Digital Lab Notebook of Kevin Murray

Honours Project, 2013

Jointly supervised by Justin Borevitz and Barry Pogson

Last updated at 15:00 on Monday 15th July, 2013

1 Mon 2012-12-03

1.1 Initial Harvest of Keng's RIX lines

1.1.1 Aim

Harvest lines before the 1 week repeated HL stress experiment.

1.1.2 Method

- Tissue was harvested into a 96 well tray of 8-strip ≈ 1mL tubes
- Single leaves were placed into the tubes, and snap frozen in liquid nitrogen.

1.1.3 Results

The following table details the collection, including the plate layout

Well	Line	Comments
A1	78	
B1	80	
C1	85	Possible cross contamination from D1
D1	74	
E1	73	Leaf shatered, lost a lot of tissue
F1	76	
G1	83	
H1	81	Had to crush leaf while warm to fit it into tube
A2	89	Had to crush leaf while warm to fit it into tube
B2	90	
C2	91	
D2	87	
E2	108	
F2	92	
G2	osb2	
H2	93	
A3	94	
B3	4	
C3	99	
D3	16	
E3	17	
F3	18	may be 17, hard to read label
G3	18	
H3	21	
A4	23	
B4	25	
C4	57	
D4	28	

Well	Line	Comments
E4	29	
F4	30	
G4	31	
H4	32	
A5	63	
B5	98	may be 97, hard to read
C5	98	
D5	20	
E5	67	
F5	99	
G5	99	
H5	100	
A6	65	
B6	72	
C6	1	
D6	1	a second fully shaded leaf from same plant as C6
E6	2	
F6	13	
G6	5	
H6	6	
A7	7	
B7	8	
C7	9	
D7	10	
E7	11	
F7	33	
G7	36	
H7	39	
A8	38	
B8	39	
C8	40	
D8	41	
E8	43	
F8	42	
G8	45	
H8	45	second leaf from unlabeled plant broke leaf while handling minutes before sampling
A9	71	
B9	46	
C9	47	
D9	99	
E9	50	
F9	51	
G9	52	
H9	52	
A10	54	
B10	55	
C10	56	
D10	58	
E10	59	
F10	71	
G10	61	

Well	Line	Comments
H10	21	
A11	68	
B11		
C11		
D11		
E11		
F11		
G11		
H11		
A12		
B12		
C12		
D12		
E12		
F12		
G12		
H12		

Attachments:

- `./2012-12/20121203-harvest-pictures.tar.bz2` MD5SUM:2843946f8cae888a60dcd2226feb874f

2 Mon 2012-12-10

2.1 Final Harvest of Keng's RIX lines

2.1.1 Aim

Harvest lines after 1 week of HL stress.

2.1.2 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.

2.1.3 Results

The following table describes the plate layout.

Well	Line	Comments
A1	87	
B1	100	
C1	92	
D1	OSB2	
E1	93	
F1	94	
G1	1*cvi	
H1	99	
A2	63	
B2	98	
C2	98	2nd plant with orange label
D2	70	
E2	67	
F2	99	
G2	66	
H2	100	
A3	65	
B3	72	
C3	12	
D3	11	
E3	10	
F3	12	
G3	9	

Well	Line	Comments
H3	8	
A4	7	
B4	6	
C4	5	
D4	13	
E4	2	
F4	1*cvi	
G4	49	
H4	47	
A5	46	
B5	42	
C5	45	
D5	71	
E5	39	
F5	40	
G5	41	
H5	43	
A6	38	
B6	39	
C6	36	
D6	33	
E6	61	
F6	62	
G6	68	
H6	58	
A7	65	
B7	71	
C7	53	
D7	55	
E7	56	
F7	50	
G7	51	
H7	52	
A8	78	NPQ4
B8	80	
C8	85	
D8	74	
E8	73	
F8	76	
G8	83	
H8	81	
A9	89	
B9	90	
C9	91	
D9	16	
E9	17	
F9	18	
G9	21	
H9	23	
A10	25	
B10	57	

Well	Line	Comments
C10	28	
D10	29	
E10	30	
F10	31	
G10		
H10		
A11		
B11		
C11		
D11		
E11		
F11		
G11		
H11		
A12		
B12		
C12		
D12		
E12		
F12		
G12		
H12		

Attachments:

- `./2012-12/20121210-harvest-photos.tar.bz2` MD5SUM:40dae2cad3babaa3c32f0d35a9d9442c

3 Mon 2013-01-14

3.1 MAKE: Washed Ball Bearings

3.1.1 Method

- Aliquot approx 15mL of 3mm diameter steel ball bearings into 50mL falcon tube
- Add clean 100% ethanol
- Vortex for $\approx$ 5 minutes
- Remove ethanol, wash beads with milliQ or sterile water
- Dry in fume cupboard overnight

3.2 TissueLyser grinding of practice samples

3.2.1 Aims

To grind tissue from the excess tissue of Keng's RIX lines collected on 3/12/12.

3.2.2 Method

- Remove pre-frozen TissueLyser blocks from -80 freezer.
- Add one cleaned bead to each Eppendorf tube (beads were not pre-cooled)
- Pour LN_2 into the TissueLyser block
- Add Eppys with beads and sample, and run for 3x 1min runs at 29Hz
- Replace samples in -80

4 Mon 2013-01-21

4.1 Quantification of RNA samples

4.1.1 Aim

- Determine qty of RNA in previously extracted samples

4.1.2 Method

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

4.1.3 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 `./2013-01/20130121-PracticeRNASamples.ods`)

4.1.4 Attachments

- `./2013-01/20130121-PracticeRNAExtractionSamples.csv`
- `./2013-01/20130121-PracticeRNAExtractionSamples.ndv`
- `./2013-01/20130121-PracticeRNASamples.ods`

4.2 MADE: 10x MOPS Solution

4.2.1 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

4.3 MADE: RNA Denaturing Gel (MOPS)

Method

- Melt 1g RNAase-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 RNAase-zap

5 Tue 2013-01-22

5.1 Denature RNA for RNA gels

5.1.1 Method

- Dilute RNA to 100ng/µL
- 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

5.2 TBE Gel

5.2.1 Aim

- To compare TBE and denaturing/MOPS gels for RNA

5.2.2 Method

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

5.2.3 Result

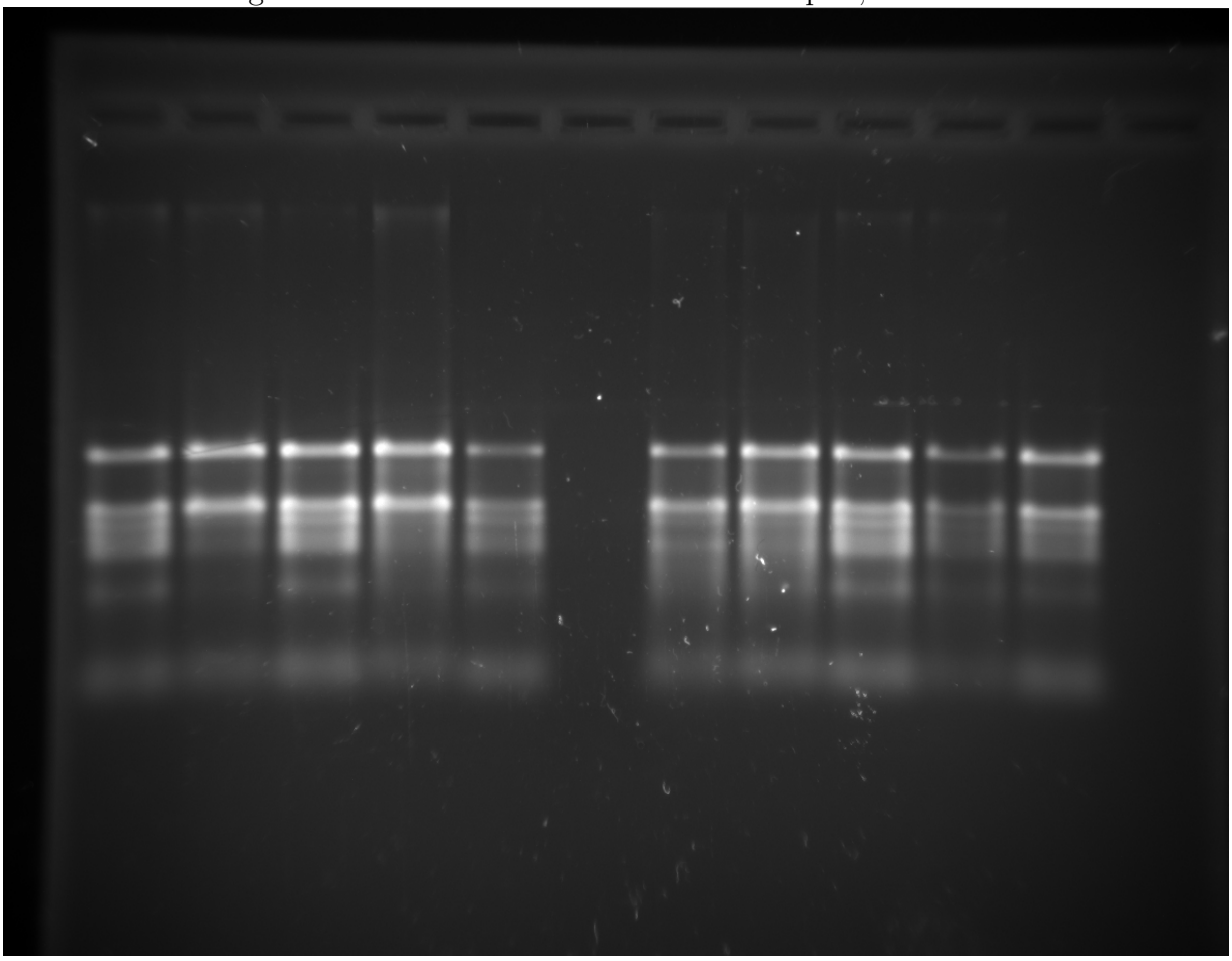
See Figure 5.1 below.
Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a mis-loading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

5.3 MOPS gel

5.3.1 Aim

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

Figure 5.1: TBE Gel of Practice RNA samples, 2013-01-22



5.3.2 Method

- Load samples after denaturing as above. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7.
- Run gel in RNAase 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.
- De-stain on orbital shaker gel in 1x MOPS, and photograph. Gel disintegrated whilst de-staining.

5.3.3 Results

See Figures 5.2 and 5.3
Mops gel confirms that the RNA was of reasonable quality. The MOPS gel appears to be of less use than the TBE gel.

Figure 5.2: MOPS Gel of Practice RNA samples, 2013-01-22

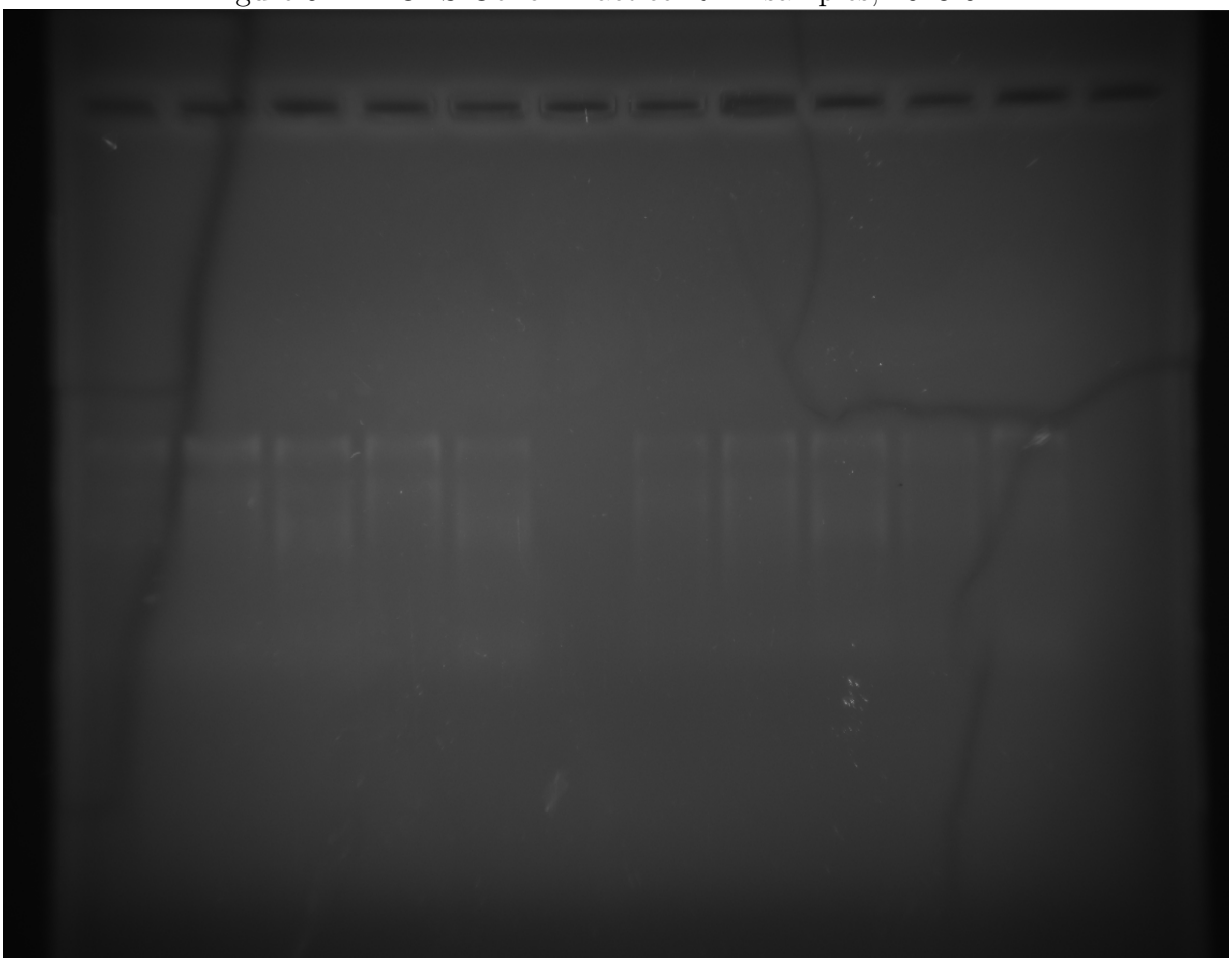
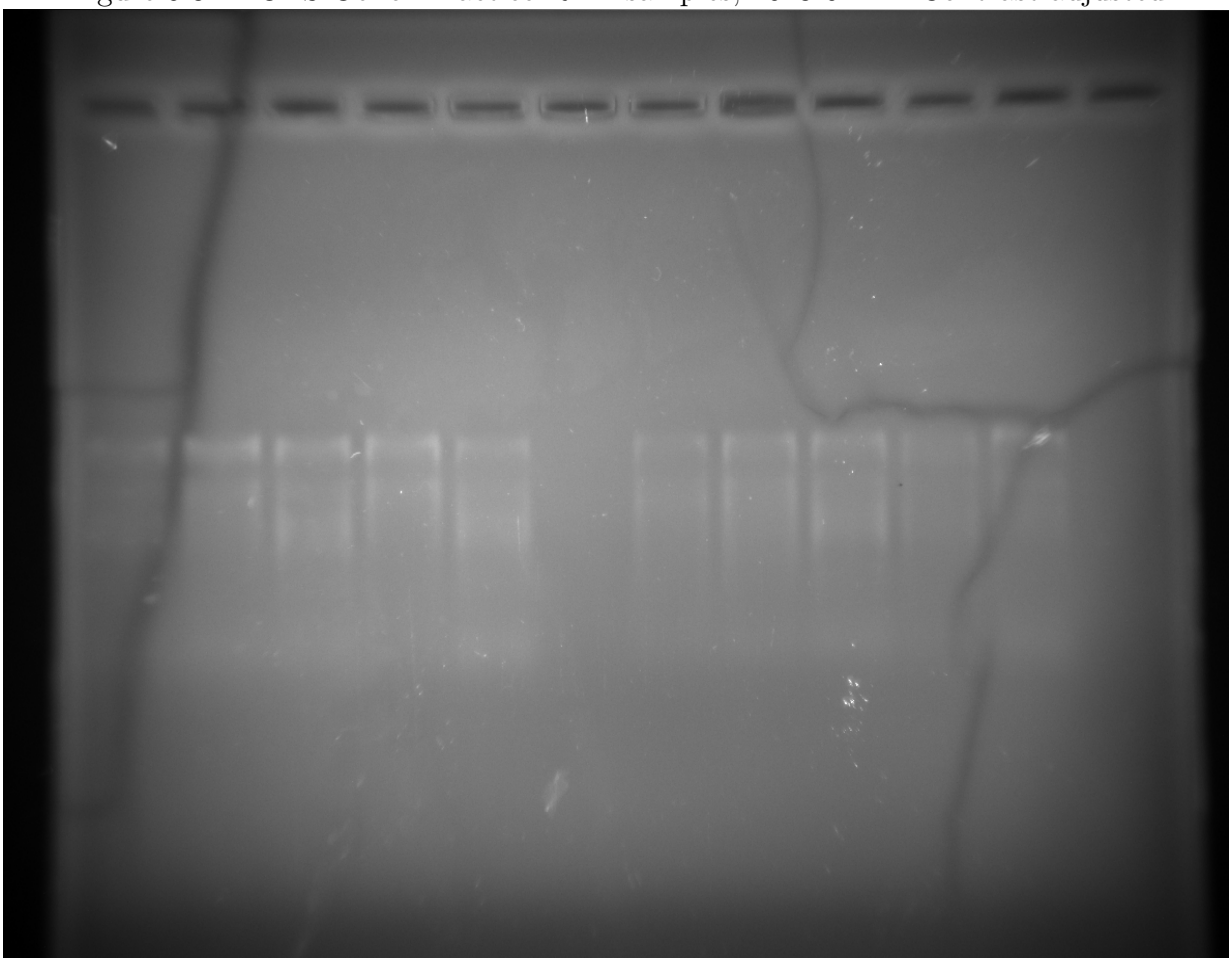


Figure 5.3: MOPS Gel of Practice RNA samples, 2013-01-22. Contrast adjusted.



6 Tue 2013-01-29

6.1 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
1	Col-0_1915	not in box
2	Col-0_4936	not in box
3	Cvi x Cvi	0
4	1 x Cvi	not in box
5	1 x 146	0
6	10 x 26	0
7	101 x 176	0
8	Ll-1	not in box
9	105 x 145	not in box
10	107 x 124	not in box
11	109 x 185	not in box
12	109 x 47	not in box
13	110 x 32	?
14	112 x 30	0
15	113 x 141	0
16	114 x 3	?
17	114 x 60	0
18	115 x 126	0
19	117 x 73	?
20	118 x 108	0
21	118 x 164	0
22	119 x 177	0
23	12 x 142	0
24	122 x 42	?
25	125 x 117	0
26	128 x 6	0
27	132 x 129	not in box
28	133 x 35	0
29	134 x 29	0
30	135 x 10	0
31	135 x 140	?
32	136 x 102	0
33	165 x 137	not in box
34	139 x 162	0
35	139 x 36	0
36	14 x 4	0
37	146 x 64	not in box

Line	Desc	Count
38	147 x 50	0
39	147 x 69	0
40	149 x 165	0
41	150 x 37	0
42	152 x 42	0
43	153 x 108	0
44	153 x 20	?
45	154 x 144	0
46	156 x 166	0
47	16 x 4	0
48	16 x 66	0
49	164 x 7	not in box
50	166 x 25	0
51	168 x 22	0
52	169 x 175	not in box
53	17 x 21	0
54	170 x 24	0
55	171 x 143	0
56	174 x 34	0
57	180 x 157	?
58	183 x 118	0
59	186 x 27	0
60	187 x 190	0
61	187 x 69	not in box
62	189 x 133	0
63	19 x 173	0
64	19 x 67	0
65	190 x 176	0
66	191 x 31	not in box
67	192 x 189	0
68	20 x 138	0
69	21 x 22	0
70	24 x 171	0
71	25 x 9	0
72	26 x 74	0
73	33 x 58	not in box
74	35 x 120	0
75	38 x 35	0
76	39 x 27	not in box
77	40 x 74	0
78	npq4	not in box
79	43 x 131	not in box
80	44 x 50	0
81	45 x 23	0
82	46 x 29	0
83	48 x 160	0
84	49 x 158	0
85	5 x 172	not in box
86	5 x 188	not in box
87	51 x 111	0
88	51 x 18	0

Line	Desc	Count
89	54 x 183	0
90	55 x 18	0
91	59 x 116	0
92	6 x 131	0
93	61 x 162	0
94	63 x 151	not in box
95	7 x 46	0
96	8 x 61	not in box
97	Ler x Ler	0
98	Ler self	0
99	Cvi x Ler	0
100	Ler x Cvi	0

7 Thu 2013-02-07

7.1 Prepare Trays for Planting

Trays were filled with steamed seed raising mix, with 1mL/L Osmocote® added and mixed before dispensing. Trays were filled by pouring potting mix over tall 5cm square pots, and compacting it with hands. 41 trays of 24 and one tray of 20 pots were made.
Once trays were made, they were watered with ≈ 1.5L RO water, containing ≈ 1mL/L AzaMax™, covered with cling film and stored at 4 degrees C.

8 Fri 2013-02-08

8.1 Planting of RIX lines

8.1.1 Aim:

Plant (ideally) 9 plants of each of the RIX lines, for the experiment Keng and I will conduct

8.1.2 Method:

Seeds were planted in pre-prepared trays, by shaking from a piece of paper. Either 6 or 12 plants of each line, and some mutants, were planted in contiguous blocks. Once planted, trays were sprayed with a small amount of water and labelled by row, i.e. each row of plants consisted of one genotype, and only one pot was labelled per row. Plants were not randomised at this point. If the tray was dry, approx 0.5-1L of RO water was added.
The following table describes the lines which were planted. 12 plants of each line were planted, unless otherwise stated in the "Qty" columns below. From now on, lines will be referred to by their number in the following table.

Line Number	Line Name	Quantity
1	Col-0	
2	cvi self	
3	1 x 146	
4	10 x 26	
5	101 x 176	
6	LL-1	
7	145 x 105	
8	107 x 121	
9	18 x 109	
10	109 x 48	
11	114 x 3	6
12	110 x 32	6
13	112 x 30	
14	115 x 12	
15	118 x 108	
16	118 x 164	
17	12 x 142	
18	122 x 42	
19	125 x 117	
20	128 x 6	
21	129 x 132	
22	133 x 35	
23	134 x 29	
24	135 x 10	
25	136 x 102	

Line Number	Line Name	Quantity
26	165 x 137	
27	139 x 36	
28	14 x 4	
29	146 x 64	
30	147 x 50	
31	147 x 69	
32	149 x 165	
33	150 x 37	
34	152 x 42	
35	153 x 108	
36	153 x 20	6
37	156 x 106	
38	119 x 60	6
39	16 x 4	
40	16 x 66	
41	16 x 7	
42	166 x 25	
43	168 x 22	
44	17 x 21	
45	171 x 143	
46	174 x 34	
47	180 x 157	
48	186 x 27	
49	187 x 69	
50	19 x 173	
51	19 x 67	
52	190 x 176	
53	191 x 31	
54	192 x 189	
55	20 x 138	6
56	24 x 171	
57	15 x 9	
58	26 x 74	
59	33 x 58	
60	38 x 35	
61	39 x 27	
62	44 x 50	
63	45 x 23	
64	46 x 29	
65	48 x 160	
66	49 x 158	
67	5 x 172	
68	5 x 188	
69	51 x 111	
70	51 x 18	
71	54 x 183	
72	55 x 18	
73	59 x 116	
74	6 x 131	
75	61 x 162	
76	63 x 151	

Line Number	Line Name	Quantity
77	8 x 61	
78	ler self	
79	ler	
80	cvi x ler	
81	ler x cvi	
82	npq1	
83	npq4	
84	pgr5	
85	Stn8-1	
86	Cvi-1	
87	135 x 142	6

9 Thu 2013-02-14

9.1 Sun and Shade Spectra

9.1.1 Aim

Measure specta from natural sun and natural shade at midday

9.1.2 Method

- John Evan's spectroradiometer was used
- Measure every nm from 400 to 800
- Measurements taken at approx 12:30-1pm
- Measure clear, unobstructed sun with no clouds in quadrangle between forrestry, geography and Robertson buildings, ANU.
- Measure shade under elm tree in same location
- Calculations made by Pip Wilson, yielded umol photon per square meter per second per nanometer measures of intesnity. (see attached xls spreadsheet).

9.1.3 Results

Overall PAR integrations were 38.0 and 1809.5 uE for shade and sun respectively.
Spectra detailed in:

- `20130214-shade and sun spectra.xlsx`
  MD5SUM db67505144fbd20ecc317a494f80ecde
- `20130214-SunShadeSpectra.csv`
  MD5SUM 56003985e19111288384dcd5f4dc51f1

10 Fri 2013-03-01

10.1 Creation of Solarcalc files

10.1.1 Aim:

Generate the solar calc files which will be used to control the conviron growth cabinents and heliospectra lights for the duration of the latter part of the experiment.

10.1.2 Method

- SolarCalc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
- Location: Temora
- Min Temp: 5 C
- Max Humid.: 80
- Start Date: 1/9/12
- End Date: 31/12/12
- Shading: 0
- LED Ratios: 7.74 6.16 5.98 5.64 7.35 1.00 5.45
- 2010 weather

10.1.3 Results

- Solarcalc output:
  `./2013-03/20130301-KMTemora2012Sep01_A_LED-Normalised.csv`
  MD5sum b1dee0ba373a1e74956dd5c8d3ccce38
- Solarcalc preferences file:
  `./2013-03/20130301-KMTemora2012Sep01_A_LED-Normalised_prefs.srp`
  MD5SUM d01fcc5ae9ad8fba07bd9b9512c82857

11 Tue 2013-03-05

11.1 Creation of Better Normalised Shaded Solarcalc files

11.1.1 Aim:

Generate the solar calc files which will be used to control the conviron growth cabinents and heliospectra lights for the duration of the latter part of the experiment.

11.1.2 Method

- SolarCalc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
- Location: Temora
- Min Temp: 10 C
- Max Humid.: 70
- Start Date: 1/9/12
- End Date: 31/12/12
- Shading: 0 and 45%
- LED Ratios: 1.29 1.03 .94 1.22 0.17 .91
- 2010 weather

11.1.3 Results

Solarcalc outputs:

- `./2013-03/20130305-Temora2012Sep01_45shade_2010weather_LED-BetterNormalised.csv` ddff182b78d1fe10e662fb827a179804
- `./2013-03/20130305-Temora2012Sep01_0shade_2010weather_LED-BetterNormalised.csv` d41d8cd98f00b204e9800998ecf8427e

Solarcalc preferences files:

- `./2013-03/20130305-Temora2012Sep01_0shade_2010weather_LED-BetterNormalised.srp` 9e800ba2b31a5346b37bc17127515d38
- `./2013-03/20130305-Temora2012Sep01_45shade_2010weather_LED-BetterNormalised.srp` d40480b773fdaad8ecf9bd14a5d445ed

12 Thu 2013-03-07

12.1 Creation of Brighter Normalised Shaded Solarcalc files

12.1.1 Aim:

Generate the solar calc files which will be used to control the conviron growth cabinents and heliospectra lights for the duration of the latter part of the experiment.
These files are to increase the brightness of the high light treatment.

12.1.2 Method

- SolarCalc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
- Location: Temora
- Min Temp: 10 C
- Max Humid.: 70
- Start Date: 1/9/12
- End Date: 31/12/12
- Shading: 0
- LED Ratios:
  2x: 2.6 2.06 2 1.96 2.44 0.34 1.82
  4x: 5.2 4.12 4 3.92 4.88 0.68 3.64
- 2010 weather

12.1.3 Results

Solarcalc outputs:

- `./2013-03/20130307-KMTemora2012Sep01_0shade_2010weather_LED-2xBetterNormalised.csv` 59fcd4aacdf20eeb750c22b18ba4f118
- `./2013-03/20130307-KMTemora2012Sep01_0shade_2010weather_LED-4xBetterNormalised.csv` 3d79e511b4367d1c5807b247fffa3b48

Solarcalc preferences files:

- `./2013-03/20130307-KMTemora2012Sep01_0shade_2010weather_LED-2xBetterNormalised.srp` bf276b2cf5658f00fbd02bce55f186f7
- `./2013-03/20130307-KMTemora2012Sep01_0shade_2010weather_LED-4xBetterNormalised.srp` 3f124559d1553f68384218509196992d

13 Sun 2013-03-10

13.1 Creation of Intermittent Bright/Shaded Solarcalc files

13.1.1 Aim:

Generate the solar calc files which will be used to control the conviron growth cabinents and heliospectra lights for the duration of the latter part of the experiment.
These files are to increase the brightness of the high light treatment.

13.1.2 Method

- SolarCalc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
  - Location: Temora
  - Min Temp: 10 C
  - Max Humid.: 70
  - Start Date: 1/9/12
  - End Date: 31/12/12
  - Shading: High Light file: 0, Low Light files: 45
  - LED Ratios:
    1x: 1.29 1.03 .94 1.22 0.17 .91
    2x: 2.6 2.06 2 1.96 2.44 0.34 1.82
  - 2010 weather
- Then, the spliceSolarCalc.py script was used to splice between them, using the command line:

```
python spliceSolarCalc.py -o KMTemora2012Sep01_Intermittent2hLL1hHL.csv \
-f 2013-03/20130305-Temora2012Sep01_45shade_2010weather_LED-BetterNormalised.csv \
-F 2013-03/20130307-KMTemora2012Sep01_0shade_2010weather_LED-2xBetterNormalised.csv \
-1 120 -2 60 # 2h Low, 1h High
```

13.1.3 Results

Solarcalc inputs:

- `./2013-03/20130305-Temora2012Sep01_45shade_2010weather_LED-BetterNormalised.csv` ddff182b78d1fe10e662fb827a179804
- `./2013-03/20130307-KMTemora2012Sep01_0shade_2010weather_LED-2xBetterNormalised.csv` 59fcd4aacdf20eeb750c22b18ba4f118

Solarcalc preferences files:

- `./2013-03/20130305-Temora2012Sep01_45shade_2010weather_LED-BetterNormalised.srp` d40480b773fdaad8ecf9bd14a5d445ed

- `./2013-03/20130307-KMTemora2012Sep01_0shade_2010weather_LED-2xBetterNormalised.srp` bf276b2cf5658f00fbd02bce55f186f7

Solarcalc Output:

- `2013-03/20130310-KMTemora2012Sep01_Intermittent2hLL1hHL.csv` c415ed3654b071ad6fbcbd044b60f732

Solarcalc Splice Script:
`./2013-03/20130310-spliceSolarCalc.py` 6cd8ea9895d19b7851c41c086535f318

14 Fri 2013-03-19

14.1 Enter plants in trayscan database

14.1.1 Aim

Allow trayscan to see and process out plants

14.1.2 Method

Using the tray registrator, the attached csv was registered in the database. The registration may have failed, PSI will fix the database to reflect it.

14.1.3 Results

`./2013-03/20130320-TrayscanImportSheet.csv`
`MD5SUM: 69b97ec5cd3f30c10c0d7b21da59a35b`

15 Mon 2013-03-25

15.1 Harvesting of practice tissue

15.1.1 Aim

Harvest unimportant tissue from extra plants to use for pracice RNA preps.

15.1.2 Method

- Plants had been grown since germination in convirons 8 & 9 under standard growth conditions
- Leaves were harvested from each plant into a 96-well 1.2mL plate on dry ice
- the plant number as per ./2013-02/20130208-KMPlantedLines.csv was recorded

15.1.3 Results

- The plate was labelled "Harvest Tissue Plate", and stored in -80.
- The samples are laid out as described below:

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	62	50	48 med lf	46	54	46						
B	62	50	48 lrg lf	46	54	46						
C	23	50	19	21	46	46						
D	48	???	61	61	46	46						
E	48	27	61	61	46	46						
F	48	23 caul	61	25	46	46						
G	48	23 caul	61	25	46	46						
H	48	48 sml	61	16	46	46						

16 Tue 2013-03-26

16.1 Harvesing the First Replicate across each chamber

16.1.1 Aim

Harvest the actual experimental samples. Today, the first replicate will be harvested.

16.1.2 Method

- Leaves were taken from plants grown under experiment conditions (recorded by Keng, to be summarised later)
- Leaves were excised and genly rolled (where possible) between fingers, without breaking or squashing them. This allowed them to unroll to fill the edges of the wells in most cases, for faster freezing.
- Approx 1.5x1.5cm sections were taken when leaves exceeded this size. Tip end of leaves were taken if a leaf was torn. Leaves were torn once rolled by quckly plucking them with tweezers, using tweezer blades to cut the leaf.
- Harvesting started at 12:00. Detailed in the table below.

Time	Chamber	Condtion
12:00:00	2	0h
12:40:00	5	0h
13:00:00	4	0h
13:40:00	2 tray 1	1h HL (1h05 in HL)
13:55:00	5 trays 2 and 3	1h HL (30m in HL)
14:05:00	2 tray 2 and 3	1h HL (1h25m in HL)
14:15:00	5 tray 1	1h HL (1h15m in HL)
14:30:00	2	0h
14:45:00	4	0h
15:00:00	5	0h
15:40:00	2	1h HL (1h10m in HL)
16:00:00	4	1h HL (1h15m in HL)
16:15:00	5	1h HL (1h15m in HL)

16.1.3 Results

The plate layout is described below. Abbreviations are: S, small, 2S, 2x small (and so on), C, cauline leaves, sq, squashed leaf while harvesting, sen, some senescence on harvested leaf (only a small amount of senescence was allowed), P, purple/anthocyanin accumulation.
Plate 1:

	1	2	3	4	5	6	7	8	9	10	11	12
A	2 1a1	2 1c1 S ???	2 1e4	2 2c3	5 1a1	5 1c3	5 2a2 Pur	5 3c1	5 2e4	4 1d1	4 3b1	4 3e1 M
B	2 1b1	2 1c3	2 3b1	2 3d1	5 1b1	5 1c4	5 3a3	5 2c4	4 1a1	4 1d2	4 2b2 sq	4 2d3
C	2 1a3 sen	2 1d1 S	2 2a3	2 2d2	5 1a3	5 1d3	5 2a4 L	5 2d1	4 1a2 CL	4 1d4 L	4 2b3 sq	4 2d3
D	2 1b4	2 1d2 L	2 2a4	2 3e1	5 1a4	5 1d4	5 2b1	5 2d3	4 1a3	4 1e1 L	4 2b4 2S	4 2d4
E	2 1b2 L	2 1d3 L	2 2b2 L	2 2e1	5 1a2	5 1e1	5 2b2	5 2d4 sq	4 1b1	4 1e3	4 2c1	4 3a1
F	2 1b3 S	2 1e1	2 2b3	2 2e2	5 1b2	5 1e2	5 3b1	5 2e1	4 1b2 CL	4 1e4	4 2c3 sq	4 3d1 sq
G	2 1c4 L	2 1e2	2 2b4	2 2e3	5 1b4	5 1e3	5 3b4	5 3e1	4 1b4	4 2a1 L	4 2c4	4 2e4 sq
H	2 1c1	2 1e3	2 2c1	2 2e4	5 1c1	5 2a1	5 2c1	5 3d1	4 1c1 2S	4 2a3 basal ½	4 2d1	4 3c1 sq

Plate 2:

	1	2	3	4	5	6	7	8	9	10	11	12
A	2 1a1	2 1c2 sq	2 1e4 CL	5 2b4	5 3e1	2 2b4	2 2e3	5 1b4	5 1e2	4 1c1 sq	4 2a3	4 2d1
B	2 1a3	2 1c3 2S	5 2a1	5 2c1 CL	5 3d1 ½	2 2c1	2 2e4	5 1c1	5 1e3	4 1d1	4 3b1 sq ½L	4 2e1 ½L
C	2 1b4	2 1a4 L CL sq	5 2a2 2S	5 3c1 L	5 2e4	2 2c3	5 1a1 sq ½L	5 1c3 2S sq	4 1a1	4 1d2 ½L sq	4 2b2	4 2d3 ½L sq
D	2 1b1	2 1d2 3S	5 3a1 3S	5 2c4	2 3b1	2 3d1	5 1b1 CL	5 1c4	4 1a2 CL sq	4 1d4 ½L	4 2b3	4 2d4 ½L sq
E	2 1b2	2 1d3 M	5 2a4	5 2d1	2 2a3	2 2d2	5 1a3 ½L	5 1d3	4 1a3	4 1e2 ½L	4 2b4	4 3a1 ½L
F	2 1b3	2 1e1 M	5 2b1 CL	5 2d3	2 2a4	2 3e1	5 1a4	5 1d4 sq	4 1b1 3S	4 1e3	4 2c1	4 3d1
G	2 1b4 2S	2 1e2 sq	5 2b2 CL	5 2d4	2 2b2	2 2e1 CL sq	5 1a2 L sq	5 1e1 CL	4 1b2 2S	4 1e4	4 2c3	4 3c1 sq
H	2 1c1	2 1e3	5 3b1	5 3e1 2S 1CL	2 2b3	2 2e2	5 1b2 ½L	5 1e1 S	4 1b4 ½L	4 2a1	4 2c4	4 2e4

Plate 3:

	1	2	3	4	5	6	7	8	9	10	11	12
A	2 8a1	2 8c2	2 9a1	2 9c1 ½	2 9e1	4 8b3	4 9a3 C	4 9e3	5 8d1	5 9a3 2S	5 9d2	
B	2 8a2 sq	2 8c3 ½L	2 9a2 ½L	2 9c3 ½ sq	2 9e2	4 8b4	4 9b3 2SC	5 8a3 ½	5 8d2	5 9a4 ½L	5 9d3	
C	2 8a3 sq	2 8c4	2 10c1	2 9c4 2S	2 9e3 sq	4 8c1	4 9b4 ½L	5 8b1 sq	5 8d3	5 10b1 2S	5 9d4 2C	
D	2 8a4	2 8d1	2 10b1 sq	2 9d1 sq	2 9e4	4 8c4 2S	4 10a1 2S	5 8b2	5 8e2	5 10e1 2S	5 10B1 2S	
E	2 8b1	2 8d2 ½L	2 9b1	2 9d2 ½	4 8a1 sq	4 8d1 2S	4 9c3	5 8b3	5 8e3	5 9b4	5 9e2	
F	2 8b2	2 8d4	2 10a1 ½L	2 9d3	4 8a3	4 8e1	4 9d1 2M	5 8b4 sq	5 8e4	5 10c1	5 9e3	
G	2 8b4	2 8e1	2 10d1 ½L	2 9d4 sq	4 8b1	4 10e1	4 9d4	5 8c4	5 9a1	5 9c3	5 9e4	
H	2 8c1	2 8e3 ½L	2 9b4 ½L		4 8b2	4 9a2 2S	4 9e1		5 9a2	5 9d1		

Plate 4:

	1	2	3	4	5	6	7	8	9	10	11	12
A	2 8a1	2 8c2	2 9A1	2 9b4	2 9e1 C	4 8b3 2S	4 9a3	4 9e3	5 8d1	5 9a3	5 9d2	
B	2 8a2 sq	2 8c3 ½L	2 9a2	2 9c1 ½	2 9e2	4 8b4	4 9b3	5 8a3	5 8d2	5 9a4	5 9d3	
C	2 8a3	2 8c4 2S sq	2 10c1	2 9c3 ½	2 9e3 C	4 8c1	4 9b4	5 8b1	5 8d3 2S	5 10b1 a1	5 9d4 C	
D	2 8a4	2 8d1 2S	2 10b1	2 9c4 2S	2 9e4	4 8c4 C	4 10a1 2S	5 8b2 ½	5 8e2 ½	5 10e1 2S	5 10d1 2S sq	
E	2 8b1 sq 3S	2 8d2 ½L	2 9b1	2 9d1	4 8a1 ½	4 8d1 2S	4 9c3 ½	5 8b3 C	5 8e3	5 9b4	5 9e2	
F	2 8b2 2S	2 8d4	2 10a1	2 9d2	4 8a3	4 8e1	4 9d1 2S	5 8b4 ½	5 8e4 2S	5 10c1	5 9e3 2C	
G	2 8b4 2S	2 8e1 ½	2 10d1 ½	2 9d3 sq	4 8b1 sq	4 10e1 ½ sq	4 9d4	5 8c2	5 9a1 sq	5 9c3	5 9e4	
H	2 8c1	2 8e3 2S	2 9b4 ½	2 9d4	4 8b2	4 9a2 3S	4 9e1	5 8c4 2S	5 9a2	5 9d1		

17 Wed 2013-03-27

17.1 Harvesing the Second Replicate across each chamber

17.1.1 Aim

Harvest the actual experimental samples. Today, the second replicate will be harvested.

17.1.2 Method

- Leaves were taken from plants grown under experiment conditions (recorded by Keng, to be summarised later)
- Procedure was as per yesterday
- Leaves were excised and genly rolled (where possible) between fingers, without breaking or squashing them. This allowed them to unroll to fill the edges of the wells in most cases, for faster freezing.
- Approx 1.5x1.5cm sections were taken when leaves exceeded this size. Tip end of leaves were taken if a leaf was torn. Leaves were torn once rolled by quckly plucking them with tweezers, using tweezer blades to cut the leaf.
- Harvesting started at 11:30. Detailed in the table below.

Time	Chamber	Condtion
11:30:00	4 part 1	0h
11:45:00	5 p1	0h
12:00:00	2 p1	0h
12:15:00	4 p2	0h
12:30:00	5 p2	0h
12:45:00	2 p2	0h
13:25:00	4 p1	1hHL
13:40:00	5 p1	1hHL
14:00:00	2 p1	1hHL
14:15:00	4 p2	1hHL
14:30:00	5 p2	1hHL
14:45:00	2 p2	1hHL

17.1.3 Results

The plate layout is described below. Abbreviations are: S, small, 2S, 2x small (and so on), C, cauline leaves, sq, squashed leaf while harvesting, sen, some senescence on harvested leaf (only a small amount of senescence was allowed), P, purple/anthocyanin accumulation.
Plate 5:

	1	2	3	4	5	6	7	8	9	10	11	12
A	4 4a1	4 4c4	4 3b1	4 3e3 S	5 3b4 P	5 4a2	5 4d1 ½	5 4c1	2 3b4 C	2 4a2 2S	2 4d2	2 5c1 ½
B	4 4a2 C	4 4e1	4 3b4	4 3e4	5 3c3 P	5 4a3 ½ C	5 4d2	5 4c2 ½	2 3c2	2 4a3	2 4d3	2 5c2
C	4 4a3 sq	4 4e3	4 5b1 sq	4 5e1 sq	5 3d2	5 4b1	5 4d3 P	5 4d1 sq	2 3c4	2 4a4 sq	2 4e1	2 5d1
D	4 4a4	4 3c2	4 5b2 sq	4 5e2	5 3d4 ½	5 4b2	5 4d4 P	5 4d2 ½ sq	2 3d3 ½	2 4b1 ½	2 4e3 sq	2 5d2
E	4 4b3	4 3a3	4 5c4	5 3a2	5 3e2 ½ sen	5 4b4 sq	5 4e2	5 4e2 ½	2 3d4	2 4b3	2 4e4 ½	2 5e1
F	4 4b4	4 3a4	4 5c2	5 3a3	5 3e3 1/3 sq	5 4c1 P	5 4e3	2 3a3	2 3e2 sq	2 4c1	2 5a1	2 5e2 2v.sml
G	4 3b2	4 5a1 sq	4 5d1 sq	5 3b2	5 3e4	5 4c1 ½ sq	5 4e4	2 3a4 ½	2 3e4	2 4c3	2 5a2	
H	4 4c3	4 5a2 sq	4 5d2 ½	5 3b3 ½	5 4a1 C	5 4c4 ½	5 5a1 ½	2 3b2 ½	2 4a1	2 4c4 ½ sq	2 5b1 sq C	

Plate 6:

	1	2	3	4	5	6	7	8	9	10	11	12
A	4 10a2	4 10d3 sen	4 11c2 sq ½	5 10a3 P sen	5 10d4	5 11c4	5 12c1 ½	2 10c2	2 11a2	2 11d4	2 12d1	
B	4 10a3	4 10d4 C	4 11e2 ½	5 10b2 ½	5 10e2	5 11d3	5 12c2	2 10c3	2 11a3	2 11e2	2 12d2	
C	4 10a4 sq	4 10e2 ½	4 11e4 ½	5 10b3	5 10e3	5 11d4	5 12d1	2 10c4	2 11b1	2 11e3	2 12e1	
D	4 10b2	4 10e3	4 12b1	5 10a4 p sq	5 11a1	5 11e1	5 12d2	2 10d3	2 11b2	2 12a2	2 12e2	
E	4 10b3	4 11a1	4 12d1	5 10c2	5 11a2	5 11e2 ½ sen	2 10a2	2 10d4	2 11c1	2 12b1		
F	4 10b4 ½ sq	4 11a3 C	4 12e1 ½ eq	5 10c3	5 11b1	5 12a2 sq	2 10a3	2 10e2	2 11c3	2 12b2		
G	4 10c2 ½	4 11a4 ½	5 10a2 ½	5 10d2 ½ sq	5 11b2 sq	5 12b1 sq	2 10a4	2 10e3	2 11d1	2 12c1		
H	4 10d2	4 11b1 2S	5 10a3 C	5 10d3	5 11b4	5 12b2 ½	2 10b4	2 10e4	2 11d3	2 12c2		

Plate 7:

	1	2	3	4	5	6	7	8	9	10	11	12
A	4 4a1	4 4b4	4 3b3	4 3e3 2S	5 3b3	5 4a1 P	5 4c4 S	5 4e4 2S	2 4a2 2S	2 4d2 sq	2 3b4 2S	2 5a2
B	4 4a2 sen	4 4e1	4 5e1 2S sen	4 5e1 sq	5 3b4 P	5 4a2	5 4d1 ½	5 5a1	2 4a3 2S	2 4d3 sq	2 3c2 ½ sen	2 5b1 S
C	4 4a3 sq	4 4e2	4 5b1	4 5e2 ½	5 3c3 P	5 4a3 ½	5 4d2 3S sq	5 5c1	2 4a4 2S	2 4e1	2 3c4 2S	2 5c1
D	4 4a4	4 3c2	4 5b2	5 3a2 sq	5 3d2 2S	5 4b1	5 4d3	5 5c2 ½	2 4b1 ½	2 4e3	2 3d3 ½	2 5c2
E	4 4b3 sq	4 3a3	4 3c4	5 3a3 sq sen	5 3d4 sq	5 4b2	5 4d3 P ?	5 5d1	2 4b3	2 4e4	2 3d4 ½shaded	2 5d1
F	4 4b4 sq	4 3a4 sq	4 5c2	5 3a3 2S	5 3e2 ½	5 4b4	5 4d4 P	5 5d2 ½	2 4c1	2 3b2 sq ½	2 3e2 wounded	2 5d2
G	4 4b2	4 5a1	4 5d1	5 3b2	5 3e3 ½ sq	5 4c1 P	5 4e2 caul	5 5e2 ½	2 4c2	2 3a3	2 3e4	2 5e1
H	4 4b3 sq	4 5a2 ½ sq	4 5d2	?	5 3e4 2S	5 4c3	5 4e3 sq	2 4a1	2 4c4 ½	2 3a4	2 5a1 ½	2 5e2 3S

Plate 8:

	1	2	3	4	5	6	7	8	9	10	11	12
A	4 11a1	4 12d1 ½	4 10c2 ½	5 10a3 2S	5 10d4 ½	5 11c4 ½	5 12b2	2 11b2	2 10a2 ½	2 10d4 caul	2 12c2 ½	
B	4 11a3 C	4 12e1 ½	4 10d2 sq	5 10a4 P	5 10e2 ½	5 11d3	5 12c1	2 11c1 ½	2 10a3	2 10e2 ½	2 12d1	
C	4 11a4 ½	4 10a2	4 10d2 sen	5 10b2	5 10e3	5 11d4 S sen	5 12c2	2 11c3	2 10a4 ½	2 10e3 ½	2 12d2	
D	4 11b1 2S	4 10a3	4 10d2 caul	5 10b3	5 11a1 sq	5 11d4	5 12d1	2 11d1 ½	2 10b4	2 10e4 ½	2 12e1 ½	
E	4 11c2 ½	4 10a4 sq	4 10d4 dam	5 10c2 ½	5 11a2 sq	5 11e1 sq	5 12d2	2 11d3 ½	2 10c2 sq	2 12a2 ½	2 12e2 ½ sq	
F	4 11e2 ½	4 10b2	4 10e2	5 10c3	5 11b1 ½	5 11e2	2 11a2 ½	2 11d4 ½	2 10c3	2 12b1		
G	4 11e4	4 10b3 S	4 10e3	5 10d2 ½	5 11b2	5 12a2	2 11a3 ½	2 11e2 ½	2 10c4	2 12b2		
H	4 12b1	4 10b4 ½	5 10a2 ½	5 10d3 ½	5 11b4 ½ sq	5 12b1	2 11b1 sen	2 11e3	2 10d3 ½	2 12c1		

18 Thu 2013-03-28

18.1 Harvesing the Third Replicate across each chamber

18.1.1 Aim

Harvest the actual experimental samples. Today, the third replicate will be harvested.

18.1.2 Method

- Leaves were taken from plants grown under experiment conditions (recorded by Keng, to be summarised later)
- Procedure was as per yesterday
- Leaves were excised and genly rolled (where possible) between fingers, without breaking or squashing them. This allowed them to unroll to fill the edges of the wells in most cases, for faster freezing.
- Approx 1.5x1.5cm sections were taken when leaves exceeded this size. Tip end of leaves were taken if a leaf was torn. Leaves were torn once rolled by quckly plucking them with tweezers, using tweezer blades to cut the leaf.
- Harvesting started at 11:30. Detailed in the table below.

StartTime	Chamber	Tray	Condtion
1135	5	5+6	0h
1140	5	7	0h
1150	2	6	0h
1155	2	5+7	0h
1203	4	5	0h
1205	4	6	0h
1210	4	7	0h
1217	5	12	0h
1223	5	13	0h
1229	5	14	0h
1235	2	12	0h
1237	2	13	0h
1242	2	14	0h
1255	4	12	0h
1258	4	13	0h
1300	4	14	0h
1252	5	5+6	1hHL (1h05)
1257	5	7	1hHL (1h10)
1305	2	5+7	1hHL (1h05)
1310	2	6	1hHL (1h10)
1318	4	6	1hHL (1h03)
1330	4	7	1hHL (1h15)
1423	5	14	1hHL (1h10)
1430	5	13	1hHL (1h15)
1440	5	12	1hHL (1h15)
1455	2	12	1hHL (1h0)
1500	2	13	1hHL (1h05)
1505	2	14	1hHL (1h10)
1517	4	12	1hHL (57m)
1520	4	13	1hHL (1h0)
1526	4	14	1hHL (1h06m)

18.1.3 Results

The plate layout is described below. Abbreviations are: S, small, 2S, 2x small (and so on), C/caul, cauline leaves, sq, squashed leaf while harvesting, sen, some senescence on harvested leaf (only a small amount of senescence was allowed), P, purple/anthocyanin accumulation.
Plate 9:

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 5a3 dam	5 5e3	5 7b3	2 6a2	2 6e1 ½	2 7c2 ½ sq	4 5e3 ½	4 6d3	4 7b2 ½			
B	5 5a4	5 5d3 1/3 sq	5 7c1 ½	2 6a3 ½	2 6e2 ½	2 7d3 basal	4 5e4 ½	4 6d4 ½	4 7c1			
C	5 6a3 ½	5 6d4 2S	5 7c2 1/3	2 6a4	2 5b3 ½	2 7d3 ½	4 6a1 ½ sq	4 6e1 ½	4 7c2 dam			
D	5 6a4 P	5 6e1	5 7c3 ½	2 6b2	2 5c4	2 7e1	4 6a4	4 6e3 ½	4 7e2			
E	5 5b3 ½	5 5e4 P	5 7d2 ½	2 6b3	2 5d4	2 7e2	4 6b1 ½	4 6e4				
F	5 5c4 sq	5 6e4	5 7e2 P	2 6b4 ½	2 7b1 ½	2 7e3 ½ sq	4 6c1 ½	4 7a1				
G	5 6b4	5 7a2 ½	5 7e3 ½ sq P	2 6c2	2 7b3 ½	4 5a4 ½	4 6c3 ½ dam	4 7a3 ½ sq				
H	5 6c1	5 7a3 P	2 6a1	2 6d4	2 7c1 ½	4 5d4 ½	4 6c4 S	4 7b1 sq				

Plate 10:

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 12a3 ½	5 13a4 P	5 13d2 ½	5 14d2 ½ P	2 13a1	2 13d2 ½	2 14c2 ½	4 12c4	4 13e1	4 14e2		
B	5 12a4	5 13b1 2S	5 13e2	5 14d3 S	2 13a3	2 13e1 dam	2 14c3	4 12e3	4 13e2	4 14e3		
C	5 12b4	5 13b2 P	5 13e4	2 12a3 ½	2 13a4	2 13e3 ½	2 14d2	4 12e4	4 13e4			
D	5 12d3 ½	5 13b3 P	5 14a2 P	2 12a4	2 13b1 3S	2 13e4	2 14d3 ½	4 13a2	4 14c1			
E	5 12d4 ½	5 13c1 dam	5 14b1 ½	2 12b3 2x	2 13b3 ½	2 14a1	2 14e2 sq	4 13b1	4 14c3			
F	5 12e3	5 13c2	5 14b2 ½ dam	2 12c3 ½ sq	2 13b4 2S	2 14b2	2 14e3	4 13b2	4 14d1			
G	5 13a1	5 13c3 2S	5 14b3 ½ P	2 12c4	2 13c3 ½	2 14b3 sq	4 12b3	4 13c2	4 14d2			
H	5 13a2 P	5 13c4 ½	5 13c3 ½	2 12d4 ½	2 13d1 ½	2 14c1 ½	4 12c3	4 13d3	4 14e1			

