

Development of a High-Density SNP Genotyping Panel as a Community Resource for Genetic Analysis in Oat

Shiaoman Chao

USDA-ARS Fargo, ND



CORE Project

(Collaborative Oat Research Enterprise)

- Project director: Eric Jackson.
- Large-scale SNP discovery based on Roche 454 reads, high throughput genotyping.
- Phenotyping CORE breeding lines, and association mapping.
- Implementing SNPs in marker assisted breeding.
- Database development

Resources for Oat SNP Discovery

- cDNAs – derived from roots, shoots, immature and mature embryos of 20 diverse hexaploid cultivars.
- DArTs – complexity reduced genomic fragments derived from 25 diverse hexaploid cultivars.
- Complexity reduced genomic fragments derived from two tetraploid accessions.

SNP Discovery Pipelines

- Single template approach (Gerry Lazo) - raw reads from Ogle, Hurdal, Assiniboia, and TAM O-301 were assembled to form four reference templates, which were used to align with reads from other accessions individually.
- Composite template approach (Nick Tinker) - raw reads from each accessions were assembled, composite templates were formed to align with condensed reads from each accession individually.
- Filtering steps, read depth ≥ 5 to call SNP candidates.

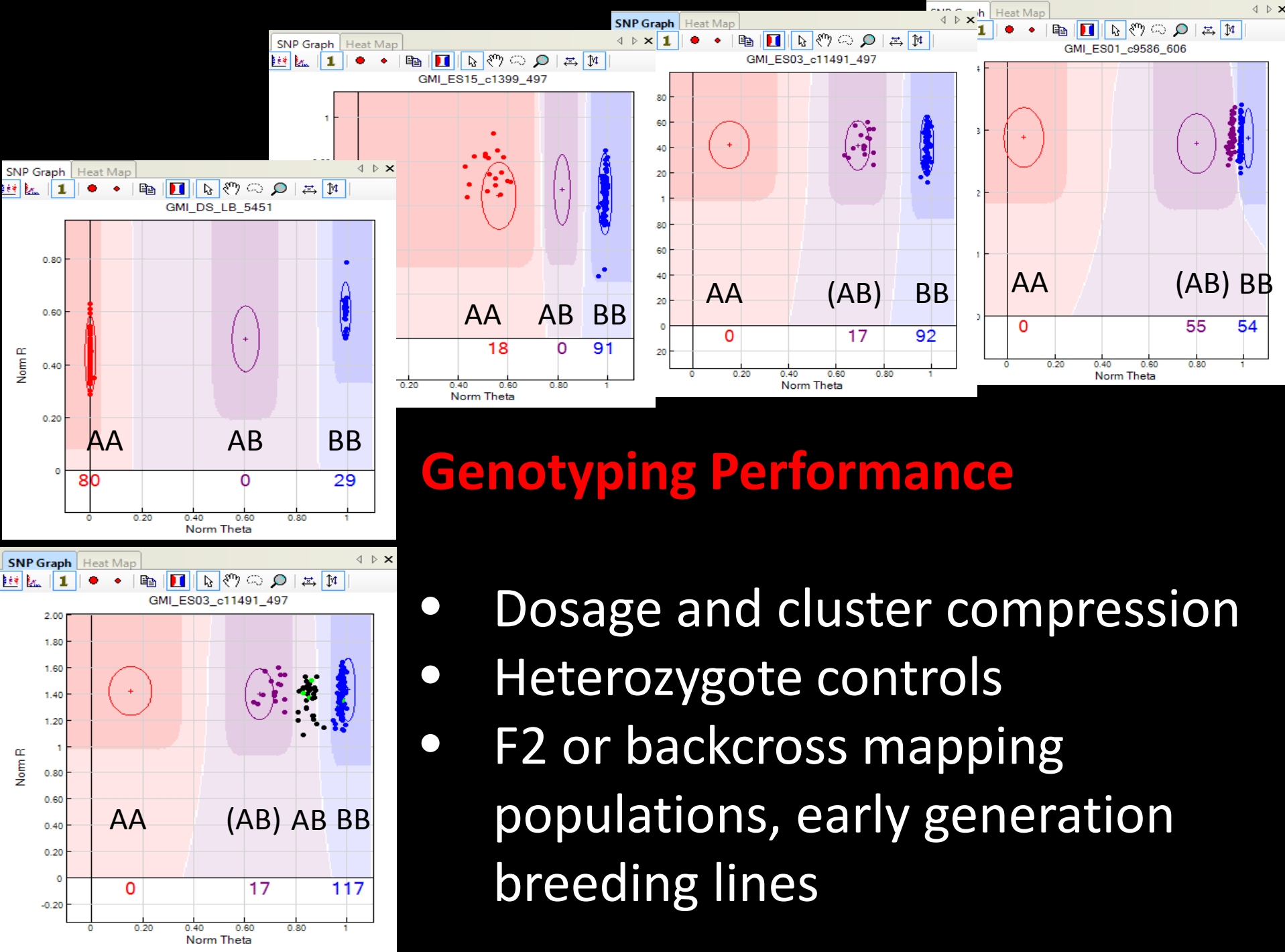
OPA Development and SNP Genotyping

- Illumina GoldenGate genotyping assay.
- Sent ~11,000 *in silico* SNPs for assay design.
- Developed 4 pilot oat OPAs.
- Oat OPA1, 2 and 4 each had 1536 SNPs.
- Oat OPA3 had 3072 SNPs, but assay failed.
- Evaluated a total of 4,608 SNPs in three OPAs,
 - 3,930 cDNAs derived
 - 578 DArTs derived
 - 100 tetraploid derived

Samples Used for Evaluation

Total 576 samples evaluated,

- 109 germplasm originated from wide geographic regions in the world.
- 6 mapping populations (partial).
- A set of monosomic lines.



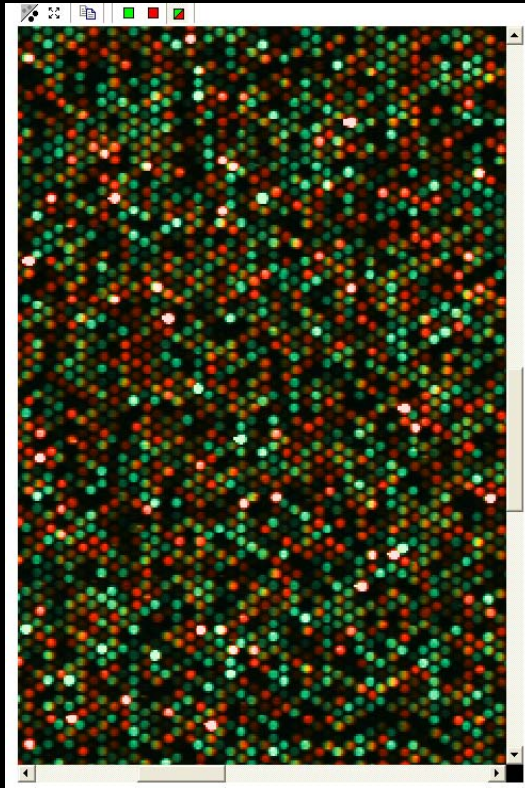
SNP Discovery Methods

Pilot Oat OPA1 and 2 Performance - based on 109 germplasm

Discovery method	No. Tested	Good SNPs	Conversion rate (%)	No . SNP mapped	%	Polymorphic SNPs	%
cDNA-STA	2270	991	44	738	33	878	89
cDNA-CTA	336	144	43	98	29	133	92
DArT-STA	300	121	40	86	29	108	89
DArT-Sanger's	66	48	73	36	55	43	90
Genomic Tetraploid	100	76	76	7	7	7	9
Total	3072	1380	45	965	31	1169	85

