Supplementary Method 4 Automated RNA extraction using Biomek FX

Equipment & Consumables

Consumables	Used/ run
Eppendorf 96-well skirted plates LoBind (Eppendorf, Cat.no. 0030129512)	1
NUNC 96-well deep well plates (ThermoScientific, Cat.no. 260251)	3
Axygen squared 96-well deep well plates (Fisher Scientific Cat.no. P-2ML-SQ-C)	1
Reservoir (VWR, Cat. no. 613-1175)	3
Adhesive PCR Plate Seals (Thermo Fisher, Cat. no. AB0558)	1
BIOMEK FX 250 μL tips, Filtered, Sterile (Cat. no. 717253)	4
BIOMEK FX 50 μL tips, Filtered, Sterile (Cat. no. A21586)	3
Equipment	
BIOMEK FX workstation and associated equipment	1
Alpaqua Magnum FLX (Cat. no. A000400)	1

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Reagents

Reagents (note: some of these reagents are used to make the buffers below)
Water for HPLC (Fisher Scientific, 2.5 L, 270733-2.5L, 4 GBP)
1 M Tris-HCl pH 8.0 (AppliChem, 500 mL, A4577,0500, 47 GBP)
Guanidine hydrochloride (Sigma, 1 kg, G3272-1KG, 168 GBP) BATCH CERTIFIED
Isopropanol (e.g. Merck Millipore, 2.5 L, 1096342500, 13 GBP)
3 M sodium acetate pH 5.2 (Sigma, 500 mL, S7899-500ML, 47 GBP)
PE buffer (Qiagen, 100 mL, 19065, 53 GBP)
Ethanol (e.g. Merck Millipore, 1 L, 1009832500, 17 GBP)
Tween-20 (Sigma Aldrich, 100 mL, T2700-100ML, 23 GBP)
0.5 M EDTA pH 8.0 (Applichem, 1 L, A4892,1000, 72 GBP)
Silica beads (VWR--G-BioSciences, 5 mL, 786-915, 127 GBP) BATCH CERTIFIED

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Buffers

***TET buffer [10mL]* See Supplementary Method 10**

PRE-PREPARED AND **BATCH CERTIFIED**

Reagent	Volume	Final concentration
Water	~49.4 mL	
0.5 M EDTA, pH 8.0	100 μL	1 mM
1 M Tris-HCl, pH 8.0	500 μL	10 mM
Tween-20	25 μL	0.05%

Binding buffer [BB] [50mL] See Supplementary Method 9

PRE-PREPARED AND **BATCH CERTIFIED**

Reagent	Volume/amount	Final concentration
Guanidine hydrochloride	23.88 g	5 M
Water	to 30 mL	
Isopropanol	to 50 mL	40%
Tween 20	25 μL	0.05%
3 M sodium acetate	2 mL	115 mM

