

DIGITAL LAB NOTEBOOK OF KEVIN MURRAY

HONOUS PROJECT, 2013

Jointly supervised by Justin Borevitz and Barry Pogson

Last updated at 17:56 on Tuesday 29th January, 2013

Mon 2013-01-21

1 Quantification of RNA samples

1.1 Aim

- Determine qty of RNA in previously extracted samples

1.2 Method

- Nanodropped RNA extraction 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

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 further 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.

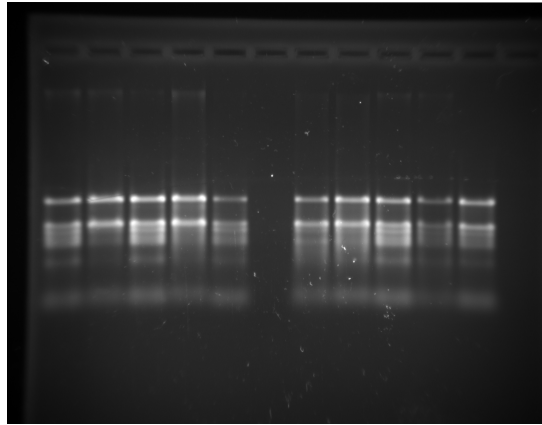
Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a misloading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

3 MOPS gel

3.1 Aim

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

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

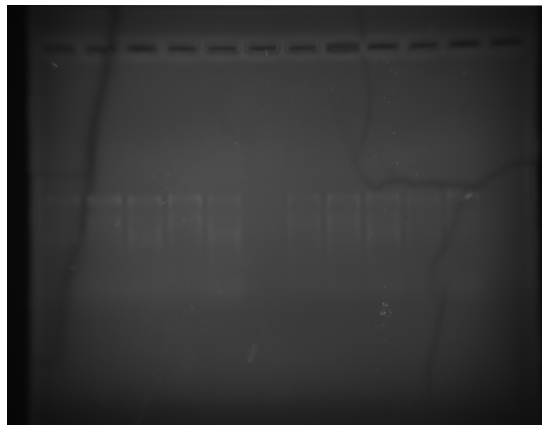


3.2 Method

- 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 $0.5\mu g/ml$ Ethidium Br in DEPC water?? for 10 min on orbital shaker.
- Destain on orbital shaker in 1x MOPS, and photograph. Gel disintegrated whilst destaining.

3.3 Results

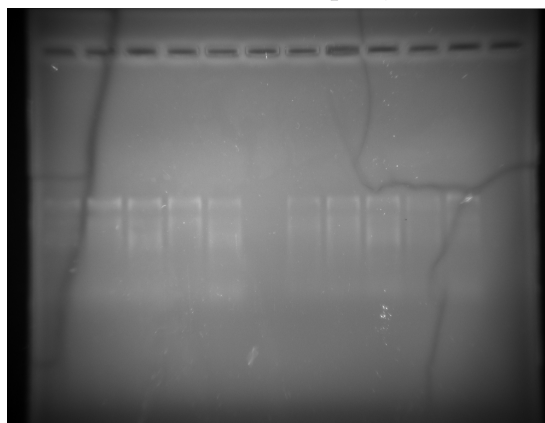
Figure 2: MOPS Gel of Practice RNA samples, 2013-01-22



See Figures 2 and 3

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

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