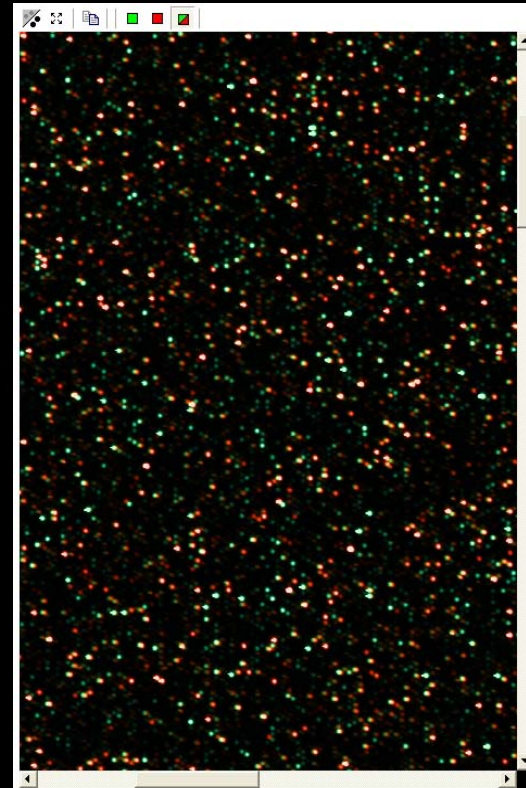
Pilot Oat OPA3 (3072-plex) Design Problem

Pilot Oat OPA2



300 DArT }
100 4X } 400 genomic SNPs
1136 cDNA SNPs
(1:3)

Pilot Oat OPA3



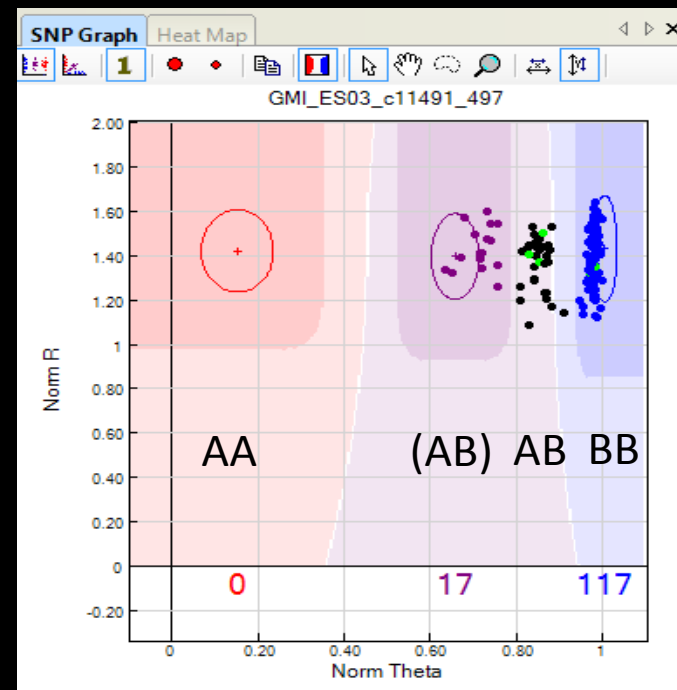
1476 genomic SNPs }
1596 cDNA SNPs } 1176 DArT
300 4X }
(1:1)

Pilot Oat OPA4 (1536-plex) Design and Performance

- Filtered SNPs derived from highly abundant sequences by *in silico* prediction,
 - Searched 3 oligos designed by Illumina against all sequence reads
 - Searched 'predicated' oligos against all sequence reads
- Selected 212 DArT SNPs and 1324 cDNA SNPs (1:6).
- Scored 1348 good SNPs, 201 (95%) DArT SNPs and 1147 (87%) cDNA SNPs, **88% conversion rate (2X improvement)**.

