DRIVING OAT INTO THE MODERN ERA OF SCIENTIFIC DISCOVERY

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Jamie Sheridan · Emir Islamovic ·Nick Tinker · Gerard Lazo · Rick Jellen
· CORE Group ·

9th International Oat Conference Beijing, China

MOTIVATION(TO DRIVE OAT)

Scientists should be motivated by the adventure of discovery.

"Equipped with his five senses, man explores the universe around him and calls the adventure Science."

- Edwin Powell Hubble

We as oat scientists should be motivated by the discovery of novel traits leading to inventions in…

Agronomics
Stress resistance
Milling quality
Nutrition

"I find out what the world needs. Then I go ahead and try to invent it"

- Thomas Edison

INVENTIONS(OAT)

Livestock and human food

"eaten by people in Scotland, but fit only for horses in England"
- Samuel Johnson's dictionary

"That's why England has such good horses, and Scotland has such fine men!"
- Scotsman

Cereals

Hot oatmeal
Cheerios

Snacks

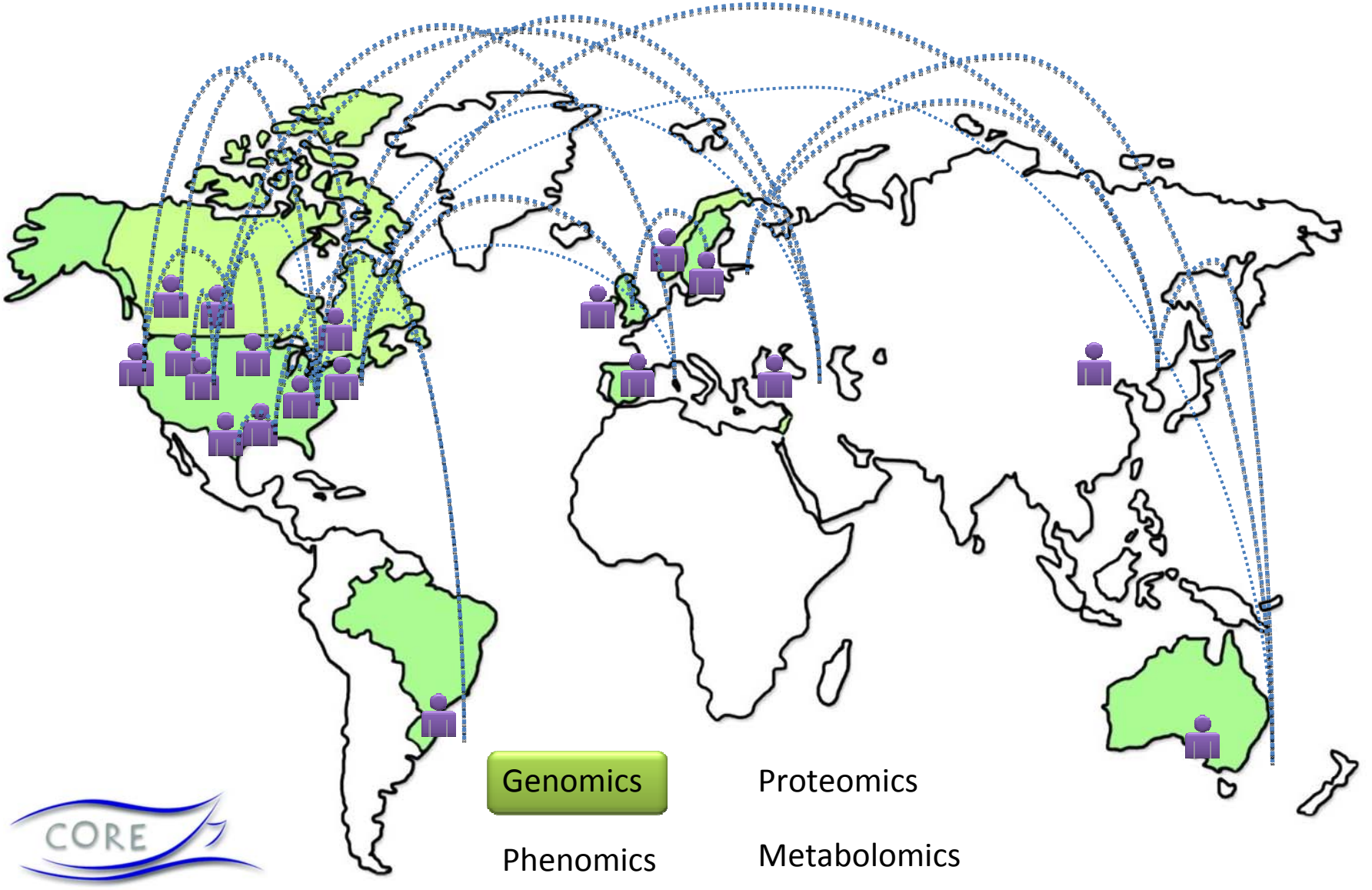
Granola bars
Cookies

Where will the next new oat invention come from?

COLLABORATION(CORE)

"Great discoveries and improvements invariably involve the cooperation of many minds."

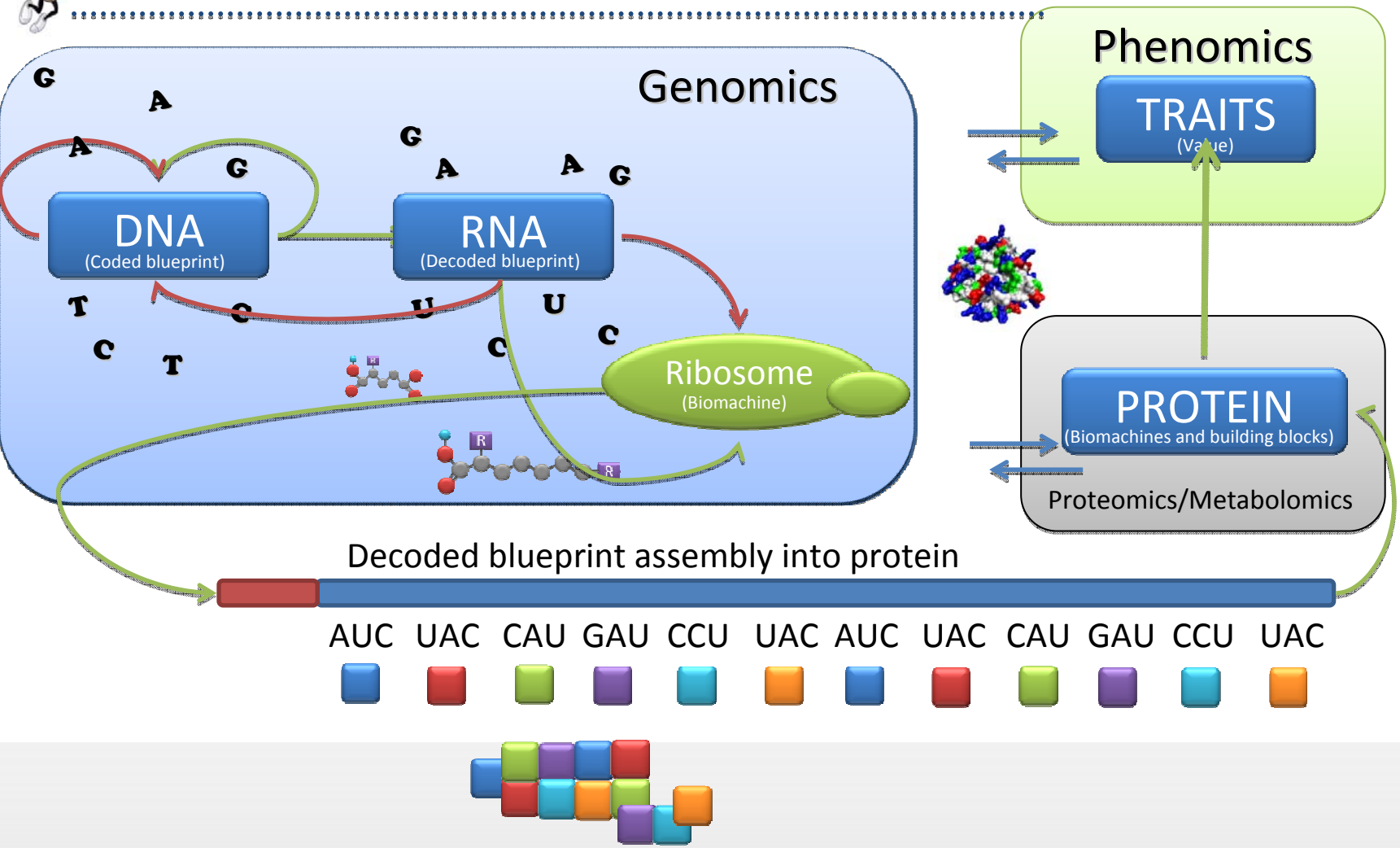
- Alexander Graham Bell



CORE

Collaborative Oat Research Enterprise

GENETIC DIVERSITY (CENTRAL DOGMA-BIOLOGY)



GENOMICS(OAT)

Annotated Mapped Sequence Resource

Develop a set of genomic resources

- Sequence
  - Transcriptome (*Expressed sequence tags*)
  - DArTome (*Complexity reduced*)
  - Microme (*small RNAs*)
- Sequence-based SNP markers
  - Illumina oOPA
    - GoldenGate®(1,536)
    - *Infinium® (6,000)*
  - KASPar
  - Genotype-by-sequencing (GbyS)
- Physically anchored consensus map

SEQUENCING(THE OMES)

9Mb (Mars)
(DArTome)

64Mb (Neptune)
(Transcriptome)

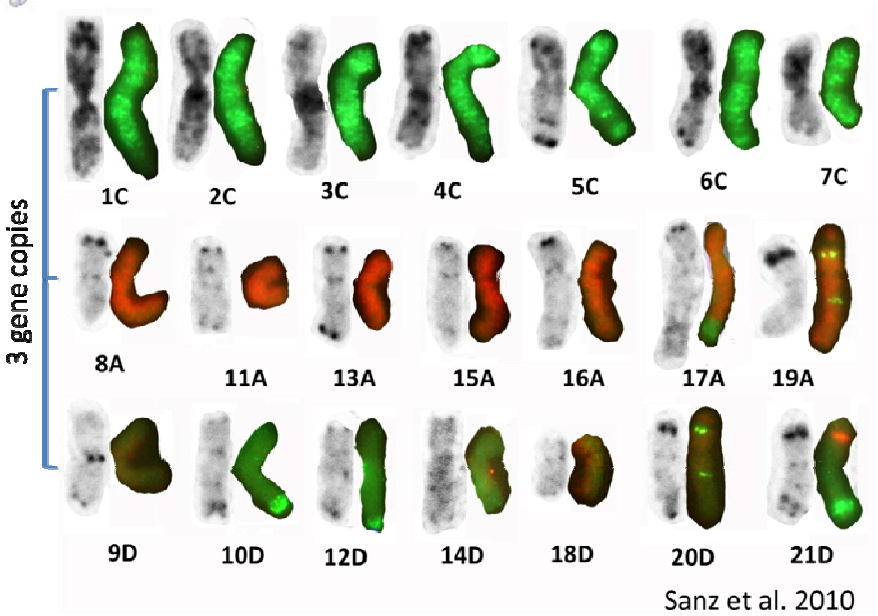
15,000Mb (Sun)
(Genome)

