Digital Lab Notebook of Kevin Murray

Honours Project, 2013

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Last updated at 12:09on Friday 8th March, 2013

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 ≈ 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	
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	
G3	18	
H3	21	
A4	23	
B4	25	
C4	57	
D4	28	
E4	29	

Well	Line	Comments
F4	30	
G4	31	
H4	32	
A5	63	
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	
H10	21	
A11	68	

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

Attachments:

- `./dec12/20121203-harvest-pictures.tar.bz2` MD5SUM:2843946f8cae888a60dcd2226feb874f

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 ≈ 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	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	

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 2013-01-14

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)
- 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

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 `./jan/20130121-PracticeRNASamples.ods`)

Attachments

- `./jan/20130121-PracticeRNAExtractionSamples.csv`
- `./jan/20130121-PracticeRNAExtractionSamples.ndv`
- `./jan/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/L
- 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 $\approx$ 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.

Result

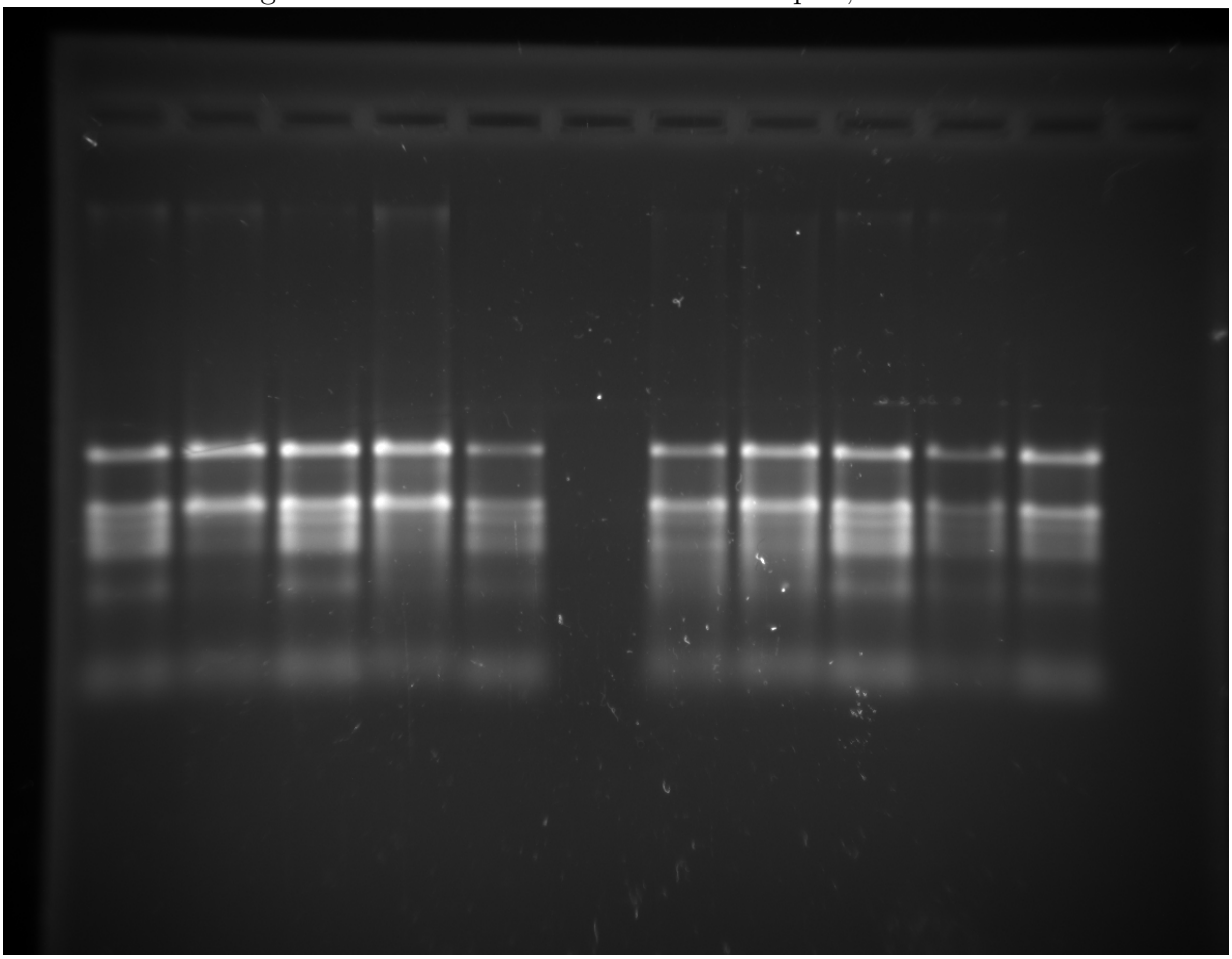
See Figure 1 below.
Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a mis-loading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

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 $\approx$ 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.

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.

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

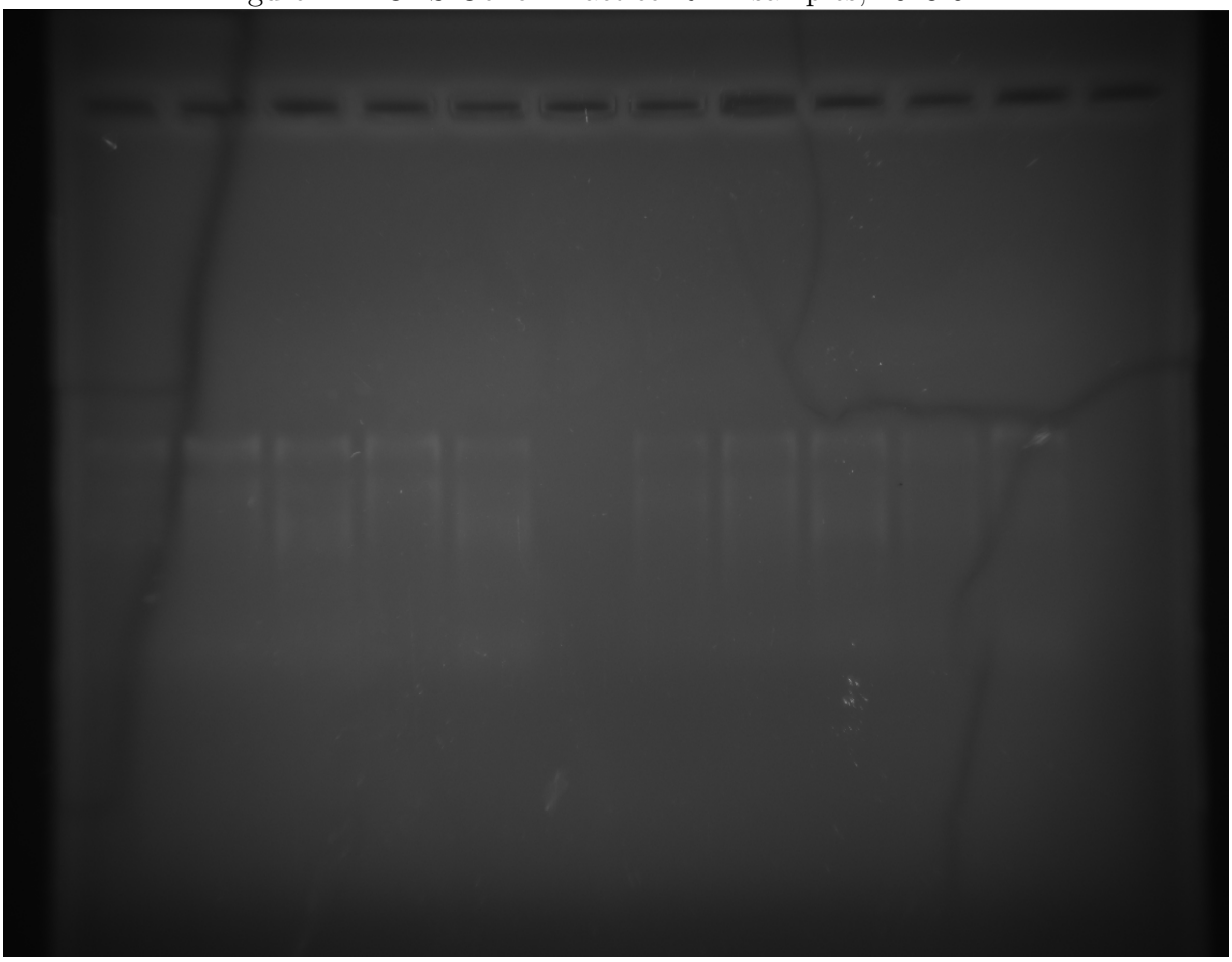
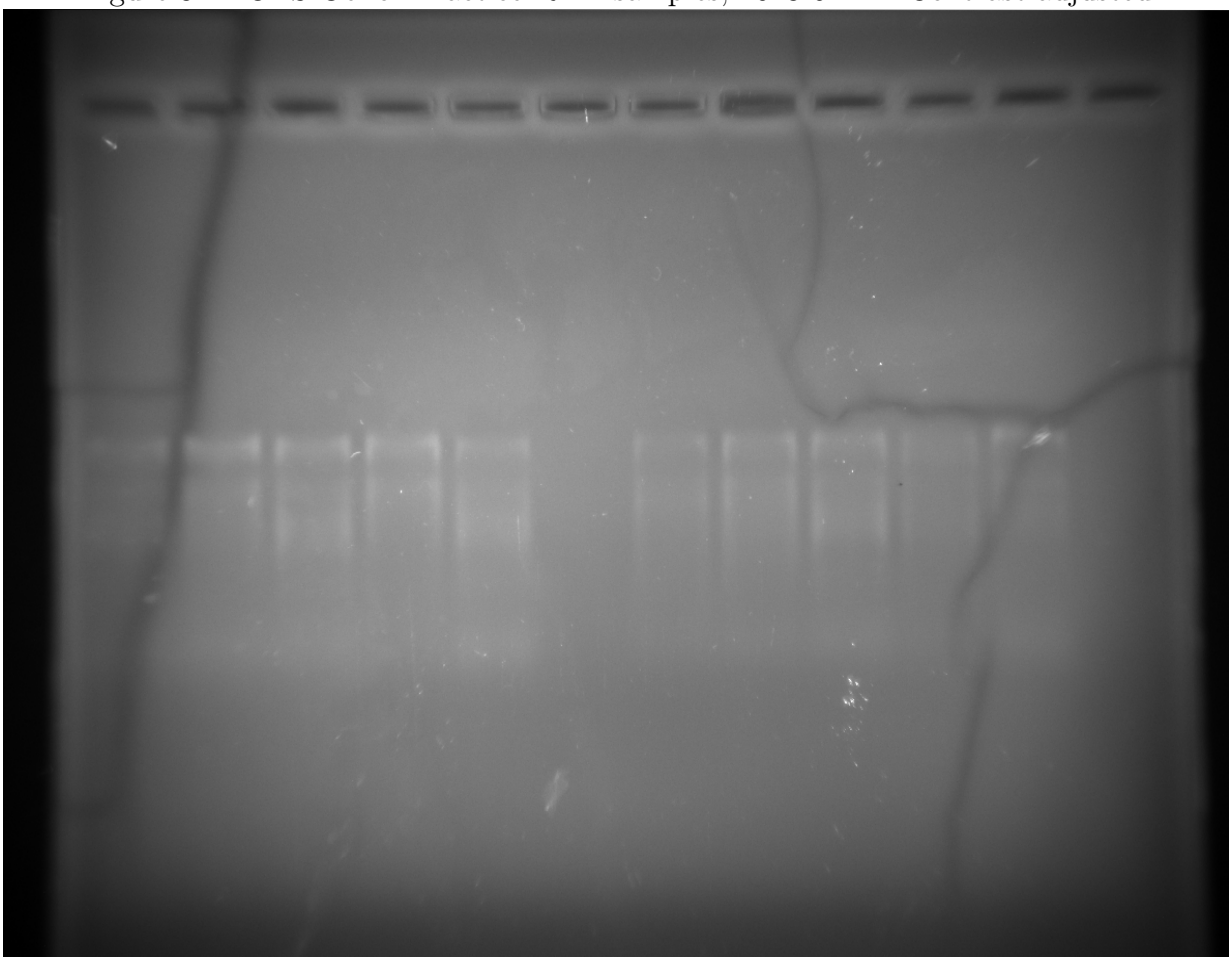


