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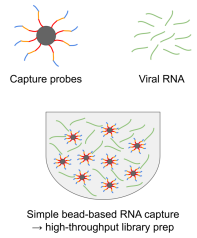
A protocol for massively parallel diagnosis and genome sequencing of SARS-CoV-2



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Coronavirus Method De...

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Protocol status: In development
This protocol is currently in the early stages of testing. Feedback and suggestions are welcomed.

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Keywords: strategy for rna capture, genome sequencing of sar, cov genome, rna capture, viral rna, dna probe, rna extraction, diagnostic testing at an unprecedented scale, patient swab samples via dna, genome sequencing, stranded dna probe, sequencing data, many thousand diagnostic test, rna hybridisation, rna, sequencing protocol, sample reverse transcription, genome, whole genome, patient swab sample, parallel diagnosis, need for sar, reverse transcription, diagnostic testing, barcoded probe set, diagnostic test, stranded dna, entire sar, transcription

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

PDF Workflow Overview.pd... 73KB

PDF Library Prep Schemat... 79KB

Materials

Reagents

	ITEM	SUPPLIER	CATALOGUE NUMBER
		General consumables, chemicals and equipment	
	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,	Sigma-	E7023-

	200 proof for molecular biology (500 mL)	Aldrich	500 mL
	DNA LoBind tubes, 1.5 mL (250 tubes)	Eppendorf	3010 8051
	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 (Epp	Sigma-Aldrich	EP0030129512-25EA

	end orf)		
	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	See Buff	-

	buffer	er 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	See Buff	-

	buffer A	er 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	Invitrogen	Q32230

	Assay Kit		
	PrimeSTAR GXL Polymerase	TaKaRa	R050A
	SPRIselect beads	Beckman Coulter	B23318
		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)
		150 mM LiCl

Individual primers [version 1]

	NAME	SEQUENCE
	[Biotin]-barcoded bead tether_1	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGGACGTAATCGGCTCGGGTCGTCTG
	[Biotin]-barcoded bead tether_2	/5biosg/TCTAGAGCCACCAGCGGCATAGTAATCGTCGGCAGCGTCAGATGTGTATAAGAGACAGATGA

		TTA CGG GCT CGG GTC GTC TG
	[Biotin]-barcoded bead tether_3	/5biosg/ TCT AGA GCC ACC AGC GGC ATA GTA ATC GTC GGC AGC GTC AGA TGT GTA TAA GAG ACA GTC CGT CAT CTG GCT CGG GTC GTC TG
	[Biotin]-barcoded bead tether_4	/5biosg/ TCT AGA GCC ACC AGC GGC ATA GTA ATC GTC GGC AGC GTC AGA TGT GTA TAA GAG ACA GAC TAG TTG CGG

		GCT CGG GTC GTC TG
	[Biotin]-barcoded bead tether_5	/5biosg/ TCT AGA GCC ACC AGC GGC ATA GTA ATC GTC GGC AGC GTC AGA TGT GTA TAA GAG ACA GAC CAT ATA AGG GCT CGG GTC GTC TG
	[Biotin]-barcoded bead tether_6	/5biosg/ TCT AGA GCC ACC AGC GGC ATA GTA ATC GTC GGC AGC GTC AGA TGT GTA TAA GAG ACA GAT CTC GAT GAG GCT CGG

		GTC GTC TG
	[Biotin]-barcoded bead tether_7	/5biosg/ TCT AGA GCC ACC AGC GGC ATA GTA ATC GTC GGC AGC GTC AGA TGT GTA TAA GAG ACA GAC TTA TAG TTG GCT CGG GTC GTC TG
	[Biotin]-bead tether	/5biosg/ TCT AGA GCC ACC AGC GGC ATA GTA ATC GTC GGC AGC GTC
	Barcoded linker_1	CAG ACG ACC CGA GCC TAC CAT GCT TCT GTC TCT TAT

		ACACATCTGACGCTGCCGACGA
	Barcoded linker_2	CAGACGACCCGAGCCAATGGAGCCGCTGTCTCTATACACATCTGACGCTGCCGACGA
	Barcoded linker_3	CAGACGACCCGAGCCCTAATCCGGTCTGTCTCTATACACATCTGACGCTGCCGACGA
	Barcoded linker_4	CAGACGACCCGAGCCTGCCTAACTCCTGTCTCTAT

		ACACATCTGACGCTGCCGACGA
	Barcoded linker_5	CAGACGACCCGAGCCAATAGTAGGCCTGTCTCTATACACATCTGACGCTGCCGACGA
	Barcoded linker_6	CAGACGACCCGAGCCGCAACTTGGACTGTCTCTATACACATCTGACGCTGCCGACGA
	Barcoded linker_7	CAGACGACCCGAGCCGAGATCGTCACTGTCTCTAT

		ACA CAT CTG ACG CTG CCG ACG A
	Illumina P5 index primer_1	AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT CGC TGA ATC GTC GGC AGC GTC
	Illumina P5 index primer_2	AAT GAT ACG GCG ACC ACC GAG ATC TAC ACC TCC AGG ATC GTC GGC AGC GTC
	Illumina P7 index primer_1	CAA GCA GAA GAC GGC ATA CGA GAT AAC CGC GGG TGA CTG GAG TTC AGA CGT GTG

		CTC TTC CGA TCT
	Illumina P7 index primer_2	CAA GCA GAA GAC GGC ATA CGA GAT GGT TAT AAG TGA CTG GAG TTC AGA CGT GTG CTC TTC CGA TCT
	Illumina P5 flow cell primer	AAT GAT ACG GCG ACC ACC GAG ATC TAC AC

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

	NAME	SEQUENCE
	ncov_cap_1_RIGHT	CAG CAC ATC TAG GTT TCG TCC GCA GAC

		GAC CCG AGC C
	ncov _cap _3_R IGH T	CCT CAT GGT CAT GTT ATG GTT GAG CCA GAC GAC CCG AGC C
	ncov _cap _5_R IGH T	GTT ACC CGT GAA CTC ATG CGT GCA GAC GAC CCG AGC C
	ncov _cap _7_RI GHT	GCA GAC ACC TTT TGA AAT TAA ATT GGC CAG ACG ACC CGA GCC
	ncov _cap _9_R IGH T	GGT GAA ACT TCA TGG CAG ACG GCA GAC GAC CCG AGC C

	ncov_cap_11_RIGHT	GTAAGGGTGGTCGCACTATTGCCAGACGACCCGAGCC
	ncov_cap_13_RIGHT	CTGCTTCCACAAGTGCTTTTGTCAGACGACCCGAGCC
	ncov_cap_15_RIGHT	CGTGTTTTACAGAAGGCCGCTACAGACGACCCGAGCC
	ncov_cap_17_RIGHT	AGAGACGGTTGGGAAATTGTTAAATTTCAGACGACCCGAGCC
	ncov_cap_19_RIGHT	AGAAGAAACTGGCCTACT

		CAT GCC AGA CGA CCC GAG CC
	ncov_cap_21_RIGHT	TGG TAA CAA ACA ATA CCT TCA CAC TCA CAG ACG ACC CGA GCC
	ncov_cap_23_RIGHT	ACC ACT GGG CAT TGA TTT AGA TGA CAG ACG ACC CGA GCC
	ncov_cap_25_RIGHT	AGA GCA AGA AGA AGA TTG GTT AGA TGA CAG ACG ACC CGA GCC
	ncov_cap_27_RIGHT	GTG GTT GTT AAT GCA GCC AAT GTC AGA CGA CCC

		GAG CC
	ncov _cap _29_ RIG HT	CAG CAC GAA GTT CTA CTT GCA CCA GAC GAC CCG AGC C
	ncov _cap _31_ RIG HT	ACC TTC AGT TGA ACA GAG AAA ACA AGC AGA CGA CCC GAG CC
	ncov _cap _33_ RIG HT	AAA GGC TGG TGG CAC TAC TGA ACA GAC GAC CCG AGC C
	ncov _cap _35_ RIG HT	CAG AAG AAA CAC GCA AAT TAA TGC CCA GAC GAC CCG AGC C
	ncov _cap	AAG TGC

	_37_ RIG HT	CAG CTA CAG TTT CTG TCA GAC GAC CCG AGC C
	ncov _cap _39_ RIG HT	GTT ATC ACC TTT GAC AAT CTT AAG ACA CTC AGA CGA CCC GAG CC
	ncov _cap _41_ RIG HT	TGA TCC TAG TTT TCT GGG TAG GTA CAC AGA CGA CCC GAG CC
	ncov _cap _43_ RIG HT	GAA GCT GCT AAC TTT TGT GCA CTC AGA CGA CCC GAG CC
	ncov _cap _45_ RIG HT	CGT GTG GTA AAC AAG CTA

		CAAAATATCTCAGACGACCCGAGCC
	ncov_cap_47_RIGHT	CAAAGGTCCTATTACGGATGTTTTCTACCAGACGACCCGAGCC
	ncov_cap_49_RIGHT	ACATTTTTCCCTGACTTAAATGGTGATGCAGACGACCCGAGCC
	ncov_cap_51_RIGHT	TGAAGAAGTAGTGGAAAATCCTACCATCAGACGACCCGAGCC
	ncov_cap_53_RIGHT	TGCTGTTAATAGTGTCCC

