

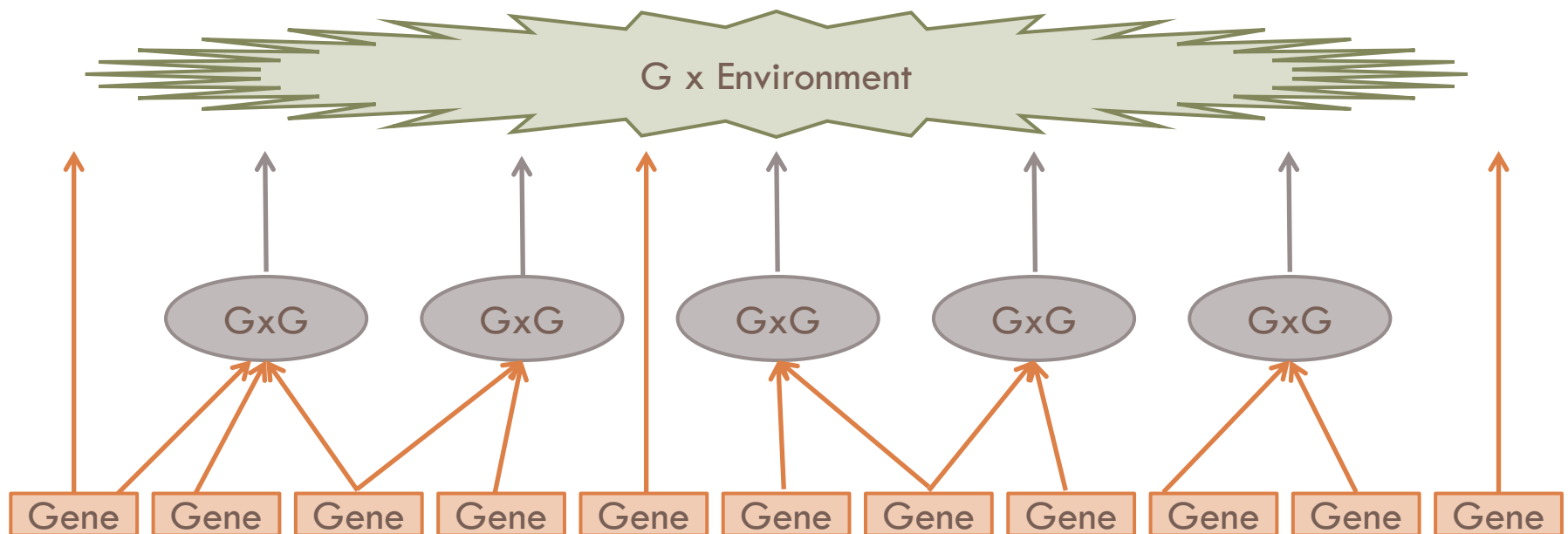
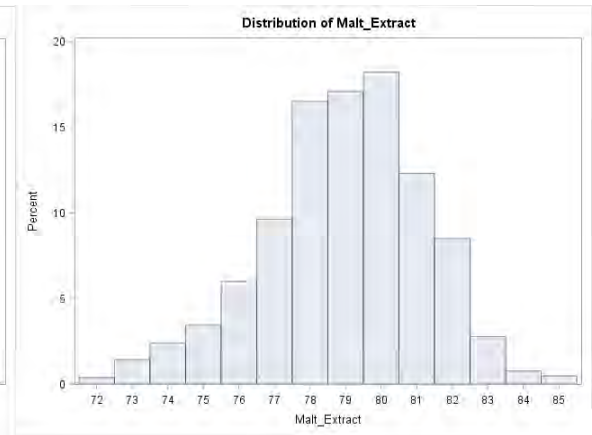
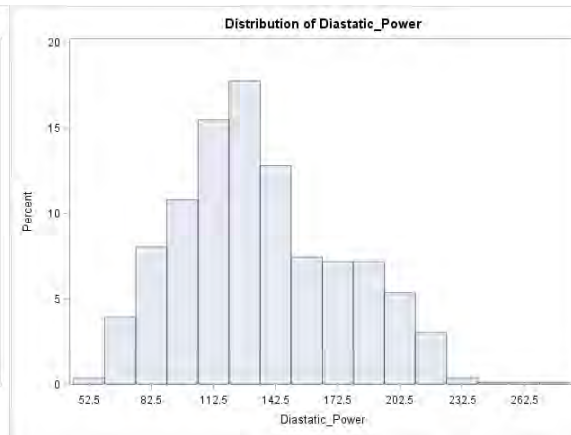
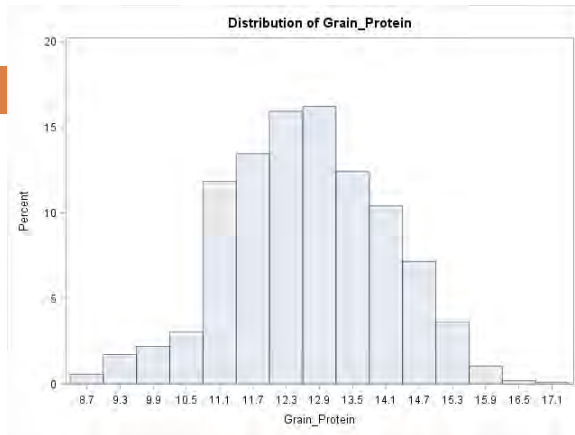
# GENE EXPRESSION SCREENING OF MUTAGENIZED BARLEY FOR MALTING QUALITY CANDIDATE GENES.