64Mb (Mercury)
(Transcriptome)

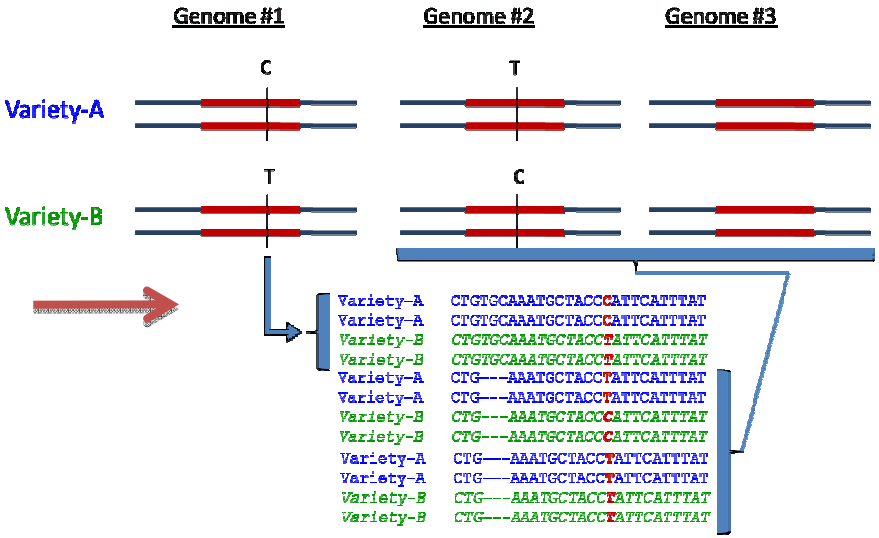
6.6Kb (½ Pluto)
(Microme)

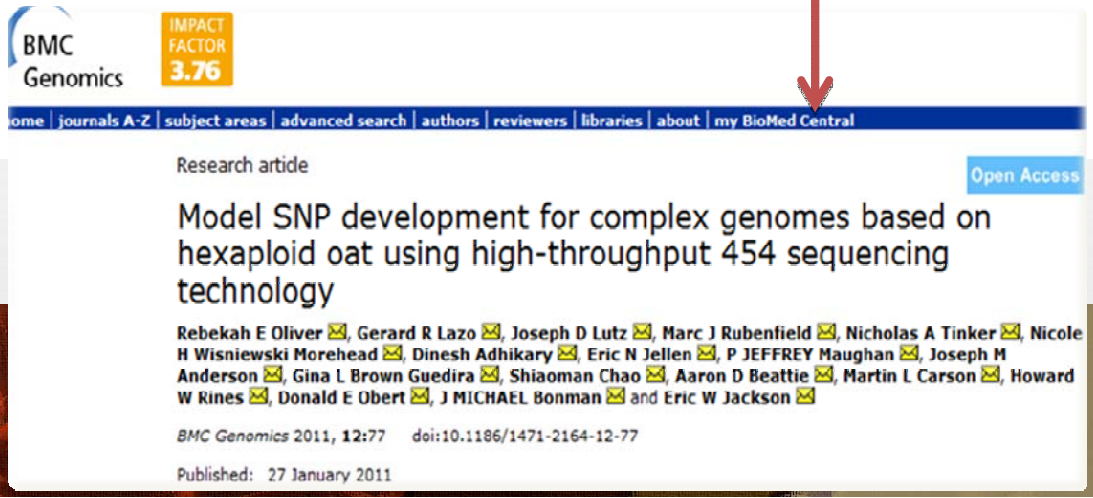
9Mb (Sun)
(DArTome)

SNP MARKERS(DEVELOPMENT)



Sanz et al. 2010





BMC Genomics

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Research article

Open Access

Model SNP development for complex genomes based on hexaploid oat using high-throughput 454 sequencing technology

Rebekah E Oliver, Gerard R Lazo, Joseph D Lutz, Marc J Rubenfield, Nicholas A Tinker, Nicole H Wisniewski Morehead, Dinesh Adhikary, Eric N Jellen, P JEFFREY Maughan, Joseph M Anderson, Gina L Brown Guedira, Shiaoman Chao, Aaron D Beattie, Martin L Carson, Howard W Rines, Donald E Obert, J MICHAEL Bonman and Eric W Jackson

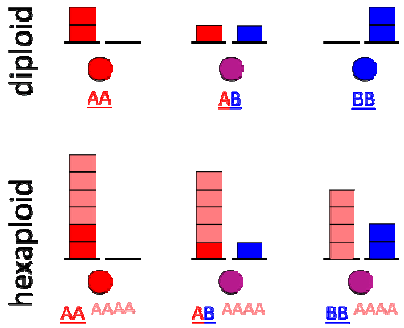
BMC Genomics 2011, **12**:77 doi:10.1186/1471-2164-12-77

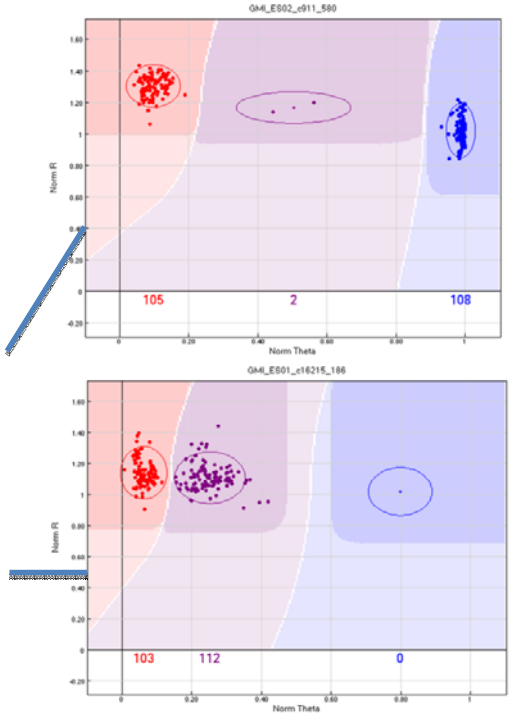
Published: 27 January 2011

SNP ASSAYS(VALIDATION)

Novel SNP discovery and chromosome-deficient hybrid anchoring strategies provide the first physically-anchored integrated map of oat

Oliver et al. *Submitted PNAS*





Summary of SNPs by marker discovery method

Discovery method*	Prefix	No. tested	Total good	Conversion rate (%)	No. SNPs Mapped	%	No. calls Diversity	%
cDNA – STA	GMI_ES01-17	2270	991	44%	738	33%	878	39%
cDNA – CTA	GMI_ES_CC	336	144	43%	98	29%	133	40%
DArT – CTA	GMI_DS_CC	300	121	40%	86	29%	108	36%
DArT - Sanger	GMI_DS_A, oPt	66	48	73%	36	55%	43	65%
Genomic Tetraploid	GMI_grs	100	7	7%	7	7%	7	7%
Totals		**3072**	**1311**	**43%**	**965**	**31%**	**1169**	**38%**

CONSENSUS MAP

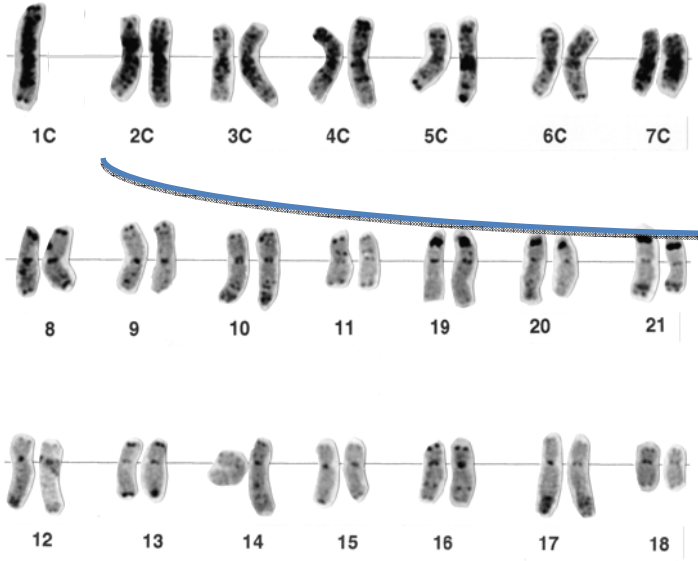
Population size, marker statistics, map characteristics, and key traits for RIL populations used for consensus mapping

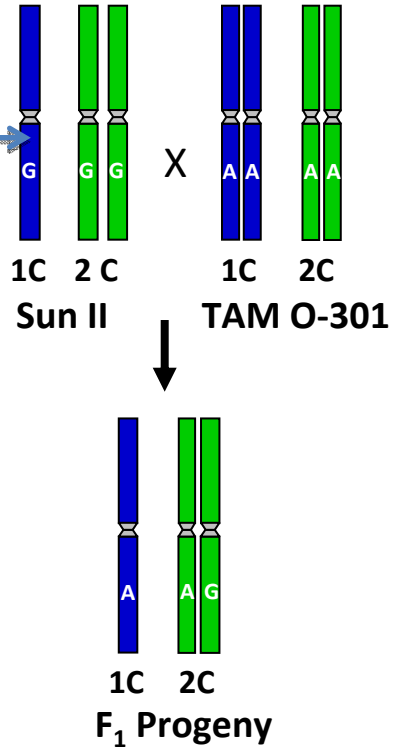
Population	RILs	Polymorphic markers	Marker type*	Framework loci†	Map size (cM)	Largest gap (cM)	No. LG	Key traits‡
Kanota/Ogle	52	320	DS, ES	111	1,387.7	36.2	25	O/Cr/Sr/H
Hurdal/Z-595-1	51	410	BA, DS, ES	370	903.8	35.1	27	DON
Ogle/TAM O-301	49	647	AB, AF, AM, BA, BM, DS, ES, Pc, M, TLP	214	1,576.2	39.0	37	Tocol/Cr/H
Otana/PI 269616	90	487	BA, DS, ES	323	1,516.7	32.6	23	PCr
Provena/94197A1-9-2-2-2-5	97	402	BA, DS, ES	278	1,775.7	30.3	24	PCr
SolFi/HiFi	52	401	BA, DS, ES	135	1,344.9	32.9	22	BG