Plate 11:

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 5a3 S	5 5e3	5 7b3 sq	2 5b3	2 7e1	2 6b3	4 6a4 2S	4 6e2 ½	4 7e2 juv			
B	5 5a4	5 5d3 ½ dam	5 7c1 ½	2 5c4 ½	2 7e2	2 6b4 ½	4 6b1 ½	4 6e4 2C	4 7e2			
C	5 6a3 ½	5 6d4	5 7c2 ¼	2 5d4 2S 1caul	2 7e3 ½	2 6d4 sq	4 6c1	4 7a1	4 5a4 ½			
D	5 6a4 P	5 6e1 ½	5 7c3 ½	2 7b1	2 6a1 ½	2 6d4	4 6c3	4 7a3 ½	4 5d4			
E	5 5b3 ½	5 5e4 P	5 7d2 ½ sq	2 7b3 ½	2 6a2	2 6c2 2S	4 6c4	4 7b1 sq	4 5e3			
F	5 5c4	5 6e4 sq	5 7e2 P	2 7c1 ½	2 6a3	2 6e1	4 6d3 sq	4 7b2 ½	4 5e4 ½			
G	5 6b4 ½	5 7a2 ½ P	5 7e3 juv	2 7c2 ½	2 6a4 caul	2 6e2	4 6d4 ½	4 7c1				
H	5 6c1	5 7a3 P	5 7e3 P	2 7d3 ½	2 6a4 2S	4 6a1 ½	4 6e1 sq	4 7c2 2S sq				

Plate 12:

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 14a2 p	5 13a1	5 13c2	5 12a3 juv	2 12b3 ½ shaded	2 13b1 2S sq	2 13e3 ½	2 14c2 ½	4 12c3 ½ sen	4 13b2 ½ sq	4 13e4 sen	4 14e2 2S
B	5 14b1 ½	5 13a2 P	5 13c3 2S	5 12a4 ½ sq	2 12b3 juv	2 13b3 ½	2 13e4 sq	2 14c3	4 12c4 ½	4 13c2	4 13e4 dam	
C	5 14b2	5 13a4 P	5 13c4	5 12b4 ½	2 12c3	2 13b4 sq	2 13e4 juv	2 14d2	4 12e3 caul	4 13d3 sq	4 14c1	
D	5 14b3	5 13b1 2S	5 13c4 juv	5 12c3	2 12c4	2 13b4	2 14a1	2 14d3 ½	4 12e3 ½ sen	4 13e1 sq	4 14c3	
E	5 14c3	5 13b2 p	5 13d2 juv	5 12d4 ½	2 12d4 ½	2 13c3 ½	2 13b2 part shaded	2 14e2	4 12e4	4 13e1 juv	4 14d1 ½	
F	5 14d2 ½ P	5 13b3 p	5 13d2 p sen	5 12e2	2 13a1	2 13d1 ½	2 14b2 juv	2 14e3 sq	4 13a2 ½	4 13e2 outer ½	4 14d2	
G	5 14d3 C	5 13b3 P juv	5 12e2	2 12a3 sq	2 13a3	2 13d2 ½	2 14b3	2 14e3	4 13b1 juv	4 13e2 inner ½ of same leaf	4 14e1 juv	
H	5 14d3 P	5 13c1	5 12e4 ½	2 12a4	2 13a4	2 13e1	2 14c1	4 12e3 2Juv	4 13b1		4 14e1 v.sen	

19 Fri 2013-04-26

19.1 Trayscan Test

19.1.1 Aim

Test the tray registrator function of the trayscan, as instructed by Petr Ent.

19.1.2 Method

A test tray was made, and placed in the trayscan for PSI to test.
The tray registration was as per the attached file:
`./2013-04/20130426-TrayscanTest.csv`
`MD5SUM: b6716d2b3c2b63adc36ef6d6a1a11104`

19.2 Corrected Trayscan Import File

19.2.1 Aim

Fix difference between the file which we imported into trayscan and the tray labels by which the phenotypic data was recorded.

19.2.2 Method

The format of the tray identifiers was changed to reflect the label format. No other changes were made.
File attachement:
`./2013-04/20130426-CorrectedTrayscanImportSheet.csv`
MD5SUM: ba68cc9c73d63b648899dac35129ace5

19.3 Count of all sampled plants

19.3.1 Aim

To determine the number of plants (for tim's abstract)

19.3.2 Method

The number of RIX, mutant and parental lines which survived to sampling week were counted.

19.3.3 Results

Chamber:	**2**	**4**	**5**
	Fluctuating	Sufficient	Excess
overall	185	148	172
rix	158	122	145
Parental	15	14	15
mutant	12	12	12

20 Mon 2013-05-06

20.1 Development of Undistortion script

20.1.1 Aim

Trial parameters to Imagemagick's convert binary to get best undistortion of barrel distortion.

20.1.2 Method

To test the different parameters to the barrel undistortion model, the following command was run, with params subsituted for the model parameters below. The original image was from the stardot 5mp webcams.
`convert -distort Barrel <params> orig.jpeg undist.jpeg`
The parameters below were tested:

- "0.0 0.0 -0.15 1.15"
- "0.0 0.0 -0.10 1.10"
- "0.0 0.0 -0.05 1.05"
- "0.0 0.0 -0.5 1.5"
- "0.0 0.0 -0.11 1.11"

20.1.3 Results

The parameters "0.0 0.0 -0.15 1.15" appeared to work best. Chromatic aberration is problematic. Will try to use fulla from the hugin toolkit.

21 Wed 2013-05-15

21.1 Creation of Caroline Chong's Solarcalc files

21.1.1 Aim

Create solarcalc files for Caroline Chong's Pelargonium.

21.1.2 Method

Using the parameters below, solarcalc output for her new pelargonium experiment was generated.
BotriverCurrent.csv:

- SolarCalc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
- Location: Botrivier -34.219136°S, 19.195707°E
- Min Temp: 5 C
- Max Humid.: 85
- Start Date: 1/11/12
- End Date: 31/12/13
- Shading: 0
- LED Ratios:
  4x: 5.2 4.12 4 3.92 4.88 1 3.64
- 2010 weather (current)

BotriverFuture.csv:

- SolarCalc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
- Location: Botrivier -34.219136°S, 19.195707°E
- Min Temp: 5 C
- Max Humid.: 85
- Start Date: 1/11/12
- End Date: 31/12/13
- Shading: 0
- LED Ratios:
  4x: 5.2 4.12 4 3.92 4.88 1 3.64
- 2040 weather

TilbaCurrent.csv:

- SolarCalc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
- Location: Tilba -36.377685°S 150.069347°E
- Min Temp: 5 C
- Max Humid.: 85
- Start Date: 1/11/12
- End Date: 31/12/13
- Shading: 0
- LED Ratios:
  4x: 5.2 4.12 4 3.92 4.88 1 3.64
- 2010 weather (current)

TilbaFuture.csv:

- SolarCalc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
- Location: Tilba -36.377685°S 150.069347°E
- Min Temp: 5 C
- Max Humid.: 85
- Start Date: 1/11/12
- End Date: 31/12/13
- Shading: 0
- LED Ratios:
  4x: 5.2 4.12 4 3.92 4.88 1 3.64
- 2040 weather

21.1.3 Results

The following files have been attached:

- ./2013-05/20130515-CarolineSolarcalcLocations.kmz md5sum 9a5be15bd5bcc854e2f27f33fb1e34e0
- ./2013-05/20130515-BotrivierCurrent.csv md5sum fbe6cea104e11eb9a35448b8be45613d
- ./2013-05/20130515-BotrivierCurrent.srp md5sum bdc0b67c73750e5c774e63d7a66aaaa1
- ./2013-05/20130515-BotriverFuture.csv md5sum 3669016016212516037162097692 3986
- ./2013-05/20130515-BotriverFuture.srp md5sum 646c0bb13b39868ba0426000af9532aa
- ./2013-05/20130515-TilbaCurrent.csv md5sum 4875aa65f83b86ead8f7008d724d54dc
- ./2013-05/20130515-TilbaCurrent.srp md5sum 878d3193b493a2ced5d69a32bfe8e3bf
- ./2013-05/20130515-TilbaFuture.csv md5sum 4ae75c0b7ff77eef4f7355cbc42fddb6
- ./2013-05/20130515-TilbaFuture.srp md5sum 3544f9404cc1687f609b674767ac2f48

21.2 Testing of tissuelyzer settings and protocol

21.2.1 Aim

Test which settings in the tissuelyzer are optimal for grinding tissue in 96 well plates.

21.2.2 Method/Results

- Remove plate containing tissue from -80, and place in tray with LN2.
- Remove silicone tray mat, and repalce with Qiagen collection tube strip caps
- As the plates did not have ball bearings, I added LN2-frozen balls to the top of each plate.
- Freeze tissuelyzer blocks in LN2
- Pour LN2 into tissuelyzer base (the section with the rubber)
- Place plate with bottom in base, and put in machine. Don't add ln2 to the top of the plate
- Run machine (with counterbalance) for 1 min at 25hz
- Remove plate. After inspection, some wells were not pulverised, due to the bead being stuck, so i banged the plate against the desk quite hard about 5 times to dislodge it
- Run for another minute. The tissues appeared to have been ground more thoroughly.
- Spin plate at 6k RPM for 1 minute. This got most of the tissue to the bottom to the tube. Some remained around the lids, and seemed to thaw slightly.
- The plate was put back into the -80

22 Thu 2013-05-16

22.1 Practice Kumar et al Library Prep

22.1.1 Aim

Take two samples up to first strand synthesis

22.1.2 Method

Make fresh LBB and additives (hereafter LBBMA):

- Add 6mL LBB from dynabeads kit to 15mL falcon tube
- add 30uL mercapto ethanol and ≈ 90uL antifoam A. (antifoam A is V. viscous, could not be accurately pippeted)

Preps:

- Using practice plate from march harvest harvest, tissuelysed wed 15/5/13
- A2: Add 1mL LBBMA, pippete to mix, breif vortex, pipette into eppie, vortex again. Sample had many chunks which did not resuspend, spilt some lysates.
- A3: Add 500uL LBBMA, use 200uL pippetor to mix, then add extra 500uL and mix w/ 1000ul pippettor. Tfr to new tube. May not have added 500uL the second time, so added ≈ 200uL extra.
- Left A1 8min and A2 5min at RT
- Tfr -> qiashredder, spin 10k rpm, 10min
- Prepare dynabeads:
  Resuspend bottle w/ v. gentle vortex
  150uL -> fresh eppie
  vortex, spin, mag sep, remove supn.
  add 100uL LBBMA, resusp., centrifuge, resusp, 50uL ea to 2 new tubes
- mag sep, remove supn
- 200uL lysates -> each tube, rest of lysates onto dry ice
- pip to mix, incubate @ rt w/ hand mixing, 10min
- sep on mag sep, 15 min
- wash per protocol (steps 1.3 5-6) w/ LBBMA, not straight LBB
- leave in WBB 20min while DNAse MM was made
- Make 20uL DNAse MM per protocol, with 5% extra, using DEPC mQ water
- wash beads again w/ WBB (NOT Low Salt Buffer)
- add 18uL TrisHCL from dynabeads kit, resusp. 2uL -> extra tubes, for bioanalyser
- add 10uL DNAase MM to 15uL, incubate 10min at 37C in pcr machine
- add 1.5uL 0.22uM filtered EDTA, pip to mix, incubate 75C for 10min
- samples -> ice for 10min, add 175 LBBMA, mix
- incubate RT with hand mixing 10min
- mag sep beads for 10min
- wash per steps 1.3 5-6, but w/ 150uL WBA for sample A3 in step 5
- Wash w/ 200uL WBB again (not LSB)
- Make 13uL primer/dntp mix:
  10mM Tris from dynabeads kit, 11.6uL
  25mM DNTPs from Pete
  Random primers from sigma

- incubate, prepare FS cDNA MM, add sample and run synthesis rxn in PCR machine as per protocol, steps 1.3 step 18 - 2.1 step 5
- Freeze sample at -20C

22.1.3 Results

None yet

23 Thu 2013-06-06

23.1 Create Condtions for keng

23.1.1 Aim

Make solarcalc conditions for keng.

23.1.2 Method

as described in the .srp files attached.

23.1.3 Results

2013-06/20130606-GriffithCurrent100Light.srp 2013-06/20130606-GrifithCurrent100Light.csv 2013-06/20130606-MilduraCurrent100Light.csv
2013-06/20130606-MilduraCurrent100Light.srp 2013-06/20130606-TemoraCurrent100Light.csv 2013-06/20130606-TemoraCurrent100Light.srp
2013-06/20130606-TemoraCurrent50Light.csv 2013-06/20130606-TemoraCurrent50Light.srp 2013-06/20130606-UmeaCurrent100Light.csv
2013-06/20130606-UmeaCurrent100Light.srp 2013-06/20130606-UmeaCurrent100LightTZ.srp 2013-06/20130606-WollongongCurrent100Light.csv
2013-06/20130606-WollongongCurrent100Light.srp 2013-06/20130606-WollongongCurrent35Light.csv 2013-06/20130606-WollongongCurrent35Light.sr
2013-06/20130606-ZaragozaCurrent100Light.csv 2013-06/20130606-ZaragozaCurrent100Light.srp

23.2 Spanish conditions

23.2.1 Aim

Create some inland spanish inland/coastal conditions for comparison.

23.2.2 Method

See .srp files attached

23.2.3 Results

The inland condition is too cold, so we won't use these.