Figure 3: MOPS Gel of Practice RNA samples, 2013-01-22. Contrast adjusted.



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	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	+

Line	Desc	Count	Line	Desc	Count
38	147 x 50	+	88	51 x 18	+
39	147 x 69	+	89	54 x 183	+
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	+

Thu 2013-02-07

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 $\approx$ 1.5L RO water, containing $\approx$ 1mL/L AzaMax, 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"	"Line Number"	"Line Name"	"Quantity"
1	"Col-0"		45	"171 x 143"	
2	"cvi self"		46	"174 x 34"	
3	"1 x 146"		47	"180 x 157"	
4	"10 x 26"		48	"186 x 27"	
5	"101 x 176"		49	"187 x 69"	
6	"LL-1"		50	"19 x 173"	
7	"145 x 105"		51	"19 x 67"	
8	"107 x 121"		52	"190 x 176"	
9	"18 x 109"		53	"191 x 31"	
10	"109 x 48"		54	"192 x 189"	
11	"114 x 3"	6	55	"20 x 138"	6
12	"110 x 32"	6	56	"24 x 171"	
13	"112 x 30"		57	"15 x 9"	
14	"115 x 12"		58	"26 x 74"	
15	"118 x 108"		59	"33 x 58"	
16	"118 x 164"		60	"38 x 35"	
17	"12 x 142"		61	"39 x 27"	
18	"122 x 42"		62	"44 x 50"	
19	"125 x 117"		63	"45 x 23"	
20	"128 x 6"		64	"46 x 29"	
21	"129 x 132"		65	"48 x 160"	
22	"133 x 35"		66	"49 x 158"	
23	"134 x 29"		67	"5 x 172"	
24	"135 x 10"		68	"5 x 188"	
25	"136 x 102"		69	"51 x 111"	

"Line Number"	"Line Name"	"Quantity"	"Line Number"	"Line Name"	"Quantity"
26	"165 x 137"		70	"51 x 18"	
27	"139 x 36"		71	"54 x 183"	
28	"14 x 4"		72	"55 x 18"	
29	"146 x 64"		73	"59 x 116"	
30	"147 x 50"		74	"6 x 131"	
31	"147 x 69"		75	"61 x 162"	
32	"149 x 165"		76	"63 x 151"	
33	"150 x 37"		77	"8 x 61"	
34	"152 x 42"		78	"ler self"	
35	"153 x 108"		79	"ler"	
36	"153 x 20"	6	80	"cvi x ler"	
37	"156 x 106"		81	"ler x cvi"	
38	"119 x 60"	6	82	"npq1"	
39	"16 x 4"		83	"npq4"	
40	"16 x 66"		84	"pgr5"	
41	"16 x 7"		85	"Stn8-1"	
42	"166 x 25"		86	"Cvi-1"	
43	"168 x 22"		87	"135 x 142"	6
44	"17 x 21"				

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

- 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

Results

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