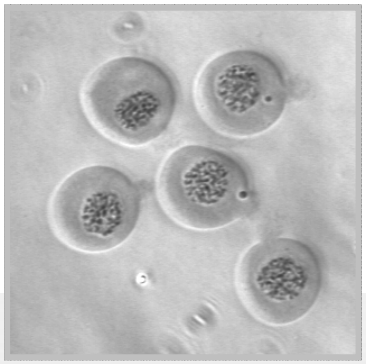
Pairwise comparison of common SNP markers across hexaploid oat populations

RIL population	SolFi/HiFi	Provena/94197A1-9-2-2-2-5	Otana/PI 269616	Ogle/TAM O-301	Hurdal/Z-595-1
Kanota/Ogle	100	113	133	214	111
Hurdal/Z-595-1	163	141	162	178	--
Ogle/TAM O-301	203	172	201	--	--
Otana/PI 269616	164	184	--	--	--
Provena/94197A1-9-2-2-2-5	148	--	--	--	--

MONOSOMIC-HYBRID (DELETION ANALYSIS)





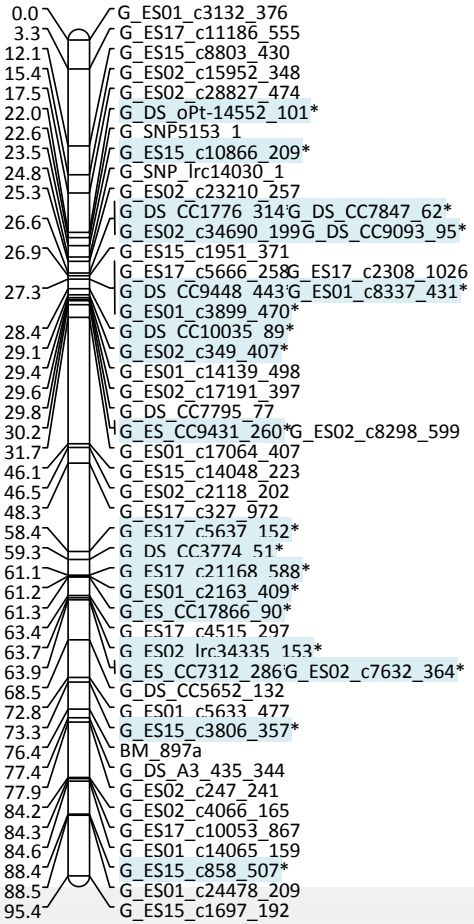


PHYSICAL MAPPING(MHDA-SNP)

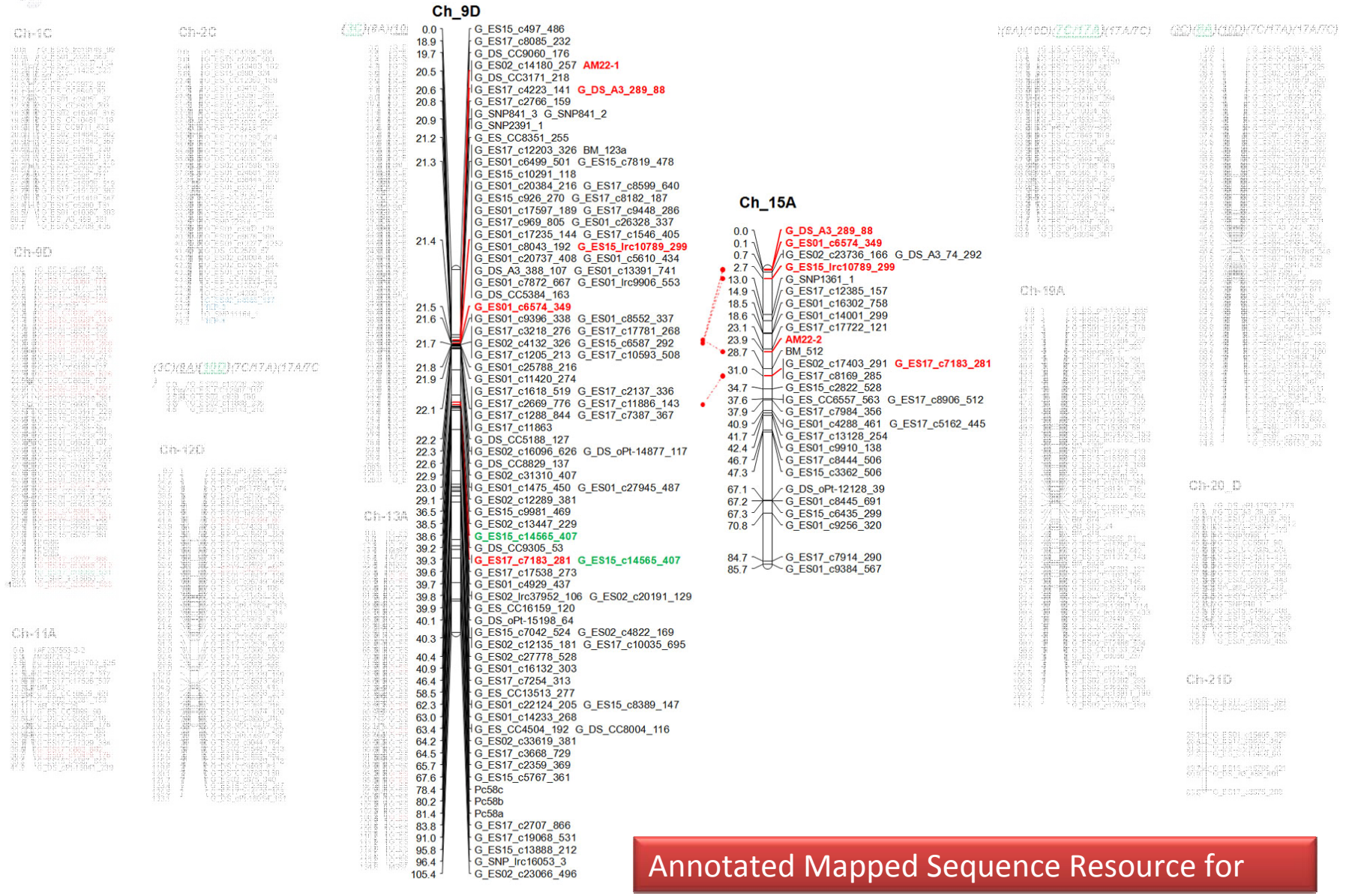
SNP ID	Sun II-1	Ogle	MHP 221 1C	MHP 99 2C	MHP 34 4C	MHP 53 5C	MHP 52 5C	MHP 185 6C	MHP 199 8A	MHP 196 8A	MHP 189 9D	MHP 205 11A	MHP 61 12D
GMI_DS_oPt-14552_101	GG	AA	AG	AG	AG	AG	AG	A-	AG	AG	AG	AG	AG
GMI_ES15_c10866_209	AA	GG		AG	AG	AG	AG	G	AG	AG	AG	AG	AG
GMI_DS_CC1776_314	AA	CC	AC	AC	AC	AC	AC	C-	AC	AC	AC	AC	AC
GMI_DS_CC7847_62	CC	GG	CG	CG	CG	CG	CG	G-	CG	CG	CG	CG	CG
GMI_ES02_c34690_199	GG	AA	AG	AG	AG	AG	AG	A-	AG	AG	AG	AG	AG
GMI_DS_CC9093_95	AA	GG	AG	AG	AG	AG	AG	G-	AG	AG	AG	AG	AG
GMI_DS_CC9448_443	AA	GG	–	AG	AG	AG	AG	G-	AG	AA	AG	AG	AG
GMI_ES01_c8337_431	GG	AA	AG	AG	AG	AG	AG	A-	AG	AG	AG	GG	AG
GMI_ES01_c3899_470	AA	TT	AT	AT	AT	AT	AT	T-	AT	AT	AT	AT	AT
GMI_DS_CC10035_89	AA	GG	AG	AG	AG	AG	AG	G-	AG	AG	AG	AG	AG
GMI_ES17_c5637_152	AA	CC	AC	AC	AC	AC	AC	C-	AC	AC	AC	AA	AC
GMI_DS_CC3774_51	GG	AA	AG	AG	AG	AG	AG	A-	AG	AG	AG	AG	AG
GMI_ES17_c21168_588	CC	GG	CG	CG	CG	CG	CG	G-	CG	CG	CG	CG	CG
GMI_ES01_c2163_409	AA	GG	AG	AG	AG	AG	AG	G-	AG	AG	AG	AG	AG
GMI_ES_CC17866_90	AA	GG	AG	AG	AG	AG	AG	G-	AG	AG	AG	AG	AG
GMI_ES02_lrc34335_153	AA	GG	AG	AG	AG	AG	AG	G-	AG	AG	AG	AG	AG
GMI_ES_CC7312_286	AA	GG	AG	AG	AG	AG	AG	G	AG	AG	AG	AG	AG
GMI_ES02_c7632_364	AA	GG	AG	AG	AG	AG	AG	G	AG	AG	AG	AG	AG
GMI_ES15_c3806_357	TT	AA	AT	AT	AT	AT	AT	A-	AT	AT	AT	AT	AT
GMI_ES15_c858_507	AA	GG	AG	AG	AG	AG	AG	G-	AG	AG	AG	AG	AG

SNP ID	Sun II-1	TAM 0-301	MHP 7 1C	MHP 97 2C	MHP 43 4C	MHP 42 4C	MHP 46 5C	MHP 100 6C	MHP 110 8A	MHP 111 8A	MHP 137 9D	MHP 192 11A	MHP 193 11A
GMI_ES15_c10866_209	AA	GG	AG	–	AG	AG	AG	G-	–	AG	AG	AG	AG
GMI_DS_CC7847_62	CC	GG	CG	CG	CG	CG	CG	G-	CG	CG	CG	CG	CG
GMI_ES02_c34690_199	GG	AA	AG	GG	AG	AG	AG	A-	AG	GG	AG	AG	AG
GMI_DS_CC9093_95	AA	GG	AG	AG	AG	AG	AG	G-	AG	AG	AG	AG	AG
GMI_ES02_c349_407	GG	AA	AG	AG	AG	AG	AG	A-	AG	GG	AG	AG	AG
GMI_ES_CC9431_260	CC	GG	CG	CG	CG	CG	CG	G-	CG	GG	CG	CG	CG
GMI_ES17_c21168_588	CC	GG	CC	CC	CC	CC	CC	G-	CC	CC	CC	CC	CC
GMI_ES_CC7312_286	AA	GG	AG	AA	AG	AG	AG	G-	AG	AA	AG	AG	AG