		TTG GGC AGA CGA CCC GAG CC
	ncov _cap _55_ RIG HT	CTC AAC CGC TGC TTT AGG TGT TCA GAC GAC CCG AGC C
	ncov _cap _57_ RIG HT	TGG TTT TTG GCA TATA TTC TTT TCA CTA GGC AGA CGA CCC GAG CC
	ncov _cap _59_ RIG HT	TCA ACT TGT ATG ATG TGT TAC AAA CGT AAC AGA CGA CCC GAG CC
	ncov _cap _61_ RIG HT	ACA GTG AAG AAT GGT TCC ATC CAT CAG

		ACG ACC CGA GCC
	ncov_cap_63_RIGHT	TGT TAC TAG ATC AGG CAT TAG TGT CTG CAG ACG ACC CGA GCC
	ncov_cap_65_RIGHT	AGG GTT TGT TGA TTC AGA TGT AGA AAC CAG ACG ACC CGA GCC
	ncov_cap_67_RIGHT	ACG AAA ACA AAT ACG TAG TGC TGC CAG ACG ACC CGA GCC
	ncov_cap_69_RIGHT	TGT CAT GTC TAA ACA TAC TGA CTT TTC AAG CAG ACG ACC

		CGA GCC
	ncov _cap _71_ RIG HT	ATT ACG CAC AAC TAA TGG TGA CTT TTC AGA CGA CCC GAG CC
	ncov _cap _73_ RIG HT	ACG TTA TGT GCT CAT GGA TGG CCA GAC GAC CCG AGC C
	ncov _cap _75_ RIG HT	GGT GCT TTG GAC ATA TCA GCA TCT CAG ACG ACC CGA GCC
	ncov _cap _77_ RIG HT	AGC ACA TAT TCA GTG GAT GGT TAT GTT CAG ACG ACC CGA GCC
	ncov _cap	TCT AAA

	_79_ RIG HT	GTT GCG TAG TGA TGT GCT ACA GAC GAC CCG AGC C
	ncov _cap _81_ RIG HT	CCC ATC TGG TAA AGT TGA GGG TTG CAG ACG ACC CGA GCC
	ncov _cap _83_ RIG HT	GCT TAA GGT TGA TAC AGC CAA TCC TCA GAC GAC CCG AGC C
	ncov _cap _85_ RIG HT	TCT TTT TGT TAC ATG CAC CAT ATG GAA TTC AGA CGA CCC GAG CC
	ncov _cap _87_ RIG HT	CCT CTA ACA CAA GAC

		CAT GTT GAC ACA GAC GAC CCG AGC C
	ncov_cap_89_RIGHT	CAC ACC ACT GGT TGT TAC TCA CAC AGA CGA CCC GAG CC
	ncov_cap_91_RIGHT	TGC TAG TTG GGT GAT GCG TAT TCA GAC GAC CCG AGC C
	ncov_cap_93_RIGHT	TCT CTG TTA CTT CTA ACT ACT CAG GTG TAC AGA CGA CCC GAG CC
	ncov_cap_95_RIGHT	ACT GAC TCT TGG TGT TTA TGA TTA CTT

		AGT CAG ACG ACC CGA GCC
	ncov_cap_97_RIGHT	GGG CTC AAT GTG TCC AGT TAC ACA GAC GAC CCG AGC C
	ncov_cap_99_RIGHT	TGC TAA TGG TGA TTC TGA AGT TGT TCT CAG ACG ACC CGA GCC
	ncov_cap_101_RIGHT	GCA AGA GAT GGT TGT GTT CCC TCA GAC GAC CCG AGC C
	ncov_cap_103_RIGHT	GCA TGG CCT CTT ATT GTA ACA GCC AGA CGA CCC GAG CC

	ncov_cap_105_RIGHT	ATGGAACTGGTACTATCTATACAGAACTGCAGACGACCCGAGCC
	ncov_cap_107_RIGHT	ACACACTACTGGTACTGGTCAGGCAGACGACCCGAGCC
	ncov_cap_109_RIGHT	AAACACAGTCTGTACCGTCTGCCAGACGACCCGAGCC
	ncov_cap_111_RIGHT	AGGACGAAGATGACAATTTAATTGATTCTTCAGACGACCCGAGCC
	ncov_cap_113_	GCATTTTGA

	RIG HT	TGA AGG TAA TTG TGA CAC ACA GAC GAC CCG AGC C
	ncov _cap _115_ RIG HT	GGT ATG ATT TCG GTG ATT TCA TAC AAA CCC AGA CGA CCC GAG CC
	ncov _cap _117_ RIG HT	TGT TAA CTG TTT GGA TGA CAG ATG CCA GAC GAC CCG AGC C
	ncov _cap _119_ RIG HT	TGT ATG CTG CTG ACC CTG CTA TCA GAC GAC CCG AGC C
	ncov _cap _121_ RIG HT	CCA ACA ATG TGT GAT

		ATC AGA CAA CTA CTC AGA CGA CCC GAG CC
	ncov _cap _123 _RIG HT	CGT AAT GTC ATC CCT ACT ATA ACT CAA ATG CAG ACG ACC CGA GCC
	ncov _cap _125 _RIG HT	TTA TGG CCT CAC TTG TTC TTG CTC AGA CGA CCC GAG CC
	ncov _cap _127_ RIG HT	AAT GCA CTT TTA TCT ACT GAT GGT AAC AAA CAG ACG ACC CGA GCC
	ncov _cap _129 _RIG HT	GGC TAG CAT AAA GAA CTT

		TAAGTCAGTTCAGACGACCCGAGCC
	ncov_cap_131_RIGHT	GGTACACTTATGATTGAACGGTTCGCAGACGACCCGAGCC
	ncov_cap_133_RIGHT	ACACACCGCATACAGTCTTACAGCAGACGACCCGAGCC
	ncov_cap_135_RIGHT	TGTGCTAATGGACAAGTTTTTGGTTCAGACGACCCGAGCC
	ncov_cap_137_RIGHT	TGCTGTCTGACAGAGAATTACATCTTC

		AGA CGA CCC GAG CC
	ncov_cap_139_RIGHT	GCC ATT AAG TGC ACC TAC ACT AGT CAG ACG ACC CGA GCC
	ncov_cap_141_RIGHT	CGC TGT TGA TGC ACT ATG TGA GAC AGA CGA CCC GAG CC
	ncov_cap_143_RIGHT	CAA TGC CAG ATT ACG TGC TAA GCC AGA CGA CCC GAG CC
	ncov_cap_145_RIGHT	AGG GTG TTA TCA CGC ATG ATG TCA GAC GAC CCG AGC C

	ncov_cap_147_RIGHT	CAGCTCACTCTTGTAATGTAAACAGATTCAGACGACCGAGCC
	ncov_cap_149_RIGHT	TCAAAACTGAAGGTTTATGTGTTGACACAGACGACCGAGCC
	ncov_cap_151_RIGHT	TACCTTTACAGCTAGGTTTTCTACAGGCAGACGACCGAGCC
	ncov_cap_153_RIGHT	GGCTTTGAGTTGACATCTATGAAGTATTTTCAGACGACCGAGCC

	ncov_cap_155_RIGHT	AGCTAGTTGTGATGCAATCATGACTCAGACGACCCGAGCC
	ncov_cap_157_RIGHT	GTAGAATGGAAGTTCTATGATGCACAGCAGACGACCCGAGCC
	ncov_cap_159_RIGHT	CATTCCACACACCAGCTTTTGATAACAGACGACCCGAGCC
	ncov_cap_161_RIGHT	AGCTTGTGGGTTTACAAACAATTTGACAGACGACCCGAGCC
	ncov_cap_163	AATGTAGCA

	_RIG HT	TTT GAG CTT TGG GCC AGA CGA CCC GAG CC
	ncov _cap _165 _RIG HT	AGA AAT GCC CGT AAT GGT GTT CTC AGA CGA CCC GAG CC
	ncov _cap _167_ RIG HT	AGC TAT GGA TGA ATT CAT TGA ACG GTA CAG ACG ACC CGA GCC
	ncov _cap _169 _RIG HT	CCC AAG ATT TAT CTG TAG TTT CTA AGG TTG CAG ACG ACC CGA GCC
	ncov _cap _171_ RIG HT	GTC GCA AAA TAT ACT CAA CTG

		TGT CAC AGA CGA CCC GAG CC
	ncov_cap_173_RIGHT	GAT CTC ATT ATT AGT GAT ATG TAC GAC CCT CAG ACG ACC CGA GCC
	ncov_cap_175_RIGHT	AAT TGG ATG TAA TTA TCT TGG CAA ACC ACA GAC GAC CCG AGC C
	ncov_cap_177_RIGHT	TGA TGT TCT TGT TAA CAA CTA AAC GAA CAC AGA CGA CCC GAG CC
	ncov_cap_179_RIGHT	TGG GAC CAA TGG TAC TAA GAG

		GTT CAG ACG ACC CGA GCC
	ncov _cap _181_ RIG HT	CAA AAG TTG GAT GGA AAG TGA GTT CAC AGA CGA CCC GAG CC
	ncov _cap _183 _RIG HT	ACT AGG TTT CAA ACT TTA CTT GCT TTA CAC AGA CGA CCC GAG CC
	ncov _cap _185 _RIG HT	AGA GTC CAA CCA ACA GAA TCT ATT GTC AGA CGA CCC GAG CC
	ncov _cap _187_ RIG HT	GCA GAT TCA TTT GTA ATT AGA GGT GAT GAC

		AGACGACCCGAGCC
	ncov_cap_189_RIGHT	ATCTATCAGGCCGGTAGCACACCAGACGACCGAGCC
	ncov_cap_191_RIGHT	ACAAAAAGTTTCTGCCTTTCCAACACAGACGACCGAGCC
	ncov_cap_193_RIGHT	CAAACACGTGCAGGCTGTTTAACAGACGACCGAGCC
	ncov_cap_195_RIGHT	TCTACCAGTGTCTATGACCAAGACACAGACGACCGAGCC

	ncov_cap_197_RIGHT	CCA TCA AAA CCA AGC AAG AGG TCA CAG ACG ACC CGA GCC
	ncov_cap_199_RIGHT	TCT GGT TGG ACC TTT GGT GCA GCA GAC GAC CCG AGC C
	ncov_cap_201_RIGHT	ACG CTT GTT AAA CAA CTT AGC TCC CAG ACG ACC CGA GCC
	ncov_cap_203_RIGHT	TGG AAA GGG CTA TCA TCT TAT GTC CTC AGA CGA CCC GAG CC
	ncov_cap_205_RIGHT	GTC TGG TAA CTG TGA TGT

		TGT AAT AGG ACA GAC GAC CCG AGC C
	ncov_cap_207_RIGHT	CTC ATC GAT CTC CAA GAA CTT GGA CAG ACG ACC CGA GCC
	ncov_cap_209_RIGHT	GGA ACT GTA ACT TTG AAG CAA GGT GCA GAC GAC CCG AGC C
	ncov_cap_211_RIGHT	AAC AGT TTA CTC ACA CCT TTT GCT CCA GAC GAC CCG AGC C
	ncov_cap_213_RIGHT	TCA TTA CTT CAG GTG ATG GCA CAA CAG

		ACG ACC CGA GCC
	ncov _cap _215 _RIG HT	CCA ATT TAT GAT GAA CCG ACG ACG CAG ACG ACC CGA GCC
	ncov _cap _217 _RIG HT	AAA ACC TTC TTT TTA CGT TTA CTC TCG TCA GAC GAC CCG AGC C
	ncov _cap _219 _RIG HT	TGT CTT CTA CAA TTT GCC TAT GCC ACA GAC GAC CCG AGC C
	ncov _cap _221 _RIG HT	CTT CTC AAC GTG CCA CTC CAT GCA GAC GAC CCG AGC C

	ncov_cap_223_RIGHT	GTC GCT ACA GGA TTG GCA ACT ATC AGA CGA CCC GAG CC
	ncov_cap_225_RIGHT	TGA AGA GCA ACC AAT GGA GAT TGA CAG ACG ACC CGA GCC
	ncov_cap_227_RIGHT	TGT TCA TCA GAC AAG AGG AAG TTC ACA GAC GAC CCG AGC C
	ncov_cap_229_RIGHT	TTT TCT TAG GAA TCA TCA CAA CTG TAG CCA GAC GAC CCG AGC C
	ncov_cap_231	TGC CAG GAA CCT

	_RIG HT	AAA TTG GGT AGC AGA CGA CCC GAG CC
	ncov _cap _233 _RIG HT	GGC CCC AAG GTT TAC CCA ATA ATC AGA CGA CCC GAG CC
	ncov _cap _235 _RIG HT	TGG ACT TCC CTA TGG TGC TAA CAA CAG ACG ACC CGA GCC
	ncov _cap _237 _RIG HT	TTC AAC TCC AGG CAG CAG TAG GCA GAC GAC CCG AGC C
	ncov _cap _239 _RIG HT	GGT CCA GAA CAA ACC CAA GGA ACA GAC GAC CCG

		AGCC
	ncov_cap_241_RIGHT	CGCATACAACAACATTCCCACCAACAGACGACCCGAGCC
	ncov_cap_243_RIGHT	CGTTTTCGCTTTTCCGTTTACGACAGACCCGAGCC
	ncov_cap_245_RIGHT	TGGAAGAGCCCTAATGTGTAAAATTAATTTTCAGACGACCCGAGCC
	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	GGAATATTGGTGAACAGAAATCAATACTGACAGACGACCGAGCC
	ncov_cap_16_RIGHT	AGGTGGTGTTGTTCAGTTGACTCAGACGACCCGAGCC
	ncov_cap_18_	TTTGGCTTT

	RIG HT	GTG TGC TGA CTC TCA GAC GAC CCG AGC C
	ncov _cap _20_ RIG HT	TTA GAA CAA CCT ACT AGT GAA GCT GTT CAG ACG ACC CGA GCC
	ncov _cap _22_ RIG HT	GCC TAT ACA GTT GAA CTC GGT ACA GCA GAC GAC CCG AGC C
	ncov _cap _24_ RIG HT	TGT GAA GAA GAA GAG TTT GAG CCA CAG ACG ACC CGA GCC
	ncov _cap _26_ RIG HT	GGA ACT TAC ACC AGT TGT TCA GAC

		TCA GAC GAC CCG AGC C
	ncov _cap _28_ RIG HT	CTT AAA GTG GGT GGT AGT TGT GTT TTC AGA CGA CCC GAG CC
	ncov _cap _30_ RIG HT	CAC AAA TGT CTA CTT AGC TGT CTT TGA TCA GAC GAC CCG AGC C
	ncov _cap _32_ RIG HT	CAA TCT TCA TCC AGA TTC TGC CAC TCA GAC GAC CCG AGC C
	ncov _cap _34_ RIG HT	CAG TGC TTA AAA AGT GTA AAA GTG CCC AGA

		CGA CCC GAG CC
	ncov _cap _36_ RIG HT	GTA AAA CAA CTG TAG CGT CAC TTA TCA CAG ACG ACC CGA GCC
	ncov _cap _38_ RIG HT	GTT CCT ATA AAG ATT GGT CCT ATT CTG GAC AGA CGA CCC GAG CC
	ncov _cap _40_ RIG HT	ACT TAT TTG GAT GGA GCT GAT GTT ACT AAC AGA CGA CCC GAG CC
	ncov _cap _42_ RIG HT	CTT CTA TTA AAT GGG CAG ATA ACA ACT GTC AGA

		CGA CCC GAG CC
	ncov_cap_44_RIGHT	AAG AGT CTT GAA CGT GGT GTG TCA GAC GAC CCG AGC C
	ncov_cap_46_RIGHT	TGG TAC ATT TAC TTG TGC TAG TGA GTA CAG ACG ACC CGA GCC
	ncov_cap_48_RIGHT	AAC CAA CCA TAT CCA AAC GCA AGC AGA CGA CCC GAG CC
	ncov_cap_50_RIGHT	CCT GGT GTA TAC GTT GTC TTT GGA CAG ACG ACC CGA GCC

	ncov_cap_52_RIGHT	CACACAGATCTAATGGCTGCTTATGTCAGACGACCCGAGCC
	ncov_cap_54_RIGHT	GCAAAGAATACTGTTAAGAGTGTCGGTACAGACGACCGAGCC
	ncov_cap_56_RIGHT	GTAGTGTTTGTCTTAGTGGTTTAGATTCTTCAGACGACCCGAGCC
	ncov_cap_58_RIGHT	ATGGCCCCGATTTCAGCTATGGCAGACGACCCGAGCC
	ncov_cap	CTGTGC

	_60_ RIG HT	TGG TAG TAC ATT TAT TAG TGA TGC AGA CGA CCC GAG CC
	ncov _cap _62_ RIG HT	AGC TAA TAA CAC TAA AGG TTC ATT GCC TAC AGA CGA CCC GAG CC
	ncov _cap _64_ RIG HT	ACT CAA AAC ACT AGT TGC AAC TGC CAG ACG ACC CGA GCC
	ncov _cap _66_ RIG HT	GCT TGT ATT GAC TGT AGT GCG CCA GAC GAC CCG AGC C
	ncov _cap _68_ RIG HT	AGC ACT TAA GGG TGG

		TAA AAT TGT TAA TCA GAC GAC CCG AGC C
	ncov_cap_70_RIGHT	GCT GAT TTT GAC ACA TGG TTT AGC CCA GAC GAC CCG AGC C
	ncov_cap_72_RIGHT	GTG TTT TGG CTG CTG AAT GTA CAC AGA CGA CCC GAG CC
	ncov_cap_74_RIGHT	GCT GGT GTT TGT GTA TCT ACT AGT GGC AGA CGA CCC GAG CC
	ncov_cap_76_RIGHT	GAG CTT TTG GTG AAT ACA GTC ATG

		TAG CAG ACG ACC CGA GCC
	ncov _cap _78_ RIG HT	TCC ACA AAG CAT TTC TAT TGG TTC TTT CAG ACG ACC CGA GCC
	ncov _cap _80_ RIG HT	GCT TGT TGT CAT CTC GCA AAG GCA GAC GAC CCG AGC C
	ncov _cap _82_ RIG HT	TGA AGA CAT GCT TAA CCC TAA TTA TGA AGA CAG ACG ACC CGA GCC
	ncov _cap _84_ RIG HT	TGC TAT GAG GCC CAA TTT CAC TCA GAC GAC CCG

		AGCC
	ncov_cap_86_RIGHT	TTTTAGCTTGGTTGTACGCTGCCAGACGACCGAGCC
	ncov_cap_88_RIGHT	CCATATTGGGTAGTGCTTTATTAGAAGATGCAGACGACCCGAGCC
	ncov_cap_90_RIGHT	TGTCTGCTTTTGCAATGATGTTTGTCAGACGACCGAGCC
	ncov_cap_92_RIGHT	ACTGTGTATGATGATGGTGCTAGGCAGACGACCCGAGCC

	ncov_cap_94_RIGHT	TGCCCTATTTTCTTCATAACTGGTAATACACAGCACGACCCGAGCC
	ncov_cap_96_RIGHT	GGCAAACCTTGTATCAAAGTAGCCCAGACGACCCGAGCC
	ncov_cap_98_RIGHT	GCTTTGTGAAGAAATGCTGGACACAGAGACGACCCGAGCC
	ncov_cap_100_RIGHT	TGACCCAAATGTATAACAGGCTAGACAGAGACGACCCGAGCC
	ncov_cap_102	AAATACGTGTGA

