# Digital Lab Notebook of Kevin Murray

## Honous Project, 2013

*Jointly supervised by Justin Borevitz and Barry Pogson*

*Last updated at 17:56on Wednesday 30th January, 2013*

# Mon 2012-12-10

## 1 Final Harvest of Keng's RIX lines

### 1.1 Aim

Harvest lines after 1 week of HL stress.

### 1.2 Method

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

### 1.3 Results

The following table describes the plate layout.

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

| 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

## 1 MAKE: Washed Ball Bearings

## 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

## 2 Tissuelyser grinding of practice samples

## 2.1 Aims

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

## 2.2 Method

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

# Mon 2013-01-21

## 1 Quantification of RNA samples

### 1.1 Aim

- Deterime qty of RNA in previously extracted samples

### 1.2 Method

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

### 1.3 Result

- Of the 14 samples, 10 had reasonable amounts of RNA, and 260/280 ratios were above 1.8 in all but one case. (see ./jan/20130121-PracticeRNASamples.ods)

### 1.4 Attachements

- ./jan/20130121-PracticeRNAExtractionSamples.csv
- ./jan/20130121-PracticeRNAExtractionSamples.ndv
- ./jan/20130121-PracticeRNASamples.ods

## 2 MADE: 10x MOPS Solution

*Method*

- Add 41.8g RNA only MOPS to beaker
- Add  450mL DEPC H2O, mix w/ stirrer bar on mag stirrer
- Add 26.6mL 3M Sodium Acetate (0.22um Filtered before use)
- Add 10mL RNA only 0.5M EDTA
- pH to 7 with 5M NaOH
- Top up to 500 mL with DEPC H2O
- Use 10ml per 100mL MOPS gel

*Mon 2013-01-21*

# 3 MADE: RNA Denaturing Gel (MOPS)

*Method*

- Melt 1g RNAse-free Agarose in 72ml DEPC H2O
- Add 10mL 10x MOPS
- Add 18mL 37% Formaldehyde
- Pour in RNA-only gel tank, previously washed with 0.5% SDS and RNAse-zap

# Tue 2013-01-22

## 1 Denature RNA for RNA gels

## 1.1 Method

- Dilute RNA to 100ng/uL
- Add RNA gel loading buffer (Obtained from Pete Crisp)
- Incubate at 65 degrees for 10 minutes. The samples were incubated for 10 minutes on the evening of 2013-01-21, but the gels were not run until 2013-01-22, so they were denatured for a futher 2 minutes at 65 degrees

## 2 TBE Gel

## 2.1 Aim

- To compare TBE and denaturing/MOPS gels for RNA

## 2.2 Method

- Dissolve 1g RNAase-free agarose in 90mL DEPC water
- Add 10mL RNAse-free TBE (prepared using DEPC Water, obtained from Pete Crisp)
- Pour in RNA-only gel tank, previously washed with 0.5% SDS or RNAse-zap
- Then, load denatured samples, and run in RNAse-free 1x TBE
- Run at $\approx$ 80V, $\approx$ 40-50mA for $\approx$ 1.75h
- Stain gel in 0.5ug/ml Ethidium Br in DEPC water?? for 10 min on orbital shaker, and photograph.