Ch_6C



PHYSICALLY ANCHORED MAP(21CH)



Annotated Mapped Sequence Resource for

FUNCTIONAL EVOLUTIONARY APPROACH (FUNCY EVO)

Model Systems

- Arabidopsis
- Maize
- Barley
- Wheat

Target Systems

- Oat

Model Systems	Target Systems
Annotated genome	Partial annotated transcriptome
Physical map	Physically anchored map
Defined biosynthetic pathways	Partially defined biosynthetic pathways
Traits	Traits

Three Stories of FUNCY EVO discovery

1. Hulless theory – Understanding the genetics of hulless oats
2. Vitamin E synthesis in barley – From QTL to gene discovery
3. Oat beta glucan – The problematic genome

HULLESS THEORY

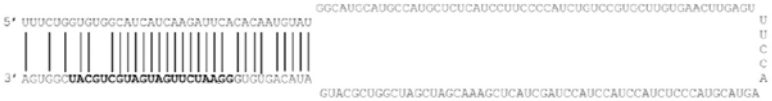
BARLEY OAT COMPARIONS(INDETERMINATE SPIKELET)

Determinate spikelet

Indeterminate spikelet

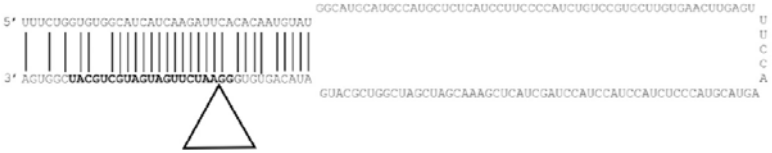
miR172 MUTANT

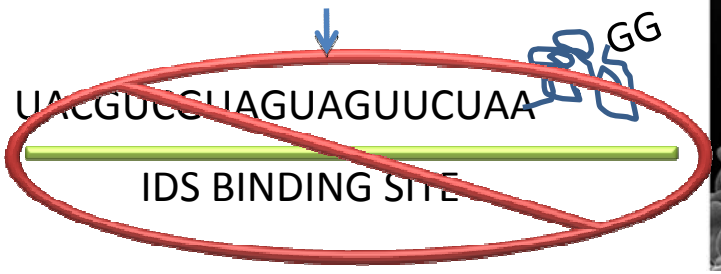
Expressed miR172 preRNA

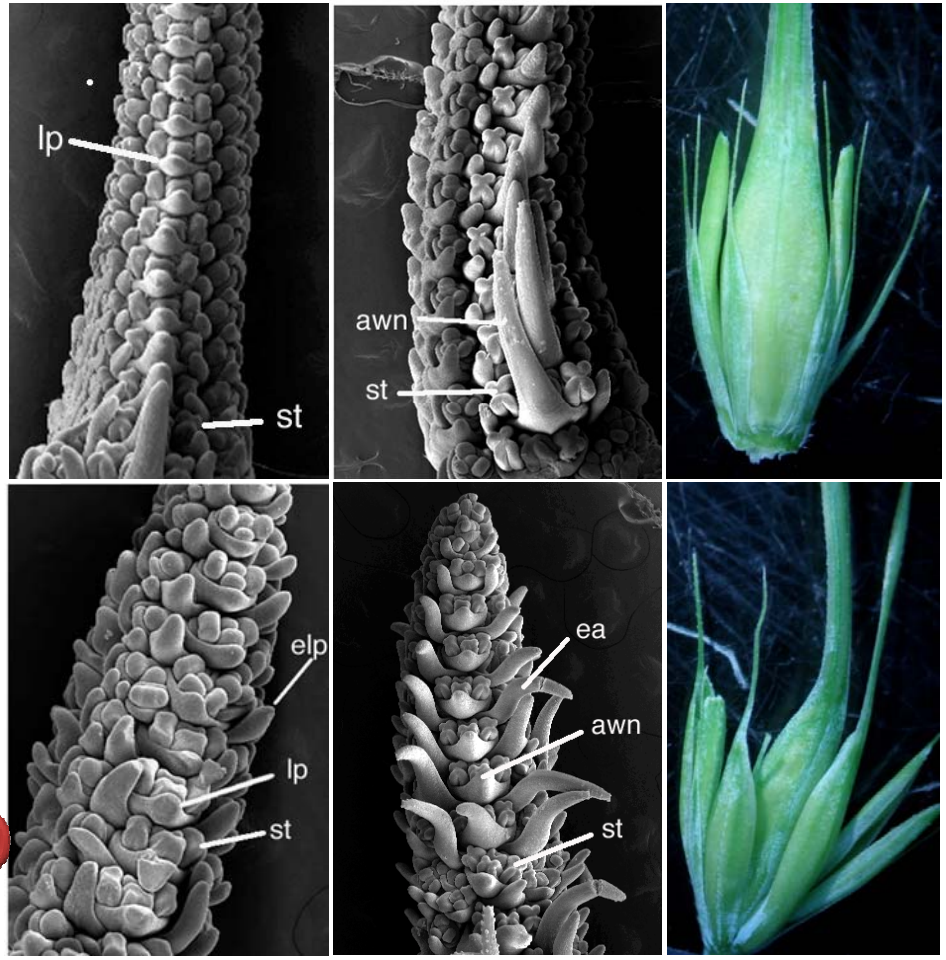


UACGUCGUAGUAGUUCUAAGG

IDS (Q Gene) BINDING SITE

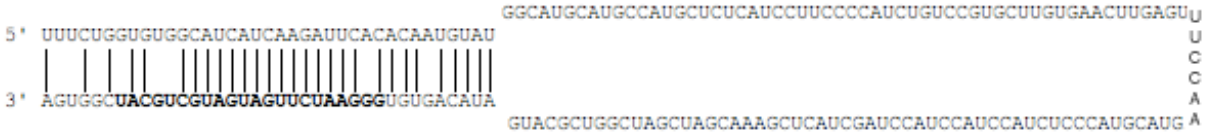






miR172 BARLEY AND OAT (COMPARISON)

miR172 pre-microRNA highly expressed in pistillate structures

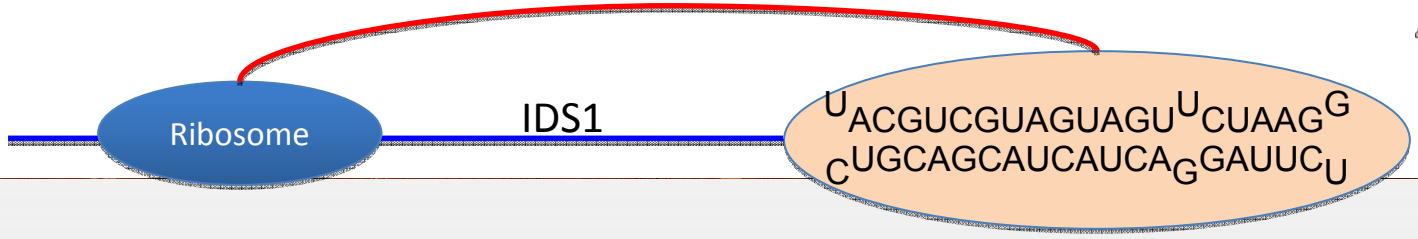


miR172 pre-microRNA is processed into mature microRNA

Barley – GGAAUCUUGAUGAUGCUGCAU

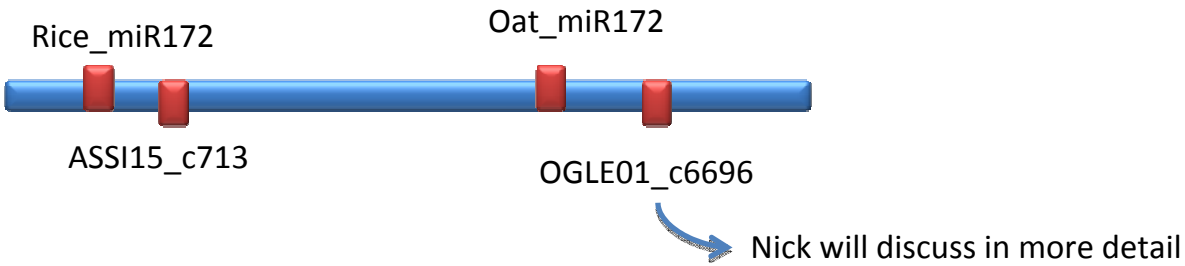
Oat – GGAAUCUUGAUGAUGCUGCAU

miR172 mature microRNA binds to IDS mRNA and silences IDS



miR172 OAT (Attractive Hypothesis)

Microsynteny with Rice Chromosome 1



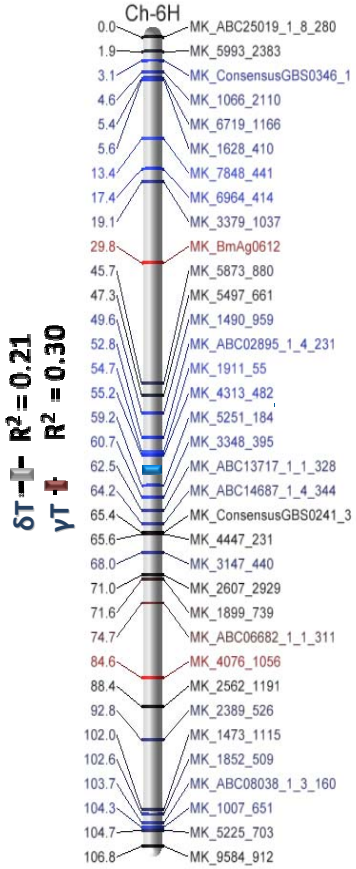
Hypothesis:

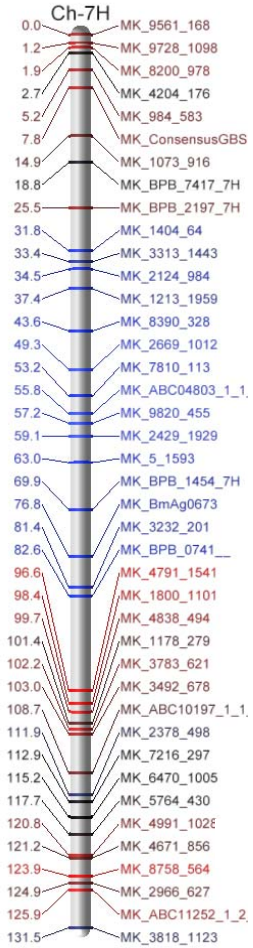
miR172 is the major gene involved in hulless trait