	_RIG HT	TGG TAC AAC ATT TAC TTC AGA CGA CCC GAG CC
	ncov _cap _104 _RIG HT	ACA AAC TGC TTG CAC TGA TGA CAC AGA CGA CCC GAG CC
	ncov _cap _106 _RIG HT	CTT GGT AGT TTA GCT GCC ACA GTC AGA CGA CCC GAG CC
	ncov _cap _108 _RIG HT	TTT GGT GGT GCA TCG TGT TGT CCA GAC GAC CCG AGC C
	ncov _cap _110_ RIG HT	GTA TAC AGG GCT TTT GAC ATC TAC AAT

		GCA GAC GAC CCG AGC C
	ncov _cap _112_ RIG HT	AAT AGA CGG TGA CAT GGT ACC ACC AGA CGA CCC GAG CC
	ncov _cap _114_ RIG HT	CGC CAA CTT AGG TGA ACG TGT ACA GAC GAC CCG AGC C
	ncov _cap _116_ RIG HT	TGT TGA CAC TGA CTT AAC AAA GCC TTC AGA CGA CCC GAG CC
	ncov _cap _118_ RIG HT	GTG AGA AAA ATA TTT GTT GAT GGT GTT CCC AGA CGA CCC

		GAG CC
	ncov _cap _120 _RIG HT	GCT TTT CAA ACT GTC AAA CCC GGC AGA CGA CCC GAG CC
	ncov _cap _122 _RIG HT	CCT AGA CAA ATC AGC TGG TTT TCC ACA GAC GAC CCG AGC C
	ncov _cap _124 _RIG HT	TTG GAA CAA GCA AAT TCT ATG GTG GCA GAC GAC CCG AGC C
	ncov _cap _126 _RIG HT	TCA CTA TAT GTT AAA CCA GGT GGA ACC CAG ACG ACC CGA GCC

	ncov_cap_128_RIGHT	AGACTTTGTGAATGAGTTTACGCATCAGAACGACCCGAGCC
	ncov_cap_130_RIGHT	CTCTCAACATACAATGCTAGTTAAACAGGCAGACGACCCGAGCC
	ncov_cap_132_RIGHT	AAGCTACATGATGAGTTACAGGACACAGACGACCCGAGCC
	ncov_cap_134_RIGHT	TCTTGTCTGTTAATCCGTATGTTTGCCAGACGACCCGAGCC
	ncov_cap	TTTAGC

	_136_RIGHT	TAACACCTGTACTGAAAGACTCAGACGACCCGAGCC
	ncov_cap_138_RIGHT	CAAATAGGAGAGTACACCTTTGAAAAAGGCAGACGACCCGAGCC
	ncov_cap_140_RIGHT	AAGGTTGGTATGCAAAAGTATTCTACACCAGACGACCGAGCC
	ncov_cap_142_RIGHT	GTACTGTAAATGCATTGCCTGAGACCAGACGACCGAGCC
	ncov_cap_144	TAGGTCCAG

	_RIGHT	ACATGTTCCTCGGCAGACGACCGAGCC
	ncov_cap_146_RIGHT	GCCTCAAAGATTTTGGGACTACCACAGACGACCGAGCC
	ncov_cap_148_RIGHT	CGTAGGAATGTGGCAACTTTACAAGCAGACGACCGAGCC
	ncov_cap_150_RIGHT	CCCGCGAAGAAGCTATAAGACACAGACGACCGAGCC
	ncov_cap_152_RIGHT	CCTCATACCACTTATGTACAAGGACT

		CAG ACG ACC CGA GCC
	ncov _cap _154 _RIG HT	GCA TCA TTC TAT TGG ATT TGA TTA CGT CTC AGA CGA CCC GAG CC
	ncov _cap _156 _RIG HT	CTT GTA GAA AGG TTC AAC ACA TGG TTC AGA CGA CCC GAG CC
	ncov _cap _158 _RIG HT	TGC AAT GTC GAT AGA TAT CCT GCT AAC AGA CGA CCC GAG CC
	ncov _cap _160 _RIG HT	CCA CTA AAG TCT GCT ACG TGT ATA ACA CAG ACG

		ACC CGA GCC
	ncov _cap _162 _RIG HT	TGA TGG ACA ACA GGG TGA AGT ACC AGA CGA CCC GAG CC
	ncov _cap _164 _RIG HT	CTC CAG CAC ATAT ATC TAC TAT TGG TGT CAG ACG ACC CGA GCC
	ncov _cap _166 _RIG HT	AGA AGC CGT AAA AAC ACA GTT CAA TTC AGA CGA CCC GAG CC
	ncov _cap _168 _RIG HT	TGG TTT ACA TCT ACT GAT TGG ACT AGC CAG ACG ACC CGA GCC

	ncov_cap_170_RIGHT	AATCTAGTCAAGCGTGGCAACCACGACGACCGAGCC
	ncov_cap_172_RIGHT	TTGCACCAGGTACAGCTGTTTTCAGACGACCGAGCC
	ncov_cap_174_RIGHT	TGGAGGTTCCGTGGCTATAAAGATCAGACGACCCGAGCC
	ncov_cap_176_RIGHT	CAAATCCAATTCAGTTGTCTTCCTATTCTCAGACGACCCGAGCC

	ncov_cap_178_RIGHT	CAGTGTGTTAATCTTACAACCAGACTCCAGACGACCGAGCC
	ncov_cap_180_RIGHT	TTCGAAGACCCAGTCCCTACTTCAGACGACCGAGCC
	ncov_cap_182_RIGHT	GCCTATTAATTTAGTGCGTGATCTCCCAGACGAGCC
	ncov_cap_184_RIGHT	TGAAAATGGAACCATTACAGATGCTGCAGACGAGCC
	ncov_cap_186	ACTGTGTTG

	_RIG HT	CTG ATT ATT CTG TCC TCA GAC GAC CCG AGC C
	ncov _cap _188 _RIG HT	CGT TAT AGC TTG GAA TTC TAA CAA TCT TGA CAG ACG ACC CGA GCC
	ncov _cap _190 _RIG HT	TTC TTT TGA ACT TCT ACA TGC ACC AGC AGA CGA CCC GAG CC
	ncov _cap _192 _RIG HT	AAC AAA TAC TTC TAA CCA GGT TGC TGC AGA CGA CCC GAG CC
	ncov _cap _194 _RIG HT	ACG TAG TGT AGC TAG

		TCA ATC CAT CAC AGA CGA CCC GAG CC
	ncov_cap_196_RIGHT	ACT GGA ATA GCT GTT GAA CAA GAC ACA GAC GAC CCG AGC C
	ncov_cap_198_RIGHT	ATT GCT GCT AGA GAC CTC ATT TGT CAG ACG ACC CGA GCC
	ncov_cap_200_RIGHT	GAA CCA AAA ATT GAT TGC CAA CCA ATC AGA CGA CCC GAG CC
	ncov_cap_202_RIGHT	AAG TTT GCA GAC ATA TGT GAC TCA ACC

		AGA CGA CCC GAG CC
	ncov_cap_204_RIGHT	TGG AAA AGC ACA CTT TCC TCG TCA GAC GAC CCG AGC C
	ncov_cap_206_RIGHT	TTG ATT TAG GTG ACA TCT CTG GCA CAG ACG ACC CGA GCC
	ncov_cap_208_RIGHT	GCT GTA TGA CCA GTT GCT GTA GTC AGA CGA CCC GAG CC
	ncov_cap_210_RIGHT	CTT ATT GTT GGC GTT GCA CTT CTT CAG ACG ACC CGA GCC

	ncov_cap_212_RIGHT	GCCGTTCCAAAAACCCATTACTTTCAGACGACCCGAGCC
	ncov_cap_214_RIGHT	TGTATTACACAGCAGTTACTTCACTTCAGACTCAGACGACCCGAGCC
	ncov_cap_216_RIGHT	TTCTTGCTTTCGTGGTATTCTTGCCAGACGACCGAGCC
	ncov_cap_218_RIGHT	CATGGCAGATTCCAACGGTACTCAGACGACCGAGCC

	ncov_cap_220_RIGHT	GGATCACCGGTGGAATTGCTATCAGACGACCGAGCC
	ncov_cap_222_RIGHT	ACGCTGTGACATCAAGGACCTGCAGACGACCGAGCC
	ncov_cap_224_RIGHT	GTTGACTTTCAGGTTACTATAGCAGAGACAGACGACCGAGCC
	ncov_cap_226_RIGHT	ATTCACCATTTCATCCTCTAGCTGATCAGACGACCCGAGCC
	ncov_cap_228	ATTTGTGCTTTT

	_RIG HT	TAG CCT TTC TGC TAC AGA CGA CCC GAG CC
	ncov _cap _230 _RIG HT	CGT GTC CTA TTC ACT TCT ATT CTA AAT GGC AGA CGA CCC GAG CC
	ncov _cap _232 _RIG HT	CCC AAA ATC AGC GAA ATG CAC CCA GAC GAC CCG AGC C
	ncov _cap _234 _RIG HT	GCA GTC CAG ATG ACC AAA TTG GCC AGA CGA CCC GAG CC
	ncov _cap _236 _RIG HT	CCT CAA GGA ACA ACA TTG CCA AAC

