# DIGITAL LAB NOTEBOOK OF KEVIN MURRAY

# Honours Project, 2013

Jointly supervised by Justin Borevitz and Barry Pogson

# Mon 2012-12-03

# Initial Harvest of Keng's RIX lines

## Aim

Harvest lines before the 1 week repeated HL stress experiment.

## Method

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

## 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	
В3	4	
C3	99	
D3	16	
E3	17	
F3	18	may be 17, hard to read label
G3	18	
Н3	21	
A4	23	
B4	25	
C4	57	

Well	Line	Comments
D4	28	Comments
E4	29	
F4	30	
G4	31	
H4	$\frac{32}{62}$	
A5	63	1 07 1 1 1
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 G7	33 26	
H7	36 39	
A8 B8	38 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	second four from disassered plant stone four winter narrating immutes sectore sampling
В9	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	

Well	Line
G10	61
H10	21
A11	68
B11	
C11	
D11	
E11	
F11	
G11	
H11	
A12	
B12	
C12	
D12	
E12	
F12	
G12	
H12	

## Attachments:

 $\bullet \ ./2012 - 12/20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 888a60 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 88460 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 88460 dcd 2226feb 874feb 20121203 - \texttt{harvest-pictures.tar.bz2} \ MD5SUM: 2843946f8 cae 88460 dc$ 

Comments

# Mon 2012-12-10

# Final Harvest of Keng's RIX lines

### Aim

Harvest lines after 1 week of HL stress.

## Method

- An Eppendorf 1.2mL deep well plate was placed on dry ice for  $\approx 10$  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.

## 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	
В3	72	
C3	12	
D3	11	
E3	10	
F3	12	

Well	Line	Comments
G3	9	
НЗ	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 C7	71	
D7	53 55	
Ε7		
F7	56 50	
G7	51	
H7	52	
A8	78	NPQ4
B8	80	111 & 1
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	

Well	Line	Comments
B10	57	
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:

# Mon 2013-01-14

# MAKE: Washed Ball Bearings

## 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

# TissueLyser grinding of practice samples

## Aims

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

## Method

- Remove pre-frozen TissueLyser blocks from -80 freezer.
- Add one cleaned bead to each Eppendorf tube (beads were not pre-cooled)
- $\bullet$  Pour LN<sub>2</sub> into the TissueLyser block
- Add Eppys with beads and sample, and run for 3x 1min runs at 29Hz
- Replace samples in -80

# Mon 2013-01-21

## Quantification of RNA samples

### Aim

• Determine qty of RNA in previously extracted samples

### Method

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

### 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)

### Attachments

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

## 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

# 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

# Tue 2013-01-22

# Denature RNA for RNA gels

### 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

## TBE Gel

## Aim

• To compare TBE and denaturing/MOPS gels for RNA

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

### 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 mis-loading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

