STATION B AUTOMATED RNA EXTRACTION PROTOCOL FOR 96 WELL PLATE

Using Bomb Bio magnetic beads (link to page and protocol, plus original RNA extraction protocol we modified and validated first)

If viral samples have been inactivated with a lysis buffer in station A this work can be safely undertaken at containment level 2. At 20 ± 2 °C (68 ± 4 °F) and <= 55% relative humidity [UKAS lab environment] and >= 50% [to minimise errors - ISO-8655-2 is for calibration at 50% rh].

PRIOR TO STATION B

- Put on PPE - lab coat, and gloves.
- Samples from station A should be in 96 deep well plate. Each well should hold 240ul of inactivated sample in a guanidine isothiocyanate based lysis buffer.

INVENTORY

Fetch for each run:

- Filter tips (1000 ul x16 tips total) - to prepare the reagents
- Filter tips (200 ul x6 *per sample*) *[i.e. 6x box of 96 filter tips for 96 samples]*
- Filter tips (20 ul x2 tips total) - to prepare the reagents
- 96 well PCR plate
- 2x 96 deep well plates (for reagents) (requirements: 2 ml, example)

Permanent:

- Gloves
- 10% bleach in spray bottle
- RNAseZap if available
- Pipette (handheld) 1ml multichannel (ethanol and IPA), 20ul single channel (ONLY used at Station B)
- OT2 P300 multichannel
- OT2 Magdeck

Reagents (*quantities quoted for a full 96 well plate):*

- 4.4 ml Silica-Coated magnetic beads (1:10)
- 80 ml 100% Isopropanol (IPA)
- 80 ml 80% Ethanol (80% EtOH) (prepare fresh before each run - diluted with ddH2O)
- 4.4 ml Nuclease-free water

ACTION SEQUENCE

PREPARATION:

1. Check all necessary PPE available
2. Check Inventory for permanent items
3. Fetch reagents (beads and nuclease-free water in fridge, IPA and EtOH in rack opposite station)

CLEANING:

4. Soapy water - clean every surface, pipettes, self, gloves, lab coats
5. Clean workspace (10% bleach)
6. Clean desk with RNAseZap

OPENTRONS SETUP:

Reagent setup:

[2x 96 deep well plates]

7. Add 1 ml 80% EtOH (1 ml per well) into all wells in a 96 deep well plate (slot 6), using manual 8-channel 1ml pipette. *[Filter tips in slot 3 are used exclusively for EtOH and reused. Automation code maps tip → EtOH well → sample, such that no cross-sample contamination occurs during washing cycles. Note: Bomb Bio protocol uses 70% but higher concentration used to compensate for evaporation]*
8. Add reagents to deep well plate (slot 8), using manual 8-channel 1 ml pipette (IPA) and manual single channel 20 ul pipette (otherwise):
    - IPA in *columns 6-8 and 10-12* inclusive (1.8 ml per well)
    - Silica-coated magnetic beads in *column 9* (0.55 ml per well)
    - Nuclease-free water in *column 5* (0.55 ml per well)

Set up OT2:

9. Connect via laptop (wifi or ethernet)
    - If first run, calibrate deck
10. Load automation protocol code
11. Follow calibration instructions for each piece of equipment (see diagram below).

[2x 96 deep well plates with reagents (slot 8) and EtOH (slot 6)]

