untitled protocol

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Abstract

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Protocol

Step 1.
http://omegabiotek.com/store/product/e-z-n-a-microelute-dna-clean-up-kit/

Step 2.
DNeasy® Blood & Tissue kit ((www.qiagen.com/handbooks)

Step 3.
RNeasy® Min kit ((www.qiagen.com/handbooks)

Step 4.
Thermal cycling conditions were as follows:

Step 5.
95°C for 2 min an initial denaturation step,

Step 6.
then 35 cycles at 95°C for 1 min denaturation,

Step 7.
55-60°C for 1 min annealing,

Step 8.
72°C for 1 min extension,

Step 9.
72°C for 5 min final extension and

Step 10.
at 4°C for every holding tube.

Step 11.
Standard qPCR profile for CNV detections and gene expression

Step 12.
Initial Denaturation: The reaction temperature is increased to 95 °C and the sample is incubated for 1 min (the time depends on the polymerase enzyme hot start mechanism) to ensure that all complex targets (dsDNA) are separated and are single stranded and available for amplification.

Step 13.
Denaturation: The reaction temperature is increased to 95 °C for 10 sec to melt all dsDNA.

Step 14.
Annealing: The temperature is lowered to 60 °C for 30 sec to promote primer and probe (if included) binding to the template.

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Step 15.
Extension: 68°C for 10 s. Duration of extension was dependent upon amplicon size (30 sec per 1 kb). The period of elongation depends upon the desired length of the amplicon and the enzyme used. Since qPCR amplicons are short, this is typically 5–30 sec.

Step 16.
TRIzol® Reagent

Step 17.
Chloroform

Step 18.
Isopropyl alcohol

Step 19.
75% ethanol

Step 20.
RNase free water

Step 21.
Determine your sample type, and perform homogenization at room temperature according to the table below. The sample volume should not exceed 10% of the volume of TRIzol® Reagent used for homogenization. Be sure to use the indicated amount of TRIzol® Reagent, because an insufficient volume can result in DNA contamination of isolated RNA.Phase separation

Step 22.
Incubate the homogenized sample (see Homogenizing samples) for 5 minutes at room temperature to permit complete dissociation of the nucleoprotein complex.

Step 23.
Add 0.2 mL of chloroform per 1 mL of TRIzol® Reagent used for homogenization. Cap the tube securely.

Step 24.
Shake tube vigorously by hand for 15 seconds.

Step 25.
Incubate for 2–3 minutes at room temperature.

Step 26.
Centrifuge the sample at 12,000 × g for 15 minutes at 4°C.Note: The mixture separates into a lower red phenol-chloroform phase, an interphase, and a colorless upper aqueous phase. RNA remains exclusively in the aqueous phase. The upper aqueous phase is 50% of the total volume.

Step 27.
Remove the aqueous phase of the sample by angling the tube at 45° and pipetting the solution out. Avoid drawing any of the interphase or organic layer into the pipette when removing the aqueous phase.

Step 28.
Place the aqueous phase into a new tube and proceed to the RNA Isolation Procedure.

Step 29.
Save the interphase and organic phenol-chloroform phase if isolation of DNA or protein is desired. See DNA Isolation Procedure and Protein Isolation Procedure for details. The organic phase can be stored at 4°C overnight.RNA Isolation ProcedureAlways use the appropriate precautions to avoid RNase contamination when preparing and handling RNA.RNA precipitation

Step 30.

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(Optional) When precipitating RNA from small sample quantities (<106 cells or <10 mg tissue), add 5–10 µg of RNase-free glycogen as a carrier to the aqueous phase.Note: Glycogen is co-precipitated with the RNA, but does not inhibit first-strand synthesis at concentrations ≤4 mg/mL, and does not inhibit PCR.

Step 31.
Add 0.5 mL of 100% isopropanol to the aqueous phase, per 1 mL of TRIzol® Reagent used for homogenization.

Step 32.
Incubate at room temperature for 10 minutes.

Step 33.
Centrifuge at 12,000 × g for 10 minutes at 4°C.Note: The RNA is often invisible prior to centrifugation, and forms a gel-like pellet on the side and bottom of the tube.

Step 34.
Proceed to RNA wash.RNA wash

Step 35.
Remove the supernatant from the tube, leaving only the RNA pellet.

Step 36.
Wash the pellet, with 1 mL of 75% ethanol per 1 mL of TRIzol® Reagent used in the initial homogenization.Note: The RNA can be stored in 75% ethanol at least 1 year at –20°C, or at least 1 week at 4°C.

Step 37.
Vortex the sample briefly, then centrifuge the tube at 7500 × g for 5 minutes at 4°C. Discard the wash.

Step 38.
Vacuum or air dry the RNA pellet for 5–10 minutes. Do not dry the pellet by vacuum centrifuge.Note: Do not allow the RNA to dry completely, because the pellet can lose solubility. Partially dissolved RNA samples have an A260/280 ratio <1.6.

Step 39.
Proceed to RNA resuspension.

Step 40.
gDNA Eraser 100 µL

Step 41.
5X gDNA Eraser Buffer □ 200 µLl

Step 42.
PrimeScript RT Enzyme Mix I 100 µL

Step 43.
5X PrimeScript Buffer 2 (for Real Time) □ 400

Step 44.
RT Primer Mix □ 400 µL

Step 45.
RNase Free dH2O 1 ml x 2

Step 46.
EASY Dilution (for Real Time PCR) □ 1 ml

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