# MOPS gel

## Aim

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

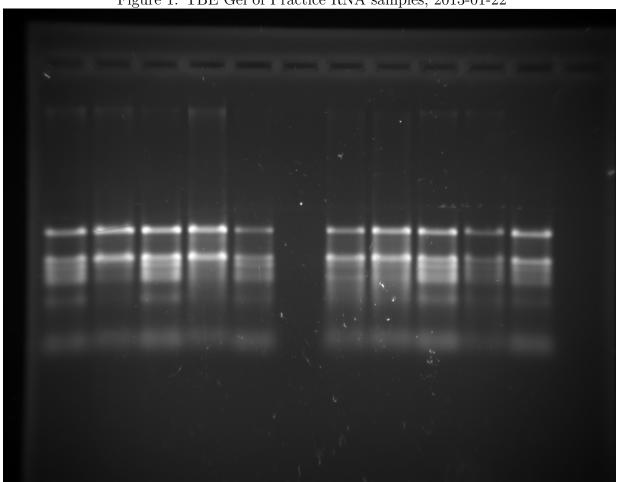


Figure 1: TBE Gel of Practice RNA samples, 2013-01-22

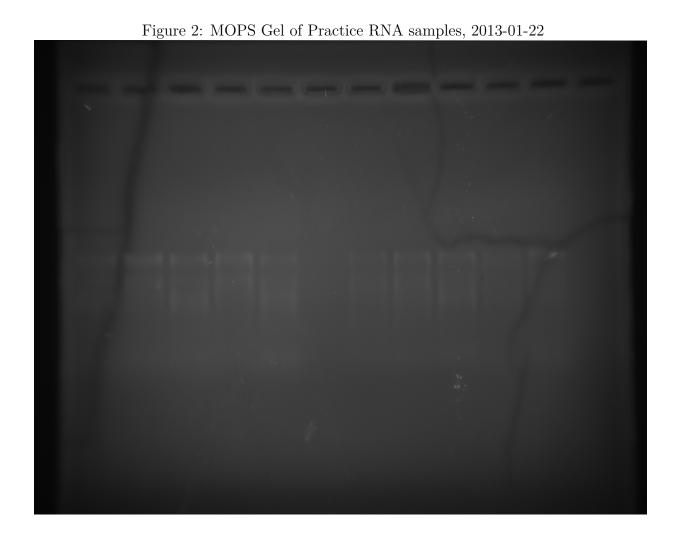
## Method

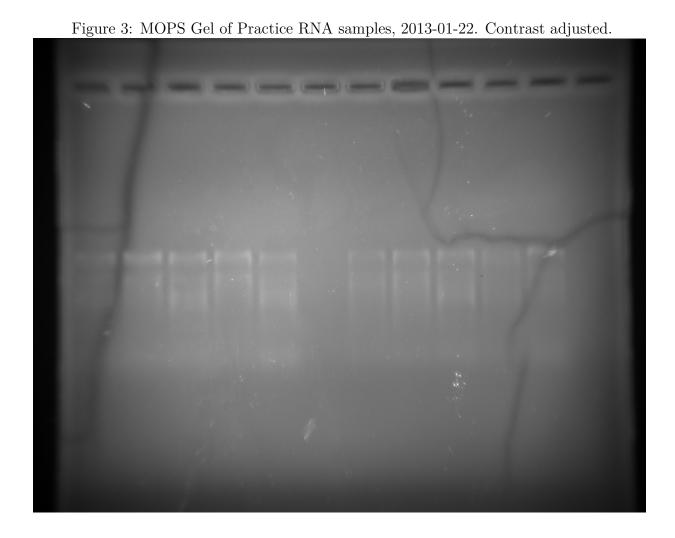
- Load samples after denaturing as above. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7.
- Run gel in RNAase free 1x MOPS at  $\approx 80 \text{V}$ ,  $\approx 100 \text{mA}$  for  $\approx 1.75 \text{h}$  as per TBE gel above.
- Stain gel in 0.5ug/ml Ethidium Br in DEPC water?? for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

## Results

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.





# Tue 2013-01-29

# 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	$\mathbf{Desc}$	Count
1	Col-0_1915	not in box
2	Col-0_4936	not in box
3	Cvi x Cvi	0
4	$1 \times Cvi$	not in box
5	$1 \times 146$	0
6	$10 \times 26$	0
7	$101 \times 176$	0
8		not in box
9		not in box
10		not in box
11		not in box
12		not in box
	$110 \times 32$	?
14		0
	113 x 141	0
	114 x 3	?
17		0
	115 x 126	0
19		?
20		0
21		0
22		0
	12 x 142	0
24	122 x 42	?
25		0
26	128 x 6	0
	132 x 129	not in box
	133 x 35	0
	134 x 29 135 x 10	0
30 31		0 ?
31 32	136 x 140 136 x 102	0
33	$165 \times 102$	not in box
34	$139 \times 162$	0
	139 x 102 139 x 36	0
36		0
	14 x 4 146 x 64	not in box
91	110 7 01	HOU III DOX

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	Line	Desc	Count
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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			
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56  174 x 34			
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			
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			
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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	61	$187 \pm 69$	not in box
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	62	$189 \times 133$	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	63	$19 \times 173$	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	64	$19 \times 67$	0
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	65	$190 \times 176$	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	66		not in box
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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			
71  25 x 9			
72  26 x 74			
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			
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85 5 x 172 not in box 86 5 x 188 not in box 87 51 x 111 0			
86 5 x 188 not in box 87 51 x 111 0			
87 $51 \times 111$ 0			

Line	$\mathbf{Desc}$	Count
89	$54 \times 183$	0
90	$55 \times 18$	0
91	$59 \times 116$	0
92	$6 \times 131$	0
93	$61 \times 162$	0
94	$63 \times 151$	not in box
95	$7 \times 46$	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

# Thu 2013-02-07

# Prepare Trays for Planting

Trays were filled with steamed seed raising mix, with 1 mL/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  $\approx 1.5 L$  RO water, containing  $\approx 1 mL/L$  AzaMax<sup>TM</sup>, covered with cling film and stored at 4 degrees C.

# Fri 2013-02-08

# Planting of RIX lines

## Aim:

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

## 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	$1 \times 146$	
4	$10 \times 26$	
5	$101 \times 176$	
6	LL-1	
7	$145 \times 105$	
8	$107 \times 121$	
9	$18 \times 109$	
10	$109 \times 48$	
11	$114 \times 3$	6
12	$110 \times 32$	6
13	$112 \times 30$	
14	$115 \times 12$	
15	$118 \times 108$	
16	$118 \times 164$	
17	$12 \times 142$	
18	$122 \times 42$	
19	$125 \times 117$	
20	$128 \times 6$	
21	$129 \times 132$	
22	$133 \times 35$	
23	$134 \times 29$	
24	$135 \times 10$	

