Digital Lab Notebook of Kevin Murray

Honous Project, 2013

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Last updated at 16:55on Wednesday 30th January, 2013

Mon 2012-12-10

1 Final Harvest of Keng's RIX lines

1.1 Aim

Harvest lines after 1 week of HL stress.

1.2 Method

- An Eppendorf 1.2mL deep well plate was placed on dry ice for ≈ 10 minutes before sampling to allow to cool.
- Whole leaves were excised and placed into 1.2mL Eppendorf 96 deep well plate.
- Where possible, the largest mature leaf was taken. In some cases, this was hard to determine, so the youngest of the fully-expanded leaves was taken (as this was generally also the largest leaf). Some plants were very small, and had only juvenile leaves, in which case the largest juvenile leaf was taken.

1.3 Results

The following table describes the plate layout.

	Well	Line	Comments	Well	Line	Comments
A1	87			A7	65	
B1	100			B7	71	
C1	92			C7	53	
D1	OSB2			D7	55	
E1	93			E7	56	
F1	94			F7	50	
G1	1*cvi			G7	51	
H1	99			H7	52	
A2	63			A8	78	NPQ4
B2	98			B8	80	
C2	98	2nd plant with orange label		C8	85	
D2	70			D8	74	
E2	67			E8	73	
F2	99			F8	76	
G2	66			G8	83	
H2	100			H8	81	
A3	65			A9	89	
B3	72			B9	90	
C3	12			C9	91	
D3	11			D9	16	
E3	10			E9	17	
F3	12			F9	18	

Mon 2012-12-10

Well	Line	Comments	Well	Line	Comments
G3	9		G9	21	
H3	8		H9	23	
A4	7		A10	25	
B4	6		B10	57	
C4	5		C10	28	
D4	13		D10	29	
E4	2		E10	30	
F4	1*cvi		F10	31	
G4	49		G10		
H4	47		H10		
A5	46		A11		
B5	42		B11		
C5	45		C11		
D5	71		D11		
E5	39		E11		
F5	40		F11		
G5	41		G11		
H5	43		H11		
A6	38		A12		
B6	39		B12		
C6	36		C12		
D6	33		D12		
E6	61		E12		
F6	62		F12		
G6	68		G12		
H6	58		H12		

Attachments:

- dec12/20121210-harvest_photos.tar.bz2 MD5SUM:40dae2cad3babaa3c32f0d35a9d9442c

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

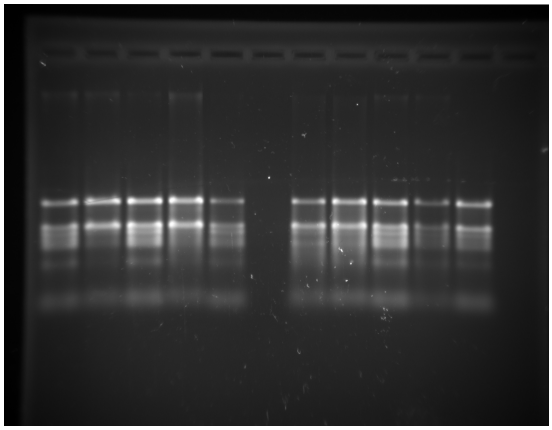
See Figure **??** below.
Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a misloading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

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

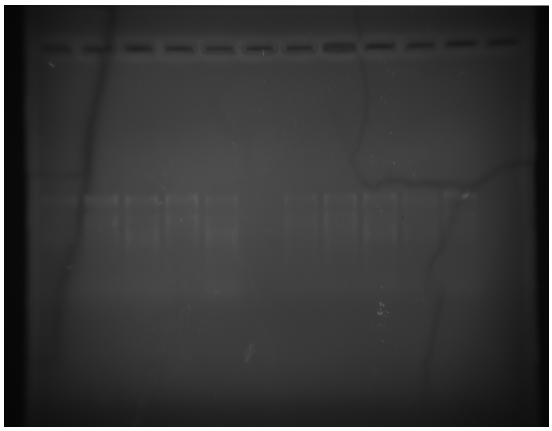


3.2 Method

- 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.

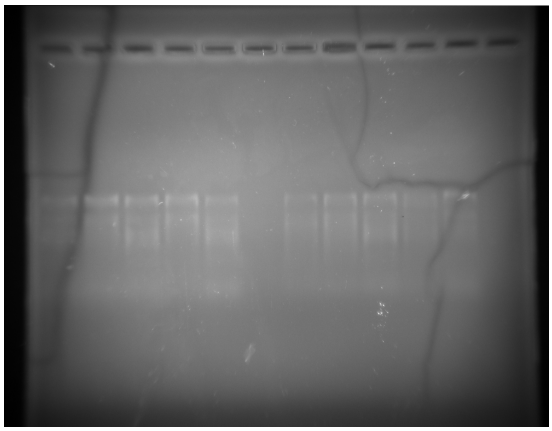
3.3 Results

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



See Figures **??** and **??**
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	+
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	+