At all times local, regional, and national laws and guidelines will be adhered to.

✏️ Edit this document to make it better ✏️

Corona virus detection

Open source experiment to recreate the comparison of PCR primers for SARS-CoV-2 detection from this paper.
This will **only be conducted using samples from the experimenter**.
If the experimenter thinks they're infected this experiment must be carried out at home / their place of self isolation.
If the experimenter has no reason to believe they're sick then they could do this in a community biolab though perhaps they'd not do this experiment and want to save the reagents (as they're not that cheap).
At all times will local, regional, and national laws and guidelines be adhered to.
This document is open for anyone to improve it.

Parent folder:
https://drive.google.com/open?id=1eSJD2E7eWmZNh8z_-0KnHD6FcQpBiBWj

Related projects:
https://groups.google.com/forum/#!msg/diybio/F_y2WsfEJog
Corona Virus Tech Handbook
https://app.jogl.io/project/118
https://groups.google.com/forum/#!topic/london-hack-space/Bw4Hn6Ej-gI

Slack groups:
http://biohackspace.org/slack join the #corona channel
https://jogl-covid-19.slack.com join the #repro-jung2020feb-primer-comparison channel

Tasks remaining

- ~~Request advice from respiratory doctor regarding how to take samples~~
- clearly describe all the steps in the protocol.
    - Including reagents, equipment, volumes, decision flow etc needed
- find sources of reagents including links to suppliers and prices
    - ~~Struggling to find a supplier of RT-PCR (WELLS BIO Inc.). Their website only lists kits that include RT-PCR as tests for things like Zika, HPV etc.~~
        - ~~Have contacted WELLS BIO Inc.~~
        - ~~Have contacted authors requesting link to kit~~
- anything else?

Recreating comparison of primer sets for SARS-CoV-2 detection

Paper to recreate

https://www.biorxiv.org/content/10.1101/2020.02.25.964775v1.full

Sample prep: Uses QIAamp viral RNA extraction Kit

Detection: 300 nM of primers and probes for the target detection. The qRT-PCR was performed with a CFX 96 touch real-time PCR detection system (Bio-rad, Hercules, CA, USA). The qRT-PCR conditions applied in this study were programmed as follows: UNG incubation, RT incubation, and enzyme activation were serially performed at 25 °C for 2 minutes, at 55 °C for 10 minutes, at 94 °C for 3 minutes respectively. Thermal cycling was then performed at 94 °C for 15 seconds (denaturation), and at 60 °C for 30 seconds (annealing and amplification) for forty-five cycles.

Sequences used

Target	Country	Name	Type	Sequence (5' → 3')	Position	Ref	Source
N	China	N-F	F	GGG GAA CTT CTC CTG CTA GAA T	28881 - 28902	[17]	COVID-19-3 $4.18
		N-R	R	CAG ACA TTT TGC TCT CAA GCT G	28958 - 28979		COVID-19-4 $4.18
		N-P	P	TTG CTG CTG CTT GAC AGA TT	28934 - 28953		
	Hong Kong	HKU-NF	F	TAA TCA GAC AAG GAA CTG ATT A	29145 - 29166	[9]	TODO: get supplier
		HKU-NR	R	CGA AGG TGT GAC TTC CAT G	29235 - 29254		TODO: get supplier
		HKU-NP	P	GCA AAT TGT GCA ATT TGC GG	29177 - 29196		
	Japan	NIID_2019-nCOV_N_F2	F	AAA TTT TGG GGA CCA GGA AC	29125 - 29144	[18]	TODO: get supplier
		NIID_2019-nCOV_N_R2	R	TGG CAG CTG TGT AGG TCA AC	29263 - 29282		TODO: get supplier
		NIID_2019-nCOV_N_P2	P	ATG TCG CGC ATT GGC ATG GA	29222 - 29241		
	Thailand	WH-NIC N-F	F	CGT TTG GTG GAC CCT CAG AT	28320 - 28339	[19]	TODO: get supplier
		WH-NIC N-R	R	CCC CAC TGC GTT CTC CAT T	28358 - 28376		TODO: get supplier
		WH-NIC N-P	P	CAA CTG GCA GTA ACC A	28341 - 28356		
	USA	2019-nCoV_N1-F	F	GAC CCC AAA ATC AGC GAA AT	28287 - 28306	[20]	TODO: get supplier
		2019-nCoV_N1-R	R	TCT GGT TAC TGC CAG TTG AAT CTG	28335 - 28358		TODO: get supplier
		2019-nCoV_N1-P	P	ACC CCG CAT TAC GTT TGG TGG ACC	28309 - 28332		
		2019-nCoV_N2-F	F	TTA CAA ACA TTG GCC GCA AA	29164 - 29183		TODO: get supplier

