Digital Lab Notebook of Kevin Murray

Honous Project, 2013

Jointly supervised by Justin Borevitz and Barry Pogson

Last updated at 15:30on Tuesday 29th 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

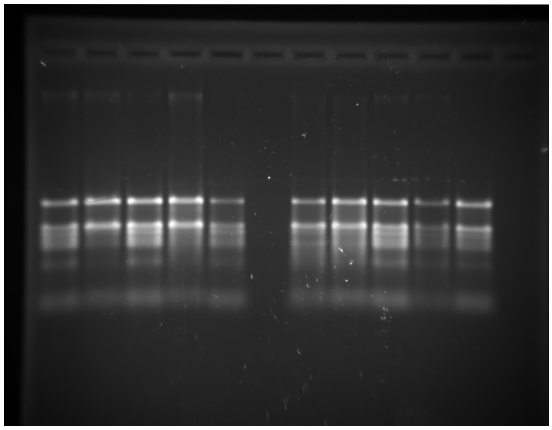
See Figure 1 below.
Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a misloading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

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

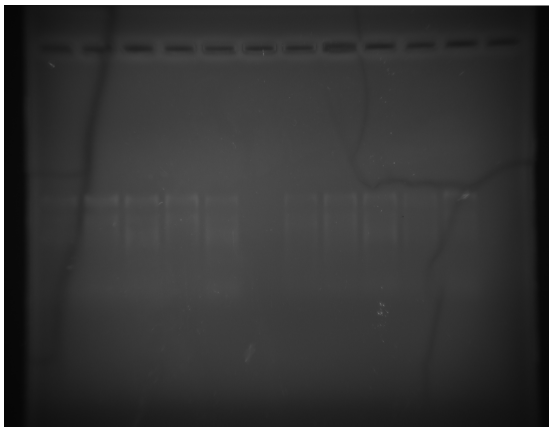


3.2 Method

- 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.

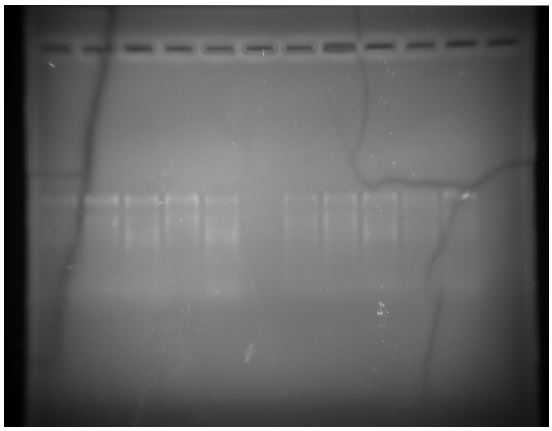
3.3 Results

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.



Tue 2013-01-29

1 Seed Stock Levels

The stocks of Joost's RIX set were checked. Seed lines were classified as having either plenty (+), limited(¡) or no (-) seed. The levels of each line are shown in the table below.

Line	Desc	Count	Line	Desc	Count
1	Col-$0_1$915	not in box	51	168 x 22	not in box
2	Col-$0_4$936	not in box	52	169 x 175	not in box
3	Cvi x Cvi	not in box	53	17 x 21	not in box
4	1 x Cvi	not in box	54	170 x 24	not in box
5	1 x 146	not in box	55	171 x 143	not in box
6	10 x 26	not in box	56	174 x 34	not in box
7	101 x 176	not in box	57	180 x 157	not in box
8	Ll-1	not in box	58	183 x 118	not in box
9	105 x 145	not in box	59	186 x 27	not in box
10	107 x 124	not in box	60	187 x 190	not in box
11	109 x 185	not in box	61	187 x 69	not in box
12	109 x 47	not in box	62	189 x 133	not in box
13	110 x 32	not in box	63	19 x 173	not in box
14	112 x 30	not in box	64	19 x 67	not in box
15	113 x 141	not in box	65	190 x 176	not in box
16	114 x 3	not in box	66	191 x 31	not in box
17	114 x 60	not in box	67	192 x 189	not in box
18	115 x 126	not in box	68	20 x 138	not in box
19	117 x 73	not in box	69	21 x 22	not in box
20	118 x 108	not in box	70	24 x 171	not in box
21	118 x 164	not in box	71	25 x 9	not in box
22	119 x 177	not in box	72	26 x 74	not in box
23	12 x 142	not in box	73	33 x 58	not in box
24	122 x 42	not in box	74	35 x 120	not in box
25	125 x 117	not in box	75	38 x 35	not in box
26	128 x 6	not in box	76	39 x 27	not in box
27	132 x 129	not in box	77	40 x 74	not in box
28	133 x 35	not in box	78	npq4	not in box
29	134 x 29	not in box	79	43 x 131	not in box
30	135 x 10	not in box	80	44 x 50	not in box
31	135 x 140	not in box	81	45 x 23	not in box
32	136 x 102	not in box	82	46 x 29	not in box
33	165 x 137	not in box	83	48 x 160	not in box
34	139 x 162	not in box	84	49 x 158	not in box
35	139 x 36	not in box	85	5 x 172	not in box
36	14 x 4	not in box	86	5 x 188	not in box
37	146 x 64	not in box	87	51 x 111	not in box
38	147 x 50	not in box	88	51 x 18	not in box
39	147 x 69	not in box	89	54 x 183	not in box

Line	Desc	Count	Line	Desc	Count
40	149 x 165	not in box	90	55 x 18	not in box
41	150 x 37	not in box	91	59 x 116	not in box
42	152 x 42	not in box	92	6 x 131	not in box
43	153 x 108	not in box	93	61 x 162	not in box
44	153 x 20	not in box	94	63 x 151	not in box
45	154 x 144	not in box	95	7 x 46	not in box
46	156 x 166	not in box	96	8 x 61	not in box
47	16 x 4	not in box	97	Ler x Ler	not in box
48	16 x 66	not in box	98	Ler self	not in box
49	164 x 7	not in box	99	Cvi x Ler	not in box
50	166 x 25	not in box	100	Ler x Cvi	not in box