		AGA CGA CCC GAG CC
	ncov_cap_238_RIGHT	ACA ACA ACA AGG CCA AAC TGT CCA GAC GAC CCG AGC C
	ncov_cap_240_RIGHT	ATT GGC ATG GAA GTC ACA CCT TCA GAC GAC CCG AGC C
	ncov_cap_242_RIGHT	TTC TTC CTG CTG CAG ATT TGG ACA GAC GAC CCG AGC C
	ncov_cap_244_RIGHT	AGT GTG TAA CAT TAG GGA GGA CTT GCA GAC GAC CCG AGC C

	RNAse_P_bead_rev	GTGGAGACAGCCGCTCACAGACGACCCGAGCC

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

	NAME	SEQUENCE
	ncov_cap_1_LEFT	ACGTGTGCTCTTCCGATCTTCTCTGTAGATCTGTTCTCTAAACGAAC
	ncov_cap_3_LEFT	ACGTGTGCTCTTCCGATCTCTGTTTTACAGGTTCGCGACGT
	ncov_cap_5_LEFT	ACGTGTGCTCTTCCGATCTCCTCATGTGGGCGAA

		AAT ACC AG
	ncov_cap_7_LEFT	ACG TGT GCT CTT CCG ATC TAT GGC TAC CCT CTT GAG TGC AT
	ncov_cap_9_LEFT	ACG TGT GCT CTT CCG ATC TGG GGA ATG TCC AAA TTT TGT ATT TCC C
	ncov_cap_11_LEFT	ACG TGT GCT CTT CCG ATC TCC ACT TGC GAA TTT TGT GGC AC
	ncov_cap_13_LEFT	ACG TGT GCT CTT CCG ATC TCT ATT GGG TTC CAC GTG

		CTAGC
	ncov_cap_15_LEFT	ACGTGTGCTCTTCCGATCTTCAAACAATTGTTGAATCCTGTGGT
	ncov_cap_17_LEFT	ACGTGTGCTCTTCCGATCTTGTTCACATCTGATTGGCTACTAACA
	ncov_cap_19_LEFT	ACGTGTGCTCTTCCGATCTCCTGTGCAAAGGAATTAAGGAGAGT
	ncov_cap_21_LEFT	ACGTGTGCTCTTCCGATCTTTCCCACAGAAGTGT

		TAA CAG AGG A
	ncov_cap_23_LEFT	ACG TGT GCT CTT CCG ATC TAG TGC AAG GTT ACA AGA GTG TGA
	ncov_cap_25_LEFT	ACG TGT GCT CTT CCG ATC TGG CTT CAC ATA TGT ATT GTT CTT TCT ACC
	ncov_cap_27_LEFT	ACG TGT GCT CTT CCG ATC TAG GAC AAT CAG ACA ACT ACT ATT CAA ACA
	ncov_cap_29_LEFT	ACG TGT GCT CTT CCG ATC TGG CTA CTA

		ACA ATG CCA TGC AAG
	ncov_cap_31_LEFT	ACG TGT GCT CTT CCG ATC TTA AGA GTT TGT GTA GAT ACT GTT CGC A
	ncov_cap_33_LEFT	ACG TGT GCT CTT CCG ATC TTC TGG AAG AAA CTA AGT TCC TCA CAG
	ncov_cap_35_LEFT	ACG TGT GCT CTT CCG ATC TTT ATAT AAC CAC TTA CCC GGG TCA GG
	ncov_cap_37_LEFT	ACG TGT GCT CTT CCG ATC TAC AAG

		AGG GTG TGG TTG ATT ATG G
	ncov _cap _39_ LEF T	ACG TGT GCT CTT CCG ATC TAC TTC TTC TTC TAA AAC ACC TGA AGA ACA
	ncov _cap _41_ LEF T	ACG TGT GCT CTT CCG ATC TCG CAA GTT GTG GAC ATG TCA ATG
	ncov _cap _43_ LEF T	ACG TGT GCT CTT CCG ATC TTG TCA GCA TTA AAT CAC ACT AAA AAG TGG
	ncov _cap _45_ LEF T	ACG TGT GCT CTT CCG ATC

		TAA TGA GTT ACT TGT TTC AAC ATG CCA
	ncov_cap_47_LEFT	ACG TGT GCT CTT CCG ATC TAC CTT TTG TTA TGA TGT CAG CAC CA
	ncov_cap_49_LEFT	ACG TGT GCT CTT CCG ATC TAG AAA GAC AAT TCT TAT TTC ACA GAG CAA
	ncov_cap_51_LEFT	ACG TGT GCT CTT CCG ATC TTG TTT GGC ATG TTA ACA ATG CAA CT
	ncov_cap_53_	ACG TGT GCT CTT

	LEFT	CCGATCTACCGAAGTTGTAGGAGACATTATACTTAA
	ncov_cap_55_LEFT	ACGTGTGCTCTTCCGATCTAATTCTAGAATTAAAGCATCTATGCCGAC
	ncov_cap_57_LEFT	ACGTGTGCTCTTCCGATCTTTTGAACTCTACTAATGTCACTATTGCAAC
	ncov_cap_59_LEFT	ACGTGTGCTCTTCCGATCTGCAATTGTTTTTCAGCTATTTG

		CAG T
	ncov _cap _61_ LEF T	ACG TGT GCT CTT CCG ATC TTG TCT ATG CTA ATG GAG GTA AAG GC
	ncov _cap _63_ LEF T	ACG TGT GCT CTT CCG ATC TAA GAC TTA TGA AAG ACA TTC TCT CTC TCA
	ncov _cap _65_ LEF T	ACG TGT GCT CTT CCG ATC TTA GTG CGG AAG TTG CAG TTA AAA TG
	ncov _cap _67_ LEF T	ACG TGT GCT CTT CCG ATC TGC GAT AGT TGT AAT

		AAC TAT ATG CTC ACC
	ncov_cap_69_LEFT	ACG TGT GCT CTT CCG ATC TAC TTA CCT TTT AAG TTG ACA TGT GCA A
	ncov_cap_71_LEFT	ACG TGT GCT CTT CCG ATC TTC ACT CGT GAC ATA GCA TCT ACA GA
	ncov_cap_73_LEFT	ACG TGT GCT CTT CCG ATC TAA CAT CTG TTA CAC ACC ATC AAA ACT T
	ncov_cap_75_LEFT	ACG TGT GCT CTT CCG ATC TTT

		CTG AGT ACT GTA GGC ACG GC
	ncov_cap_77_LEFT	ACG TGT GCT CTT CCG ATC TAT GAG GTT TAG AAG AGC TTT TGG TGA
	ncov_cap_79_LEFT	ACG TGT GCT CTT CCG ATC TTG TTC ACA CCT TTA GTA CCT TTC TGG
	ncov_cap_81_LEFT	ACG TGT GCT CTT CCG ATC TAG TAC AAG TAT TTT AGT GGA GCA ATG GA
	ncov_cap_83_LEFT	ACG TGT GCT CTT CCG ATC

		TAA CGG TCT TTG GCT TGA TGA CG
	ncov_cap_85_LEFT	ACG TGT GCT CTT CCG ATC TTA AGT TTG TTC GCA TTC AAC CAG GA
	ncov_cap_87_LEFT	ACG TGT GCT CTT CCG ATC TTG GAC CTT TTG TTG ACA GGC AA
	ncov_cap_89_LEFT	ACG TGT GCT CTT CCG ATC TGG AAT TGC CGT TTT AGA TAT GTG TGC
	ncov_cap_91_LEFT	ACG TGT GCT CTT CCG ATC TGT

		CCA GAG TAC TCA ATG GTC TTT GT
	ncov_cap_93_LEFT	ACG TGT GCT CTT CCG ATC TAC TGT GTT ATG TAT GCA TCA GCT GT
	ncov_cap_95_LEFT	ACG TGT GCT CTT CCG ATC TAC AAC TGT CAT GTT TTT GGC CAG
	ncov_cap_97_LEFT	ACG TGT GCT CTT CCG ATC TAC TAC TCC CAC CCA AGA ATA GCA
	ncov_cap_99_LEFT	ACG TGT GCT CTT CCG ATC TTG GTT

		TCA CTA CTT TCT GTT TTG CTT
	ncov_cap_101_LEFT	ACG TGT GCT CTT CCG ATC TAA TTT GAC CGT GAT GCA GCC AT
	ncov_cap_103_LEFT	ACG TGT GCT CTT CCG ATC TAC CTC TTA CAA CAG CAG CCA AAC
	ncov_cap_105_LEFT	ACG TGT GCT CTT CCG ATC TGC TTA GTC CTG TTG CAC TAC GAC
	ncov_cap_107_LEFT	ACG TGT GCT CTT CCG ATC TGG TAT GGT ACT

		TGG TAG TTT AGC TGC
	ncov_cap_109_LEFT	ACG TGT GCT CTT CCG ATC TTC AGG CAA TAA CAG TTA CAC CGG
	ncov_cap_111_LEFT	ACG TGT GCT CTT CCG ATC TAG TCA GCT GAT GCA CAA TCG TT
	ncov_cap_113_LEFT	ACG TGT GCT CTT CCG ATC TAC ACT TTC TCT AAC TAC CAA CAT GAA GA
	ncov_cap_115_LEFT	ACG TGT GCT CTT CCG ATC TAA GGA CTG GTA