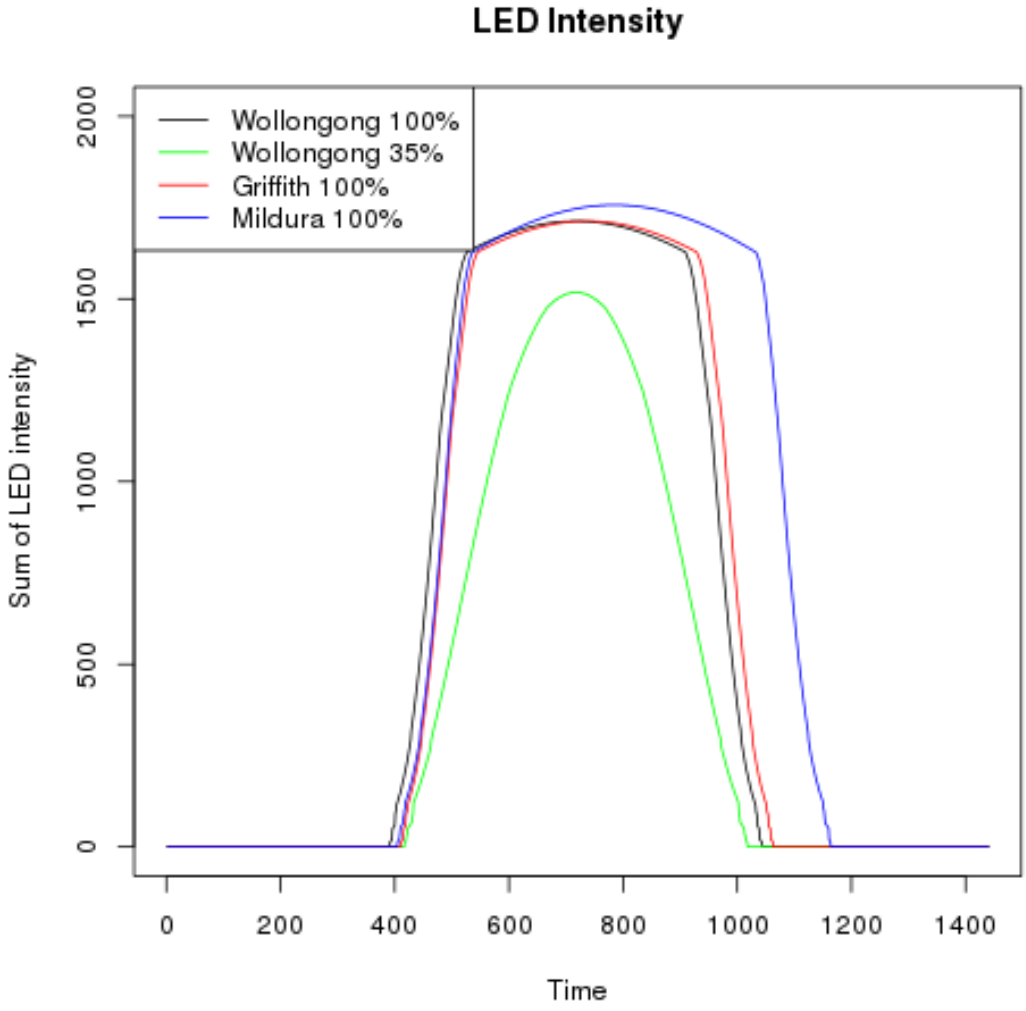


Figure 23.1: Light

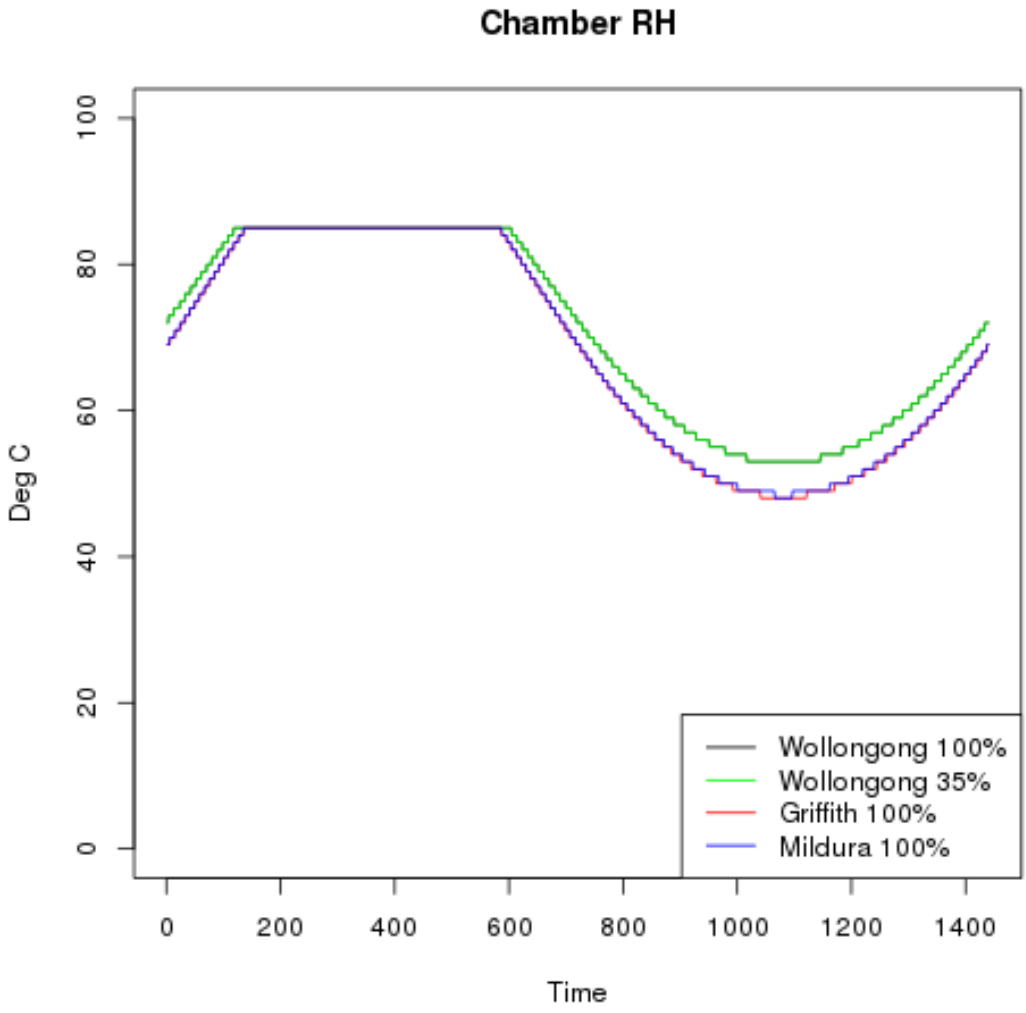


Figure 23.2: RH

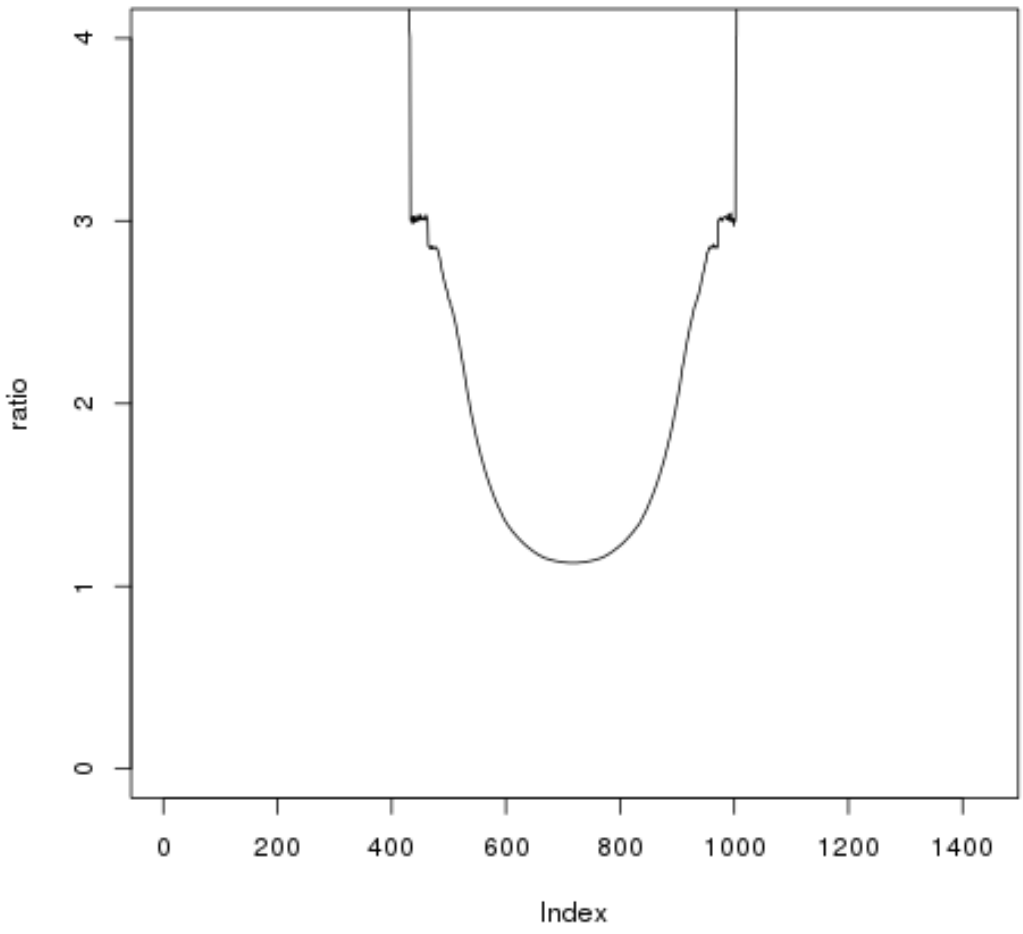


Figure 23.3: Light Ratio (WollongongCurrent100Light / WollongongCurrent35Light)

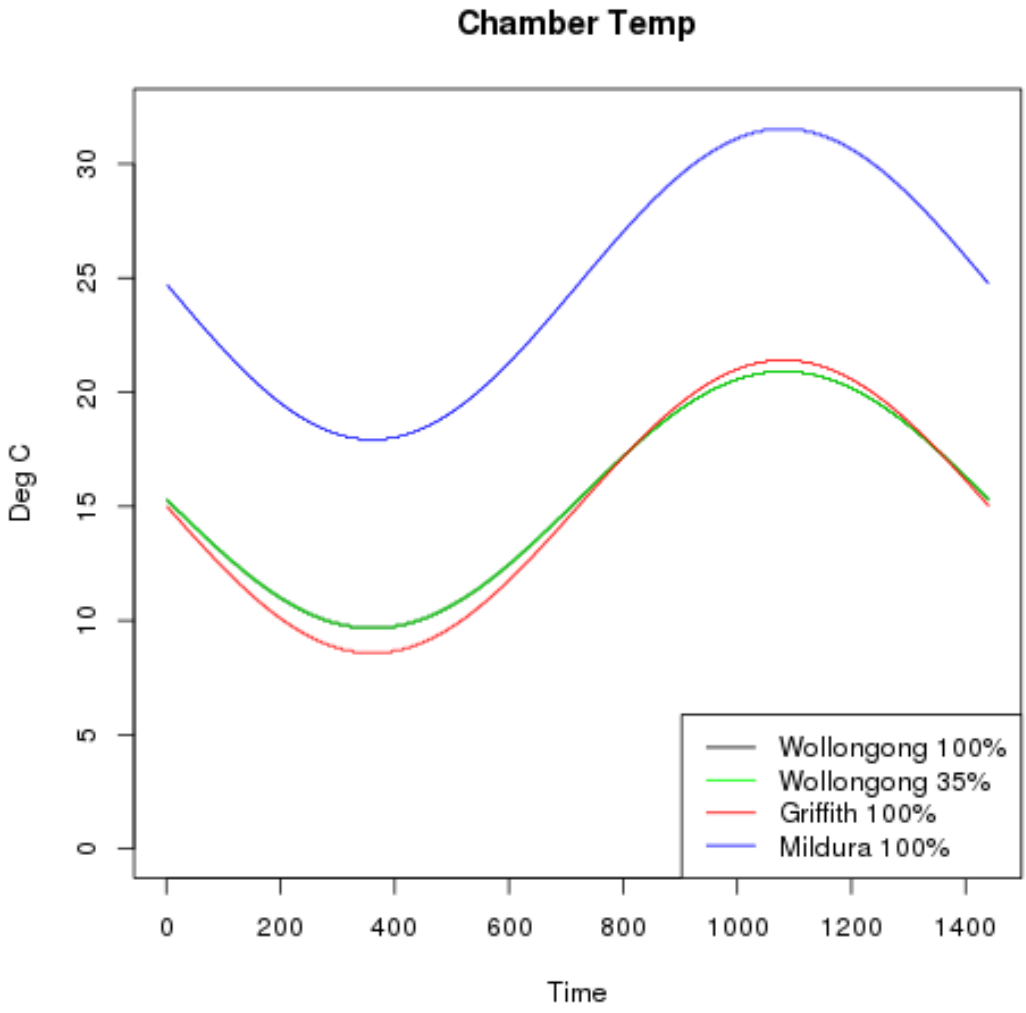


Figure 23.4: Temp

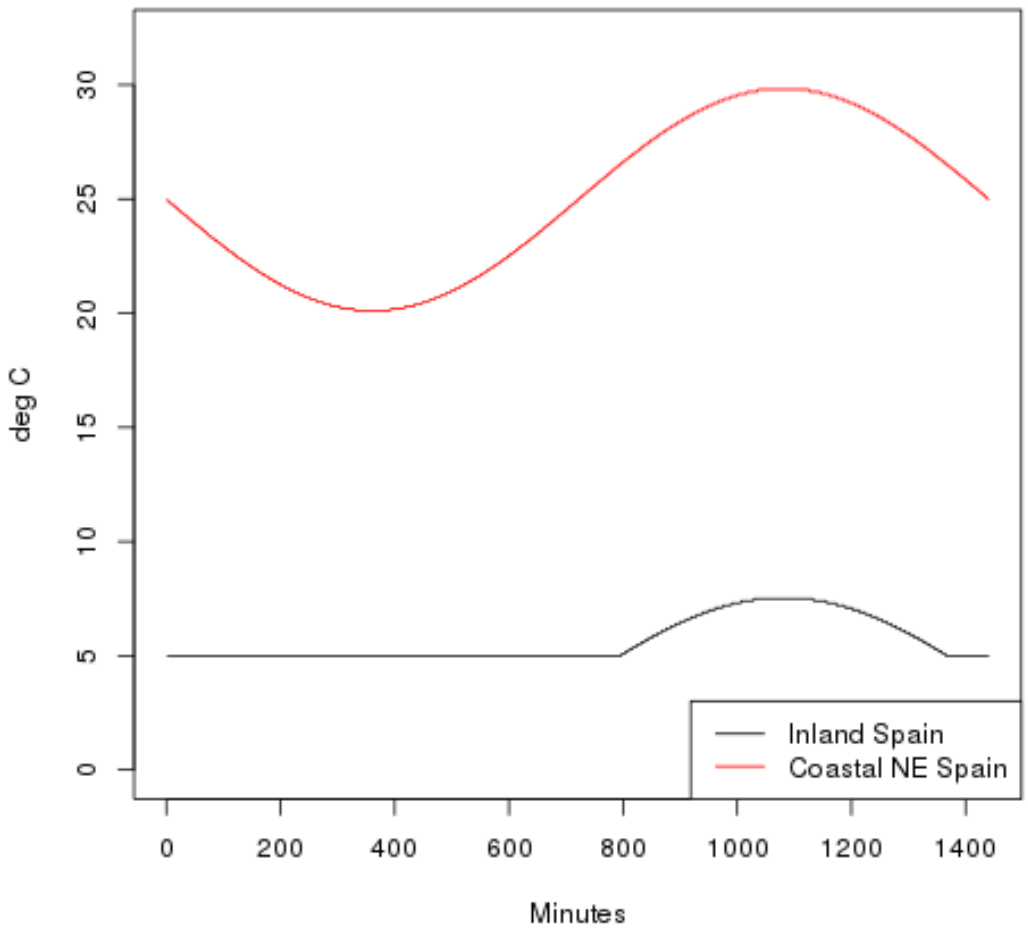


Figure 23.5: Spain Temps

24 Fri 2013-06-21

24.1 Run Degradome analyseR for Pete's poster

24.1.1 Aim

Run DegAnalyseR on the paresnip data

24.1.2 Method

Paresnip output was filtered using:
`R -f DegradomeAnalyseR/paresnip/paresnip_filter.R --args <files>`
The files paresnip_1, paresnip_2, and paresnip_3 were filtered pairwise, and all together.

24.1.3 Results

genotype	Rep	count
Col-0	1	37985
Col-0	2	41471
Col-0	3	36789
Col-0	1-2	14236
Col-0	1-3	13844
Col-0	2-3	13488
Col-0	DA	1709

25 Mon 2013-07-15

25.1 Extraction of samples from plates

25.1.1 Aim

Get the col, cvi and ler samples out of the plates for RNA extraction

25.1.2 Method

- Plates were placed in liquid N2, and allowed to cool.
- Qiagen collection tube caps were used to close the plates
- individual caps were cut away and sample disrupted w/ a ln2 frozen p1000 tip
- contents were emptied into pre labeled and pre ln2 frozen eppies (normal 1.7ml ones)
- plates were re-capped with collection tube caps, and plate mats strapped to plates w/ cotton thread to stop explosions.

25.2 Table of counts sample counts

25.2.1 Counts

I've counted the number of samples per condition, genotype and reps to see what's missing. See also attached files:
20130712-harvestPlateGenotypeLayouts.ods `f04bb7426b9d7e9048ba5757811e6ec4`
20130714-sampleGenotypeTreatmentCounts.ods `deca19773a3f8c626763a466fbbfd7d6`

		Col	Cvi	Ler
0h	Fl Ch2	2	2	2
	Su Ch4	1	2	1
	Ex Ch5	3	1	1
1h HL	Fl Ch2	2	2	3
	Su Ch4	2	1	2
	Ex Ch5	3	3	4

Table 25.1: Samples for each genotype