant containing the amplified product to a new tube.

27 Clean up each reaction by performing a left side size selection with a 1.2X volume of SPRIselect beads as per the manufacturer's instructions (Beckman Coulter). Elute the sample in 40 µl of nuclease-free water.

28 Quantify 1 µl of each purified sample using the Qubit dsDNA HS assay or similar.

29 Combine the *second-round* PCR components in separate 0.2 mL PCR tubes:

Component	Pool 1	Pool 2
Purified template (previous step)	10 ng	10 ng
5X PrimeSTAR GXL buffer	10 µl	10 µl
dNTP mixture (2.5 mM each)	4 µl	4 µl
Illumina P5 flow cell primer (10 µM)	1.25 µl	1.25 µl
Illumina P7 index primer (10 µM)	1.25 µl	1.25 µl
PrimeSTAR GXL DNA polymerase	1 µl	1 µl
Nuclease-free water	to 50 µl	to 50 µl

30 Mix well by pipetting and briefly centrifuge.

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31 Incubate the *second-round* PCR reactions as follows:

Step	Temperature	Time	Cycles
Denaturation	98 °C	00:00:10	6
Annealing	60 °C	00:00:15	6
Extension	68 °C	00:01:00	6
Hold	4 °C	Indefinite	1

32 Clean up each reaction by performing a left side size selection with a 0.8X volume of SPRIselect beads as per the manufacturer's instructions (Beckman Coulter). Elute the sample in 40 µl of nuclease-free water.

Library quantitation and sequencing

33 Determine the concentration of the final "pool 1" and "pool 2" multiplexed libraries in ng/µL using the Qubit dsDNA HS assay or similar.

34 Assess the fragment size distribution of each library and determine the average fragment length using an Agilent 2100 Bioanalyzer or similar.

35 Calculate sample molarity using the following formula:

Concentration in nM = [(concentration in ng/µL) / (660 g/mol x average size in bp)] x 10^6

36 Combine an equimolar amount of each library and mix.

If sequencing additional samples, quantitate them as above and combine at the desired relative molarity.

37 Sequence the final pooled library using an Illumina instrument, generating 2 x 150 nt paired end reads

Alternative bead preparation method

38

This alternative method enables the production of multiple uniquely barcoded bead preparations from a single biotinylated template oligo. This reduces oligo synthesis costs, but does introduce an additional level of protocol complexity.

Wash 200 µl of streptavidin-coated Dynabeads™ MyOne™ C1 magnetic beads as per the manufacturer's instructions:

38.1 Resuspend the Dynabeads™ magnetic beads in the vial (vortex for > 00:00:30).

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38.2 Transfer **200 µl** of Dynabeads™ magnetic beads to a tube.

38.3 Add an equal volume of 1X B&W buffer and resuspend.

38.4 Place the tube on a magnet for **00:01:00** and discard the supernatant.

38.5 Remove the tube from the magnet and resuspend the washed magnetic beads in **200 µl** of 1X B&W buffer.

38.6 Repeat steps 38.4–38.5 twice, for a total of 3 washes.

38.7 Pellet beads once more, and resuspend in **400 µl** of 2X B&W buffer.

39 Immobilise a generic oligonucleotide of the structure 5'-[biotin][spacer][partial Illumina p5 adapter]-3' onto the beads as follows:

39.1 Add **400 µl** of 5 µM oligonucleotide ([Biotin]-bead tether) to **400 µl** of washed beads.

39.2 Incubate for **00:15:00** at room temperature using gentle rotation.

39.3 Separate the biotinylated DNA coated beads with a magnet for 2–3 min.

39.4 Wash the coated beads 2–3 times with 1X B&W buffer.

39.5 Resuspend in **1.2 ml** of nuclease-free water.

40 For each unique barcode, generate two libraries of bead-bound SARS-CoV-2 probes via a two-stage annealing and extension process using a barcoded linker oligonucleotide of the structure 3'-[partial Illumina p5 adapter complement][sample barcode][generic stub complement]-5' and two separate oligonucleotide pools with the general structure 3'-[generic stub complement][SARS-CoV-2 target]-5':

40.1 Mix the following components in a 0.2 mL PCR tube:

Component	Pool 1	Pool 2
Bead-bound template (previous step)	**60 µl**	**60 µl**
5X PrimeSTAR GXL buffer	**20 µl**	**20 µl**
dNTP mixture (2.5 mM each)	**8 µl**	**8 µl**
Barcoded linker (10 µM)	**5 µl**	**5 µl**
SARS-CoV-2 probe pool 1 or 2 (10 µM)	**5 µl**	**5 µl**
PrimeSTAR GXL DNA polymerase	**2 µl**	**2 µl**

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40.2 Incubate the reaction as follows:

Step	Temperature	Time	Cycles
Denaturation	**98 °C**	**00:00:10**	4
Annealing	**55 °C**	**00:00:15**	4
Extension	**68 °C**	**00:02:00**	4
Hold	**4 °C**	Indefinite	4

41 For each bead sample, dissociate and remove the non-biotinylated strand as follows:

41.1 Pellet the beads on a magnet and wash once in **50 µl** 1X SSC.

41.2 Resuspend the beads in **20 µl** of freshly prepared 0.15 M NaOH and incubate at room temperature for **00:10:00** .

41.3 Pellet the beads on a magnet and discard the supernatant containing the non-biotinylated strand.

41.4 Wash the beads once with **50 µl** 0.1M NaOH, once with **50 µl** of 1X B&W buffer and once with **50 µl** TE buffer.

41.5 Resuspend beads in **50 µl** TE buffer.

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