		TGA TTT TGT AGA AAA CCC
	ncov_cap_117_LEFT	ACG TGT GCT CTT CCG ATC TTG TTA ATG CCT ATA TTA ACC TTG ACC AGG
	ncov_cap_119_LEFT	ACG TGT GCT CTT CCG ATC TTG TTT TAT TCT CTA CAG TGT TCC CAC C
	ncov_cap_121_LEFT	ACG TGT GCT CTT CCG ATC TTG TTG CTT TTC AAA CTG TCA AAC CC
	ncov_cap_123_LEFT	ACG TGT GCT CTT CCG ATC

		TGA TAA GTA CTT TGA TTG TTA CGA TGG TGG
	ncov_cap_125_LEFT	ACG TGT GCT CTT CCG ATC TTC AAA AAT TAT TGA AAT CAA TAG CCG CCA
	ncov_cap_127_LEFT	ACG TGT GCT CTT CCG ATC TTG TAG CTT GTC ACA CCG TTT CT
	ncov_cap_129_LEFT	ACG TGT GCT CTT CCG ATC TTG TCC GCA ATT TAC AAC ACA GAC T
	ncov_cap_131_	ACG TGT GCT CTT

	LEFT	CCGATCTATGTCTGAAGCAAAATGTTGGACTG
	ncov_cap_133_LEFT	ACGTGTGCTCTTCCGATCTATGCTTACCACTTACTAAACATCCT
	ncov_cap_135_LEFT	ACGTGTGCTCTTCCGATCTGTTGTAAATGCTGTTACGACATGT
	ncov_cap_137_LEFT	ACGTGTGCTCTTCCGATCTAGCGATAATGTTACTGACTTTAATGCAA
	ncov_cap	ACGTGT

	_139_LEFT	GCTCTTCCGATCTCCACTTAACCGAAATTATGTCTTACTGG
	ncov_cap_141_LEFT	ACGTGTGCTCTTCCGATCTACCACAACTCAATATCATCAGATGAG
	ncov_cap_143_LEFT	ACGTGTGCTCTTCCGATCTAATGTAGTAGAATTATACCTGCACGTGC
	ncov_cap_145_LEFT	ACGTGTGCTCTTCCGATCTACAGAATTTCAATTCAGTGT

		GTA GAC
	ncov_cap_147_LEFT	ACG TGT GCT CTT CCG ATC TGG TAA GAG AAT TCC TTA CAC GTA ACC C
	ncov_cap_149_LEFT	ACG TGT GCT CTT CCG ATC TCT GAT AGA GAC CTT TAT GAC AAG TTG CA
	ncov_cap_151_LEFT	ACG TGT GCT CTT CCG ATC TAG GAC ATG ACC TAT AGA AGA CTC ATC T
	ncov_cap_153_LEFT	ACG TGT GCT CTT CCG ATC TTT TTC CAG

		AGT TAG TGC TAA ACC ACC
	ncov _cap _155 _LEF T	ACG TGT GCT CTT CCG ATC TTA GAC GTG CCA CAT GCT TTT CC
	ncov _cap _157_ LEF T	ACG TGT GCT CTT CCG ATC TTG TTA AGC GTG TTG ACT GGA CT
	ncov _cap _159 _LEF T	ACG TGT GCT CTT CCG ATC TCT ATT CTT ATG CCA CAC ATT CTG ACA AA
	ncov _cap _161_ LEF T	ACG TGT GCT CTT CCG ATC TTC TGA CAG

		TCC ATG TGA GTC TCA
	ncov_cap_163_LEFT	ACG TGT GCT CTT CCG ATC TTG GAA CAC TTT TAC AAG ACT TCA GAG T
	ncov_cap_165_LEFT	ACG TGT GCT CTT CCG ATC TGG GTG TGG ACA TTG CTG CTA AT
	ncov_cap_167_LEFT	ACG TGT GCT CTT CCG ATC TAA CAA GCT AGT CTT AAT GGA GTC ACA
	ncov_cap_169_LEFT	ACG TGT GCT CTT CCG ATC TTC TAC TGA

		TTG GAC TAG CTA AAC GTT T
	ncov_cap_171_LEFT	ACG TGT GCT CTT CCG ATC TTG GTG TAA AGA TGG CCA TGT AGA A
	ncov_cap_173_LEFT	ACG TGT GCT CTT CCG ATC TCA TTT TGG TGC TGG TTC TGA TAA AGG
	ncov_cap_175_LEFT	ACG TGT GCT CTT CCG ATC TTT CAC TTA CAT TTG TGG GTT TAT ACA ACA
	ncov_cap_177_LEFT	ACG TGT GCT CTT CCG ATC

		TAC AAA TCC AAT TCA GTT GTC TTC CTA TTC
	ncov_cap_179_LEFT	ACG TGT GCT CTT CCG ATC TAG TCA GTG TGT TAA TCT TAC AAC CAG A
	ncov_cap_181_LEFT	ACG TGT GCT CTT CCG ATC TTT TGC TTC CAC TGA GAA GTC TAA CA
	ncov_cap_183_LEFT	ACG TGT GCT CTT CCG ATC TAC CTT GAA GGA AAA CAG GGT AAT TTC A
	ncov_cap	ACG TGT

	_185_LEFT	GCTCTTCCGATCTTGCTGCAGCTTATTATGTGGGT
	ncov_cap_187_LEFT	ACGTGTGCTCTTCCGATCTAGTTTTTAACGCCACCAGATTGC
	ncov_cap_189_LEFT	ACGTGTGCTCTTCCGATCTGGCAAACTGGAAAGATTGCTGAT
	ncov_cap_191_LEFT	ACGTGTGCTCTTCCGATCTACCACTAATGGTGTTGGTTACC
	ncov_cap_193	ACGTGTGCTCTT

	_LEFT	CCGATCTTCTTGACATTACACCATGTTCTTTG
	ncov_cap_195_LEFT	ACGTGTGCTCTTCCGATCTAGGTATATGCGCTAGTTATCAGACTCA
	ncov_cap_197_LEFT	ACGTGTGCTCTTCCGATCTCAATCTTTTGTTGCAATATGGCAGTTT
	ncov_cap_199_LEFT	ACGTGTGCTCTTCCGATCTAAGTGACACTTGCAGATGCTGG

	ncov_cap_201_LEFT	ACGTGTGCTCTTCCGATCTTGGCTTATAGGTTTAATGGTATTGGAGT
	ncov_cap_203_LEFT	ACGTGTGCTCTTCCGATCTACAAAGTTGAGGCTGAAGTGCA
	ncov_cap_205_LEFT	ACGTGTGCTCTTCCGATCTACTTATGTCCCTGCACAAGAAAAGA

	ncov_cap_207_LEFT	ACG TGT GCT CTT CCG ATC TTG ATC CTT TGC AAC CTG AAT TAG ACT
	ncov_cap_209_LEFT	ACG TGT GCT CTT CCG ATC TTG GTG ACA ATT ATG CTT TGC TGT ATG
	ncov_cap_211_LEFT	ACG TGT GCT CTT CCG ATC TGT TCG CGC TAC TGC AAC GAT AC
	ncov_cap_213_LEFT	ACG TGT GCT CTT CCG ATC TAT GCT TTA GTC TAC TTC TTG CAG AGT

	ncov_cap_215_LEFT	ACG TGT GCT CTT CCG ATC TAC ACA GTT ACT TCA CTT CAG ACT ATT ACC
	ncov_cap_217_LEFT	ACG TGT GCT CTT CCG ATC TAC AAG CTG ATG AGT ACG AAC TTA TGT
	ncov_cap_219_LEFT	ACG TGT GCT CTT CCG ATC TTC TAG AGT TCC TGA TCT TCT GGT CT
	ncov_cap_221_LEFT	ACG TGT GCT CTT CCG ATC TGG CCA GTA ACT TTA GCT TGT

		TTT GT
	ncov _cap _223 _LEF T	ACG TGT GCT CTT CCG ATC TGT AAT CGG AGC TGT GAT CCT TCG
	ncov _cap _225 _LEF T	ACG TGT GCT CTT CCG ATC TTG CTT GTA CAG TAA GTG ACA ACA GA
	ncov _cap _227 _LEF T	ACG TGT GCT CTT CCG ATC TAC AAC AGT ACT TTT AAA AGA ACC TTG CT
	ncov _cap _229 _LEF T	ACG TGT GCT CTT CCG ATC TTG CTT CAC ACT CAA AAG

		AAA GAC AGA
	ncov _cap _231 _LEF T	ACG TGT GCT CTT CCG ATC TTG TAG TTG ATG ACC CGT GTC CT
	ncov _cap _233 _LEF T	ACG TGT GCT CTT CCG ATC TAG AGT ATC ATG ACG TTC GTG TTG T
	ncov _cap _235 _LEF T	ACG TGT GCT CTT CCG ATC TAG GAA GAC CTT AAA TTC CCT CGA GG
	ncov _cap _237 _LEF T	ACG TGT GCT CTT CCG ATC TTG AGG GAG CCT TGA ATA

		CAC CA
	ncov _cap _239 _LEF T	ACG TGT GCT CTT CCG ATC TCT CTT GCT TTG CTG CTG CTT GA
	ncov _cap _241 _LEF T	ACG TGT GCT CTT CCG ATC TCA TTG GCC GCA AAT TGC ACA AT
	ncov _cap _243 _LEF T	ACG TGT GCT CTT CCG ATC TAG GCT GAT GAA ACT CAA GCC TT
	ncov _cap _245 _LEF T	ACG TGT GCT CTT CCG ATC TTG AAT TCT CGT AAC TAC ATA GCA