Binds to IDS and (SNB) and acts as translational repressor

Environmental unstable with strong expression in primary and secondary florets

VITAMIN E SYNTHESIS (QTL TO GENE)





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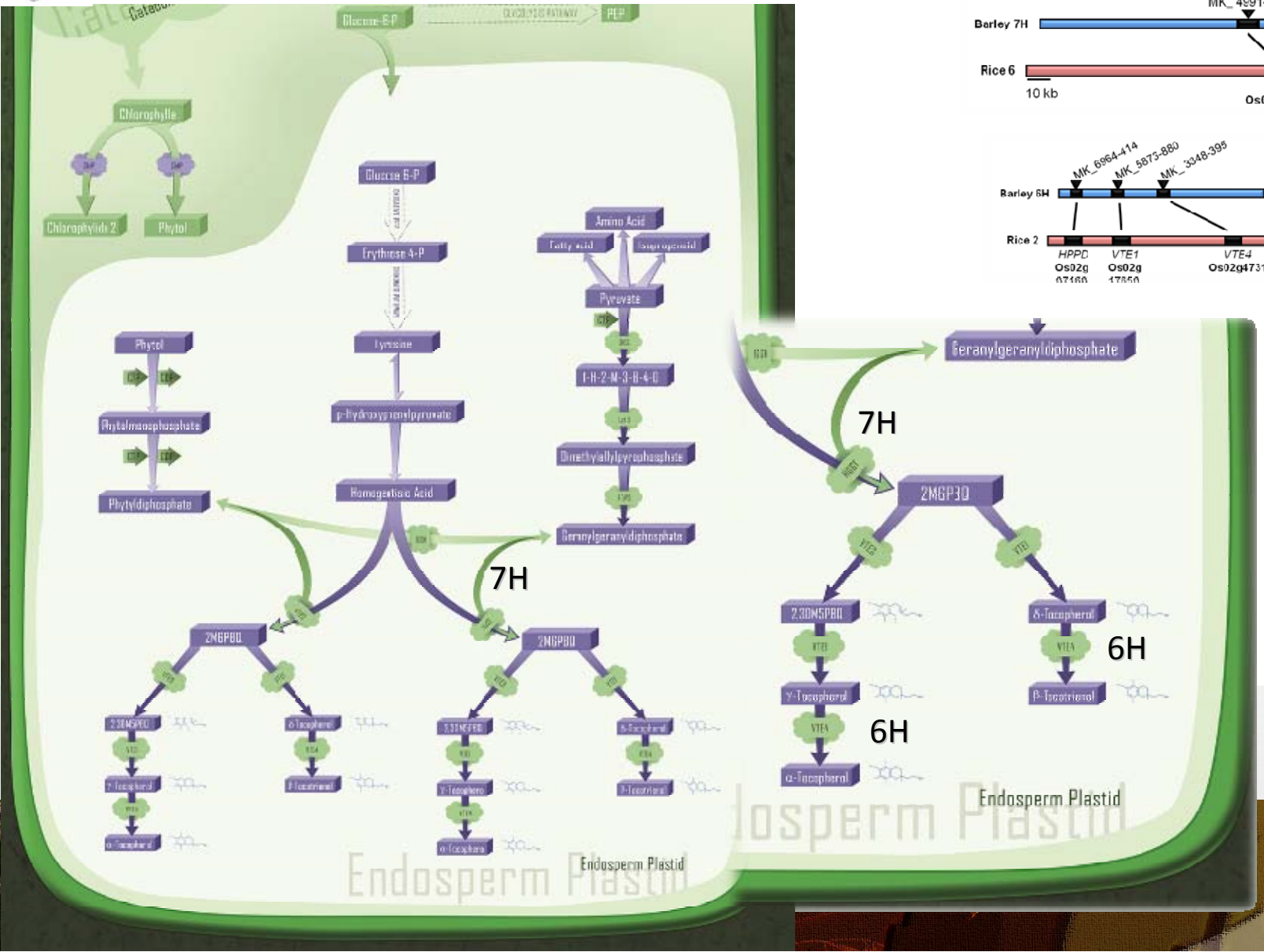

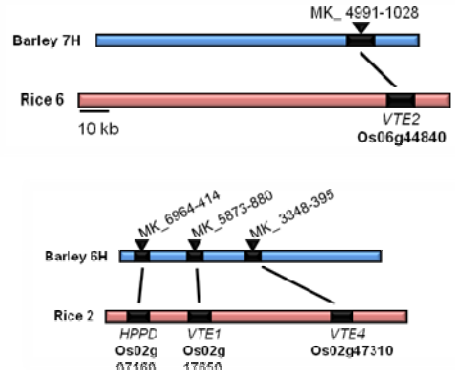

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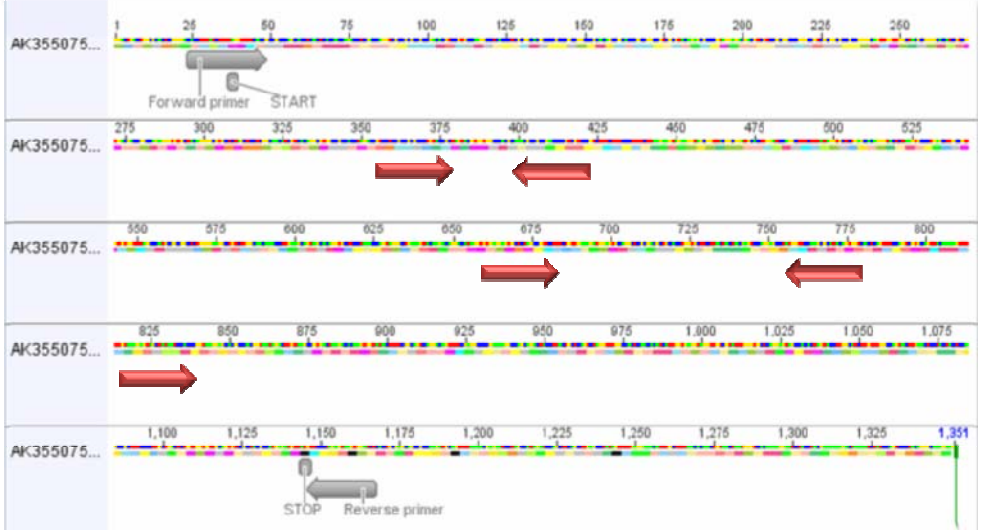
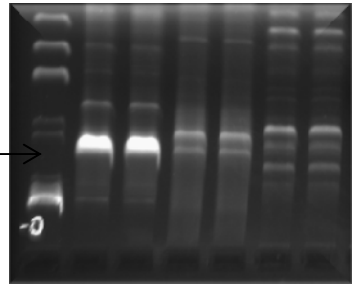
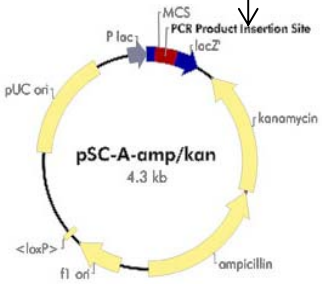


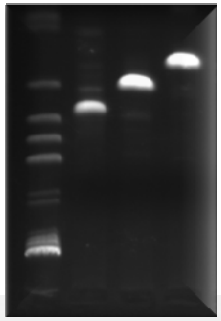
Design 'fishing primers' based on CDS



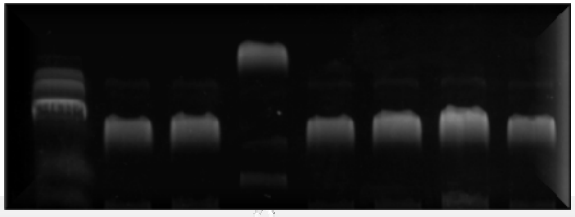
PCR-amplify full-length gene, purify from gel and clone