Development of A Working Oat OPA

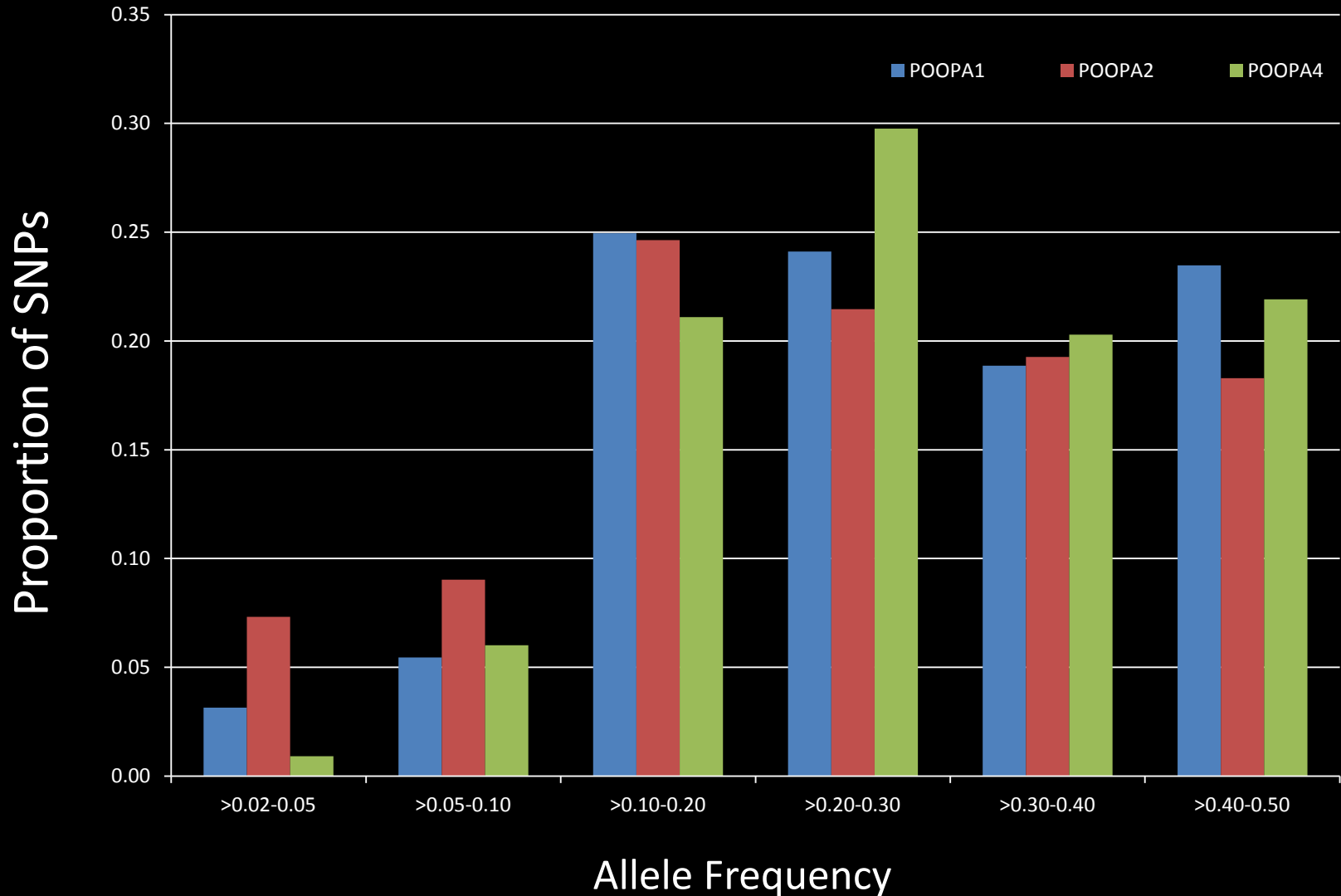
- Genotyping performance, cluster separation based on normalized theta value >0.3 .
- Minor allele frequency $\geq 2\%$.
- SNPs with less than 10% missing data among 109 germplasm, removing SNPs detecting null.
- Chromosome distribution.