## 2.3 Result

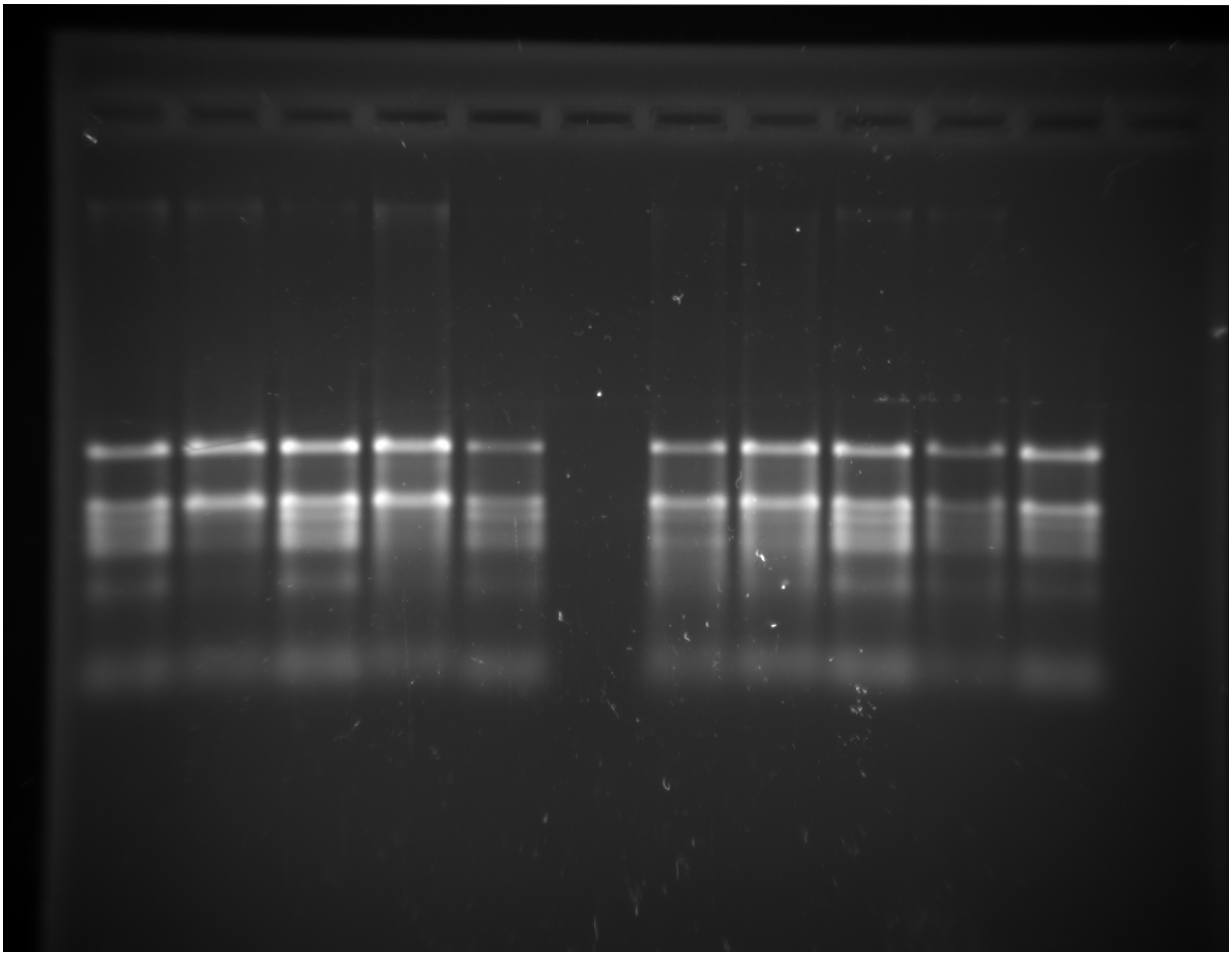
See Figure 1 below.
Gel indicates some degradation of RNA, however most samples are OK. Sample order is (left to right) A2, A3, A5, A6, A7, B3, B5, B7. A7 appears to have no RNA, although this is probably a misloading error. Overall, the TBE gel appears to be of more use than the MOPS gel.

## 3 MOPS gel

## 3.1 Aim

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

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



## 3.2 Method

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

## 3.3 Results

See Figures 2 and 3
Mops gel conifrms 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

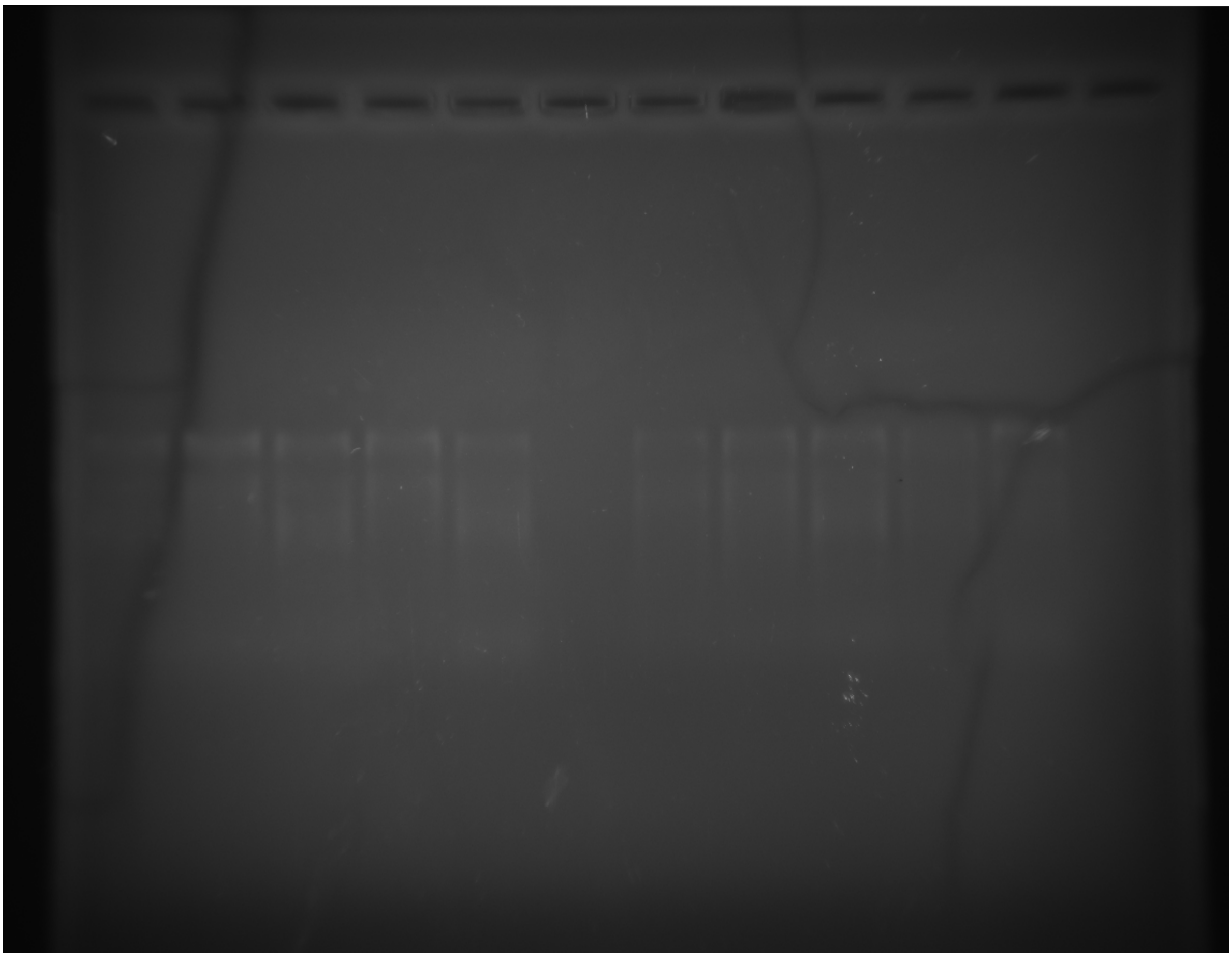
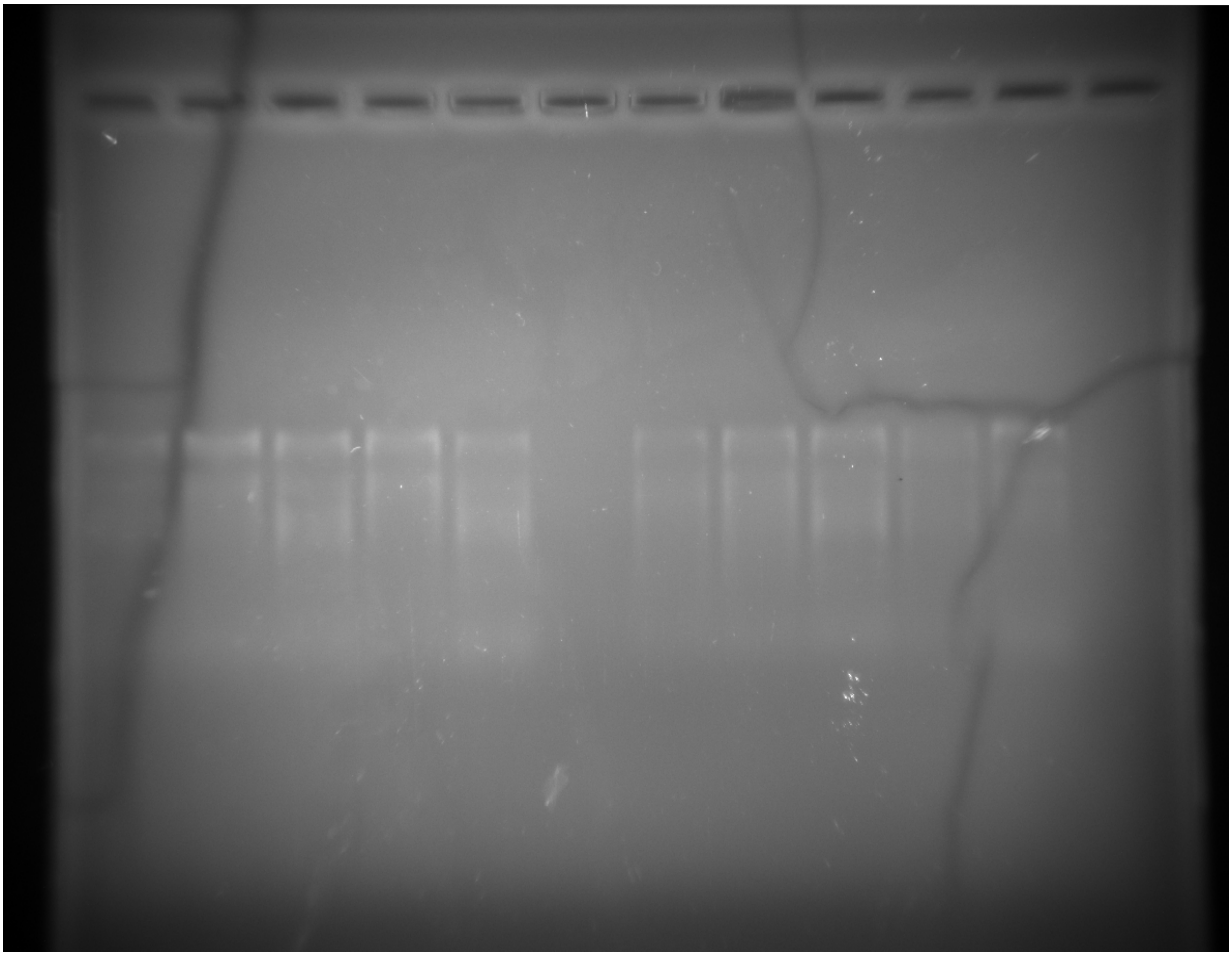