		CAAGT
	RNase_P_LEFT	ACGTGTGCTCTTCCGATCTGATTTGGACCTGCGAGCG

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

	NAME	SEQUENCE
	ncov_cap_2_LEFT	ACGTGTGCTCTTCCGATCTTCAGCACATCTAGGTTTCGTCC
	ncov_cap_4_LEFT	ACGTGTGCTCTTCCGATCTCATCAACGTTCGGATGCTCGA
	ncov_cap_6_LEFT	ACGTGTGCTCTTCCGATCTTGGCA

		CTG ATC CTT ATG AAG ATT TTC A
	ncov_cap_8_LEFT	ACG TGT GCT CTT CCG ATC TGC ATG AAA TTG CTT GGT ACA CGG
	ncov_cap_10_LEFT	ACG TGT GCT CTT CCG ATC TGC GTC ACC AAA TGA ATG CAA CC
	ncov_cap_12_LEFT	ACG TGT GCT CTT CCG ATC TAC CTG AGC ATA GTC TTG CCG AA
	ncov_cap_14_LEFT	ACG TGT GCT CTT CCG ATC TGT GAC TTT

		AAA CTT AAT GAA GAG ATC GCC
	ncov_cap_16_LEFT	ACG TGT GCT CTT CCG ATC TTG TAC GAT CAA TTT TCT CCC GCA
	ncov_cap_18_LEFT	ACG TGT GCT CTT CCG ATC TAA ACC CGT CCT TGA TTG GCT TG
	ncov_cap_20_LEFT	ACG TGT GCT CTT CCG ATC TTT TGT CAC GCA CTC AAA GGG AT
	ncov_cap_22_LEFT	ACG TGT GCT CTT CCG ATC TGC CCT TGC ACC

		TAA TAT GAT GGT
	ncov_cap_24_LEFT	ACG TGT GCT CTT CCG ATC TTG CTG TCA TAA AAA CTT TGC AAC CA
	ncov_cap_26_LEFT	ACG TGT GCT CTT CCG ATC TGG TAA ACC TTT GGA ATT TGG TGC C
	ncov_cap_28_LEFT	ACG TGT GCT CTT CCG ATC TAC ATT AAA AAT GCA GAC ATT GTG GAA GA
	ncov_cap_30_LEFT	ACG TGT GCT CTT CCG ATC TTG TCT TCA

		TGT TGT CGG CCC AA
	ncov_cap_32_LEFT	ACG TGT GCT CTT CCG ATC TAA GAT CGC TGA GAT TCC TAA AGA GG
	ncov_cap_34_LEFT	ACG TGT GCT CTT CCG ATC TGT GGG TGA TGT TGT TCA AGA GGG
	ncov_cap_36_LEFT	ACG TGT GCT CTT CCG ATC TTG GAA CTG TTT CTT GGA ATT TGC G
	ncov_cap_38_LEFT	ACG TGT GCT CTT CCG ATC TTG TAA CAC ATG

		GCT TAA ATT TGG AAG AA
	ncov _cap _40_ LEF T	ACG TGT GCT CTT CCG ATC TTC CTA CCA CAT TCC ACC TAG ATG G
	ncov _cap _42_ LEF T	ACG TGT GCT CTT CCG ATC TAA CCT CAT AAT TCA CAT GAA GGT AAA ACA
	ncov _cap _44_ LEF T	ACG TGT GCT CTT CCG ATC TAG AGT TGA AGT TTA ATC CAC CTG CT
	ncov _cap _46_ LEF T	ACG TGT GCT CTT CCG ATC TCC

		TTA AGG GTG TAG AAG CTG TTA TGT
	ncov_cap_48_LEFT	ACG TGT GCT CTT CCG ATC TCC TCA GAA TAC AAA GGT CCT ATT ACG G
	ncov_cap_50_LEFT	ACG TGT GCT CTT CCG ATC TAG AAA CCT GCT TCA AGA GAG CTT
	ncov_cap_52_LEFT	ACG TGT GCT CTT CCG ATC TGG GAA TGG ATA ATC TTG CCT GCG
	ncov_cap_54_LEFT	ACG TGT GCT CTT CCG ATC TTG

		CTA AGC CTT TTC TTA ACA AAG TTG T
	ncov_cap_56_LEFT	ACG TGT GCT CTT CCG ATC TTG GTT TTT ACT ATT AAG TGT TTG CCT AGG T
	ncov_cap_58_LEFT	ACG TGT GCT CTT CCG ATC TAA TGG GAT TTA ACT GCT TTT GGC TT
	ncov_cap_60_LEFT	ACG TGT GCT CTT CCG ATC TGC ATG TTG TAG ACG GTT GTA ATT CA
	ncov_cap_62_	ACG TGT GCT

	LEFT	CTTCCGATCTGACTTGTCACTACAGTTTAAAAGACCAA
	ncov_cap_64_LEFT	ACGTGTGCTCTTCCGATCTAGAATCATCTGCAAAATCAGCGTCT
	ncov_cap_66_LEFT	ACGTGTGCTCTTCCGATCTTCTACTTTTATTTCAGCAGCTCGGC
	ncov_cap_68_LEFT	ACGTGTGCTCTTCCGATCTTGCGCAGGTAGCAAAAAGTCACA
	ncov_cap	ACGTGT

	_70_ LEF T	GCT CTT CCG ATC TGC AGT TAA TTA AAG TTA CAC TTG TGT TCC
	ncov _cap _72_ LEF T	ACG TGT GCT CTT CCG ATC TTG CTG CAG TCA TAA CAA GAG AAG T
	ncov _cap _74_ LEF T	ACG TGT GCT CTT CCG ATC TAC CAA TGT ACT AGA AGG TTC TGT TGC
	ncov _cap _76_ LEF T	ACG TGT GCT CTT CCG ATC TGA TCT TTA CCA GGA GTT TTC TGT GGT

	ncov_cap_78_LEFT	ACGTGTGCTCTTCCGATCTACTGTACTCTGTTTAACACCAGTTTA CT
	ncov_cap_80_LEFT	ACGTGTGCTCTTCCGATCTCCTTTAGTACTTTTGAAGAAGCTGCG
	ncov_cap_82_LEFT	ACGTGTGCTCTTCCGATCTCCAACACTCTATCACCTCAGC
	ncov_cap_84_LEFT	ACGTGTGCTCTTCCGATCTGGCTGGTATGTTCAACTCAGGG

	ncov_cap_86_LEFT	ACGTGTGCTCTTCCGATCTTGGTTCATGTGGTAGTGTTG
	ncov_cap_88_LEFT	ACGTGTGCTCTTCCGATCTACACAACTCTTAATGACTTTAACCTGT
	ncov_cap_90_LEFT	ACGTGTGCTCTTCCGATCTACAATGCTCAGGTGTTACTTCCA

	ncov_cap_92_LEFT	ACGTGTGCTCTTCCGATCTTTACCTTCTCTGCCACTGTAGCT
	ncov_cap_94_LEFT	ACGTGTGCTCTTCCGATCTACACTTATGAATGTCTTGACACTCGT
	ncov_cap_96_LEFT	ACGTGTGCTCTTCCGATCTTTGTACTTGTTACTTCTTGGCCTCTTTGT
	ncov_cap_98_LEFT	ACGTGTGCTCTTCCGATCTAGTCTTACTCTCAGTTTTGCAAC

		AAC T
	ncov _cap _100 _LEF T	ACG TGT GCT CTT CCG ATC TGT TCC CTT CCA TCA TAT GCA GCT
	ncov _cap _102 _LEF T	ACG TGT GCT CTT CCG ATC TGG GCA AAA GTT ACT AGT GCT ATG CA
	ncov _cap _104 _LEF T	ACG TGT GCT CTT CCG ATC TCA GGT TGT AGA TGC AGA TAG TAA AAT TGT
	ncov _cap _106 _LEF T	ACG TGT GCT CTT CCG ATC TTG CAC TGT TAT CCG ATT

		TAC AGG A
	ncov _cap _108 _LEF T	ACG TGT GCT CTT CCG ATC TAA GTG CCT GCC AAT TCA ACT GT
	ncov _cap _110_ LEF T	ACG TGT GCT CTT CCG ATC TAA AAA CAC AGT CTG TAC CGT CTG C
	ncov _cap _112_ LEF T	ACG TGT GCT CTT CCG ATC TAA TTC CTA AAA ACT AAT TGT TGT CGC TTC C
	ncov _cap _114_ LEF T	ACG TGT GCT CTT CCG ATC TTC AAC GTC TTA

		CTA AAT ACA CAA TGG CA
	ncov _cap _116_ LEF T	ACG TGT GCT CTT CCG ATC TCG AAA TGC TGG TAT TGT TGG TGT
	ncov _cap _118_ LEF T	ACG TGT GCT CTT CCG ATC TTG ACT TCA CGG AAG AGA GGT TAA AAC
	ncov _cap _120 _LEF T	ACG TGT GCT CTT CCG ATC TCC ACT TCA GAG AGC TAG GTG TTG
	ncov _cap _122 _LEF T	ACG TGT GCT CTT CCG ATC TCA CTT CTT CTT