http://www.genomics.agilent.com/files/Media/PID96_P2.jpg

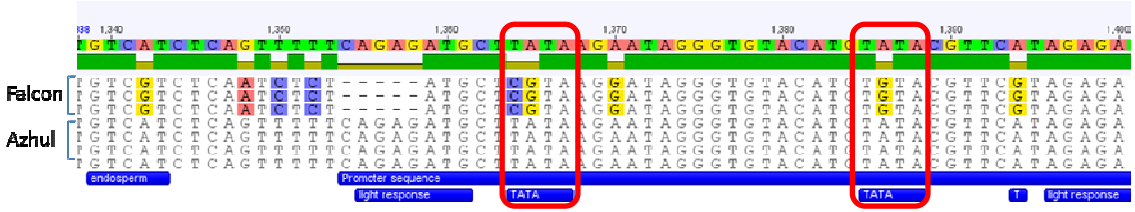


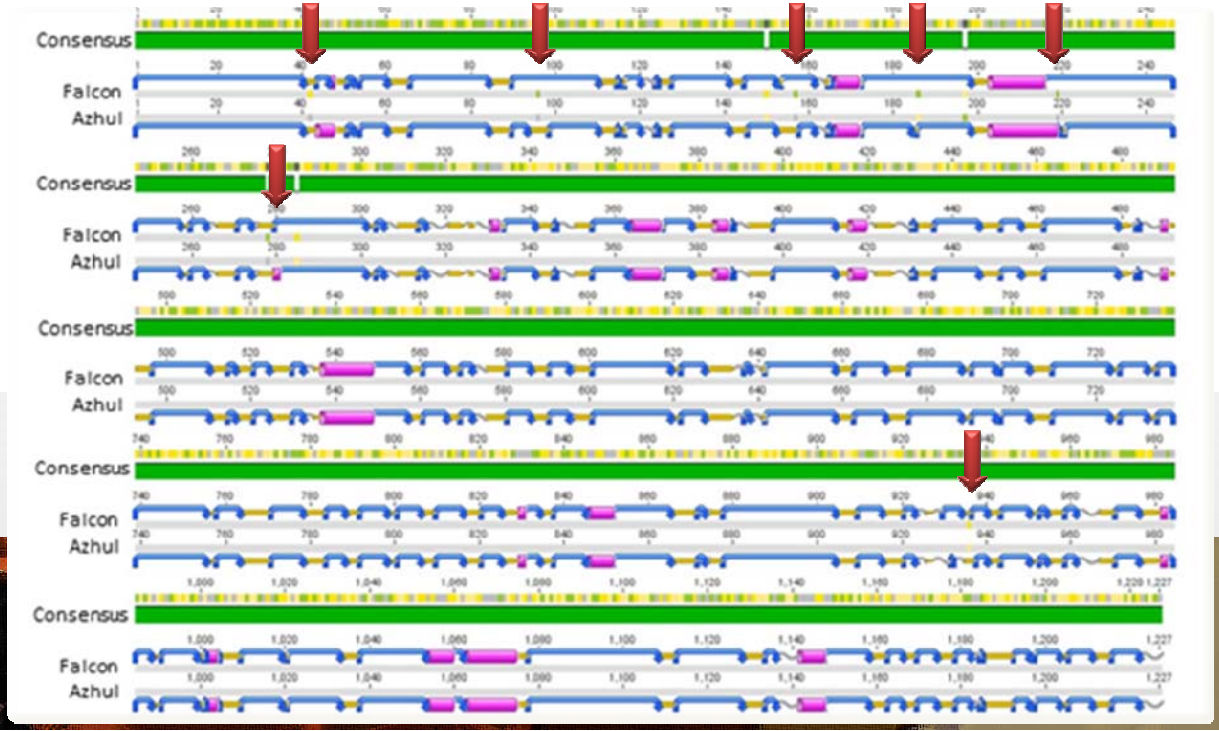
Internal primer check

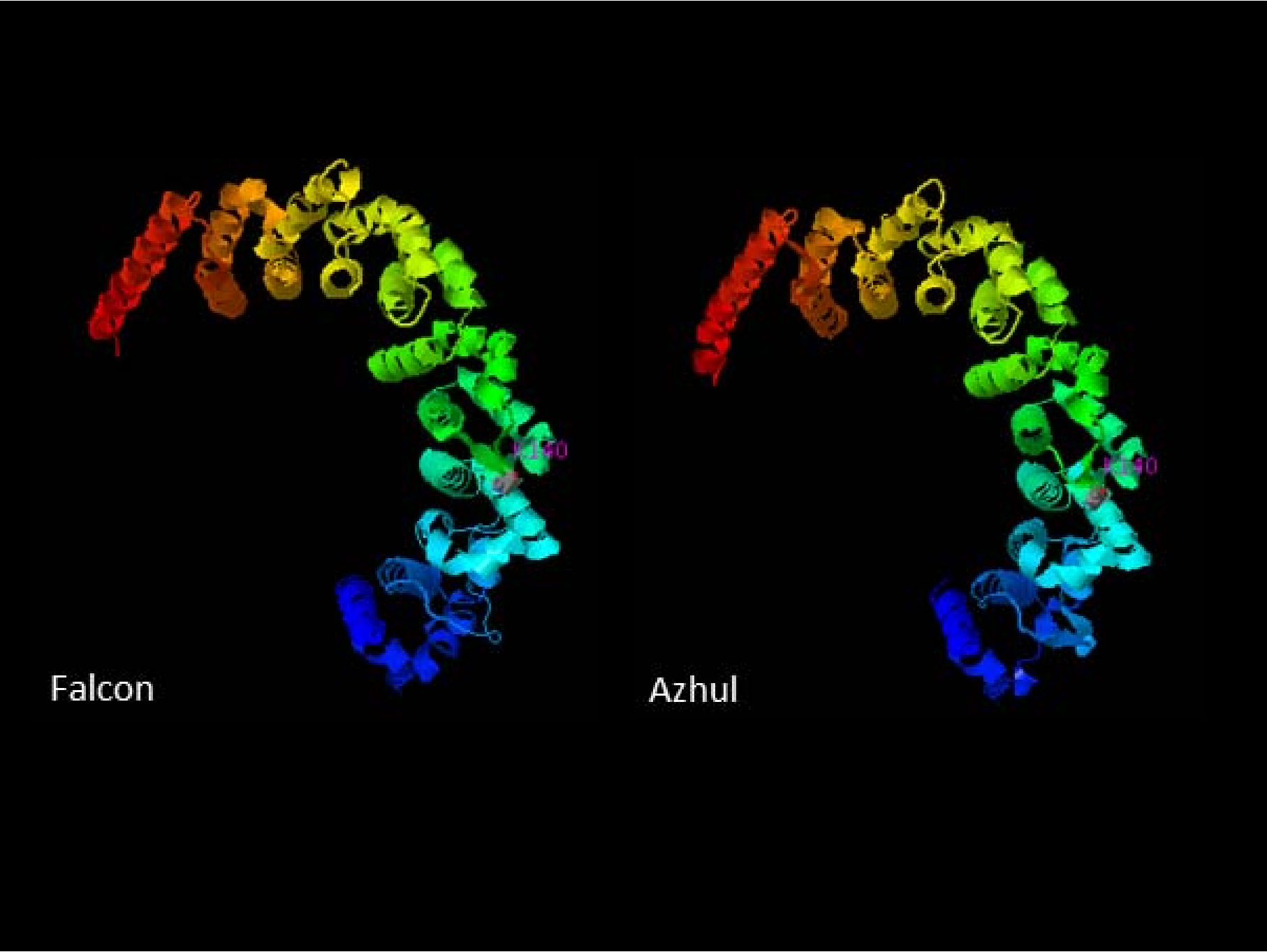


Plasmid check

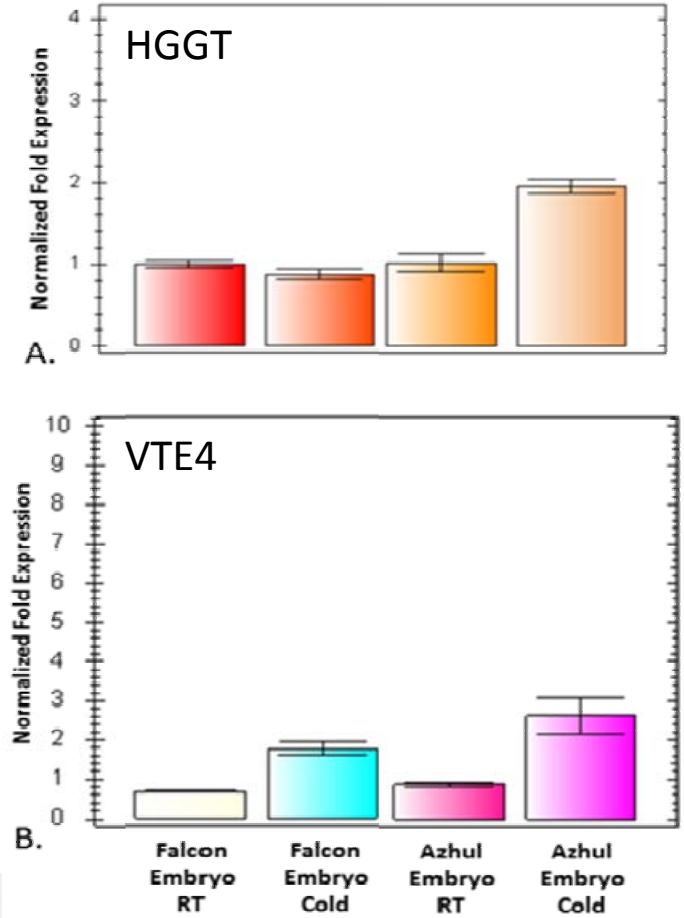
VITAMIN E SYNTHESIS (QTL TO GENE)