Kathy Esvelt Klos  
USDA-ARS

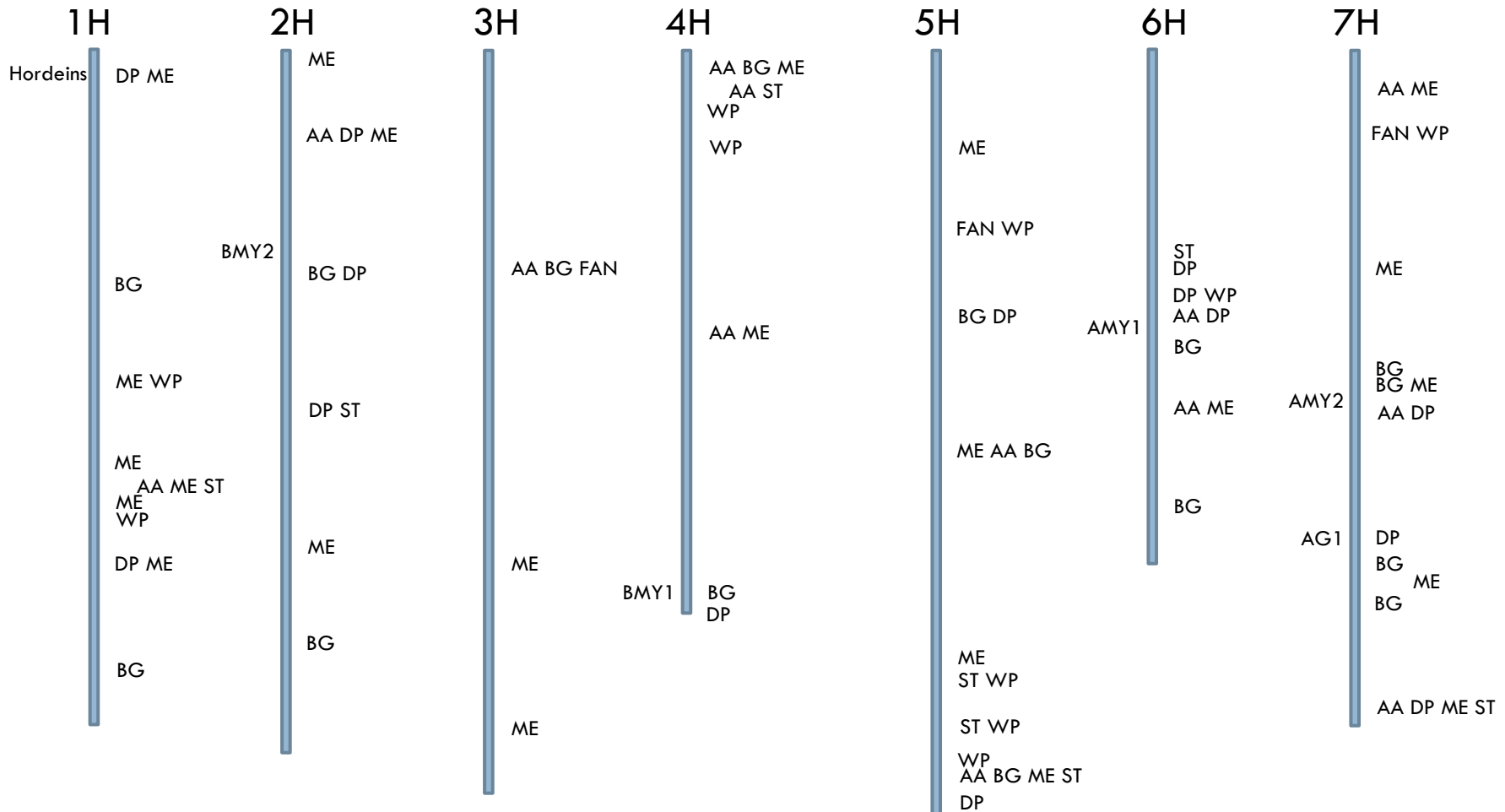
# Gene expression screening for barley mutants – summary of progress:

- As of this month, we have screened 300 of an estimated 2,000 M3 lines for changes to the expression levels of 5 genes in barley seed after 72 hours imbibition.
- We have selected 3 lines as possible mutation carriers.

# Malting quality: multiple layers of complexity



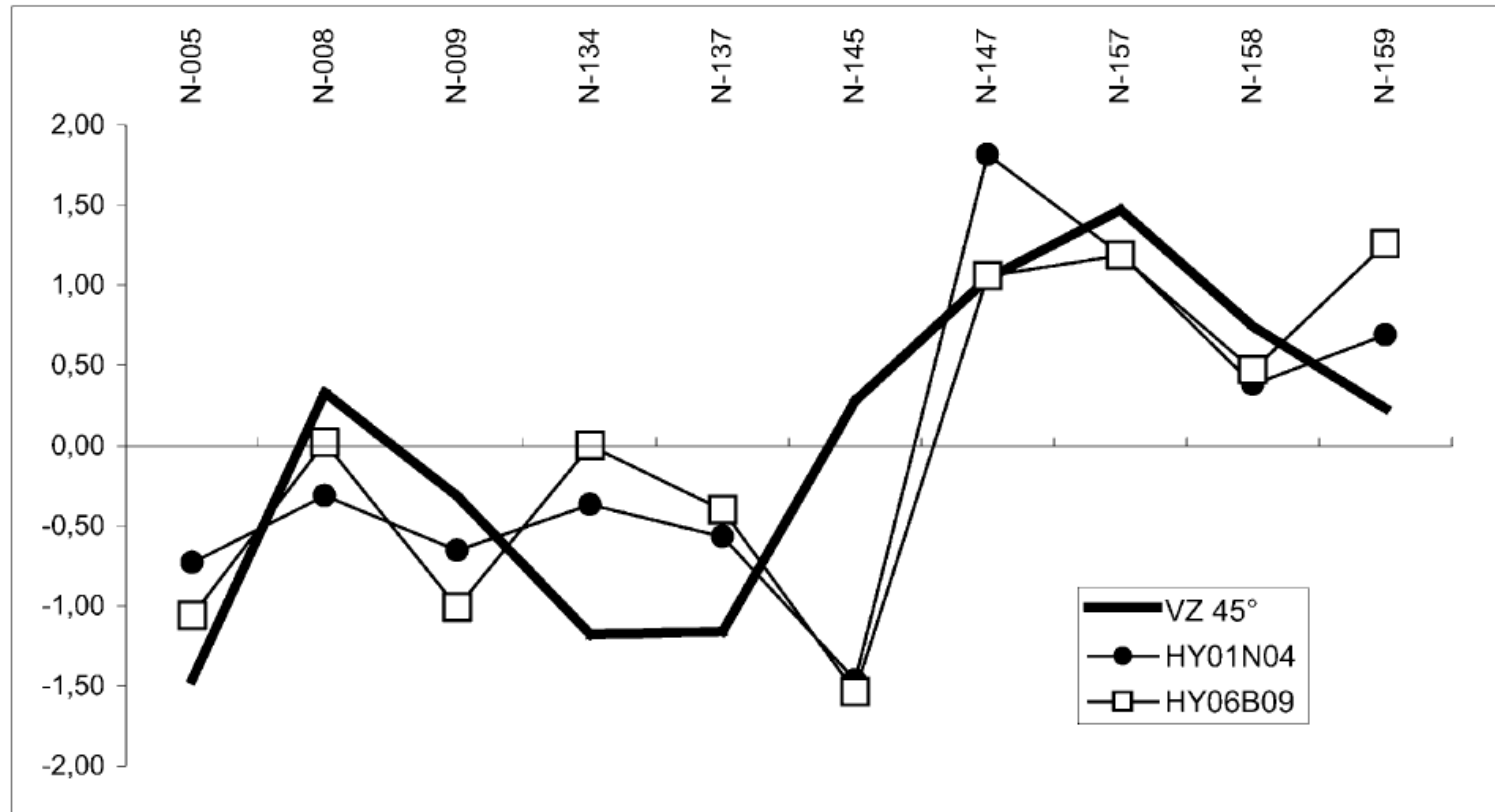
# Mapped QTL: Alpha-Amylase, Beta-Glucan, Diastatic Power, FAN, Malt Extract, Soluble/Total Protein, Wort Protein



Mohammadi et al. 2015. TAG 128:705-72.  
Islamovic et al. 2014. Mol Breed. 34:59-73.

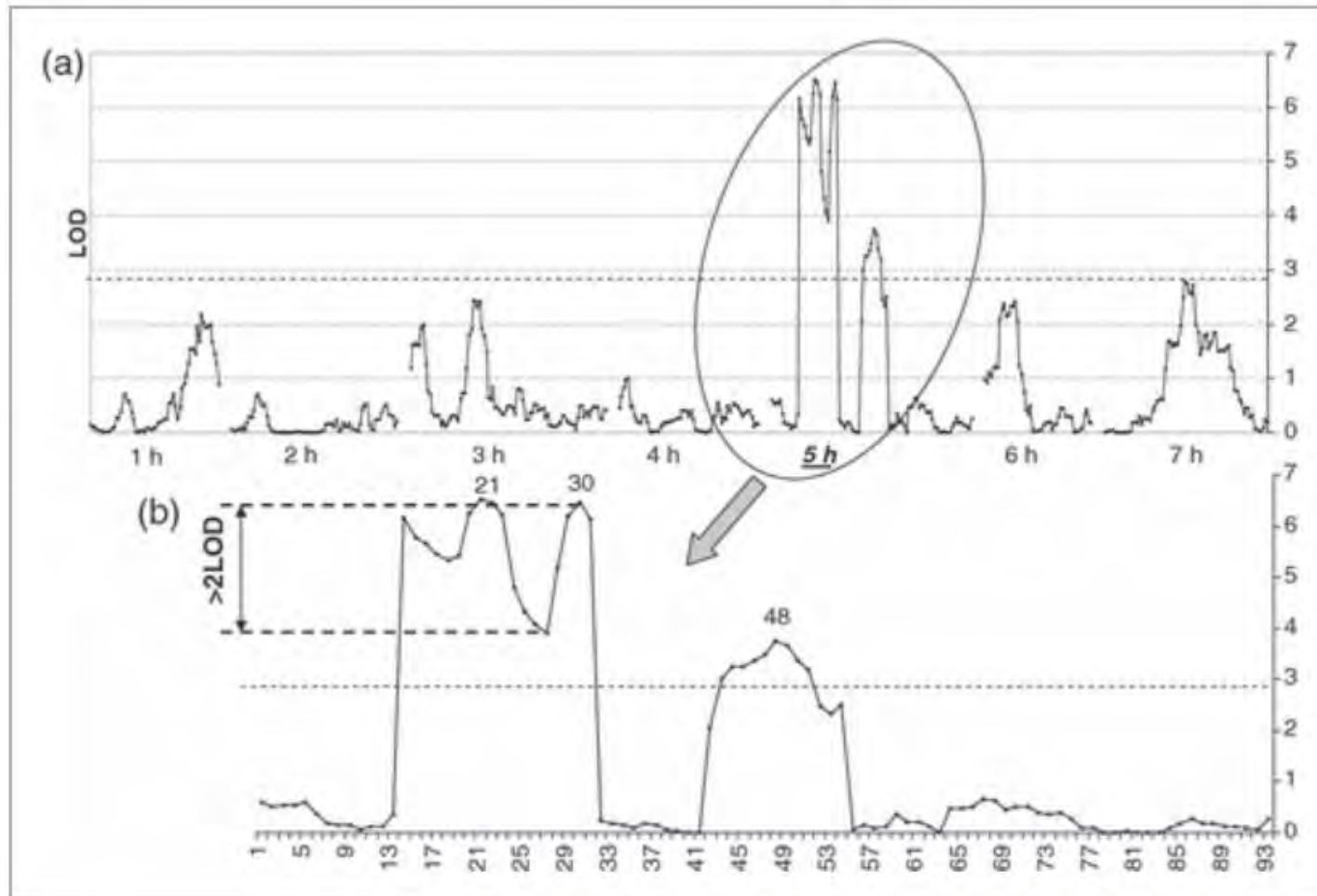
Gutierrez et al. 2011. TPG 4:256-272. Marquez-Cedillo et al. 2000. TAG 101:173-184  
Emebiri et al. 2004. Field Crops Res. 87:195-205

# Gene expression variation

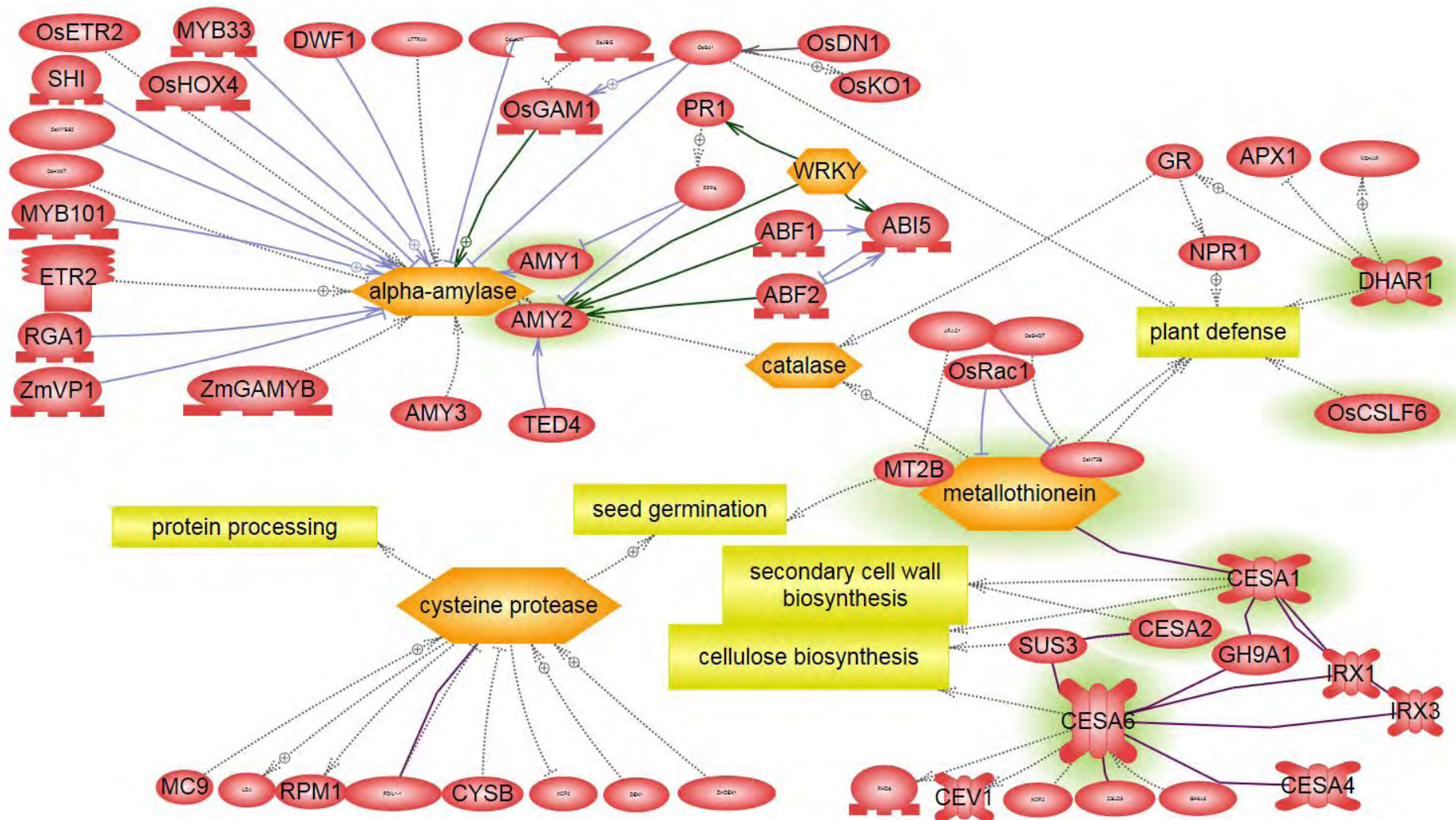


Variation among barley varieties for gene expression can be associated with variation in malting quality.