#### Line Number Line Name Quantity 25 $136 \times 102$ 26 $165 \times 137$ 27 $139 \times 36$ 28 $14 \times 4$ 29 146 x 64 $147 \times 50$ 30 31 $147 \times 69$ 32 $149 \times 165$ 33 $150 \times 37$ $152 \pm 42$ 34 35 $153 \times 108$ 36 $153 \ge 20$ 6 37 $156 \times 106$ $119 \times 60$ 6 38 39 $16 \times 4$ 40 $16 \times 66$ $16 \ge 7$ 41 42 $166 \times 25$ 43 $168 \times 22$ 44 $17 \times 21$ 45 $171 \times 143$ 46 $174 \times 34$ 47 $180 \times 157$ 48 $186 \times 27$ 49 $187 \times 69$ 50 $19 \times 173$ $19 \ge 67$ 51 52 $190 \times 176$ 53 191 x 31 54 $192 \times 189$ 55 $20 \ge 138$ 6 $24 \times 171$ 56 $15 \times 9$ 57 $26 \times 74$ 58 $33 \times 58$ 59 60 $38 \times 35$ $39 \times 27$ 61 62 $44 \times 50$ $45 \times 23$ 63 64 $46 \times 29$ 65 $48 \times 160$ 66 $49 \times 158$ 67 $5 \times 172$ 68 $5 \times 188$ $51 \times 111$ 69 70 $51 \times 18$ $54 \times 183$ 71 72 $55 \ge 18$ 73 $59 \times 116$ 746 x 131 75 61 x 162

## Line Number Line Name Quantity

- $76\quad 63\ge 151$
- 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

# Thu 2013-02-14

## Sun and Shade Spectra

### Aim

Measure specta from natural sun and natural shade at midday

### 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).

### 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

# Fri 2013-03-01

## Creation of Solarcalc files

## 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.

### Method

 - Solar Calc version 2013 Feb<br/> 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

## 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  $MD5SUM\ d01fcc5ae9ad8fba07bd9b9512c82857$ 

# Tue 2013-03-05

## Creation of Better Normalised Shaded Solarcalc files

## 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.

#### Method

 - Solar Calc version 2013 Feb<br/> 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

## Results

Solarcalc outputs:

- $\bullet ./2013-03/20130305-Temora 2012 Sep 01\_45 shade\_2010 weather\_LED-Better Normalised.csv \\ ddff 182b78d1fe 10e 662fb 827a179804$
- $\bullet ./2013-03/20130305-Temora 2012 Sep 01\_0 shade\_2010 weather\_LED-Better Normalised.csv \\ d41 d8cd 98f 00b 204e 980099 8ecf 8427e$

### Solarcalc preferences files:

- ./2013-03/20130305-Temora2012Sep01\_0shade\_2010weather\_LED-BetterNormalised.srp 9e800ba2b31a5346b37bc17127515d38
- ./2013-03/20130305-Temora2012Sep01\_45shade\_2010weather\_LED-BetterNormalised.srp  $\rm d40480b773fdaad8ecf9bd14a5d445ed$

# Thu 2013-03-07

## Creation of Brighter Normalised Shaded Solarcalc files

### 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.

## 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: 0LED 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

#### Results

### Solarcalc outputs:

- ./2013-03/20130307-KMTemora2012Sep01\_0shade\_2010weather\_LED-2xBetterNormalised.csv 59 fcd4 a acdf20 eeb750c22b18ba4f118
- ./2013-03/20130307-KMTemora2012Sep01\_0shade\_2010weather\_LED-4xBetterNormalised.csv 3d79e511b4367d1c5807b247fffa3b48

### Solarcalc preferences files:

- ./2013-03/20130307-KMTemora2012Sep01\_0shade\_2010weather\_LED-2xBetterNormalised.srp bf276b2cf5658f00fbd02bce55f186f7
- $\bullet ./2013-03/20130307-\texttt{KMTemora}2012 Sep01\_0 shade\_2010 weather\_LED-4x BetterNormalised.srp. 3 f124559 d1553 f68384218509196992 d$