		2019-nCoV_N2-R	R	GCG CGA CAT TCC GAA GAA	29213 - 29230		TODO: get supplier
		2019-nCoV_N2-P	P	ACA ATT TGC CCC CAG CGC TTC AG	29188 – 29210		
		2019-nCoV_N3-F	F	GGG AGC CTT GAA TAC ACC AAA A	28681 - 28702		TODO: get supplier
		2019-nCoV_N3-R	R	TGT AGC ACG ATT GCA GCA TTG	28732 - 28752		TODO: get supplier
		2019-nCoV_N3-P	P	AYC ACA TTG GCA CCC GCA ATC CTG	28704 - 28727		
RdRp/Orf 1	China	ORF1ab-F	F	CCC TGT GGG TTT TAC ACT TAA	13342 - 13362	[17]	TODO: get supplier
		ORF1ab-R	R	ACG ATT GTG CAT CAG CTG A	13442 - 13460		TODO: get supplier
		ORF1ab-P	P	CCG TCT GCG GTA TGT GGA AAG GTT ATG G	13377 - 13404		
	Germany	RdRp_SARSr-F	F	GTG ARA TGG TCA TGT GTG GCG G	15431 - 15452	[10]	COVID-19-9 $4.18
		RdRp_SARSr-R	R	CAR ATG TTA AAS ACA CTA TTA GCA TA	15505 - 15530		COVID-19-10 $4.94
		RdRp_SARSr-P2	P	CAG GTG GAA CCT CAT CAG GAG ATG C	15470 - 15494		
	Hong Kong	HKU-ORF1b-nsp14F	F	TGG GGY TTT ACR GGT AAC CT	18778 - 18797	[9]	TODO: get supplier
		HKU-ORF1b-nsp14R	R	AAC RCG CTT AAC AAA GCA CTC	18889 - 18909		TODO: get supplier
		HKU-ORF1b-nsp14P	P	TAG TTG TGA TGC WAT CAT GAC TAG	18849 - 18872		

Protocol

Obtain a sample

How to take a sample:
https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories/novel-coronavirus-2019-ncov-sampling-requirements
from “COVID-19: guidance for sampling and for diagnostic laboratories”:
https://www.gov.uk/government/publications/wuhan-novel-coronavirus-guidance-for-clinical-diagnostic-laboratories

Upper respiratory tract sample options:

- *combined nose and throat swab in one collection tube containing universal transport medium OR*
- *single swab used for throat then nose OR*
- *individual nose and throat swabs in separate collection tubes OR*
- *nasopharyngeal aspirate in a universal transport pot*

Lower respiratory tract sample in universal container (sputum) if obtainable
If the patient is admitted, take a sample for acute serology:

- *5mL in either serum tube or plain (no additive) tube.*

-- Advice from respiratory doc was that you need to wipe the surface by going round and round in the nose for 5 seconds and same for the throat.

Advice from CDC on sample collection said it needed to be done using polyester and not cotton or anything wooden
https://www.who.int/docs/default-source/coronaviruse/uscdcrt-pcr-panel-for-detection-instructions.pdf?sfvrsn=3aa07934_2

> *Swab specimens should be collected only on swabs with a synthetic tip (such as polyester or Dacron®) with aluminum or plastic shafts. Swabs with calcium alginate or cotton tips with wooden shafts are not acceptable.*

Isolation of the RNA

Using QIAamp viral RNA extraction Kit
Protocol for kit

Logspin: Protocol for spin columns
DIY RNA Extraction
DIY RNA Extraction No.2

Warnings and precautions
"RNA is extremely sensitive to RNases [...] Hands and dust particles may carry bacteria and molds and are the most common sources of RNase contamination.". More info

RNase alert kit for detecting / debugging RNase. £191 for 25

Sample and size: proposal 1: saliva
Quick and easy to do. Ensure you are hydrated and then avoid drinking for 15 minutes (to ensure saliva is runny but not just water).
Spit up to 140 µl up to 500 µl into 1.5ml eppendorf.

Removal of cells
As the samples will contain cells: "Samples containing cells, such as [...] most swabs, should first be [...] centrifuged for 10 minutes at 1500 x g and the supernatant used.

Equipment needed
Centrifuge up to 1 ml at 1500g for removing cells
Pipette tips (200 and 1000 ul)
Pipette (200 and 1000 ul)

Preparation of reagents
Follow "Preparation of reagents" on page 17

Make "carrier RNA mix" from "Buffer AVE" and "carrier RNA", freezing rest as aliquots of _?_ ul

Calculate and make only amount of required "Buffer AVL–carrier RNA mix
    For two samples this looks like: 1.12 ml 11.2ul
Buffer AW1
    Requires 25ml ethanol
Buffer AW2
    Requires 25ml ethanol

(Reverse Transcription) RNA to cDNA

Amplification via PCR

Paper uses "1 step RT-PCR mix" WELLS BIO INC., South Korea
ThermoFisher 25 for £286
Qiagen 200 for $672
ThermoFisher 100 for £163

Load samples in following order:

1. primers
2. negative control 1
3. positive control
4. negative control 2

Required consumables & equipment

Description	Comment	Number needed	Cost
Lab coat			
Gloves	To protect self from chemicals and avoid contaminating sample		
Goggles			
RNase free Pipette tips (20, 200 and 1000 ul)	Preferably with aerosol barrier filters. Definitely need to be RNAase free sterile packs. If at home then no way to keep sterile.	1 pack each	
Pipettes (20, 200 and 1000ul)		1 of each	

1.5 ml eppendorfs	Sterile		
Microcentrifuge	With rotor for 1.5 ml and 2 ml tubes		
80°C water bath	if checked Buffer AVL for precipitate and it's present		
Eppendorf rack			
52904 QIAamp viral RNA extraction Kit for 50 RNA preps	Sample preparation. Storage of kit at 15 to 25°C	1	$271.00
Primers	See above for source		
Ethanol (96–100%)			
ThermoFisher 100 for £163		1	£163

Sources of positive controls

https://eu.idtdna.com/pages/landing/coronavirus-research-reagents
https://www.moleculARcloud.org/How-to-detect-the-2019-novel-coronavirus.html

Related links

WHO page of protocols

https://www.who.int/emergencies/diseases/novel-coronavirus-2019/technical-guidance/laboratory-guidance