Digital Lab Notebook of Kevin Murray

Honous Project, 2013

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Last updated at 15:46on Wednesday 23rd January, 2013

Mon 2013-01-21

1 Quantification of RNA samples

1.1 Aim

- Deterime qty of RNA in previously extracted samples

1.2 Method

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

1.3 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)

1.4 Attachements

- ./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

Mon 2013-01-21

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 Denature RNA for RNA gels

1.1 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 futher 2 minutes at 65 degrees

2 TBE Gel

2.1 Aim

- To compare TBE and denaturing/MOPS gels for RNA

2.2 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
- Run at $\approx$ 80V, $\approx$ 40-50mA for $\approx$ 1.75h
- Stain gel in 0.5ug/ml Ethidium Br in DEPC water?? for 10 min on orbital shaker, and photograph.

2.3 Result

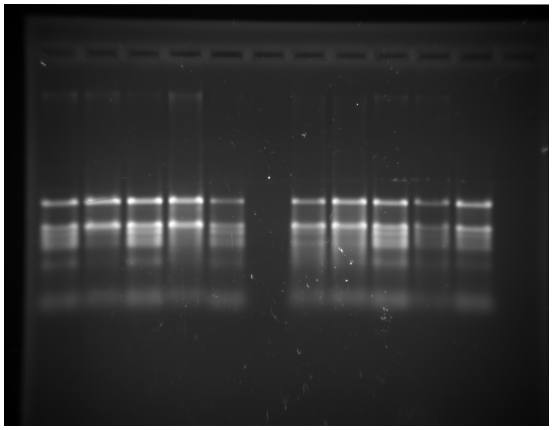
See Figure 1 below.
Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a misloading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

3 MOPS gel

3.1 Aim

- Determine qualtiy of RNA and Compare MOPS with TBE for RNA gels

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

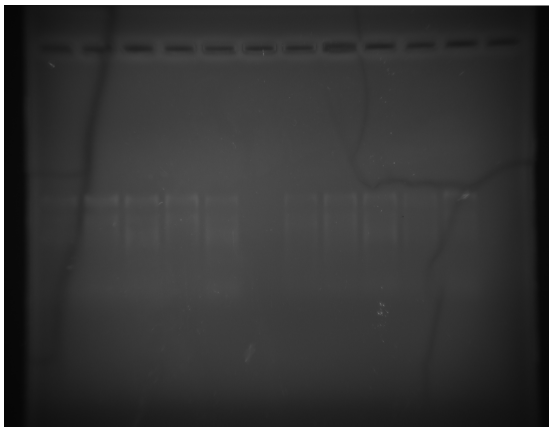


3.2 Method

- Load samples after denaturing as above. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7.
- Run gel in RNAse free 1x MOPS at $\approx$ 80V, $\approx$ 100mA for $\approx$ 1.75h as per TBE gel above.
- Stain gel in 0.5ug/ml Ethidium Br in DEPC water?? for 10 min on orbital shaker.
- Destain on orbital shaker gel in 1x MOPS, and photograph. Gel disintegrated whilst destaining.

3.3 Results

Figure 2: MOPS Gel of Practice RNA samples, 2013-01-22



See Figures 2 and 3
Mops gel conifrms that the rna was of reasonable quality. The MOPS gel appears to be of less use than the TBE gel.

Figure 3: MOPS Gel of Practice RNA samples, 2013-01-22. Contrast adjusted.