## Sun 2013-03-10

## Creation of Intermittent Bright/Shaded Solarcalc files

#### 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.

#### 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
```

#### Results

Solarcalc inputs:

- $\bullet ./2013-03/20130305-Temora2012Sep01\_45 shade\_2010 weather\_LED-BetterNormalised.csv \\ ddff182b78d1fe10e662fb827a179804$
- ./2013-03/20130307-KMTemora2012Sep01\_0shade\_2010weather\_LED-2xBetterNormalised.csv 59 fcd4 aacdf20 eeb750c22b18ba4f118

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-KMTemora 2012Sep01\_Intermittent2hLL1hHL.csv c415ed3654b071ad6fbcbd044b60f732

Solarcalc Splice Script:

./2013-03/20130310-spliceSolarCalc.py  $6{\rm cd8ea}9895{\rm d}19{\rm b}7851{\rm c}41{\rm c}086535f318$ 

# Fri 2013-03-19

## Enter plants in trayscan database

### Aim

Allow trayscan to see and process out plants

### 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.

## Results

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

# Mon 2013-03-25

## Harvesting of practice tissue

### Aim

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

### 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
- $\bullet$  the plant number as per ./2013-02/20130208-KMPlanted Lines.csv was recorded

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

	1	<b>2</b>	3	4	5	6	7	8	9	10	11	<b>12</b>
Α	62	50	48  med lf	46	54	46						
В	62	50	48 lrg lf	46	54	46						
$\mathbf{C}$	23	50	19	21	46	46						
D	48	???	61	61	46	46						
$\mathbf{E}$	48	27	61	61	46	46						
$\mathbf{F}$	48	23 caul	61	25	46	46						
G	48	23 caul	61	25	46	46						
Η	48	48  sml	61	16	46	46						

## Tue 2013-03-26

## Harvesing the First Replicate across each chamber

#### Aim

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

### 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)

		12 4 2d1 4 2d1 ½L 4 2d3 ½L sq 4 2d4 ½L sq 4 3a1 ½L 4 3d1 4 3c1 sq 4 3c1 sq 4 3c1 sq		
				12
12 4 3e1 M 4 2d3 4 2d3 4 2d4 4 3a1 4 3a1 sq 4 3c4 sq 4 3c1 sq		11 4 2a3 4 3b1 sq ½L 4 2b2 4 2b4 4 2c1 4 2c1 4 2c3	11 12 9d2 9d3 9d4 2C 10B1 2S 9e2 9e3 9e4	11 5 9d2 5 9d3 5 9d4 C 5 10d1 2S sq 5 9e2 5 9e3 2C 5 9e4
11 4 3b1 4 2b2 sq 4 2b3 sq 4 2b4 2S 4 2c1 4 2c1 4 2c3 4 2c3 a1 ½ 4 2c4		10 4 1c1 sq 4 1d1 4 1d2 ½L sq 4 1d4 ½L 4 1e2 ½L 4 1e3 4 1e4 4 2a1	10 9a3 2S 5 9a4 ½L 5 10b1 2S 5 10e1 2S 5 9b4 5 10c1 5 9c3 5	10 5 9a3 5 9a4 5 10b1 a1 5 10e1 2S 5 9b4 5 10c1 5 9c3 5 9c3
10 4 1d1 4 1d2 4 1d4 L 4 1d4 L 4 1e1 L 4 1e3 4 1e4 4 2a1 L 4 2a3 basal ½		9 5 1e2 5 1e3 4 1a1 4 1a2 CL sq 4 1a3 4 1b1 3S 4 1b2 2S 4 1b4 ½L	9 5 8d1 5 8d2 5 8e2 5 8e3 5 9a1 5 9a2	9 5 8d1 5 8d2 5 8d2 5 8e2 ½ 5 8e3 5 8e4 2S 5 9a1 sq
9 5 2e4 4 1a1 4 1a2 CL 4 1a3 q 4 1b1 4 1b2 CL 4 1b4 4 1b2 CL 4 1b4		8 5 1b4 5 1c1 5 1c3 2S sq 5 1c4 5 1d3 5 1d4 sq 5 1 e1 CL 5 1e1 CL	8 4 963 C 5 8a3 ½ 5 8b1 sq 5 8 8b2 5 8b3 1 5 8b4	8 4 9e3 5 8a3 5 8b1 5 5 8b2 % 5 8b3 C 5 8b3 C 5 8b4 % 5 8c4 2S