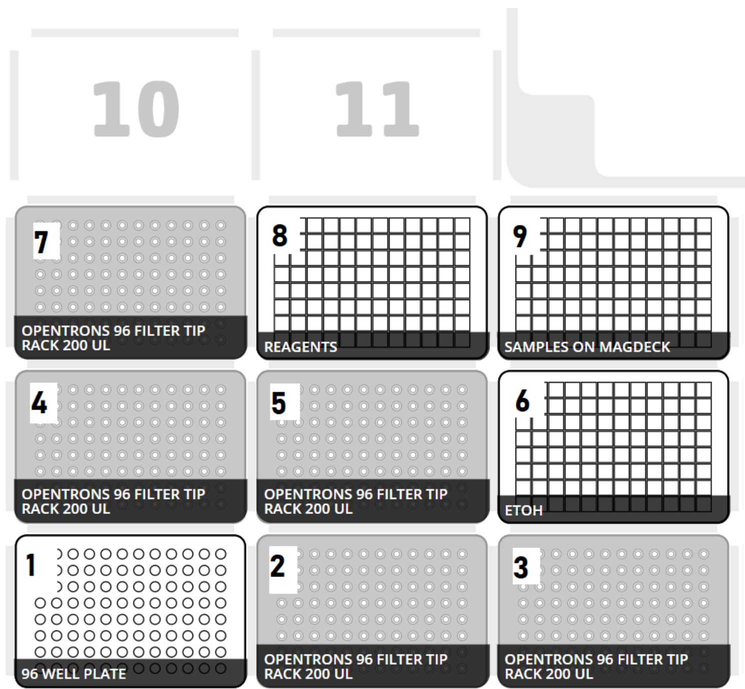
[6x 96 filter tip racks 200ul (slots 2-5 inclusive, 7, 10 if required] #NB MAY NEED EXTRA RACK IN 11

[1x 96 well plate (slot 1)]

[MagDeck (slot 9)]

[Samples in 96 deep well (slot 9)]

calibration can also be done using a “dummy” set of tips and plates which are reused for each calibration. This lowers the risk of contaminating fresh plastics.



12. *When calibrated, virtual deck should appear as above.*
13. Remove plasticware and clean OT2 deck and pippet with RNAseZap. (if using a dummy set for the calibration step replace the OT2 with the intended plasticware after cleaning)
14. Run protocol.

15. When finished, remove 96 well PCR plate (slot 1), which contains RNA extracted from samples. Move to Station C. If samples will not go through station C immediately, plate should be sealed and stored at -20℃ for short term storage or -80 for long term storage.

OVERVIEW OF AUTOMATED PROTOCOL:

1. Add IPA to samples. Mix.
2. Add mag beads to samples. Mix.
3. Magnet on. Remove supernatant (RNA stays with beads).
4. IPA wash: magnet off, add IPA, mix, magnet on, remove IPA.
5. EtOH wash: magnet off, add 80% EtOH, mix, magnet on, remove EtOH (x4)
6. Dry beads (blow air over beads for 35 mins using constant pipetting)
7. Magnet off, add nuclease-free water, mix
8. Magnet on, transfer supernatant to 96 well plate

SUMMARY OF CHANGES TO STANDARD PROTOCOL FOR AUTOMATION:

1. Removed DNase step. Our tests showed this to be unnecessary for our particular qPCR setup (which is RNA-based, i.e. DNA does not interfere). In fact, the extra step only degraded the RNA in the samples.
2. All mixing/spinning/centrifuging/vortexing has been replaced by a mix function, which pipettes the liquid up and down at a variable rate. Mixing times have been shortened drastically.

3. Time required for magbeads to congregate to the side of the well appears to be almost nil; i.e. upon magnet engaging, the magbeads move almost instantaneously to a cluster at the side. Congregation time reduced to 30 seconds, to allow for any remaining congregation.
4. Beads dry without the aid of a hotplate. Air is pipetted over the beads while they dry; our tests indicate that 35 minutes appears sufficient.

AUTOMATION NOTES:

1. Removal of EtOH before drying step is critical to successful drying. Aspiration of supernatant directed to bottom of well for this step, and excess volume aspirated.
2. To prevent dripping, extra air must be aspirated before moving a volume of liquid across sample wells, tip racks, or other reagents.
3. Excess volume must be dispensed when removing liquid from tips in order to fully void all reagent.
4. During mixing, uneven volumes may be found in the various tips within the multichannel. Excess volume must be aspirated before mixing, and dispensed after mixing, to fully void the tips of liquid.

AUTOMATION CODE:

Visit https://github.com/UK-CoVid19/OpentronDev for up to date protocols.