

DIGITAL LAB NOTEBOOK OF KEVIN MURRAY

HONOUS PROJECT, 2013

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Last updated at 19:53 on Tuesday 22nd January, 2013

2013-01-21

1 Practice RNA Extraction

1.1 Quantification of RNA samples

Aim

- Determine qty of RNA in previously extracted samples

Method

- Nanodropped RNA extraction from 15/1/13??
- Standard protocol, used sterile milliQ water as blank.

Result

- Of the 14 samples, 10 had reasonable amounts of RNA, and 260/280 ratios were above 1.8 in all but one case. (see ./jan/20130121-PracticeRNASamples.ods)

Attachments

- ./jan/20130121-PracticeRNAExtractionSamples.csv
- ./jan/20130121-PracticeRNAExtractionSamples.ndv
- ./jan/20130121-PracticeRNASamples.ods

2 MADE: 10x MOPS Solution

Method

- Add 41.8g RNA only MOPS to beaker
- Add 450mL DEPC H₂O, mix w/ stirrer bar on mag stirrer
- Add 26.6mL 3M Sodium Acetate (0.22um Filtered before use)
- Add 10mL RNA only 0.5M EDTA
- pH to 7 with 5M NaOH
- Top up to 500 mL with DEPC H₂O
- Use 10ml per 100mL MOPS gel

3 MADE: RNA Denaturing Gel (MOPS)

Method

- Melt 1g RNase-free Agarose in 72ml DEPC H₂O
- Add 10mL 10x MOPS
- Add 18mL 37% Formaldehyde
- Pour in RNA-only gel tank, previously washed with 0.5% SDS and RNase-zap

Tue 2013-01-22

1 RNA Sample Gels

1.1 Denature RNA for RNA gels

Method

- Dilute RNA to 100ng/uL
- Add RNA gel loading buffer (Obtained from Pete Crisp)
- Incubate at 65 degrees for 10 minutes. The samples were incubated for 10 minutes on the evening of 2013-01-21, but the gels were not run until 2013-01-22, so they were denatured for a further 2 minutes at 65 degrees

1.2 TBE Gel

Aim

- To compare TBE and denaturing/MOPS gels for RNA

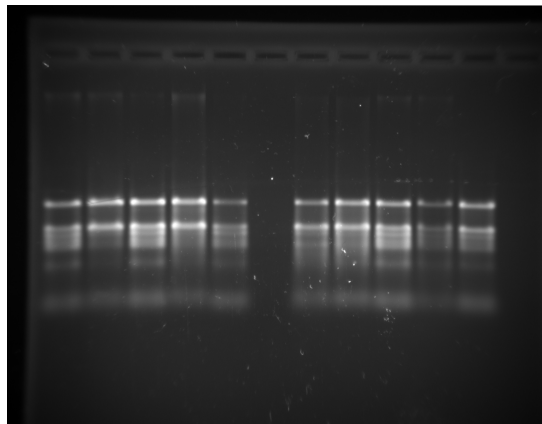
Method

- Dissolve 1g RNAase-free agarose in 90mL DEPC water
- Add 10mL RNAse-free TBE (prepared using DEPC Water, obtained from Pete Crisp)
- Pour in RNA-only gel tank, previously washed with 0.5% SDS or RNAse-zap
- Then, load denatured samples, and run in RNAse-free 1x TBE

Result

See Figure 1 below.

Figure 1: TBE Gel of Practice RNA samples, 2013-01-22



Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a misloading error. Overall, the TBE gel appears to be of more use than the MOPS gel.