8 5 3c1 5 2c4 5 2d1 5 2d3 5 2d4 sq 5 2e1 5 3e1		%F	7 4 9a3 C 4 9b4 ½L 4 10a1 2S 4 9c3 4 9c3 4 9d4 4 9d4	7 4 9a3 4 9b4 4 10a1 2S 4 9c3 ½ 4 9c4 25 4 9d1 2S 4 9d4
7 5 2a2 Pur 5 3a3 8 5 2a4 L 1 5 2b1 5 2b2 5 3b1 5 3b4			6 4 8b3 4 8b4 4 8c1 4 8c1 4 8c4 2S 4 8c4 2S 4 8c1 4 8c1 4 8c1 4 9c1 4 9c1	6 4 8b3 2S 4 8b4 4 8c1 4 8c4 C 4 8c4 C 4 8c1 2S 4 8c1 4 10e1 ½ sq 4 9a2 3S
5 1a1 5 1c3 5 1b1 5 1c4 5 1a3 5 1d4 5 1a4 5 1a2 5 1c1 5 1b2 5 1c1 5 1b4 5 1c2 5 1c1 5 1c3 5 1c1 5 2a1 5 1c1 5 2a1		6 2 2b4 2 2 2c1 2 2c3 2 3d1 2 2d2 2 3c1 2 2c1 2 2c2 2 2c2	5 2 9e1 2 9e2 2 9e3 sq 2 9e4 4 8a1 sq 4 8b1	5 2 9e1 C 2 9e2 2 9e3 C 2 9e4 4 8a1 ½ 4 8b1 sq 4 8b1 sq 4 8b2
4 2 2 2 3 2 3 3 4 1 2 2 4 2 2 2 2 1 2 2 2 2 2 2 2 2 2 3 2 2 2 3 3 3 3 3 3 3		5 3e1 5 3e1 5 3d1 ½ 2 2e4 2 2a3 2 2a4 2 2b2 1 2 2b3	4 2 9c1 ½ 2 9c3 ½ sq 2 9c4 2S 2 9d1 sq 2 9d2 ½ 2 9d3 2 9d4 sq	4 2 9b4 2 9c1 ½ 2 9c3 ½ 2 9c4 2S 2 9d1 2 9d2 2 9d3 sq 2 9d3 sq
3 2 164 2 283 2 283 2 284 2 282 2 283 2 284 2 284		4 5 2b4 5 2c1 CL 5 3c1L 5 2c4 5 2d1 5 2d3 5 2d3 5 2d3	3 2 9a1 2 9a2 ½L 2 10c1 2 10b1 sq 2 9b1 2 10a1 ½L 2 10d1 ½L 2 9b4 ½L	3 2 9A1 2 9a2 2 10c1 2 10b1 2 9b1 2 10a1 2 10d1 ½ 2 9b4 ½
2 2 1c1 S ??? 2 1c3 2 1d3 L 2 1d2 L 2 1d3 L 2 1e1 2 1e2 2 1e2 2 1e3		3 2 1e4L CL 5 2a1 5 2a2 2S 5 3a1 3S 5 2a4 5 2b1 CL 5 2b2 CL 5 3b1	2 8c2 2 8c3 ½L 2 8c4 2 8d1 2 8d2 2 8d4 2 8e4 2 8e1 2 8e3 ½L	2 2 8c2 2 8c3 ½L 2 8c4 2S sq 2 8d1 2S 2 8d2 ½L 2 8d4 2 8d4 2 8e1 ½ 2 8e3 2S
1 A 2 1a1 B 2 1b1 C 2 1a3 sen D 2 1b4 E 2 1b2 L F 2 1b3 S G 2 1c4 L H 2 1c1		2 1c2 sq 2 1c3 2S 2 1a4L CL sq 2 1d2 3S 2 1d3 M 2 1d1 M 2 1e1 M 2 1e2 sq 2 1e3	1 A 2 8a1 B 2 8a2 sq C 2 8a3 sq D 2 8a4 E 2 8b1 F 2 8b2 G 2 8b4 H 2 8c1	2 8a.1 2 8a.2 2 8a.3 2 8a.3 2 8a.3 2 8b.4 2 8b.1 sq. 3S 2 8b.4 2S 2 8b.4 2S
		1 2 1a1 2 1a3 2 1b4 2 1b1 2 1b2 2 1b2 2 1b3 2 1b4 2S		HCFEDCBA
		HGFEDCBA		
	Plate 2:		Plate 3:	Plate 4:

# Wed 2013-03-27

## Harvesing the Second Replicate across each chamber

### Aim

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

#### 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.

$\mathbf{Time}$	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	$1 \mathrm{hHL}$
13:40:00	5 p1	$1 \mathrm{hHL}$
14:00:00	2 p1	$1 \mathrm{hHL}$
14:15:00	4 p2	$1 \mathrm{hHL}$
14:30:00	5 p2	$1 \mathrm{hHL}$
14:45:00	2 p2	$1 \mathrm{hHL}$

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:

12 2 5c1 ½ 2 5c2 2 5d1 2 5d2 2 5e1 2 5e2 2v.sml	12 d1 d2 e1 e2	12 2 5a2 2 5a2 2 5c1 2 5c1 2 5c2 haded 2 5d1 anded 2 5d2 2 5e1 2 5e2	11 12 2 12c2 1/3 2 12d1 2 12d2 2 12e2 1/3 sq
11 2 4d2 2 4d3 2 4e3 2 4e3 sq 2 4e4 ½ 2 5a1 2 5a1 2 5a2 2 5a2	10 11 2 11d4 2 12d1 2 11e2 2 12d2 2 11e3 2 12d2 2 12a2 2 12e2 2 12b1 2 12b2 2 12c2 2 12c2	q 2 3b4 2S q 2 3c2 ½ sen 2 3c4 2S 2 3d3 ½ 2 3d4 ½ 2 3d4 ½shaded q ½ 2 3e2 wounded 2 3e4 2 3e4	10 2 10d4 caul 2 15 2 10e2 ½ 2 15 2 10e3 ½ 2 15 2 10e4 ½ 2 15 2 12a2 ½ 2 15 2 12b1 2 12b2 2 12c1