PE-buffer, 60 ml needed for 1 BIOMEK FX extraction run

Prepare according to instructions by Qiagen

REMEMBER TO ADD ETHANOL TO QIAGEN REAGENT AS PER QIAGEN INSTRUCTIONS ON THE PE BOTTLE

Reagent	Volume
PE buffer by Qiagen	60 mL

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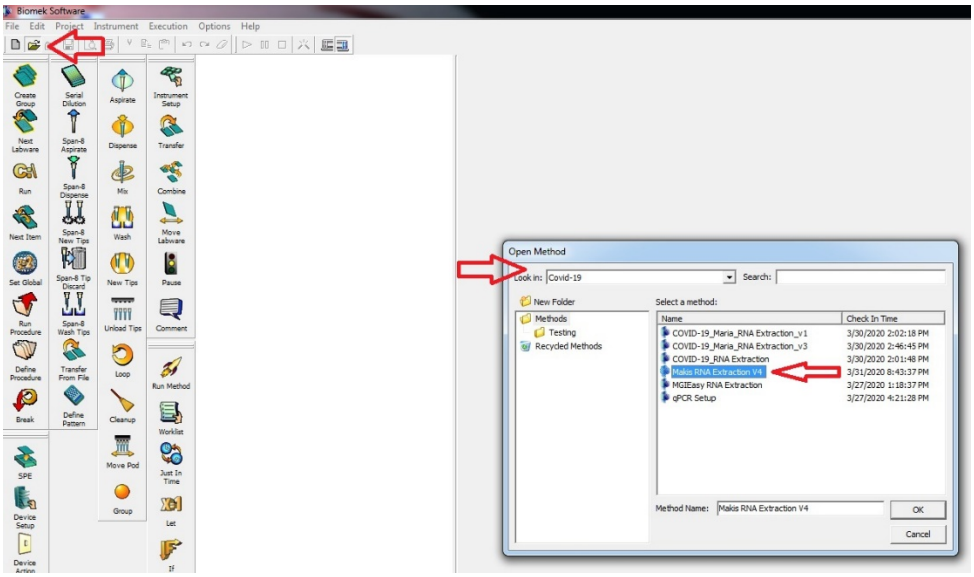
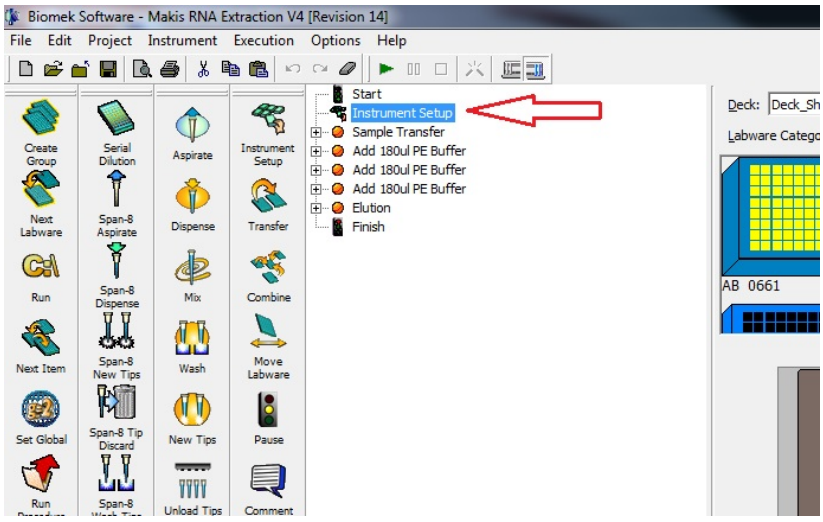
Procedure

RNA extraction using IN HOUSE protocol on BIOMEK FX
NB The deck layout is specific to the robot performing the extraction. Be sure to use the correct set-up for the correct robot!

1. Open the Biomek software, and select the relevant RNA extraction program "**(See protocol below)**



2. Click on "**Instrument Setup**" as shown below to view the deck layout:



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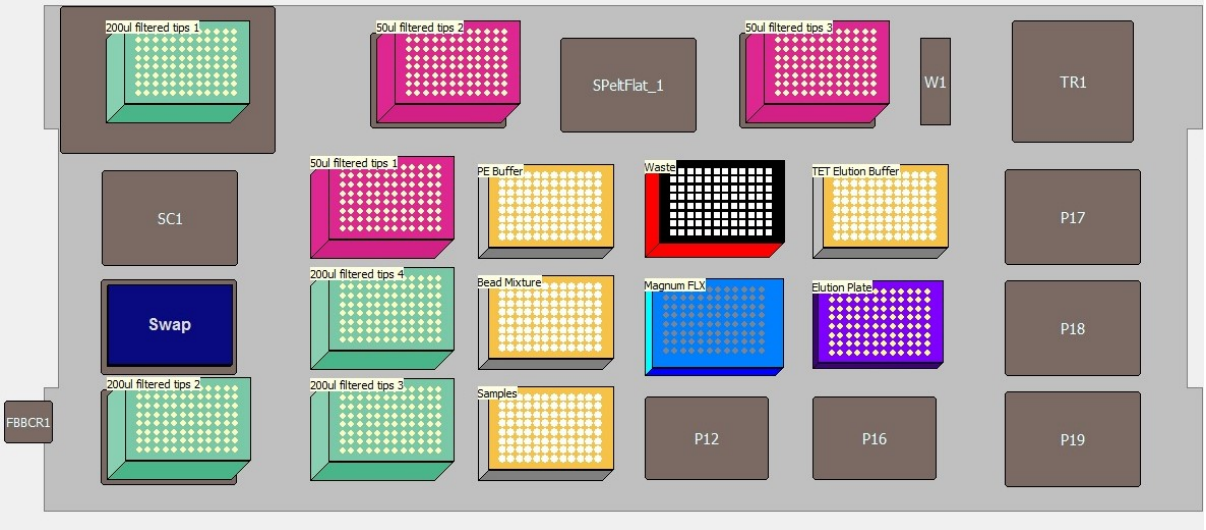
FX1 DECK LAYOUT



