**Script order:**

1) setaria\_ril\_plantcv\_shell\_script.sh

- This is a bash script that calls plantcv functions enclosed in the directory:  
~/Dropbox/setaria\_height\_paper/script/image\_analysis/

2) add\_ril\_names.R

- This is an R script that loads in the resulting phenotype .csv file, coverts time stamp to days after planting, adds genotype names and converts pixel to experimental units (cm)

3) bellweather\_ril\_height\_eval.R

- Load in data and summarize the types of height measurements using different functions (mean, median, max, min)

- Calculate correlation between height types (note the result from ril.cor.mean goes into manuscript as Table\_S1)

- Calculate heritability of each of the five height types and partitioning of the total variance (each is done on 2 day blocks)

- Values from this analysis go into the paper as Table\_S2

- Results of plant height type influence on H2, treatment sensitivity and gxt partition go into paper as Figure\_S2a-c

- Values from this analysis go into the paper as Table\_S3 (treatment variance %)

- Evaluate the influence of summarization technique on measurement type (results suggest no major effect)

- Results of summary statistic effect on H2 go into paper as Figure\_S3

4) reformat\_of\_illinois\_data.R

- Load in the data from Illinois field team and filter based upon their flagging system (removed flag=2; aka definite outlier). Summarize using mean of the three measurements collected at each time point on each plot.

- Save raw data in QTL format

5) ril\_heritability\_height.R

- Load in data and calculate broad sense heritability of plant height as described by Alex Lipka, also calculate % variance explained by genotype within treatment blocks using a linear model and total variance partitioning using a linear model.

- Write out .csv files that contain heritability and total variance explained calculated in each experiment

- Naming conventions for experiment DR14 (Drought 2014)  
 \* **DR14.h2** is broad sense heritability

\* **DR14.h2\_in\_treatment** is broad sense heritability calculated within treatment blocks

\* **DR14.all** is a combined file that contains the results both **DR14.h2** and **DR14.h2\_in\_treatment**

\* **DR14.total\_var** is the total variance partitioned without plot as a factor, **DR14.total\_var.l** denotes the data.frame is in long form for ggplot2

\* **DR14.total\_var\_field** is the total variance partitioned including plot as a factor, **DR14.total\_var\_field.l** denotes the data.frame is in long form for ggplot2

- Change in proportion of variance through time observed in Bellweather trial goes into paper as: Figure\_1c

- Comparison of height heritability between years (H2 improved in 2014)

- This script produces the output that goes into Table\_S5 (Total variance partition across all experiments, includes all factors) and Table\_S6 (H2 across and within treatment blocks)

6) ril\_heritability\_flowering.R

- Same as above but for flowering time

- Comparison of flowering heritability between years (H2 did not improve in 2014)

7) anova\_analysis.R

- Filter data from the Bellweather RIL experiment to remove empty pots and plants not in bound.

- Get a subset of each experiment representative of final height

- Perform a type III ANOVA on all drought experiments to see order of factor importance (genotype, location, treatment)

- Check the importance of each factor in drought experiments (genotype, treatment, plot and year)

- Do same thing for each year

- Same for Bellweather experiment

- Have an issue with DL experiment (discuss with Ivan)

- Same thing for density experiments

- Perform ANOVA analysis on each day to determine what day treatment factor contributes significant effect

- Results of ANOVA tables are written out as .csv files and included in Table\_S7

8) ril\_logistic\_fit\_height.R

- Now fit the Bellweather phenotyping data using three types of logistic regression (3-parameter logistic, 4-parameter logistic, gompertz)

- For each genotype-treatment combination identify the best model fit from these three and write the estimated values, rates and model parameters to a new file

- Make plots of the estimated values and rates

- Write out estimated data in QTL format

9) ril\_growth\_rate.R

- Extract the rate data and format it for the QTL pipeline

- Writes files for average growth rate per day, maximal growth rate and day of maximal growth rate

- Plots growth rate per day, maximal growth rate and day of maximal growth rate as measured in treatment blocks (Figure\_8)

10) ril\_field\_blups.R

- Calculate BLUP models for each of the field experiments

- Write out derived BLUP value estimates in format for QTL pipeline

11) combined\_summary\_statistics.R

- Load in BLUP data and calculate quantiles, mean, variance and standard deviation (All of this data goes into Table\_1)

- Load in best fit logistic estimates of height from Bellweather calculate quantiles, mean, variance and standard deviation (All of this data goes into Table\_1)

- Load in the Dinneny Lab data same calculations as above

- Now load in the raw data from each experiment

- Calculate average replication within trials

- Calculate coefficient of variation for each time point in each experimental treatment (This entire data set ends up in Table\_S4), This is based upon model estimates (BLUP and logistic) not raw data

- Make Figure\_1a and Figure\_1b

12) correlation\_across\_exp\_and\_flowering.R

- Load in BLUP and logistic estimates of plant height and extract height at a time point representative of the last day

- Combine all measurements representative of mature plant height into a single data frame

- Calculate correlation between experiments based upon rank order

- Make a correlation plot of this as Figure\_2 (heatmap of experiments) also see Figure\_S4 (dendrogram of individuals).