10 2 4a2 2S 2 4a3 2 4a4 sq 2 4b1 ½ 2 4c1 2 4c1 2 4c3 2 4c4 2 4c4 2 4c4	8 9 10c2 211a2 10c3 211a3 10c4 211b1 10d3 211b2 10e2 211c3 10e3 211d1 10e4 211d3	10 2S 2 4d2 sq 2S 2 4d3 sq 2S 2 4e1 ½ 2 4e3 ; 2 4e3 ; 2 4e4 2 3b2 sq ½ 2 3b3 ½ 2 3a3	9 2 10a2 ½ 2 2 10a3 1½ 2 2 10a4 1½ 2 2 10b4 2 10c2 sq 2 10c3 sq 2 2 10c3 2 10c4 2 10c4 2 2 10c4 2 10
9 2 354 C 2 3c2 2 3c4 2 3d3 % 2 3d4 2 3e2 sq 2 3e4 2 2 4a1	7 8 5 12c1 ½ 2 10c2 5 12c2 2 10c3 5 12d2 2 10c3 5 12d2 2 10d3 2 10a2 2 10d4 2 10a3 2 10e2 2 10a4 2 10e3 2 10b4 2 10e4	8 9 4e4 2S 2 4a2 2S 5a1 2 4a3 2S 5c1 2 4a4 2S 5c2 ½ 2 4b1 ½ 5d1 2 4b3 5d2 ½ 2 4c1 5e2 ½ 2 4c1 5e2 ½ 2 4c1 5e2 ½ 2 4c2	8 2 11b2 2 11c1 % 2 2 11c1 % 2 2 11c3 % 2 2 11d3 % 2 2 11d4 % 2 2 11c2 % 2 2 11c3 % 2
8 5 4c1 5 4c2 ½ 5 4d2 ½ 5 4d2 ½ 5 4d2 ½ 5 4d2 ½ 2 3a3 2 3a4 ½ 2 3a4 ½	sq sq sq sq	S ½ 3S sq 55 50 70 70 70 70 70 70 70 70 70 7	7 5 12b2 5 12c1 5 12c2 5 12d1 5 12d2 2 11a2 ½ 2 11a3 ½ 2 11b1 sen
7 5 4d1 ½ 5 4d2 5 4d2 5 4d3 P 5 4d4 P 8q 5 4e2 P P 5 4e3 P ½ 8q 5 4e4 P ½ 8q 5 4e4 P ½ 8q 5 55a1 ½	5 6 5 10d4 5 11c4 5 10e2 5 11d3 5 10e3 5 11d4 5 11a1 5 11e1 5 11a2 5 11e2 5 11b2 sq 5 12b1 5 11b4 5 12b2	6 4a1 P 4a2 4a3 ½ 4b1 4b2 4b4 4c1 P	6 5 11c4 ½ 5 11d4 % 5 11d4 S sen 5 11d4 5 11e1 sq 5 12a2 5 12a2
6 5 4a2 5 4a3 5 4b3 5 4b1 5 4b2 sen 5 4b4 8 sq 5 4c1 5 4c4	4 5 10a3 P sen 5 1 5 10b2 ½ 5 10b2 ½ 5 10b3 5 10b3 5 10c2 5 10c2 5 10c3 5 10c3 5 10c3 5 10c3 5 10c3 5 10c3	5 3b3 5 3b4 P 5 3c4 P 5 3c2 2S 5 3d4 sq 5 3c4 ys 5 3c4 2S 5 3c4 2S	5 10d4 ½ 10e2 ½ 10e3 11a1 sq 11a2 sq 11b1 ½ 11b4 ½ sq
4 5 3e3 S 5 3b4 P 3e4 5 3c3 P 5e1 sq 5 3d2 ½ 5e2 5 3d4 ½ 3a2 5 3e2 ½ sen 3a3 5 3e3 1/3 sq 3b2 5 3e4	be defined the second of the s	4 3e3 2S 5e1 sq 5e2 ½ 3a2 sq 3a3 sq sen 3a3 2S	4 5 10a3 2S 5 10a4 P 5 10b2 5 10b3 5 10c2 ½ 5 10c3 ½
3 4 4 3b1 4 3e3 S 4 4 3b4 4 3b4 4 5b1 sq 4 5c2 4 5c2 6 3a3 4 4 5c2 5 3a3 4 5c4 5c4 5c2 5 3a3 4 5c4 5c2 5 3b3 6 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	2 3 4 10d3 sen 4 11c2 sq 4 10d4 C 4 11c2 ½ 4 10e2 ½ 4 11e4 ½ 4 11e3 4 12b1 4 11a1 4 12d1 4 11a3 C 4 12e1 ½ 4 11b1 2S 5 10a2 ½ 4 11b1 2S 5 10a3 C	3 3b3 5e1 2S sen 5b1 5b2 3c4 5c2 5d1	3 4 10c2 ½ 4 10d2 sq 4 10d2 sen 4 10d2 caul 4 10d4 dam 4 10e2 4 10e3 5 10a2 ½
4 4c4 4 4e1 4 4e3 4 3c2 4 3a3 4 3a3 4 5a1 sq 4 5a1 sq 4 5a2 sq	ъs	2 4b4 4e1 4e2 3c2 3a3 3a4 sq 5a1 5a2 ½ sq	2 4 12d1 ½ 4 12e1 ½ 4 10a2 4 10a3 4 10a3 4 10b2 6 10b3 7 10b3 8 10b3 8 10b3 8 10b3 8 10b3
1 B 44a1 C 44a3 sq D 44a4 E 44b3 F 44b4 G 43b2 H 44c3	1 A 410a2 B 410a3 C 410a4 sq D 410b2 E 410b3 F 410b4 ½ G 410c2 ½ H 410d2	1 4 4a1 4 4a2 sen 4 4 4a3 sq 4 4 4a4 4 4b3 sq 4 4 4b3 sq 4 4 4b4 sq 4 4 4b5 sq 4 4 4b5 sq 4	1 4 11a1 4 11a3 C 4 11a4 ½ 4 11b1 2S 4 11c2 ½ 4 11c2 ½ 4 11e4 4 12b1
НСТЕОСВУ		AGCOBFQH	нствосвъ
	Plate 6:	Plate 7:	Plate 8:

# Thu 2013-03-28

# Harvesing the Third Replicate across each chamber

### Aim

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

#### 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	1 hHL (1 h 05)
1505	2	14	1hHL (1h10)
1517	4	12	1hHL (57m)
1520	4	13	1hHL (1h0)
1526	4	14	1hHL (1h06m)

	. 12	