# Gene expression may be influenced by multiple loci.



A network of regulatory interactions among malting candidate genes is reported in the literature.



# Hypotheses:

- Mutagenesis may generate variation in candidate gene and their regulators that can be identified through changes to gene expression profiles.
- A single mutation has the potential to alter the expression of multiple genes, and the nature of these alterations could provide information about barley gene networks.
- Alterations to the expression profiles of malting quality candidate genes may result in changes to malting quality.

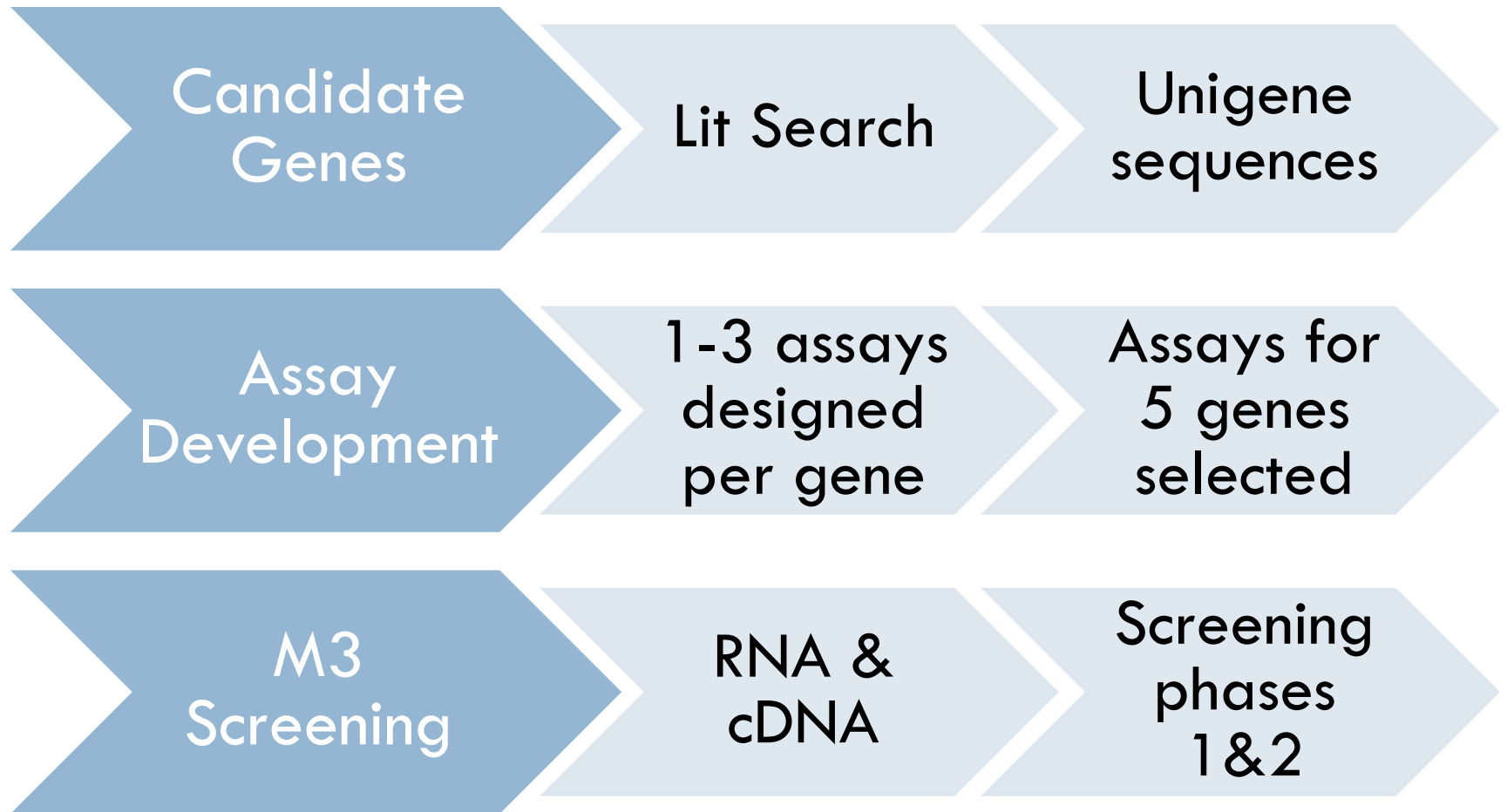


# Goals:

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- Generate a list of candidate genes hypothesized to influence enzyme levels in germinating barley seed and which, therefore, could influence malting quality.
- Identify mutant lines with altered gene expression 72 hours post imbibition.
- Map the location of mutations causing variation in gene expression.
- Characterize mutations for the changes they make to gene expression profiles and malting quality.