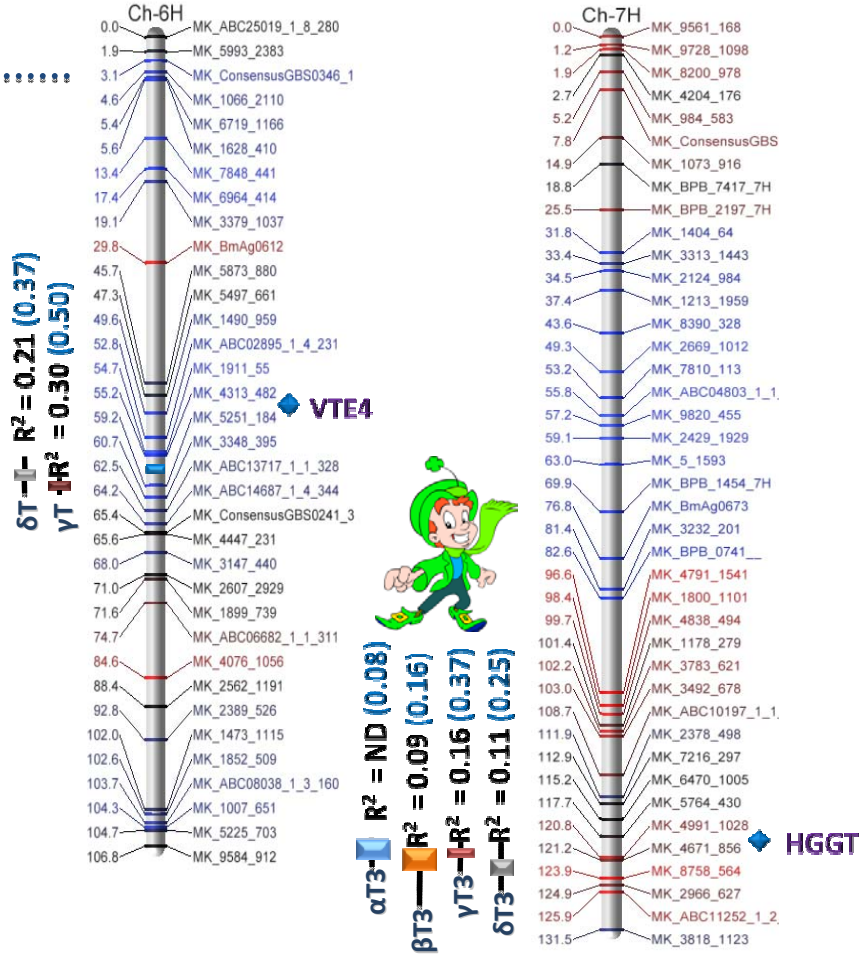


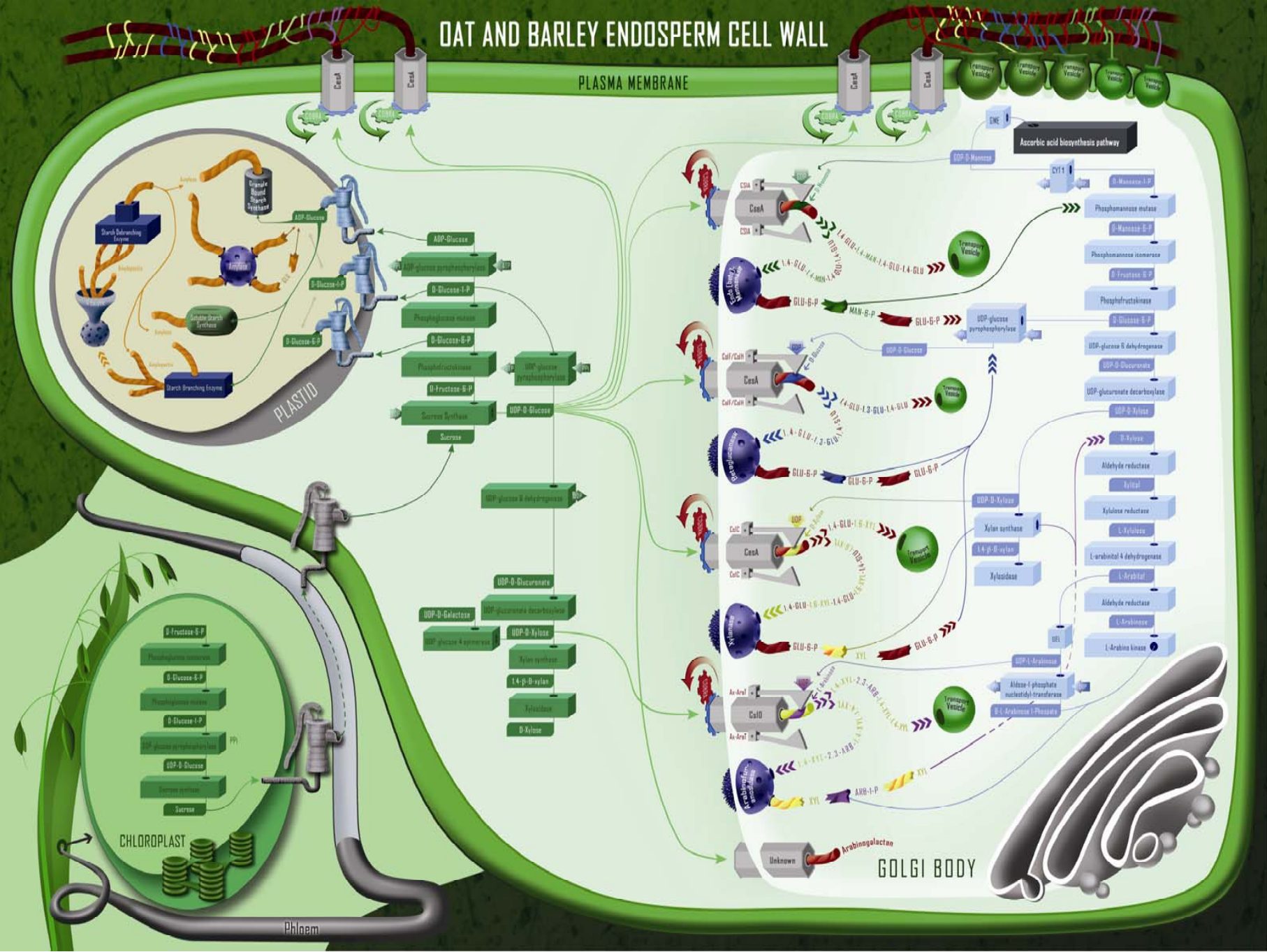



Falcon
Azhul

VITAMIN E SYNTHESIS (QTL TO GENE)

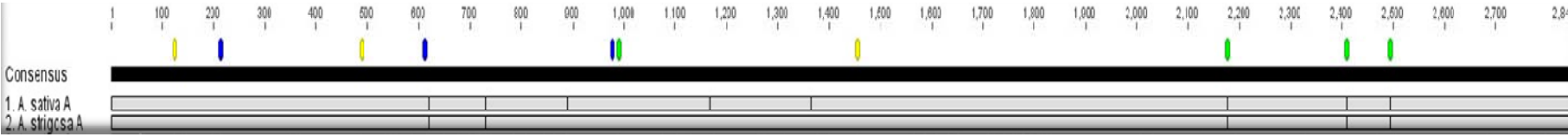




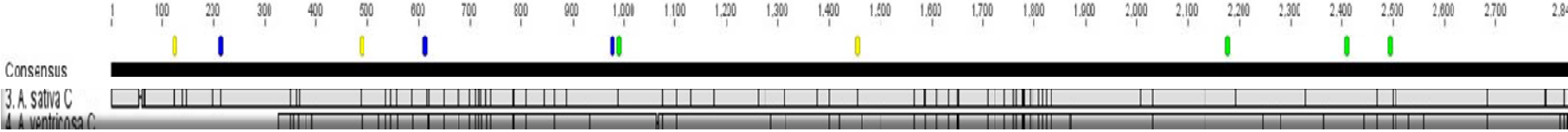

OAT AND BARLEY ENDOSPERM CELL WALL
PLASMA MEMBRANE
CesA
Transport Vesicle
Ascorbic acid biosynthesis pathway
GMP
GDP-D-Mannose
CYT1
D-Mannose-1-P
Phosphomannose mutase
D-Mannose-6-P
Phosphomannose isomerase
D-Fructose-6-P
Phosphofructokinase
D-Glucose-6-P
UDP-glucose 6 dehydrogenase
UDP-D-Glucuronate
UDP-glucuronate decarboxylase
UDP-D-Xylose
D-Xylose
Aldehyde reductase
Xylitol
Xylulose reductase
L-Xylulose
L-arabinitol 4 dehydrogenase
L-Arabitol
Aldehyde reductase
L-Arabinose
L-Arabina kinase
UEL
UDP-L-Arabinose
Aldose-1-phosphate nucleotidyl-transferase
UDP-L-Arabinose 1-Phosphate
Xylan synthase
1,4-β-D-xylan
Xylosidase
Cellulose
Mannan
Glucomannan
Glucan
Xyloglucan
Xylan
Arabinogalactan
Unknown
GOLGI BODY
ADP-Glucose
ADP-glucose pyrophosphorylase
D-Glucose-1-P
Phosphoglucose mutase
D-Glucose-6-P
Phosphofructokinase
D-Fructose-6-P
Sucrose Synthase
Sucrose
UDP-glucose pyrophosphorylase
UDP-D-Glucose
UDP-glucose 6 dehydrogenase
UDP-D-Glucuronate
UDP-glucuronate decarboxylase
UDP-D-Galactose
UDP-glucose 4 epimerase
UDP-D-Xylose
Xylan synthase
1,4-β-D-xylan
Xylosidase
D-Xylose
Granule Bound Starch Synthase
Amylase
Starch Debranching Enzyme
Soluble Starch Synthase
Starch Branching Enzyme
PLASTID
CHLOROPLAST
Phloem

OAT BETA GLUCAN(THE PROBELMATIC GENOME)

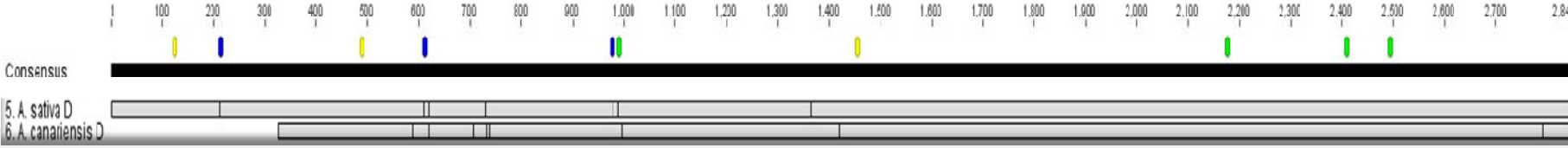
Avena sativa (AACCDD) x *A. strigosa* (AA)



Avena sativa (AACCDD) x *A. ventricosa* (CC)

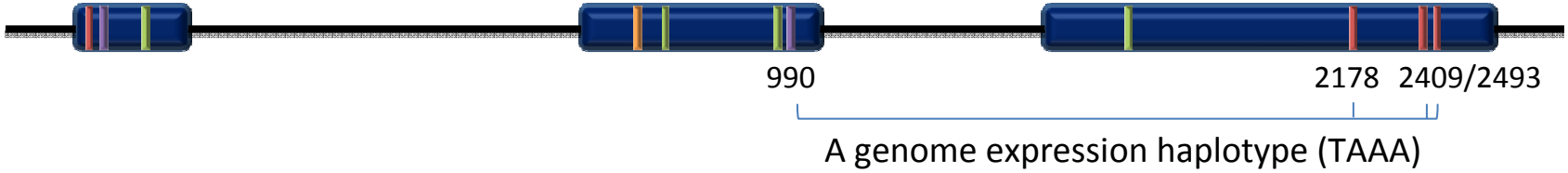


Avena sativa (AACCDD) x *A. canariensis* (DD)



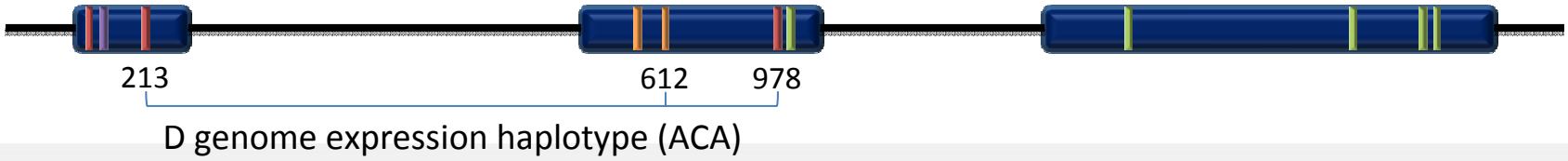
OAT BETA GLUCAN (THE PROBELMATIC GENOME)

A Genome



C Genome

D Genome



OAT BETA GLUCAN (THE PROBELMATIC GENOME)