3. Place 4 boxes (lid off) of **250 µL filter tips** (green boxes) as shown above.

4. Place 3 boxes (lid off) of **50 µL filter tips** (pink boxes) as shown above.

5. Place an **Axygen Deep well plate** in position **P10**.

At a clean bench prepare reagents as follows:

Prepare beads for 96 samples**:**

6 Take a 1.5 mL tube of pre-aliquoted silica beads (550 µL) from the fridge and vortex well.

7 Place on the single tube magnet for 1 minute or until supernatant is clear.

8 Remove and discard supernatant (into the tip waste bin).

9 Remove tube from magnet and add **500 µL TET**. Resuspend the beads by vortexing.

10 Pulse spin the tube and place on the magnet for 1 minute or until supernatant is clear.

11 Remove and discard supernatant.

12 Repeat steps 10 to 12 for a total of 3 times.

13 Remove the tube from the magnet, resuspend the beads in **500 µL TET**, vortex and spin down.

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Prepare binding buffer/bead mix:

14 In the Falcon tube containing the 50 mL binding buffer (labelled BB, pre-aliquoted) add the beads prepared in 6.

15 Mix well by inverting.

16 Pour into a reservoir and aliquot 400 µL immediately in each well of a 96 deep well Nunc plate, using a multichannel pipette. Place on the deck **P8.**

17 Add PE buffer to a new reservoir and aliquot 600 µL into a new Nunc plate and place plate in position **P7**.

18 Add TET to new reservoir and aliquot 70 µL TET (from batch certified aliquot) into a new Nunc plate and place in position **P14**.

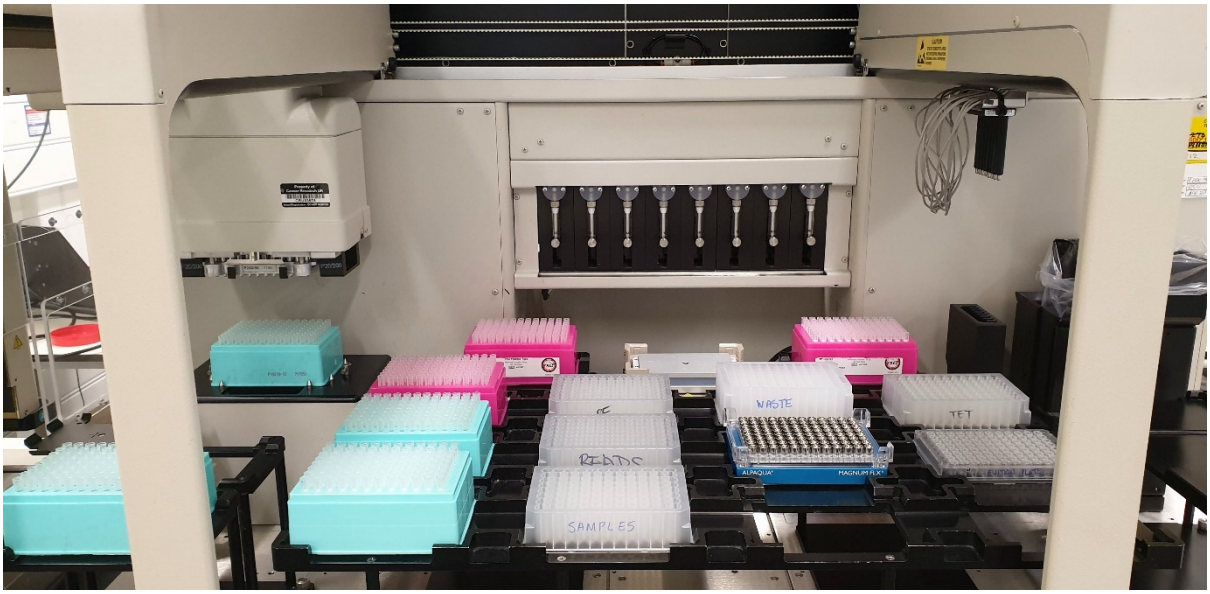
19 Place the sample plate from Supplementary Method 3 (*seal removed*) in position **P9**.

NB: If samples have been temporarily stored, briefly spin in the plate centrifuge.

20 Label an empty Eppendorf LoBind 96-well skirted plate with a pre-printed SPL barcode (e.g. SPL00000) on the front side, and place in position **P15**.

21 Place the **Alpaqua Magnum FLX** in position **P11.**

The final set up should look like the photo below:



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22 Using the laptop associated with the Biomek FX, push the samples through the Extraction step in ClarityLIMS.