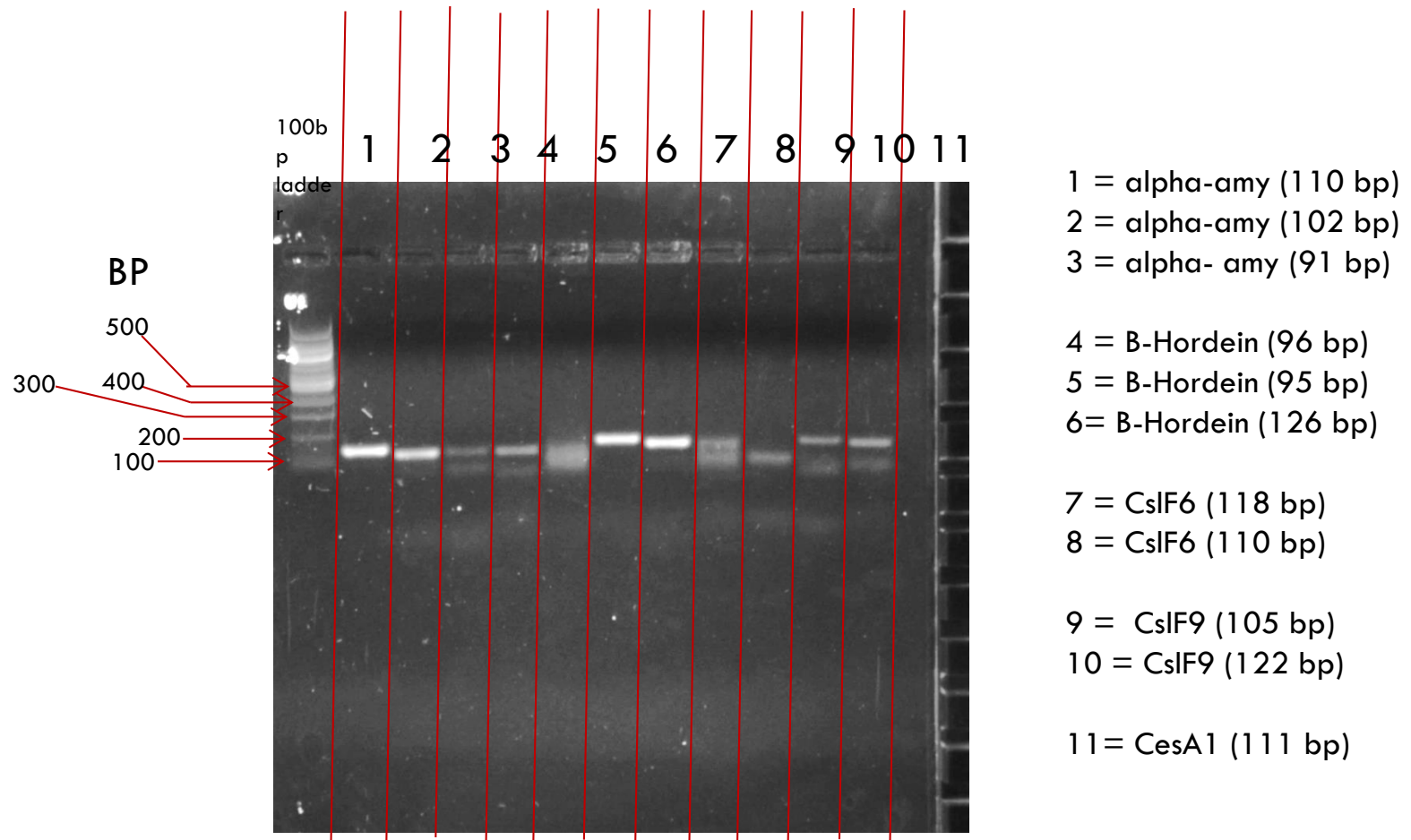
# Steps to mutant identification



# Candidate genes & assay design

GenBank Locus	Primer sets	GenBank Locus	Primer sets
<b>Alpha Amylases</b>		<b>CsIF6 &amp; CsIF9</b>	
M17126	3	EU267181	3
M17128	2	EU267184	3
FN179392	3	<b>Cellulose Synthases</b>	
<b>B &amp; C Hordeins</b>		AY483150	3
DQ267479	2	AY483152	3
JQ867075	2	AY483155	3
X03103	3	<b>Cystein Proteinases</b>	
JQ867090	3	AM941120	2
BLYHORDCA	3	AB377533	2
X60037	3	AY841792	2
<b>DHA Reductase</b>		<b>Metallothionein-Like</b>	
AK359422	1	S53707	1