- Load in BLUP estimates of flowering time

- Combine into a common data frame

- Investigate correlation between height and flowering time, add data to Table\_1 and make a few plots

13) Run scripts to make genetic map [Need to revisit this]

14) foxy\_qtl\_pipeline.py

- Run properly formatted data through the pipeline on a computer server. See instructions at: <https://github.com/maxjfeldman/foxy_qtl_pipeline>

- As part of this process you’ll also want to run the script: plot\_common\_qtl.py

15) qtl\_summary\_plots.R

- Define a few functions to condense all unique QTL into a fewer number of major QTL based upon overlapping 10 cM interval

- Transfer all QTL identified as associated with the difference of plant traits to a new data frame

- Combine these QTL data frames into sets corresponding to their experimental factors (i.e. one for drought, one for density, one for controlled environments, one for field, etc.)

- Write out the location of all QTL detected as a .csv file (Table\_S9)

- Make a plot of all the unique QTL locations NOT including QTL associated with the difference between treatments (Figure\_3)

- Write out a .csv file that contains all the QTL associated with the difference between treatments (Table\_S10)

- Condense each of these sets into a set of unique QTL using the functions defined at the top of the script

- Write out the location of all 34 truly unique QTL after collapsing within 10 cM window (Table\_2)

- Plot the location of these 34 unique QTL (Figure\_S5)

- Get a list of QTL (9) found in each of the 4 treatment blocks (dry, wet, dense, sparse) and write out to a file named “markers\_for\_fixed\_model.csv”

- Plot the location of the 34 unique QTL and the location of the difference QTL after collapsing them using the function at the top of the script (Figure\_S7)

16) foxy\_qtl\_pipeline\_known\_qtl.py

- Run properly formatted data through the pipeline on a computer server. See instructions at: <https://github.com/maxjfeldman/foxy_qtl_pipeline>

- As part of this process you’ll also want to run the script: plot\_common\_known\_qtl.py

17) known\_qtl\_fit\_at\_final\_day.R

- Read in the summary table results from the known QTL pipeline

- Combine all the experiments, subset to include only the last day

- Prepare a table that contains the % variance explained for each QTL at a representative final time point

- Calculate summary statistics across treatments and grand mean

- Prepare a heat map plot of this with the % variance explained in each cell (Figure\_5)

- Also prepare a .csv file and heat map that contains the % variance at each QTL across all time points and all experiments

18) venn\_diagram\_analysis.R

- Define a few functions to condense all unique QTL into a fewer number of major QTL based upon overlapping 10 cM interval

- Load in summary table data from QTL pipeline

- Combine all data into a common data frame, condense data and then split out into normal QTL and QTL associated with the numerical difference between treatments, then split out into individual experiments

- Get QTL (SNP locations) unique to a single treatment block in each experiment

- Make a few venn diagrams that illustrate how much these unique QTL over lap across different experiments

- Now for each experiment get QTL found only in wet and dry treatment blocks (DR13 -> DR13\_wo (wet only) or DR13\_do (dry only). This data is not plotted.

- Make a venn diagram that illustrates how much overlap there is within QTL between drought experiments (Figure\_5a), density experiments (Figure\_5b) and across a field v. controlled environment contrast (Figure\_5c)

- Make venn diagram plots of overlapping QTL between individual drought and density experiments, see how repeatable condition specific QTL are across experiments (Figure\_S6)

19) evaluate\_SLOD\_v\_MLOD\_result.R

- Define summary/condensing functions

- Load in SLOD and MLOD summary tables, collapse them to main QTL and add days after planting field

- Breakout into experiment and treatment block within experiment

- In each experiment, get list of QTL, number of QTL

- Calculate the mean, median variance and coefficient of variation for the number of QTL identified across treatment blocks within experiments

- Calculate the mean and median penalized LOD score (pLOD) and put all of these data in a common data.frame

- Load in a data.frame that contains the pLOD score of the QTL model and the penalty determined from the permutation threshold (This data was entered manually from R/qtl cross objects built as part of the QTL pipeline)

- Examined correlation between pLOD and (LOD/penalty), QTL number and (LOD/penalty), QTL number and pLOD, pLOD and QTL number, (LOD/penalty) and number of time points, pLOD and number of QTL, pLOD and number of time points, QTL number and number of time points

20) time\_series\_qtl\_analysis.R

- Define summary/condensing functions

- Load in SLOD summary tables for each experiment and put into a common data.frame, then condense using the functions described above

- Make a series of plots illustrating the proportional contribution of each QTL through time in each experiment (Figure\_S9)

- Make a series of plots illustrating the allelic effect size of each QTL through time in each experiment (Figure\_S10)

- Plot a treatment colored version to be included in the main body of manuscript (Figure\_7)

21) temporal\_qtl\_model.R

- Define summary/condensing functions, one specifically designed to reformat for plotting the results of a 3 QTL model

- Load in height estimate data from time series experiments and QTL summary table results

- For each experiment, get the 3 most significant QTL and get RIL names representative of these genotypes

- Plot the values of these genotypes over the course of the experiment in each treatment block (Figure\_9a and Figure\_9b)

- For phenotype experiment plot the rates too (Figure\_9c and Figure\_9d)

- Plot the percent of the variance that increase/decrease height from each QTL in the model at each time point and in each treatment block (Figure\_10a and Figure\_10b)

22) height\_by\_time\_and\_pot\_size.R

- Load in heights from the glass house experiment

- Assign column names to the day of the experiment where weights were taken

- Add in appropriate pot size category

- Make plot of heights through time faceted by pot size (Figure\_11)

- Make plot of heights through time faceted by genotype (Figure\_S12)