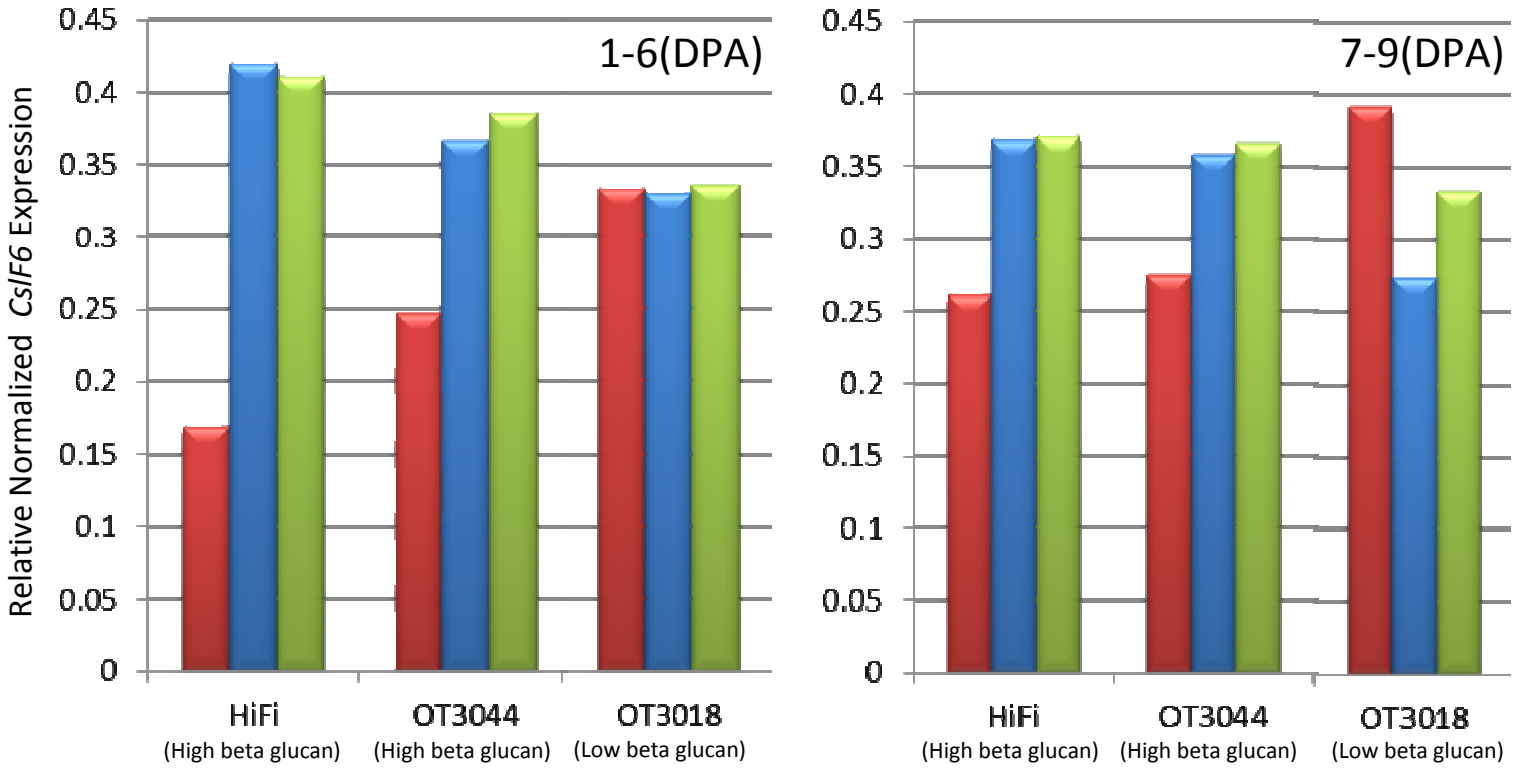
- RNA harvest from oat seed 1-3 DPA, 4-6 DPA, and 7-9 DPA
- Illumina HiSeq (DHMRI Genomics Center)
- Contig assembly and haplotype count (NC Charlotte – Robert Reid DHMRI Core Lab)

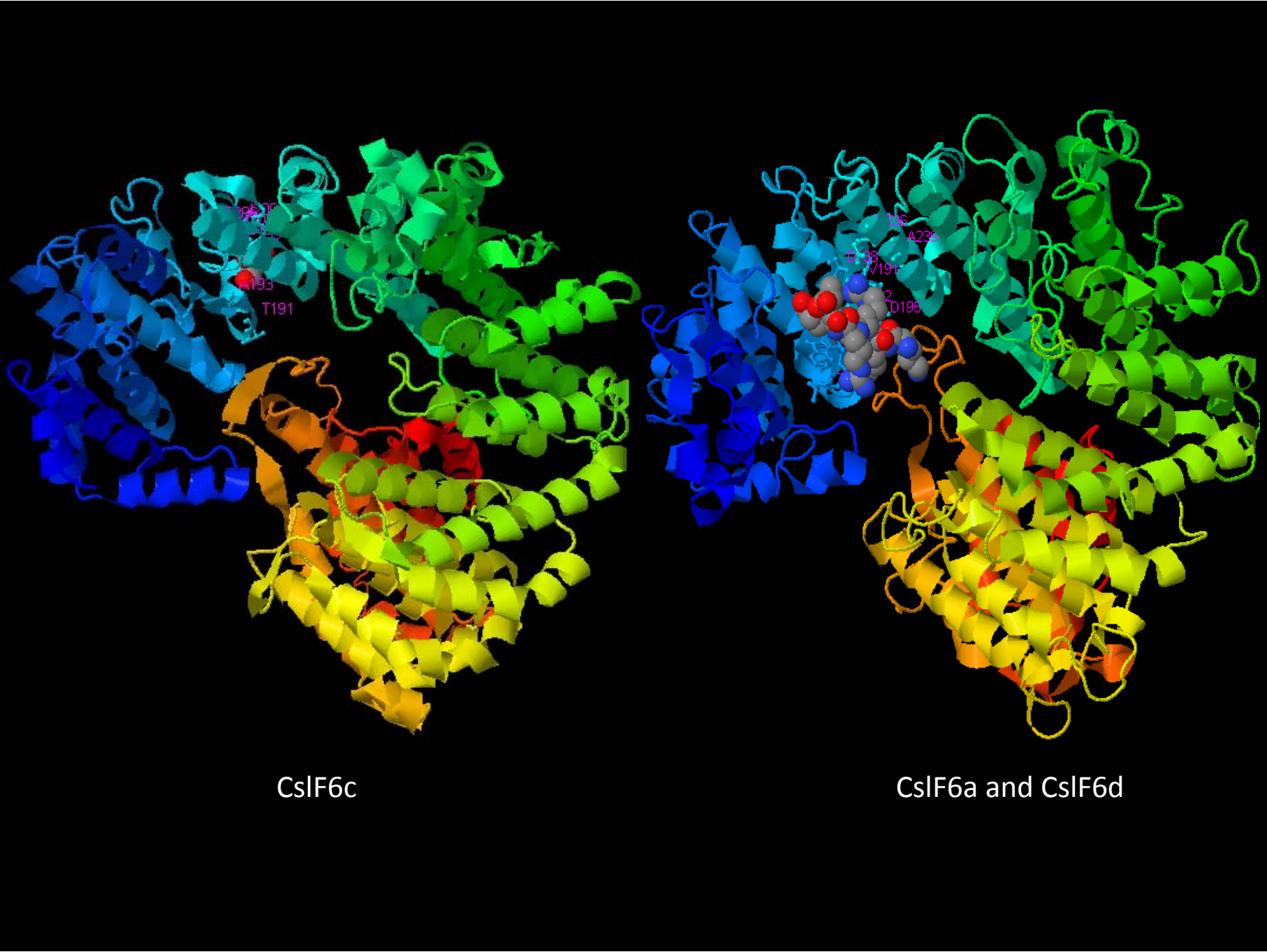
Mean exon specific expression of *CSLF6* alleles from the A, C, and D genomes in oat

	Genome Expression†		
CslF6 Exon	A	C	D
1	--	14.3 (2)	36.8 (1)
2	42.8 (1)	37.2 (1)	34.4 (1)
3	43.8 (3)	27.9 (1)	--
Abs(Dif)	2.6 (LSD)	5.7 (HSD)	6.0 (LSD)

†Expression is based on 10^4 read counts from HiSeq

OAT BETA GLUCAN(THE PROBELMATIC GENOME)

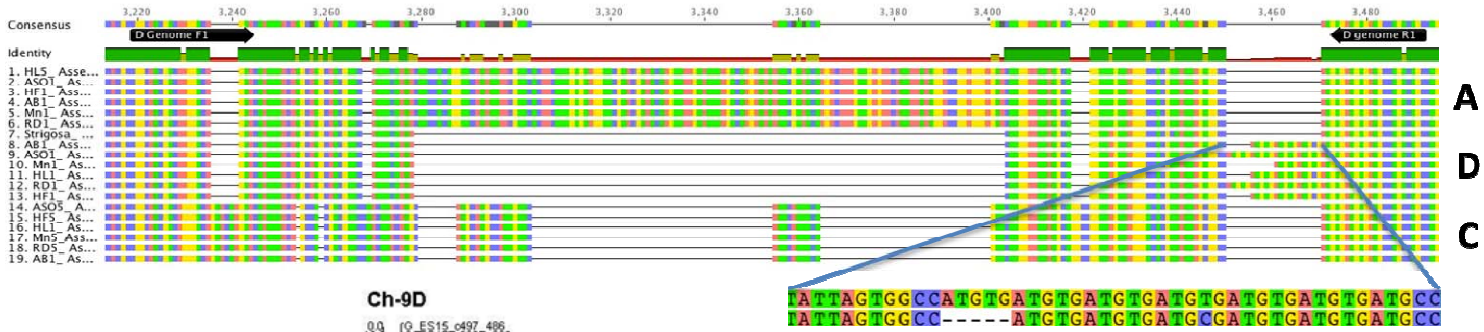


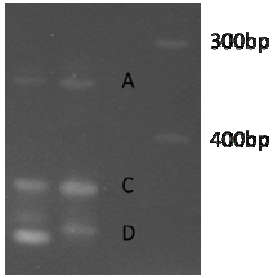


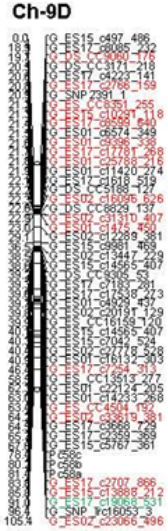
CslF6c

CslF6a and CslF6d

OAT BETA GLUCAN(THE PROBLEMATIC GENOME)

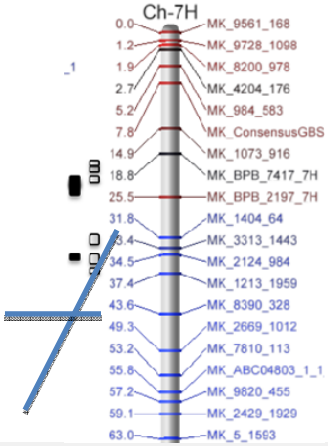






CslF6

WAXEY



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