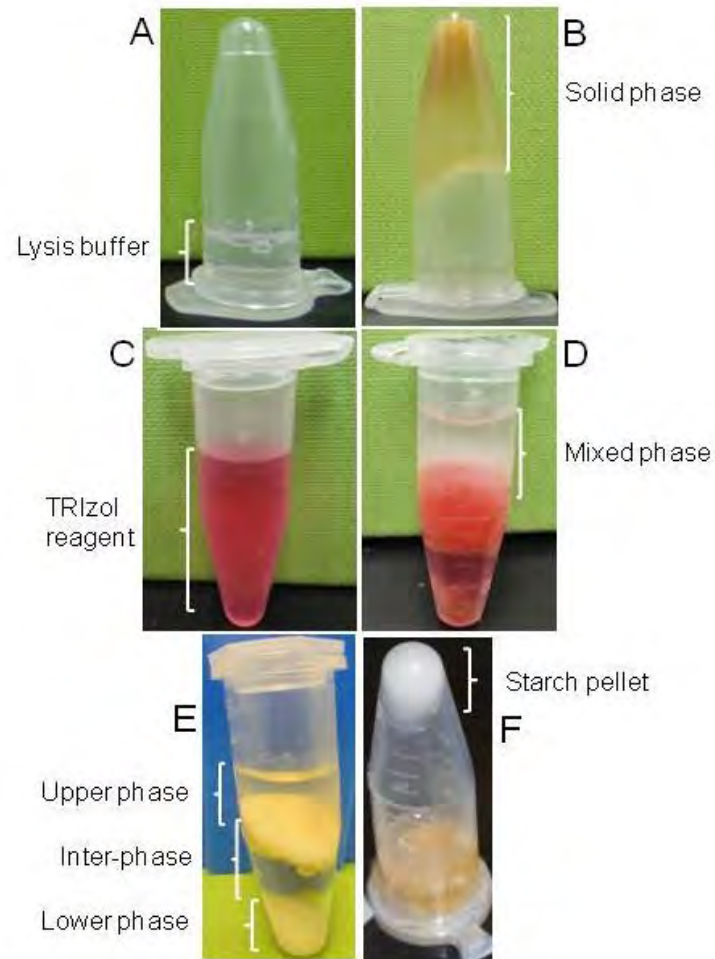
# Multiple assays were designed for each candidate gene



RT-PCR (End-point PCR) with Harrington cDNA

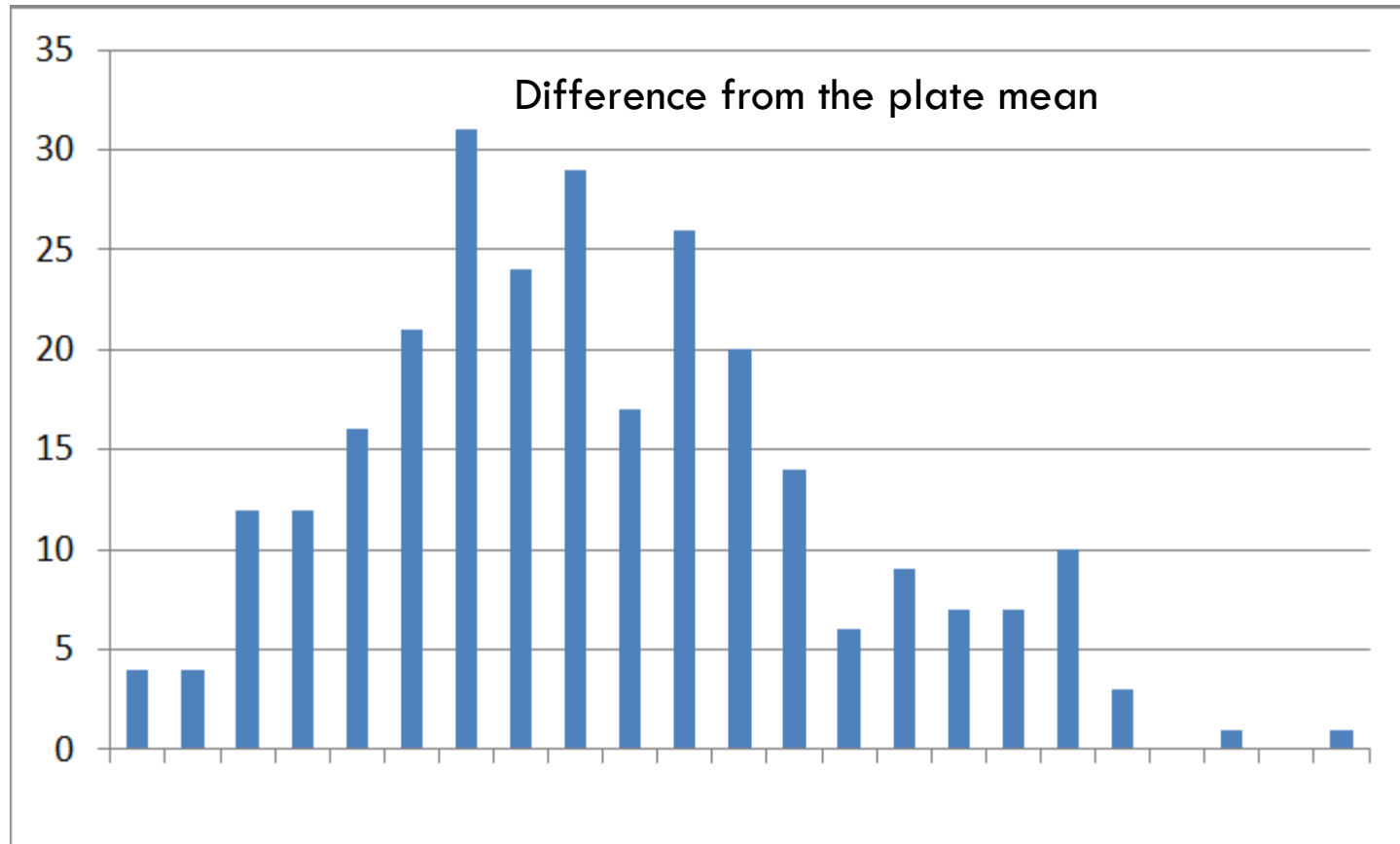
# Alterations to the RNA extraction protocol