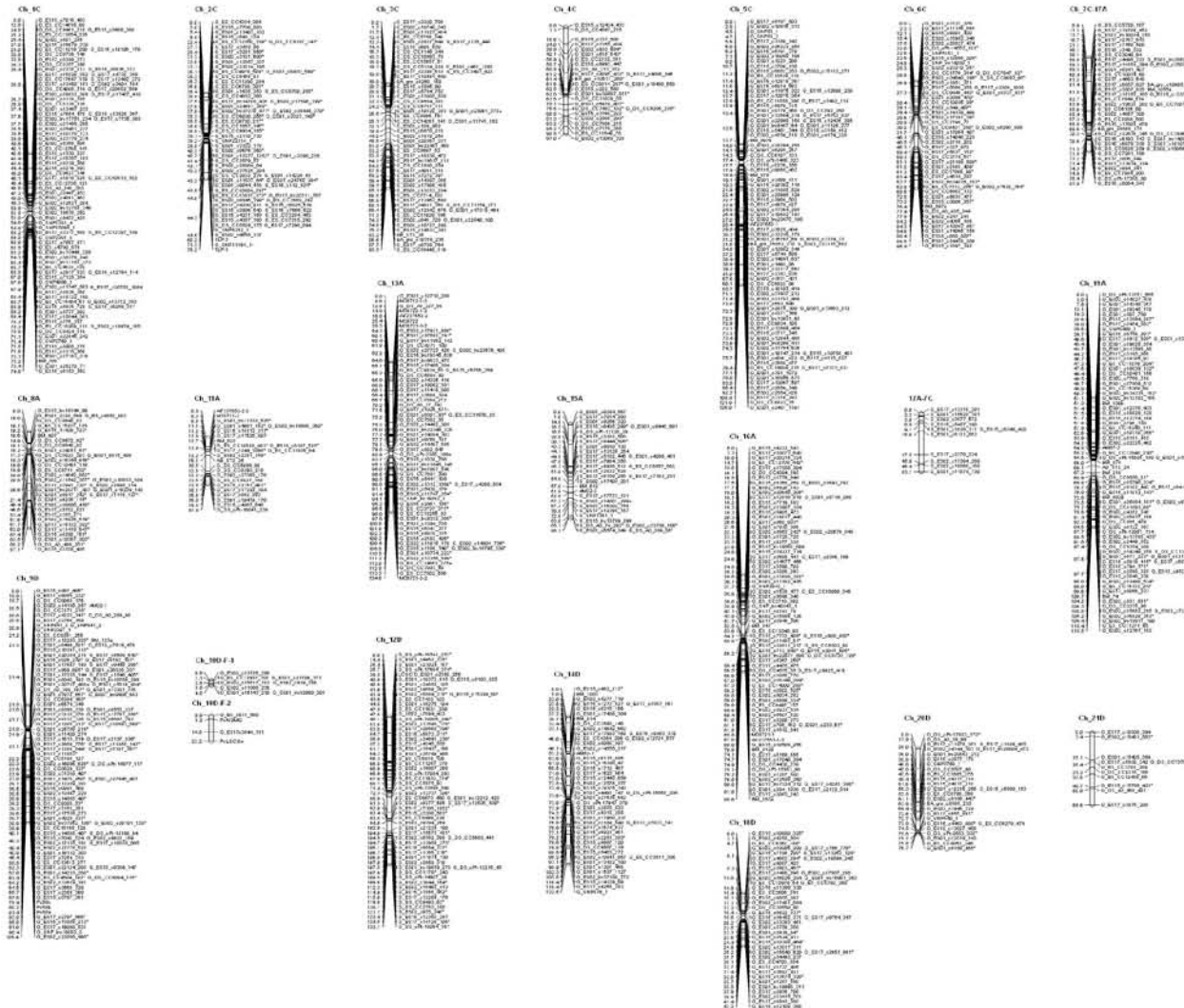
Selection of Working Oat OPA SNPs

	POOPA1	POOPA2	POOPA4	Total
cDNA	446	311	812	1569
DArT	31	94	169	294
Tetraploid	0	5	0	5
Total	477	410	981	1868

Minor Allele Frequency Distribution Working SNP Candidates



SNP-based Consensus Maps of Cultivated Oat



21 linkage groups anchored to chromosomes

1000 SNPs mapped

Acknowledgements

Rebekah Oliver, USDA-ARS, Aberdeen, ID

Gerry Lazo, USDA-ARS, Albany, CA

Nick Tinker, AAFC, Ontario, Canada

Rick Jellen, BYU, Provo, UT

Jeff Maughan, BYU, Provo, UT

Rachel Redman, ex-BYU, Provo, UT

Carol Lange, TAMU, College Station, TX

Dawn Feltus, USDA-ARS, Fargo, ND

Mary Osenga, USDA-ARS, Fargo, ND

**Funding Sources: General Mills, USDA NIFA, USDA-ARS CRIS,
NAMA**

Special thanks to Eric Jackson for making this work possible.