	10  11  12	
	6	4 7b2 ½
	œ	4 6d3
	4	4 5e3 ½
	9	27c2% sq
	ນ	$2 \text{ 6e1 } \frac{1}{2}$
	4	2 6a2
	က	5 7b3
•	7	5 5e3
	1	5 5a3 dam
		Α
late 9:		

12	12	12
<del></del>	11	11
10 am	10 4 14e2 4 14e3	10
9 4 7b2 ½ 4 7c1 4 7c2 dam 4 7c2	9 4 13e1 4 13e2 4 13e4 4 14c1 4 14c3 4 14d1 4 14d2 4 14d2	9 4 7e2 juv 4 7e2 4 5a4 ½ 4 5e3 4 5e4 ½
4 6d3 4 6d4 ½ 4 6d4 ½ 4 6e1 ½ 4 6e3 ½ 4 6e4 4 7a1 4 7a3 ½ sq 4 7b1 sq	8 4 12c4 4 12e3 4 12e4 4 13a2 4 13b1 4 13b2 4 13c3 4 13d3	8 4 6e2 ½ 4 6e4 2C 4 7a1 4 7a3 ½ 4 7b1 sq 4 7b2 ½ 4 7c1 4 7c2 2S sq
% % % % % % % % % % % % % % % % % % %	7 2 14c2 ½ 2 14c3 2 14d3 2 14d3 2 14e2 sq 2 14e3 4 12b3 4 12c3	7 4 6a4 2S 4 6b1 ½ 4 6c1 4 6c3 4 6c4 4 6d4 ½ 4 6d4 ½ 4 6d4 ½ 4 6d4 ½
6 7 7 2 1/2 1/2 1/2 1/2 1/2 1/2 1/2 1/2 1/2 1	6 2 13d2 ½ 2 13s1 dam 2 13s3 ½ 2 13s4 2 14s1 2 14b2 2 14b3 sq 2 14c1 ½	6 2 6b3 2 6b4 ½ 2 6d4 sq 2 6d4 2 6c2 2S 2 6e1 2 6e2 4 6a1 ½
	5 2 13a1 2 13a3 2 13a4 2 13b4 2 13b4 25 2 13c3 ½ 2 13c3 ½	5 2 7e1 2 7e2 2 7e3 ½ 2 6a1 ½ 2 6a2 2 6a3 2 6a4 caul 2 6a4 2S
4 5 2 6a2 2 6e1 ½ 2 6a3 ½ 2 6e2 ½ 2 6a4 2 5b3 ½ 2 6b2 2 5c4 2 6b3 2 5c4 2 6b3 ½ 2 7b1 ½ 2 6c2 2 7b3 ½ 2 6c4 2 7c1 ½	4 5 14d2 ½ P 5 14d3 % 2 12a3 ½ 2 12b3 2x 2 12c3 ½ sq 2 12c4 ½	4 2 5b3 2 5c4 ½ 2 5d4 2S 1caul 2 7b1 2 7b3 ½ 2 7c1 ½ 2 7c2 ½ 2 7c3 ½
3 5 7 b3 5 7 c2 1/3 5 7 c2 1/3 5 7 c3 ½ 5 7 e2 P 5 7 e3 ½ sq P 2 6 a1	3 5 1342 % 5 1382 5 1382 5 1442 P 5 1451 % 5 1452 % dam 5 1453 % P	3 5 7 5 3 89 5 7 6 1 ½ 5 5 7 6 2 ¼ 5 5 7 6 3 ½ 5 5 7 6 2 P 5 7 6 3 juv 5 7 6 3 P
2 5 563 5 563 1/3 sq 5 664 2S 5 664 5 664 5 7a2 ½ 5 7a3 P	2 5 13a4 P 5 13b1 2S 5 13b2 P 5 13b3 P 5 13c1 dam 5 13c2 5 13c2 5 13c3 2S	2 5 5e3 5 5d3 ½ dam 5 6d4 5 6e1 ½ 5 5e4 P 5 6e4 sq 5 7a2 ½ P 5 7a3 P
1 5 5a3 dam 5 5a4 5 6a3 ½ 5 6a4 P 5 5c4 sq 5 6c1	1 5 12a3 ½ 5 12a4 5 12b4 5 12b3 ½ 5 12d4 ½ 5 12e3 5 13a1 5 13a2 P	1 5 5a3 S 5 5a4 5 6a3 ½ 5 6a4 P 5 5b3 ½ 5 5c4 5 6b4 ½
НСЧЕОСВЪ	HGTHUCBA	HCFECCBA

Plate 11:

Plate 10:

4 14e2 3S 4 14e3

4 13e4 sen 4 13e4 dam 4 14c1 4 14c3

 $4 13b2 \frac{1}{2}$  sq 4 13c2

 $4 12c3 \frac{1}{2}$  sen

œ

7

10

4 14d2 4 14e1 juv 4 14e1 v.sen

4 13e2 outer  $\frac{1}{2}$  4 13e2 inner  $\frac{1}{2}$  of same leaf

4 13d3 sq 4 13e1 sq 4 13e1 juv

4 12c4 ½ 4 12e3 caul 4 12e3 ½ sen

2 14c2 ½ 2 14c3 2 14d2 2 14d2 2 14e2 2 14e3 2 14e3 4 12e3 2Juv

2 13e3 ½
2 13e4 sq
2 13e4 juv
2 14a1
2 13b2 part shaded
2 14b2 juv
2 14b3
2 14c1

2 13b1 2S sq 2 13b3 ½ 2 13b4 sq 2 13b4 2 13c3 ½ 2 13d1 ½ 2 13d2 ½ 2 13d2 ½

2 12b3 ½ shaded 2 12b3 juv 2 12c3 2 12c4 2 12d4 ½ 2 13a1 2 13a3 2 13a4

5 12a3 juv 5 12a4 ½ sq 5 12b4 ½ 5 12c3 5 12c4 ½ 5 12e2 2 12a3 sq 2 12a3 sq

5 13c2 5 13c3 2S 5 13c4 5 13c4 juv 5 13c2 juv 5 13d2 p sen 5 12e2 5 12e2

5 13a1 5 13a2 P 5 13a4 P 5 13b1 2S 5 13b2 p 5 13b3 p 5 13b3 P juv 5 13c1

5 14a2 p 5 14b1 ½ 5 14b2 5 14b3 5 14c3 5 14d2 ½ P 5 14d3 C 5 14d3 P

HCHECCBA

က

N

Plate 12:

4 12e4 4 13a2 ½ 4 13b1 juv 4 13b1

4 14e4 2Juv

4 14d1 ½

12

## Fri 2013-04-26

## Trayscan Test

#### Aim

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