23 Make sure you switch ON the Inheco (blue box).

24 To START the program press the green triangle 

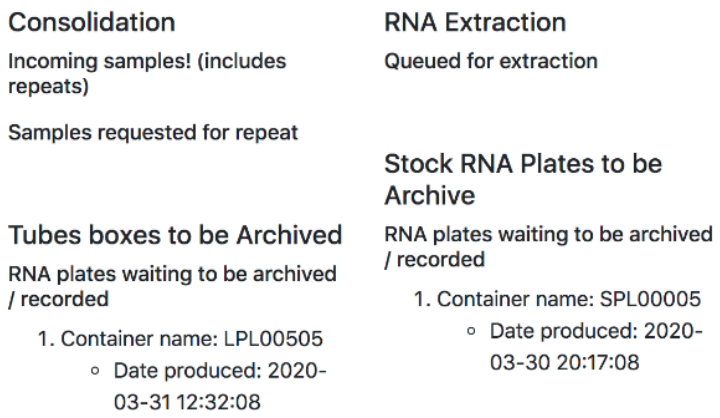
located in the software.

25 When then program is complete, remove the Eppendorf 96-well skirted plate from the deck, seal with a Thermo Adhesive PCR plate seal and place in the -80ºC until it is used for qPCR.

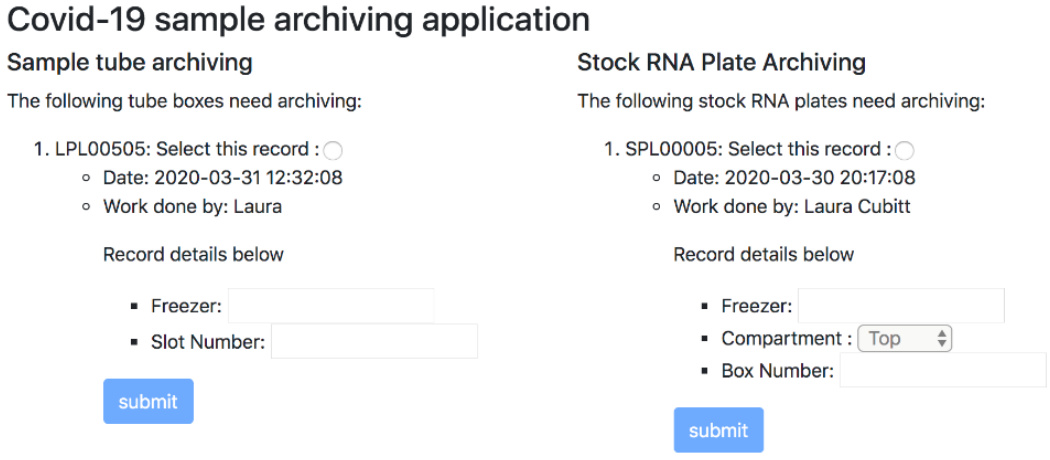
Archiving

26 Archiving of Inactivated viral samples would have already been performed in Supplementary Method 3. For archiving the stock RNA plates using the app in ClarityLims, a database table is updated with the relevant information

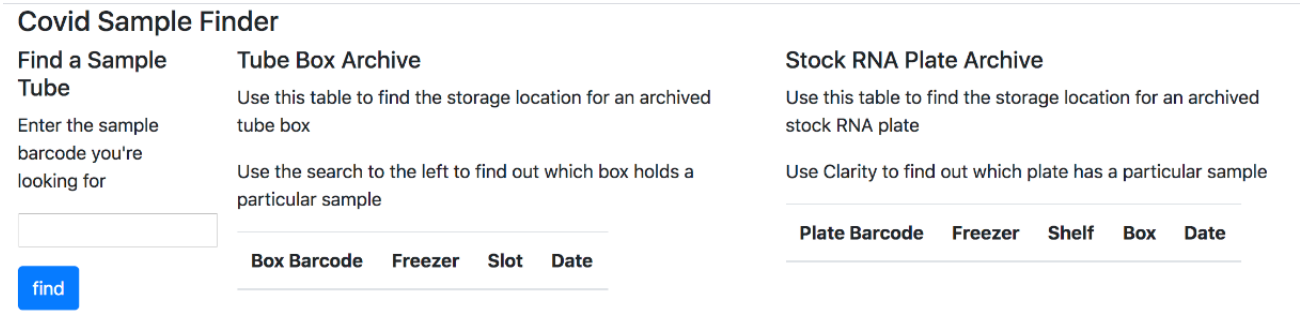
27 This results in an entry appearing on the dashboard to let the team know the samples are ready to be archived:



28 An archiving app then displays the boxes and plates that are waiting to be archived, and gives the team the chance to enter the storage information:



29 Once logged, the record disappears from this screen, and the archiving information will appear in the archive search app:



APPENDIX

The robot carries out the following automated protocol:

1. The robot transfers 150 µL of the samples to the 400 µL (2.6x Vol) of Binding Buffer (BB) containing the Silica beads and mixes 10 times by pipetting up and down.
2. Move the plate on the Shaker for 5 min
3. Move plate to position P8 and mix 20 times
4. Move plate to Shaker for 2 min
5. Move plate to P8 and incubate for 3 min
6. Move plate to Magnet for 5 min
7. Remove supernatant
8. Move plate from magnet to position P8

Wash with PE (1)

9. Transfer 180 µL of PE Buffer in each well of the Bead Mixture plate
10. Move plate to the Shaker for 20 sec
11. Move plate to the Magnet for 60 sec
12. Remove supernatant
13. Move plate from the magnet to position P8

Wash with PE (2)

14. Transfer 180 µL of PE Buffer in each well of the Bead Mixture plate
15. Move plate to the Shaker for 20 sec
16. Move plate to the Magnet for 60 sec
17. Remove supernatant
18. Move plate from the magnet to position P8

Wash with PE (3)

19. Transfer 180 µL of PE Buffer in each well of the Bead Mixture plate
20. Move plate to the Shaker for 20 sec
21. Move plate to the Magnet for 60 sec
22. Remove supernatant
23. Move plate from the magnet to position P8
24. Wait for 2 min (drying)

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Elution

25. Transfer 50 μL of TET Buffer to the Bead Mixture plate and mix 10 times
26. Move plate to the Shaker for 5 min
27. Move plate to the magnet for 60 sec
28. Transfer 40 μL from the Bead Mixture plate to the Eppendorf twin.tec plate

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Checklist Automated RNA extraction on Biomek FX

Name(s) of operator(s)	
Sample box barcode	**LPL**
RNA plate barcode	**SPL**
Bead Lot No.	
qPCR plate barcode	

Biomek FX operation

Operator name:_____ **Date/time:**_____

- ❑ Wipe down the bench.
- ❑ Initialise the FX by homing all axes.
- ❑ Open the RNA extraction program on the FX computer.
- ❑ Load the Biomek FX deck with 4x 250 µl and 3x 50 µl pipette tip boxes and a waste plate.
- ❑ Wash aliquot of beads 3x in TET, add to Binding buffer and dispense 400µl into plate. Load onto deck.
- ❑ Dispense 600µl Buffer PE into plate. Load onto deck.
- ❑ Dispense 60µl remaining TET into plate. Fill the negative control well with 60µl water. Load onto deck.
- ❑ Load LPL sample plate onto deck.
- ❑ Affix an SPL barcode to an Eppendorf Twin-tec skirted plate. Load onto deck.
- ❑ Verify that all items have been loaded onto the deck correctly. Second person sign-off:_____
- ❑ Complete the RNA extraction step in Clarity. Scan the SPL barcode in.
- ❑ Run the RNA extraction program.
- ❑ After program completion, visually check volumes in the RNA plate.
- ❑ Wait for qPCR team member to arrive with qPCR plate.
- ❑ Open the program ‘qPCR setup’ on the FX computer.
- ❑ Load the RNA plate and qPCR plate. Ensure the qPCR plate is placed on an adapter plate.
- ❑ Load pink pipette tip box. Remove the tip from position H12.

- ❑ Run the transfer program.
- ❑ Add positive control manually in H12.
- ❑ Seal the qPCR plate, hand to qPCR team member.
- ❑ Seal the RNA plate and place inside small -80°C freezer, ready to archive box.
- ❑ Staple this sheet to the corresponding Page 1 with sample reception and Hamilton steps. Place the sheets in the binder.
- ❑ Wipe down the bench.

Archiving

Note: wait a reasonable amount of time before archiving in case the qPCR is unsuccessful and needs repeating.

- ❑ Take sample box to CovFreezer 1, place in next available slot in the rack and record location details in the COVID-19 Landing page.
- ❑ Take RNA plates to CovFreezer 2, place in next available box and record location details in the COVID-19 Landing page.

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Notes – errors, variations on SOP etc