# Digital Lab Notebook of Kevin Murray

## Honours Project, 2013

*Jointly supervised by Justin Borevitz and Barry Pogson*

*Last updated at 13:01on 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:*

- `./2012-12/20121203-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:*

- `./2012-12/20121210-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 `./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/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

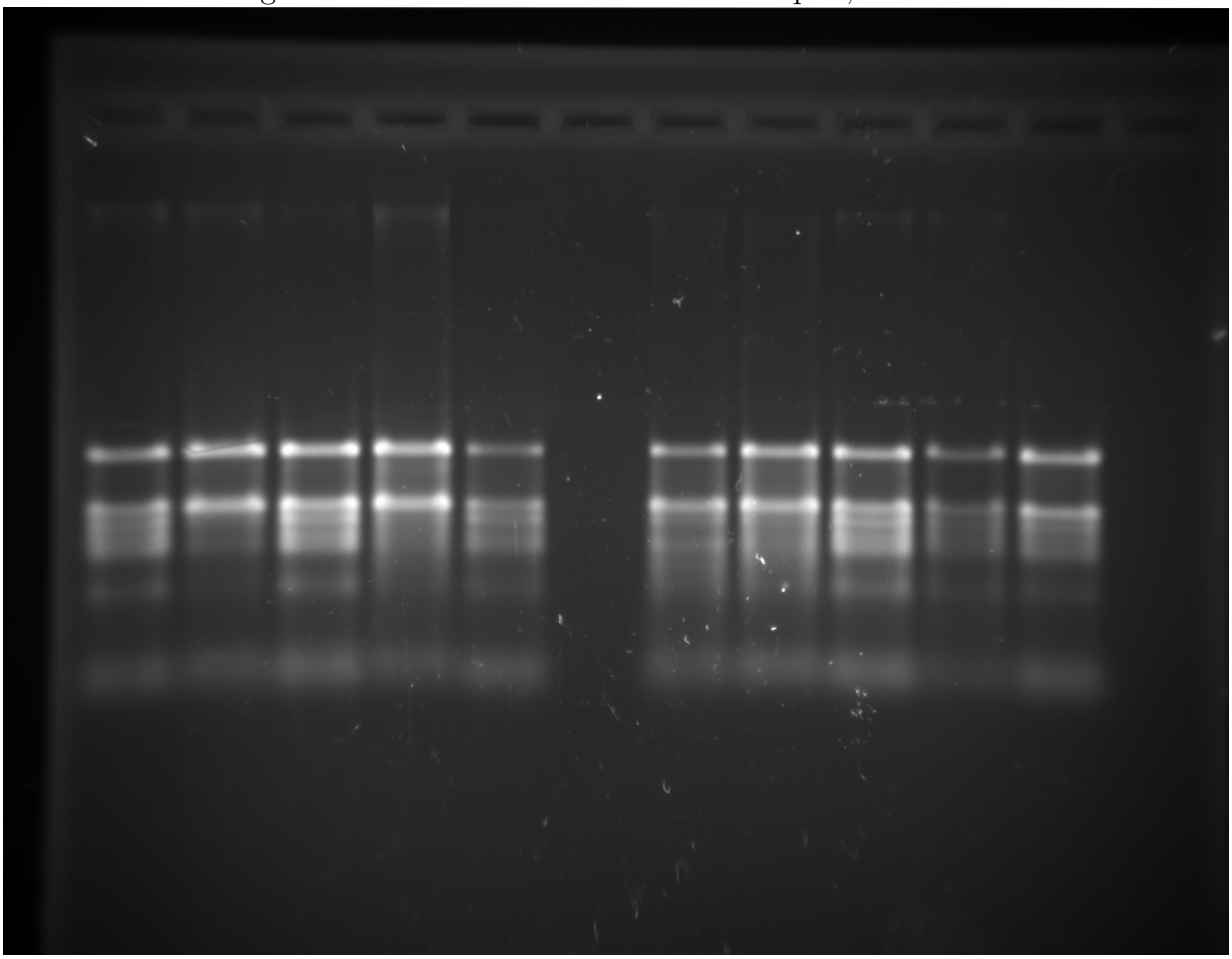
See Figure **??** below.
Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a mis-loading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

## 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 **??** and **??**
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

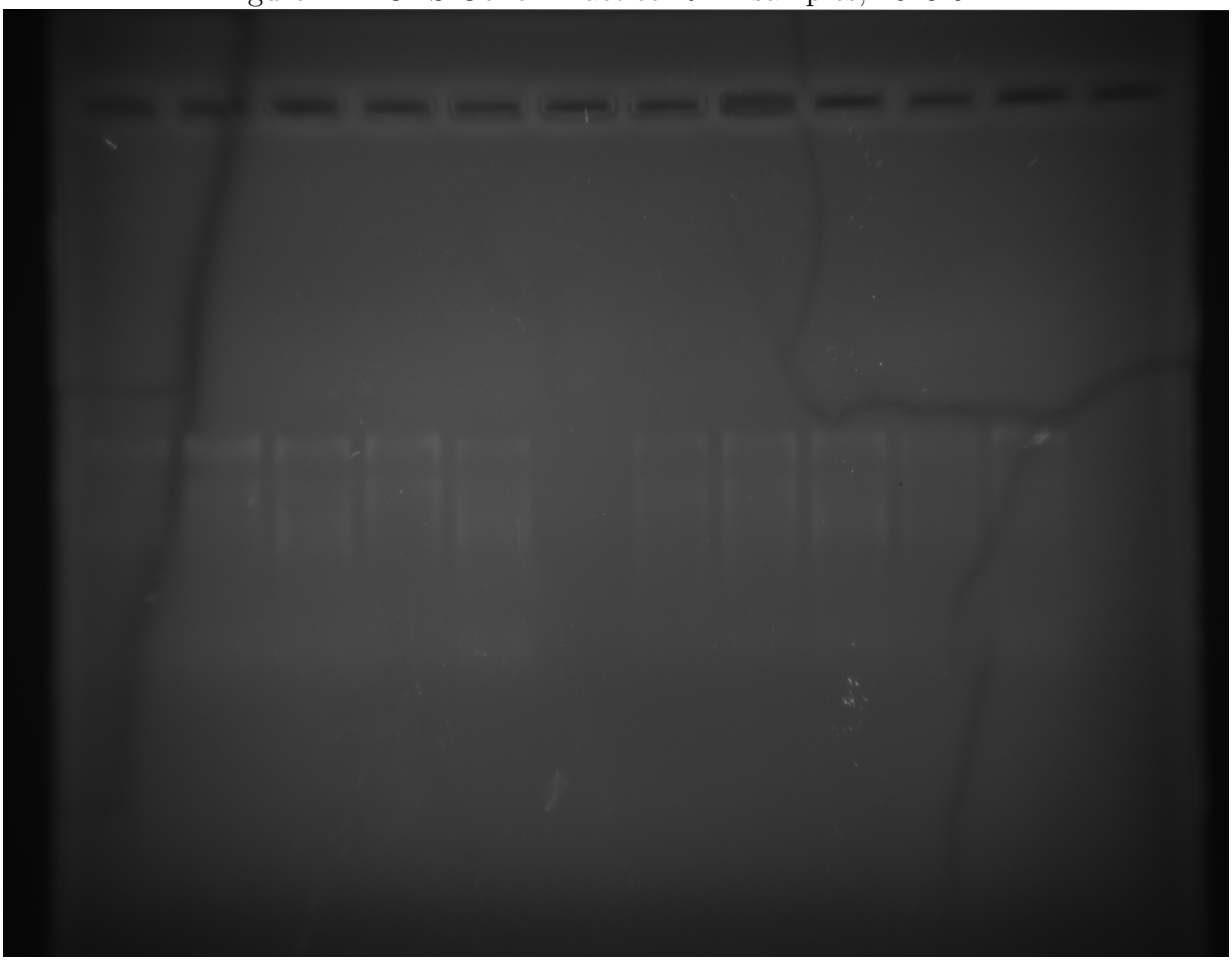
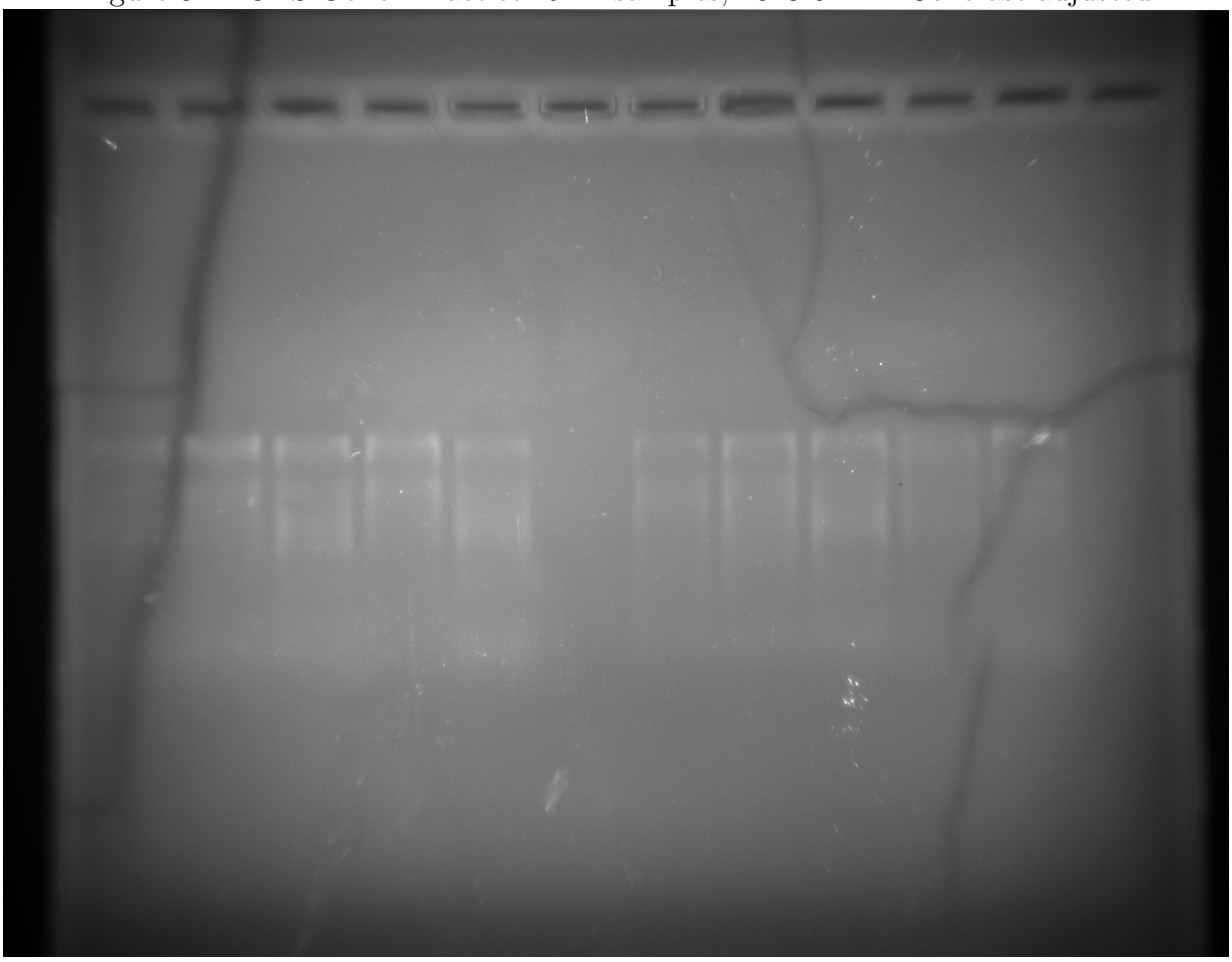


Figure 3: MOPS Gel of Practice RNA samples, 2013-01-22. Contrast adjusted.



# 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" | | | | |

# 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

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

# Thu 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

- 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

### Results

Solarcalc outputs:

- `KMTemora2012Sep01_45shade_2010weather_LED-BetterNormalised.csv` ddff182b78d1fe10e662fb827a179804
- `KMTemora2012Sep01_0shade_2010weather_LED-BetterNormalised.csv` d41d8cd98f00b204e9800998ecf8427e

Solarcalc preferences files:

- `KMTemora2012Sep01_0shade_2010weather_LED-BetterNormalised.srp` 9e800ba2b31a5346b37bc17127515d38
- `KMTemora2012Sep01_45shade_2010weather_LED-BetterNormalised.srp` d40480b773fdaad8ecf9bd14a5d445ed