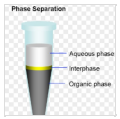

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RNA Isolation

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1 Works for me dx.doi.org/10.17504/protocols.io.6hjhb4n

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ABSTRACT

Real Time Polymerase Chain Reaction

RT PCR.docx

1 ● Get plates, aspirate media

2 ● Add 2mL ice cold DPBS

3 ○ Aspirate DPBS

4 ● Add 500uL Trizol/well in chemical hood

5 ● Bring yellow tubes to hood, collect trizol and add to tubes

6 ● Add 100uL chloroform to tubes

7 ○ hand shake 15s

8 ○ Place on ice 5min until see layers (aq/lipids/bottom)

9 ● Spin full speed 20min cold centrifuge

10 ○ During, make 70% ethanol on ice (3mL RNAse-free water + 7mL ethanol)

11 ○ Make new ep set 1-9

12 ● In hood, collect top layer after spinning and place into eppendorfs

13 ○ **take note of first volume and make all the same

14 ○ Mix 1:1 ethanol with supernatant, use same tip to transfer from eppendorfs into pink spin tubes

15 ● Spin 9000x 1min bench centrifuge

16 ○ Discard flowthrough in hood, keep tube

17 ● Add 350uL RW1 buffer to tubes

18 ○ Spin 1 min

19 ○ During, prepare DNAse soln (with syringe)

20 ● Add 70uL DNAse soln to middle of each column, leave 15min RT

21 ● Repeat RW1 and spin

22 ○ Discard collection tube and get new

23 ● Wash RPE 500uL

24 ○ Spin 1min

25 ○ Discard collection and keep tubes

26 ● Repeat RPE, spin 2min

27 ○ Discard tubes

28 ● Spin 1min empty to dry

29 ○ Get new eppendorfs, label

30 ● Place pink tubes on top of eppendorfs

31 ● Add 30uL RNAse free water, centrifuge 1min

32 ○ Close tubes as quickly as possible and keep on ice→ nanodrop