DNA isolation and detection from EVs after DNase treatment

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Verzió szám: 02
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Angol verzió: Försönits András
Ellenőrizte:

Oldaltörés

Reagents

PBS
Sigma; DNase I Kit (#AMPD1)
Sigma; ProtectRNA Inhibitor 500x Concentrate (#R7397)
Geneaid; Genomic DNA Mini Kit (#GB300)
3M sodium acetate buffer pH:5,2 (4°C)
abs. ethanol (-20°C)
70% ethanol (-20°C)
TE buffer pH:7,6-7,8
Sigma; Ribonuclease A (#R4642)
Sigma; Agarose for routine use (#A9539)
Izinta; Biotium; GelRed Nucleic Acid Stain (#41002)
Thermo Scientific; GeneRuler 1 kb Plus DNA Ladder (#SM1331)

Samples

Separated small, medium or large EVs; cells as controls (yet cells doesn’t need to be DNase treated beforehand

DNase treatment

Note: Unit Definition:One unit of DNase I, Amplification Grade completely digests 1µg of plasmid DNA to oligonucleotides in 10 minutes at 37°C. Activity is assayed in a 50µl reaction, containing 33mM Tris-acetate, pH:7.8, 66 mM potassium acetate, 10mM magnesium acetate, 0.5mM dithiothreitol, 1.0µg DNA, and 0.15-2.5 units of DNase I. This unit is equal to ~0.75Kunitz units

1. Pellet the EVs, than sip the supernatant with a needle
2. Resuspend the EV pellet in 95 µl 1x Reaction Buffer

   Note: 10x Reaction Buffer 200 mM Tris-HCl, pH 8.3, 20 mM MgCl;
   for 1 reaction: 89,5µl PBS + 10,5µl 10x Reaction Buffer
3. For big and medium EVs, use a 1,5 ml Eppendorf tube, for small vesicles, use a 1,8 ml Nunc cryotube

   Note: Do not use UC-tubes for DNase treatment!
4. Add 5µl (5 unit) DNase to the vesicles, suspend it carefully and gently vortex

   Note: DNase is to be kept cold (on ice)
5. Incubate for 10 minutes at 37°C or 15 minutes RT
6. Add 10 µl Stop Solution (50mM EDTA), mix it with pipetting only
7. Add 900 µl 1x ProtectRNA Inhibitor, mix it with pipetting

   Note: 998µl PBS + 2µl 500xProtectRNA Inhibitor; 900µl/tube from this solution
8. Add about 500 µl of PBS
9. Spin the tubes according to the vesicle protocol
10. Sip the supernatant with a needle, and resuspend the pellet in 150 µl RBC Lysis Buffer
11. Vortex it hard for 1 minute
12. Keep the samples at -20°C

DNS isolation

1. Turn the termoblock on to 70 °C
2. Preheat Elution Buffer / TE buffer / nuclease-free water to 70 °C
3. Thaw the samples
4. Add 200 µl GB Buffer and vortex it
5. Incubate samples for min. 10 minutes at 70 °C. Mix it by rotating the tubes at least 3x
6. Add 1 µl RNase and incubate it for 5 minutes RT
7. Add 200 µl absolute ethanol and vortex it.

   Note: Add the alcohol only one at a time, and than immediately vortex it. Dissolve any precipitate that may appear by pipetting it.
8. Put the whole volume onto a GD column
9. Spin it with 12000 g 2'
10. Put the column into a new collection tube
11. Add 400 µl W1 Buffer
12. Spin it with 12000g 30s and discard the flowthrough
13. Add 600 µl Wash Buffer
14. Spin it with 12000g 30s and discard the flowthrough
15. Dry the column membrane by spinning it with 12000g 3 minutes and discard the flowthrough
16. Put the column into a 1,5 ml Eppendorf tube
17. Add 50 µl preheated Elution Buffer / TE buffer / nuclease-free water and incubate it for 5 minutes RT / 37 °C
18. Measure the DNA concentration on NanoDrop

Concentrate DNA by ethanol precipitation

for instructions see Protocol!

Gel electrophoresis

50x TAE puffer

2M TRIS [121 g]
0,05M EDTA[50 ml, 0,5M]
concentrated acetic acid [28 ml]
dilute it with didestillated water until 500 ml

1,5% agarose gel

1. Measure 0,9 g agarose into a lombique
2. Add 60 ml 1x TAE buffer
3. Boil it, while mixing eventually
4. Let it cool until about 60 °C
5. Add 6 µl GelRed dye
6. Prepare the scaffold and put in the appropriate comb
7. Pour the gel into the scaffold with a single move

   Note: get rid of the bubbles with the base of a pipette tip if needed
8. Let it harden for about 20 minutes RT in dark

9. Fill a water bath with 1x TAE buffer and put in the gel
10. Put ice around the bath to prevent it from heating
11. Apply about 100V for about 20-30 minutes

    Note: DNA runs towards the red (+) side
12. Make a picture of your gel by using one of the gel documentation systems