#### 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

## Corrected Trayscan Import File

### Aim

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

### Method

The format of the tray identifiers was changed to reflect the label format. No other changes were made. File attachement:

 $./2013-04/20130426-{\tt Corrected Trayscan Import Sheet.csv}$ 

MD5SUM: ba68cc9c73d63b648899dac35129ace5

## Count of all sampled plants

#### Aim

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

### Method

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

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

# Mon 2013-05-06

## Development of Undistortion script

### Aim

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

### Method

To test the different parameters to the barrel undistortion model, the following command was run, with params substituted 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"

### 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.

# Wed 2013-05-15

## Creation of Caroline Chong's Solarcalc files

#### Aim

Create solar calc files for Caroline Chong's Pelargonium.

#### Method

- Solar Calc version 2013 Feb C (zip file MD5SUM: 0b2b456771eb44ed1fa8ed1a087bfbd0) was used.
- Location: Botrivier -34.219136°S, 19.195707°E
- Min Temp: 5 CMax Humid.: 85
- Start Date: 1/11/12End Date: 31/12/13
- End Date: 31/12Shading: 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:  $5~\mathrm{C}$
- 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:

- Solar Calc 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
- $\bullet$  2040 weather

#### Results

The following files have been attached:

- $\qquad ./2013-05/20130515- Caroline Solar calc Locations. kmz\ md5 sum\ 9a5be15bd5bcc854e2f27f33fb1e34e0$
- $\bullet \quad ./2013-05/20130515\text{-BotrivierCurrent.csv} \ \mathrm{md5sum} \ \mathrm{fbe6cea104e11eb9a35448b8be45613d}$
- ./2013-05/20130515-BotrivierCurrent.srp md5sum bdc0b67c73750e5c774e63d7a66aaaa1
- $\bullet \quad ./2013-05/20130515\text{-BotriverFuture.csv} \ \mathrm{md5sum} \ 36690160162125160371620976923986$
- $\bullet \quad ./2013-05/20130515\text{-BotriverFuture.srp} \ \, \mathrm{md5sum} \ \, 646c0bb13b39868ba0426000af9532aa$
- $\bullet \quad ./2013-05/20130515\text{-}TilbaCurrent.csv \ md5sum \ 4875aa65f83b86ead8f7008d724d54dc \\$
- $\qquad ./2013-05/20130515-TilbaCurrent.srp\ md5sum\ 878d3193b493a2ced5d69a32bfe8e3bf\\ \\ \qquad ./2013-05/20130515-TilbaFuture.csv\ md5sum\ 4ae75c0b7ff77eef4f7355cbc42fddb6\\$
- $\bullet \quad ./2013-05/20130515\text{-TilbaFuture.srp md5sum } 3544f9404cc1687f609b674767ac2f48$

## Testing of tissuelyzer settings and protocol

### Aim

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

### Method/Results

- $\bullet~$  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.
- $\bullet~$  The plate was put back into the -80