RNA Isolation (Small RNA Extraction)

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- ***What is it used for?***

Small RNA extraction is the first step prior to proceeding for microRNA RT-PCR. Before performing small RNA isolation from total RNA (extracted in previous section), two more steps are required: 1. DNase treatment and 2. Agarose gel electrophoresis, which are respectively crucial for eliminating of DNA contamination and to check the extracted RNA quality.

- ***How does DNase treatment work?***

In a 0.5 ml tube, 5 µl of DNase buffer (40 mM Tris-HCl, 60 mM MgCl2, pH 7.5) and 5µl (5U) of DNase I will be added to 5 µl of total extracted RNA. Then the mixture will be incubated in a 37 °C water bath for 30 minutes. Later, 5 µl of RQ1 DNase Stop Solution will be added and incubated in 65 °C water bath for 10 minutes. This DNase treated RNA after quality check will be used for small RNA extraction.

- ***How does agarose gel electrophoresis run?***

In order to check the RNA extraction quality, a 1% agarose gel is run. In a flask 20 mg of agarose powder is weighed and then 20 ml 1X TBE (Tris/Borate/EDTA) is added to it. The flask will then be transferred to a microwave at 20% power until all agarose is completely dissolved (usually 45 seconds). The liquid is then cooled under cold running water for 10-15 seconds or is allowed to become cool by letting it sit at RT so that it is not too hot to hold. Ethidium bromide (1 µl per 20 ml) will be added to the liquid agar in the flask. Then, the gel is poured into the gel mold held in a cassette, with the comb in place. The gel is allowed to set for about 20 minutes. After that, the comb is removed and the gel in the gel mold is transferred to a tank containing 1X TBE buffer in a way that the gel is completely submerged. Then on a sheet of parafilm 1 µl of RNA is mixed with 1 µl of 2X RNA loading dye and then is loaded into each well of the gel. In order to estimate the RNA band sites 1 µl of a 50 bp ladder is loaded. Then the tank is connected to the power supply and the gel is electrophoresed at 120V for 40 minutes. After that the RNA bands are visualized using a gel imaging system.

- ***How does gel extraction of small RNA work?***

Since DNase treatment only removes DNA contamination from total extracted RNA, small RNA gel extraction will be performed to enrich for microRNA. A 2 ml tube is filled with 0.4 M NaCl and placed in an ice box. Extracted total RNA from the previous step is run on a 2% agarose gel. According to the ladder the small RNA which was less than 250 bp located in the lower part of the gel, is cut under the UV in a dark room and the fragments are transferred to the 2 ml tube of 0.4 M NaCl. After this step,

the ice box is placed on a belly dancer for four hours. Then the tube is stored at 4 °C overnight. After that, the liquid content of the tube is transferred into a new tube and then 3 volumes of cold absolute ethanol (4 °C) and 0.1 volume of NaOAc (Sodium Acetate) are added to it. The tube is incubated at -80 °C overnight, followed by centrifugation for 30 minutes, 4 °C at 17,900 x g. The pellet then will be washed with cold 70% ethanol and centrifuged for 5min, 4°C at 17,900 x g. The supernatant is then discarded and the pellet is resuspended in 50 µl of DEPC treated dH_2O. The small RNAs are kept at -80 °C until use.

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