COMMUNITY PROTOCOL

Influenza A RNA extraction from MDCK cells or embryonated chicken egg allantoic fluid

September 04, 2019

Keller, M.W. *et al.*, Direct RNA Sequencing of the Coding Complete Influenza A Virus Genome. *Nature Scientific Reports*, **8**, 14408

Materials

- 200–2000 µl MDCK cell culture or embryonated chicken egg allantoic fluid
- TRIzol (Invitrogen)
- Phase Lock GelTM tubes (VWR) - optional
- RNase-free glycogen or GlycoBlue™ Coprecipitant (ThermoFisher) - optional
- 75% freshly-prepared ethanol
- Isopropanol
- Nuclease-free water or TE buffer
- 1.5 ml Eppendorf DNA LoBind tubes
- 1.5 ml Eppendorf tubes
- Custom Reverse Transcription Adapter (RTA) for library prep (see below)

Method

- Critical step

  Ensure you start with at least 3X more TRIzol than the volume of your starting material.

  Extract 250 µl of sample in a 1.5 ml Eppendorf DNA LoBind tube. For example, if you have 1000 µl starting material, four tubes will be used.

- Step 2

  Follow the standard TRIzol protocol, using a Phase Lock GelTM tube to trap the organic phase. Add 1 µg RNase-free glycogen to the first isopropanol precipitation. Then use standard 1.5 ml tubes for the pelleting steps.

- Optional step

  GlycoBlue Coprecipitant can be used to make the pellet visible.

- Critical step

  If the extractions were performed in multiple tubes, when resuspending the pellet in ethanol, use the same 1 ml of ethanol to serially resuspend all the pellets.

- Step 5

  Elute the RNA in 10–100 µl nuclease-free water or TE buffer.

Results

- **Yield:** 10–1100 ng

Sequencing performance

Libraries were prepared using the Direct RNA Sequencing Kit (SQK-RNA001) with a custom RTA:

Name	Sequence
RTA-A	/5phos/ GGCTTCTTCTTGCTCTTAGGTAGTAGGTTC
RTA-B	GAGGCGAGCGGTCAATTTTCCTAAGAGCAAGAAGAAGCCTTTTTTTTTT
RTA-B-U12	GAGGCGAGCGGTCAATTTTCCTAAGAGCAAGAAGAAGCCAGCAAAAGCAGG
RTA-A-U12.4	GAGGCGAGCGGTCAATTTTCCTAAGAGCAAGAAGAAGCCAGCGAAAGCAGG

The custom RTAs can be purchased from IDT, with each of the modified RTA-B strands already duplexed to the RTA-A strand. The RTA-A has a 5' phosphate modification for ligation. The regions of reverse complementarity between the RTA strands are underlined, and the target sequences are coloured.

- Typical throughput: ★☆☆ (~1 million full-length reads in 48 h)
- Read length profile: