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Isolation of extracellular vesicle RNA from cell culture supernatant

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Abstract

The study of extracellular RNA has been recently reported as an important mechanism of intercellular signaling. Extracellular vesicles which contain protein, mRNA, and non-coding RNA.can be isolated from cell culture supernatant. Extracellular vesicle RNA isolated from tumor cells in culture can be a useful tool for analyzing the role of either extracellular vesicles or extracellular vesicle RNA. This protocol presents a method of isolating extracellular vesicle RNA from human cholangiocarcinoma cells in culture.

Subject terms: Isolation, Purification and Separation | Cell biology | Cell culture | Nucleic acid based molecular biology | Tissue culture

Keywords: Extracellular vesicles | Tumor Cells | Isolation | RNA

Reagents

Reagents:
One of the following commercial kits:
• miCURY RNA Isolation Kit –Biofluids (Exiqon, #300112)
• miRNeasy Mini Kit (Qiagen, #217004)
• SeraMir Exosome RNA Purification Kit (System Biosciences, #RA806TC-1)

• Isopropanol
• Absolute ethanol
• Nuclease free H2O

Equipment

Equipment:
• Bench top centrifuge
• Vortex Mixer

- Micropipettes with sterile tips 20-1000
- Sterile collection bottle
- Microcentrifuge tubes
- 0.22 µm PES vacuum filter

Procedure

1. Grow KMBC cholangiocarcinoma cells on 32×10 cm tissue culture plates. Use Extracellular vesicle (EV)-cleared media for cultures (total 400 ml). Incubate on cells for 48 hr.
2. Collect supernatant and filter through a 0.22 µM PES filter
3. Aliquot into ultracentrifugation tubes, and centrifuge for 70 min at 100,000 x g at 4°C.
4. Discard supernatant, and resuspend pellet from each tube with 1 ml PBS.
5. Combine washed pellets into one ultracentrifuge tube, and centrifuge for 70 min at 100,000 x g at 4°C.
6. Discard supernatant, resuspend final EV pellet with 600 µl PBS. Store samples at -70°C until ready for use.
7. RNA Isolation can be performed from one of the following options.

A. miCURY RNA Isolation Kit, Exiqon
i. Transfer 200 µl of EV into 1.5 ml microcentrifuge tube.
ii. Add 60 µl Lysis Solution BF, vortex 5 sec, incubate for 3 min at room temperature.
iii. Add 20 µl Protein Precipitation Solution BF, vortex 5 sec, incubate for 1 min at room temperature.
iv. Centrifuge at 11,000g for 3 min at room temperature.
v. Transfer clear supernatant to new microcentrifuge tube.
vi. Add 270 µl Isopropanol, vortex 5 sec.
vii. Assemble microRNA Mini Spin Column BF in a new collection tube and load entire sample onto column.
viii. Centrifuge at 11,000g for 30 sec at room temperature. Discard flow-through and return column to collection tube.
ix. Add 700 µl Wash Solution 2 BF to column and centrifuge at 11,000g for 30 sec at room temperature. Discard flow-through and return column to collection tube.
x. Add 250 µl Wash Solution 2 BF to column and centrifuge at 11,000g for 2 min at room temperature. Discard flow-through and return column to collection tube.
xi. Add 50 µl rDNase directly onto membrane of spin column.
xii. Close lid and incubate for 15 min at room temperature
xiii. Add 100 µl Wash Solution 1 BF to column and centrifuge at 11,000g for 30 sec at room temperature. Discard flow-through and return column to collection tube.
xiv. Add 700 µl Wash Solution 2 BF to column and centrifuge at 11,000g for 30 sec at room

temperature. Discard flow-through and return column to collection tube.
xv. Add 250 µl Wash Solution 2 BF to column and centrifuge at 11,000g for 2 min at room temperature.
xvi. Transfer spin column into a new collection tube.
xvii. Add 50 µl RNase free H2O directly onto the membrane of spin column.
xviii. Close lid and incubate for 1 min at room temperature.
xix. Centrifuge at 11,000g for 1 min at room temperature.
xx. Discard spin column and store purified RNA at -70°C.