Figure 3: MOPS Gel of Practice RNA samples, 2013-01-22. Contrast adjusted.



# Tue 2013-01-29

## 1 Seed Stock Levels

The stocks of Joost's RIX set were checked. Seed lines were classified as having either plenty (+), limited(?) or no (-) seed. The levels of each line are shown in the table below.

| Line | Desc | Count | Line | Desc | Count |
|---|---|---|---|---|---|
| 1 | Col-$0_1$915 | not in box | 51 | 168 x 22 | + |
| 2 | Col-$0_4$936 | not in box | 52 | 169 x 175 | not in box |
| 3 | Cvi x Cvi | - | 53 | 17 x 21 | + |
| 4 | 1 x Cvi | not in box | 54 | 170 x 24 | - |
| 5 | 1 x 146 | + | 55 | 171 x 143 | + |
| 6 | 10 x 26 | + | 56 | 174 x 34 | + |
| 7 | 101 x 176 | + | 57 | 180 x 157 | ? |
| 8 | Ll-1 | not in box | 58 | 183 x 118 | - |
| 9 | 105 x 145 | not in box | 59 | 186 x 27 | + |
| 10 | 107 x 124 | not in box | 60 | 187 x 190 | - |
| 11 | 109 x 185 | not in box | 61 | 187 x 69 | not in box |
| 12 | 109 x 47 | not in box | 62 | 189 x 133 | - |
| 13 | 110 x 32 | ? | 63 | 19 x 173 | + |
| 14 | 112 x 30 | + | 64 | 19 x 67 | + |
| 15 | 113 x 141 | - | 65 | 190 x 176 | + |
| 16 | 114 x 3 | ? | 66 | 191 x 31 | not in box |
| 17 | 114 x 60 | + | 67 | 192 x 189 | + |
| 18 | 115 x 126 | + | 68 | 20 x 138 | + |
| 19 | 117 x 73 | ? | 69 | 21 x 22 | - |
| 20 | 118 x 108 | + | 70 | 24 x 171 | + |
| 21 | 118 x 164 | + | 71 | 25 x 9 | + |
| 22 | 119 x 177 | - | 72 | 26 x 74 | + |
| 23 | 12 x 142 | + | 73 | 33 x 58 | not in box |
| 24 | 122 x 42 | ? | 74 | 35 x 120 | - |
| 25 | 125 x 117 | + | 75 | 38 x 35 | + |
| 26 | 128 x 6 | + | 76 | 39 x 27 | not in box |
| 27 | 132 x 129 | not in box | 77 | 40 x 74 | - |
| 28 | 133 x 35 | + | 78 | npq4 | not in box |
| 29 | 134 x 29 | + | 79 | 43 x 131 | not in box |
| 30 | 135 x 10 | + | 80 | 44 x 50 | + |
| 31 | 135 x 140 | ? | 81 | 45 x 23 | + |
| 32 | 136 x 102 | + | 82 | 46 x 29 | + |
| 33 | 165 x 137 | not in box | 83 | 48 x 160 | + |
| 34 | 139 x 162 | - | 84 | 49 x 158 | + |
| 35 | 139 x 36 | + | 85 | 5 x 172 | not in box |
| 36 | 14 x 4 | + | 86 | 5 x 188 | not in box |
| 37 | 146 x 64 | not in box | 87 | 51 x 111 | + |
| 38 | 147 x 50 | + | 88 | 51 x 18 | + |
| 39 | 147 x 69 | + | 89 | 54 x 183 | + |

| Line | Desc | Count | Line | Desc | Count |
|---|---|---|---|---|---|
| 40 | 149 x 165 | + | 90 | 55 x 18 | + |
| 41 | 150 x 37 | + | 91 | 59 x 116 | + |
| 42 | 152 x 42 | + | 92 | 6 x 131 | + |
| 43 | 153 x 108 | + | 93 | 61 x 162 | + |
| 44 | 153 x 20 | ? | 94 | 63 x 151 | not in box |
| 45 | 154 x 144 | - | 95 | 7 x 46 | - |
| 46 | 156 x 166 | + | 96 | 8 x 61 | not in box |
| 47 | 16 x 4 | + | 97 | Ler x Ler | + |
| 48 | 16 x 66 | + | 98 | Ler self | + |
| 49 | 164 x 7 | not in box | 99 | Cvi x Ler | + |
| 50 | 166 x 25 | + | 100 | Ler x Cvi | + |