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We use this protocol and it's working

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Purification of influenza A virus RNA from cell supernatant to check sequences

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DISCLAIMER

ABSTRACT

We use this protocol to isolate RNA from influenza A virus-containing cell supernatant and monitor maintenance of sequence insertions or mutations

MATERIALS

Reagents

Ultrapure DNase/RNase free dH_2O (Thermofisher – Cat# 10-977-015)

RNA cleanup and concentrator (Zymo Research – Cat# R1015) or RNeasy MinElute Cleanup Kit (Qiagen – Cat# 74204)

QIAamp Viral RNA Mini Kit (Qiagen– Cat# 52904)

RNase-Free DNase Set (Qiagen– Cat# 79254)

100% Ethanol (we use VWR Cat#89125-170, but others will work)

iScript Reverse Transcription Supermix (BioRad - Cat #1708841)

dNTPs (we use Thermofisher – Cat#FERR0181, but others should work) – make a working stock of 25 mM

Taq DNA polymerase with standard Taq buffer (New England Biolabs – Cat#M0273)

Primers that bind on either side of the insertion/deletion/mutation

Equipment

Microcentrifuge

1 **Influenza A virus extraction from cell supernatant using QIAamp Viral RNA Mini Kit**

Follow the manufacturer's protocol using a starting cell supernatant volume of 140 µl.

Elute in 60 µl buffer AVE.

2 **DNase treatment using Qiagen RNase-free DNase set:**

Make mastermix of DNase and buffer. For each sample:

- 10 µl buffer RDD
- 2.5 µl DNase I

Add to each RNA tube, 27.5 µl H_2O , and 12.5 µl DNase master mix.

Incubate at RT for 10min.

3 **Purification of RNA using Zymo RNA clean up and concentrator kit:**

Follow the manufacturer's protocol, adding 100 µl RNA binding buffer and 300 µl ethanol as the initial step before transferring to column

Elute in 15 µl water.

Note: Can also use RNeasy MinElute kit, using manufacturer's protocol

4 **Reverse transcription with iScript.**

For each sample, mix:

- 1 µl purified RNA
- 3 µl 5x iScript reagent
- 11 µl H_2O

(total volume = 15 µl)

Also set up one no-RT reaction, same as above but using the iScript no-RT control reagent

instead.

Run RT reaction as per manufacturer's protocol:

- 25°C 5 min
- 46°C 20 min
- 95°C 1 min

Before proceeding to PCR, add 35 µl H_2O to each reaction.

5 PCR with Taq polymerase and primers flanking the insertion/deletion/mutation

Mix 4 µl virus cDNA with 46 µl of PCR mix containing :

- 0.4 µl dNTP (25 mM)
- 5 µl 10x Standard Taq buffer
- 0.25 µl Taq (5 U/ µl)
- 2 µl Forward primer (10 µM)
- 2 µl Reverse primer (10 µM)
- 36.35 µl dH2O

Note: other RT and polymerase reagents should work just as well. A proofreading polymerase may be preferable if checking for point mutations

6 Analysis of fragments

Run fragments on agarose gel to check that you have obtained the expected sizes. If appropriate, gel purify the product and send for Sanger sequencing.