		TGC TCA GGA TGG T
	ncov_cap_124_LEFT	ACG TGT GCT CTT CCG ATC TCG CAT ATA CAA AAC GTA ATG TCA TCC C
	ncov_cap_126_LEFT	ACG TGT GCT CTT CCG ATC TAT GGG TTG GGA TTA TCC TAA ATG TGA
	ncov_cap_128_LEFT	ACG TGT GCT CTT CCG ATC TTC AGG AGA TGC CAC AAC TGC TT
	ncov_cap_130_LEFT	ACG TGT GCT CTT CCG ATC TTA CTC TCT

		GAC GAT GCT GTT GTG
	ncov_cap_132_LEFT	ACG TGT GCT CTT CCG ATC TAG ATC CAT CAA GAA TCC TAG GGG C
	ncov_cap_134_LEFT	ACG TGT GCT CTT CCG ATC TGG AAC CTG AGT TTT ATG AGG CTA TGT
	ncov_cap_136_LEFT	ACG TGT GCT CTT CCG ATC TAC TCA ACT TTA CTT AGG AGG TAT GAG CT
	ncov_cap_138_LEFT	ACG TGT GCT CTT CCG ATC TAC GCT

		CAA AGC TAC TGA GGA GA
	ncov _cap _140 _LEF T	ACG TGT GCT CTT CCG ATC TAC CGA GGT ACA ACA ACT TAC AAA TTA AAT
	ncov _cap _142 _LEF T	ACG TGT GCT CTT CCG ATC TCT CTA CTA CCC TTC TGC TCG CA
	ncov _cap _144 _LEF T	ACG TGT GCT CTT CCG ATC TCT TTG ATG AAA TTT CAA TGG CCA CAA A
	ncov _cap _146 _LEF T	ACG TGT GCT CTT CCG ATC TGC

		ACA TAA AGA CAA ATC AGC TCA ATG C
	ncov_cap_148_LEFT	ACG TGT GCT CTT CCG ATC TGT TGA TTC ATC ACA GGG CTC AGA
	ncov_cap_150_LEFT	ACG TGT GCT CTT CCG ATC TTG GGT TAC ATC CTA CAC AGG CA
	ncov_cap_152_LEFT	ACG TGT GCT CTT CCG ATC TGT CGA GGG GTG TCA TGC TAC TA
	ncov_cap_154_LEFT	ACG TGT GCT CTT CCG ATC TAG TGA

		CAC ACT TAA AAA TCT CTC TGA CA
	ncov_cap_156_LEFT	ACG TGT GCT CTT CCG ATC TCA GGT AAC CTA CAA AGC AAC CAT GA
	ncov_cap_158_LEFT	ACG TGT GCT CTT CCG ATC TCC CAG TTC TTC ACG ACA TTG GT
	ncov_cap_160_LEFT	ACG TGT GCT CTT CCG ATC TAA CTT GCC TGG TTG TGA TGG TG
	ncov_cap_162_LEFT	ACG TGT GCT CTT CCG ATC TTG TAG

		ACA TCA TGC TAA TGA GTA CAG AT
	ncov_cap_164_LEFT	ACG TGT GCT CTT CCG ATC TCA CAA AAG TTG ATG GTG TTG ATG TAG A
	ncov_cap_166_LEFT	ACG TGT GCT CTT CCG ATC TAC TGA AAC GAT TTG TGC ACC ACT
	ncov_cap_168_LEFT	ACG TGT GCT CTT CCG ATC TCC AAC AAT TAC CTG AAA CTT ACT TTA CTC A
	ncov_cap_170	ACG TGT GCT CTT

	_LEFT	CCG ATC TGG TTC ATC TAA GTG TGT GTG TTC TGT
	ncov_cap_172_LEFT	ACG TGT GCT CTT CCG ATC TAG AAT GCT ATT AGA AAA GTG TGA CCT TCA
	ncov_cap_174_LEFT	ACG TGT GCT CTT CCG ATC TTG ATG CAG ATT CAA CTT TGA TTG GTG
	ncov_cap_176_LEFT	ACG TGT GCT CTT CCG ATC TGC TGA TCT TTA TAA GCT CAT GGG ACA
	ncov_cap	ACG TGT

	_178_LEFT	GCTCTTCCGATCTAGGGTACTGCTGTTATGTCTTTAAA
	ncov_cap_180_LEFT	ACGTGTGCTCTTCCGATCTAGACTTGTTCTTACCTTTCTTTTCCA
	ncov_cap_182_LEFT	ACGTGTGCTCTTCCGATCTGTTGGATGGAAAGTGAGTTCAGAGT
	ncov_cap_184_LEFT	ACGTGTGCTCTTCCGATCTTTCGGCTTTAGAACCATTGGTAGATT

	ncov_cap_186_LEFT	ACGTGTGCTCTTCCGATCTAGTGTACGTTGAAATCCTTCACTGT
	ncov_cap_188_LEFT	ACGTGTGCTCTTCCGATCTACTTTTAAGTGTTAAGTGGAGTGTCTCCT
	ncov_cap_190_LEFT	ACGTGTGCTCTTCCGATCTAGATTGTTTAGGAAGTCTAATCTCAAACCT
	ncov_cap_192_LEFT	ACGTGTGCTCTTCCGATCTATGGTTTAACAGGCACA

		GGTGT
	ncov_cap_194_LEFT	ACGTGTGCTCTTCCGATCTATCAACTTACTCCTACTTGGCGTG
	ncov_cap_196_LEFT	ACGTGTGCTCTTCCGATCTAACTCTATTGCCATACCCACAAATTTT
	ncov_cap_198_LEFT	ACGTGTGCTCTTCCGATCTAGTCAAACAAATTTACAAAACACCACA
	ncov_cap_200_LEFT	ACGTGTGCTCTTCCGATCTTTTGCCACCTTT

		GCT CAC AGA TG
	ncov_cap_202_LEFT	ACG TGT GCT CTT CCG ATC TGC ACT TGG AAA ACT TCA AGA TGT GG
	ncov_cap_204_LEFT	ACG TGT GCT CTT CCG ATC TTG CTG CTA CTA AAA TGT CAG AGT GT
	ncov_cap_206_LEFT	ACG TGT GCT CTT CCG ATC TTG TAA CAC AAA GGA ATT TTT ATG AAC CAC A
	ncov_cap_208_LEFT	ACG TGT GCT CTT CCG ATC TGT AAA

		CAT TCA AAA AGA AAT TGA CCG CC
	ncov _cap _210 _LEF T	ACG TGT GCT CTT CCG ATC TGC TCA AAG GAG TCA AAT TAC ATT ACA CA
	ncov _cap _212 _LEF T	ACG TGT GCT CTT CCG ATC TCC AAG GGT GTT CAC TTT GTT TGC
	ncov _cap _214 _LEF T	ACG TGT GCT CTT CCG ATC TGC CAA CTA TTT TCT TTG CTG GCA
	ncov _cap _216 _LEF T	ACG TGT GCT CTT CCG ATC TTT

		CAC ACA ATC GAC GGT TCA TCC
	ncov_cap_218_LEFT	ACG TGT GCT CTT CCG ATC TCC TTA CTG CGC TTC GAT TGT GT
	ncov_cap_220_LEFT	ACG TGT GCT CTT CCG ATC TAA GCT CCT TGA ACA ATG GAA CCT
	ncov_cap_222_LEFT	ACG TGT GCT CTT CCG ATC TTT CTT TCA GAC TGT TTG CGC GT
	ncov_cap_224_LEFT	ACG TGT GCT CTT CCG ATC TAA CGC TTT CTT

		ATT ACA AAT TGG GAG C
	ncov_cap_226_LEFT	ACG TGT GCT CTT CCG ATC TTC TCA ATT AGA TGA AGA GCA ACC AAT GG
	ncov_cap_228_LEFT	ACG TGT GCT CTT CCG ATC TCG TCT ATC AGT TAC GTG CCA GAT
	ncov_cap_230_LEFT	ACG TGT GCT CTT CCG ATC TAT TAT CTT TTG GTT CTC ACT TGA ACT GC
	ncov_cap_232_LEFT	ACG TGT GCT CTT CCG ATC TAC

		ATC GAT ATC GGT AAT TAT ACA GTT TCC T
	ncov_cap_234_LEFT	ACG TGT GCT CTT CCG ATC TAC CCT CAG ATT CAA CTG GCA GT
	ncov_cap_236_LEFT	ACG TGT GCT CTT CCG ATC TAA TGA AAG ATC TCA GTC CAA GAT GGT
	ncov_cap_238_LEFT	ACG TGT GCT CTT CCG ATC TGC AGT CAA GCC TCT TCT CGT TC
	ncov_cap_240_LEFT	ACG TGT GCT CTT CCG ATC

		TGCAAAAACGTACTGCCACTAAAGC
	ncov_cap_242_LEFT	ACGTGTGCTCTTCCGATCTACACAGGTGCCATCAAATTGGA
	ncov_cap_244_LEFT	ACGTGTGCTCTTCCGATCTTGCTGACTCAACTCAGGCCTAA
	RNAse_P_LEFT	ACGTGTGCTCTTCCGATCTGATTTGGACCTGCGAGCG

Preparation of barcoded beads for RNA capture

1

Note

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.

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.

4.5 Resuspend beads in 50 µL TE buffer.

Note

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.

> Note
>
> 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.

> Note
>
> 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.

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”.

Note

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.

Library amplification

22

Note

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

Note

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.

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.

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.

> Note
>
> 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 × 150 nt paired end reads

Alternative bead preparation method

38

> Note
>
> 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).

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 1.200 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

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.