B. miRNeasy Mini Kit
i. Transfer 200 µl of EV into 1.5 ml microcentrifuge tube.
ii. Add 700 µl QIAzol Lysis Reagent, vortex 5 sec, incubate for 5 min at room temperature.
iii. Add 140 µl chloroform, shake vigorously for 15 sec, incubate for 3 min at room temperature.
iv. Centrifuge sample at 12,000g for 15 min at 4°C.
v. Transfer the upper aqueous phase to a new microcentrifuge tube.
vi. Add 500 µl 100% Ethanol, mix by pipetting.
vii. Assemble RNAeasy Mini Column in a new collection tube and load entire sample onto column.
viii. Centrifuge sample at 8,000g for 15 sec at room temperature. Discard flow-through and return column to collection tube.
ix. Add 700 µl Buffer RPE to column and centrifuge at 8,000g for 15 sec at room temperature. Discard flow-through and return column to collection tube.
x. Add 500 µl Buffer RPE to column and centrifuge at 8,000g for 2 min at room temperature. Discard flow-through and return column to collection tube.
xi. Centrifuge column at 8,000 x g for 1 min at room temperature to dry membrane.
xii. Transfer spin column into a new collection tube.
xiii. Add 30 µl RNase free H2O directly onto the membrane of spin column.
xiv. Close lid and centrifuge for 1 min at 8,000 x g at room temperature.
xv. Discard spin column and store purified RNA at -70°C.

C. SeraMir Exosome RNA Purification Kit (Since EV is already isolated by ultracentrifugation, omit ExoQuick-TC precipitation step)
i. Transfer 200 µl of EV into 1.5 ml microcentrifuge tube.
ii. Add 140 µl Lysis Buffer to EV, vortex 15 sec, incubate for 5 min at room temperature.
iii. Add 80 µl 100% Ethanol, vortex 10 sec.
iv. Assemble Spin Column in a new collection tube and load entire sample onto column.
v. Centrifuge sample at 13,000 rpm for 1 min at room temperature. Discard flow-through and return column to collection tube.
vi. Add 400 µl Wash Buffer to column and centrifuge at 13,000 rpm for 1 min at room temperature. Discard flow-through and return column to collection tube.

vii. Add 400 µl Wash Buffer to column and centrifuge at 13,000 rpm for 1 min at room temperature. Discard flow-through and return column to collection tube.
viii. Centrifuge column at 13,000 rpm for 2 min at room temperature to dry membrane.
ix. Transfer spin column into a new collection tube.
x. Add 30 µl Elution Buffer directly onto the membrane of spin column.
xi. Close lid and centrifuge at 2,000 rpm for 2 min at room temperature.
xii. Increase speed and centrifuge at 13,000 rpm for 1 min at room temperature.
xiii. Discard spin column and store purified RNA at -70°C.

Associated Publications

This protocol is related to the following articles:

- Extracellular vesicle-mediated transfer of long non-coding RNA ROR modulates chemosensitivity in human hepatocellular cancer
  Kenji Takahashi, Irene K. Yan, Takayuki Kogure, Hiroaki Haga, and Tushar Patel
- Involvement of Extracellular Vesicle Long Noncoding RNA (linc-VLDLR) in Tumor Cell Responses to Chemotherapy
  K. Takahashi, I. K. Yan, J. Wood, H. Haga, and T. Patel
- Modulation of hypoxia-signaling pathways by extracellular linc-RoR
  K. Takahashi, I. K. Yan, H. Haga, and T. Patel
- Intercellular nanovesicle-mediated microRNA transfer: A mechanism of environmental modulation of hepatocellular cancer cell growth
  Takayuki Kogure, Wen-Lang Lin, Irene K. Yan, Chiara Braconi, and Tushar Patel

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Competing financial interests

None

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Readers' Comments

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