Harrington wild type seed on damp filter paper after 72 hrs at room temperature



# Screening phase 1

- 300 lines
- 1 seed per line
- 5 genes



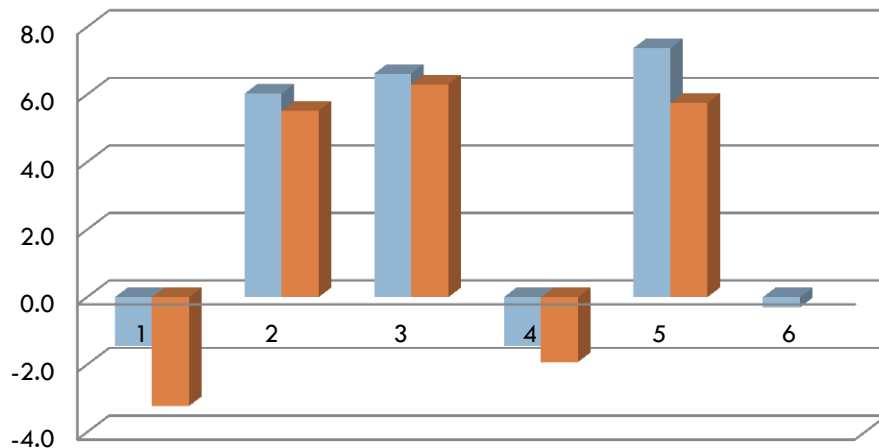
M3 lines were selected on the basis of gene expression levels 1-2 standard deviations from the plate mean

Sample	Amy1	C-Hordein	Cslf9	Cystein Proteinase	Metallothionien
15	3.1067658	-0.4819565	-1.4655435	-2.399255319	-2.272234043
23	-1.4660863	2.6480435	-2.4455435	-0.119255319	2.797765957
52	1.3895365	-0.4119565	-3.1555435	-1.249255319	-0.412234043
72	3.3501187	-2.2619565	-0.4555435	-0.409255319	-1.712234043
80	-3.1506633	4.4980435	-0.1355435	0.490744681	1.107765957
92	-3.1996531	1.8809375	3.6737634	2.896526316	0.306489362
138	3.1123489	-3.3290625	-0.1262366	-1.763473684	0.796489362
147	-9.6599569	-2.4790625	-1.6962366	-1.743473684	1.056489362
166	2.8257732	-3.4390625	-1.2862366	.	2.916489362
216	3.7787742	-1.3298958	-1.0482796	-1.848541667	-3.564270833
241	-2.0621357	4.6801042	1.3617204	4.571458333	4.895729167
253	3.8874538	1.0201042	0.8417204	-0.728541667	-2.154270833
272	-1.2645302	3.6001042	1.7617204	4.371458333	5.055729167
277	-1.2236967	2.7101042	2.1117204	3.441458333	4.145729167
286	3.3166262	0.0201042	-0.8882796	-1.068541667	-1.894270833

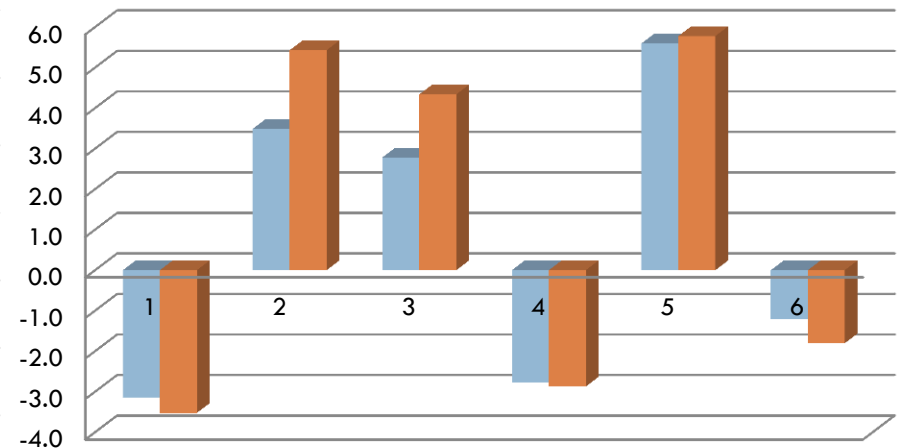
# Screening phase 2

- 15 lines
- 6 seed per line
- 2 replicates
- 5 genes

**M3 166 AMY1**



**M3 166 C-Hordein**





# Screening phase 3

- M52, M166, M277
- Merit
- 14 M3 parent plants
- Test for segregation in F2 seed



# Gene expression screening for barley mutants – summary of progress:

- As of this month, we have screened 300 of an estimated 2,000 M3 lines for changes to the expression levels of 5 genes in barley seed after 72 hours imbibition.
  - ▣ NEXT: continue screening additional M3 lines
- We have selected 3 lines as possible mutation carriers.
  - ▣ NEXT: Identify plants homozygous for mutations. Advance segregating crosses to map the mutation and evaluate effects on malting quality.

# Responsible Parties

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- Abdur Rashid, Ph.D.
- Tom Baldwin, Ph.D.
- Michael Gines