

# NimbleGen Sequence Capture: A New Technology for the Positional Cloning of QTL from Crops with Complex Genomes

QTL Cloning Workshop

Plant and Animal Genome Conference

10 January 2009

Patrick S. Schnable  
Center for Plant Genomics

IOWA STATE UNIVERSITY

# Elucidating the *Mechanisms* of Heterosis



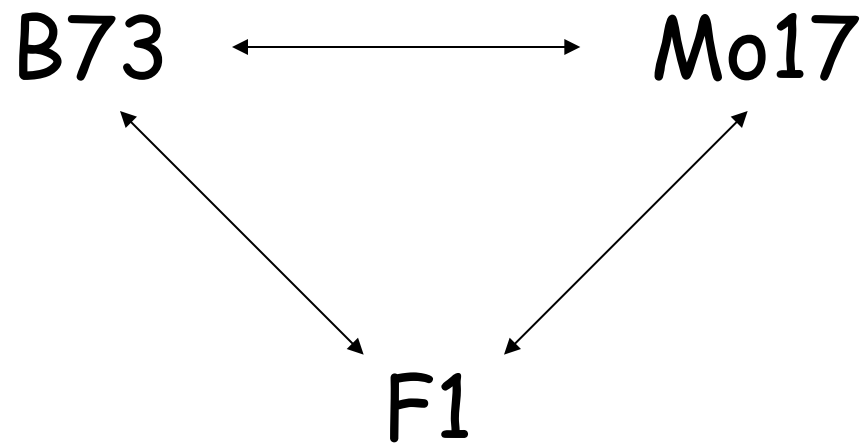
B73    F1    Mo17



B73    F1    Mo17



# Gene Expression Profiling



- Conducted gene expression profiling among three genotypes (9 biological replications)
- Characterized gene action among the ~1,500 genes from cDNA array that exhibited the most statistically significant differences in expression among the three genotypes (FDR ~15.1%)



Ruth Swanson-Wagner

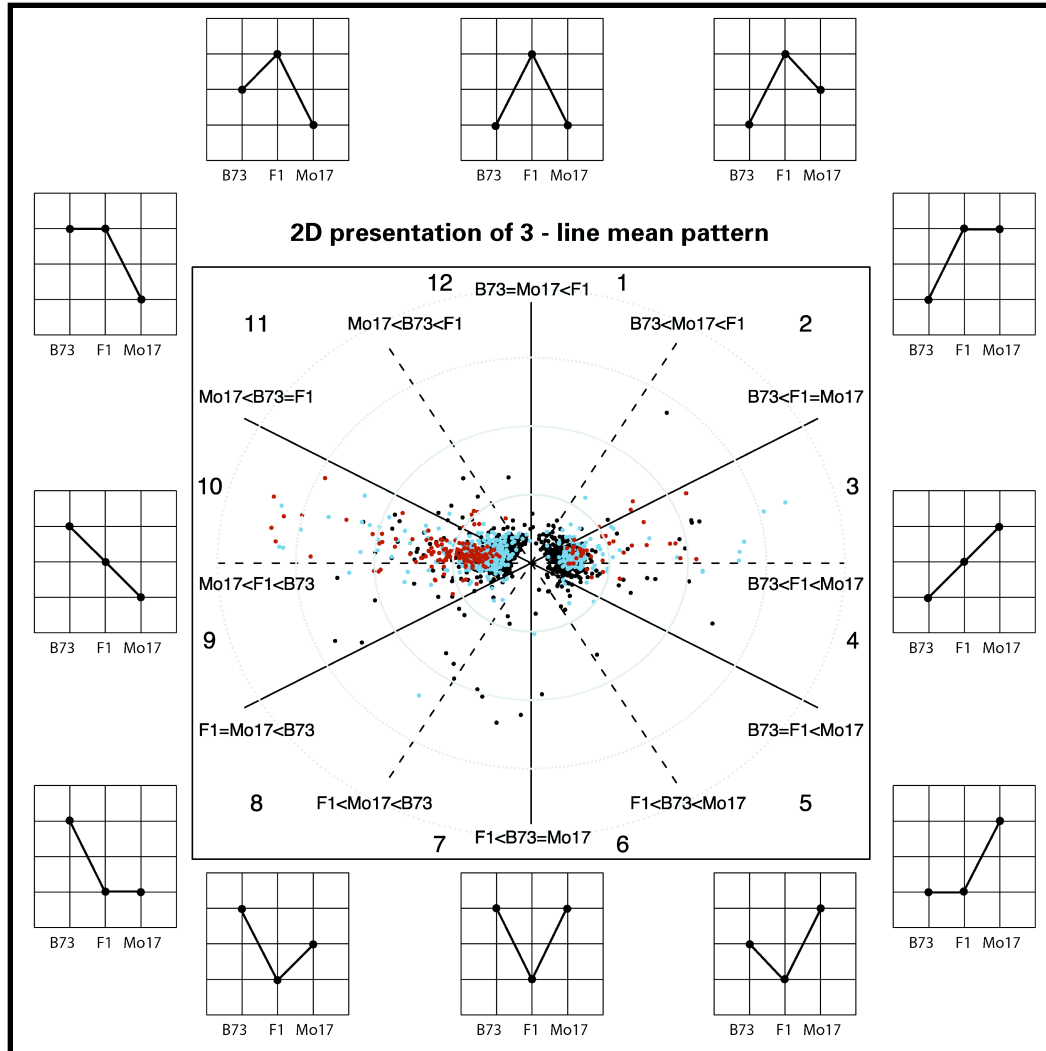


Yi Jia (贾毅)

# All possible modes of gene action are observed in a global comparison of gene expression in a maize F<sub>1</sub> hybrid and its inbred parents

Ruth A. Swanson-Wagner<sup>\*††</sup>, Yi Jia<sup>†‡§</sup>, Rhonda DeCook<sup>¶</sup>, Lisa A. Borsuk<sup>||</sup>, Dan Nettleton<sup>||\*\*</sup>, and Patrick S. Schnable<sup>\*†§||\*†††††</sup>

<sup>\*</sup>Interdepartmental Genetics Graduate Program, <sup>†</sup>Departments of Genetics, Development and Cell Biology, <sup>‡</sup>Statistics, and <sup>§</sup>Agronomy, <sup>¶</sup>Interdepartmental Plant Physiology Graduate Program, <sup>||</sup>Interdepartmental Bioinformatics and Computational Biology Graduate Program, and <sup>\*\*</sup>Center for Plant Genomics, Iowa State University, Ames, IA 50011



## Gene Action:

~1,500 differentially regulated genes

22% non-additive

78% additive

1% FDR

5% FDR

15% FDR

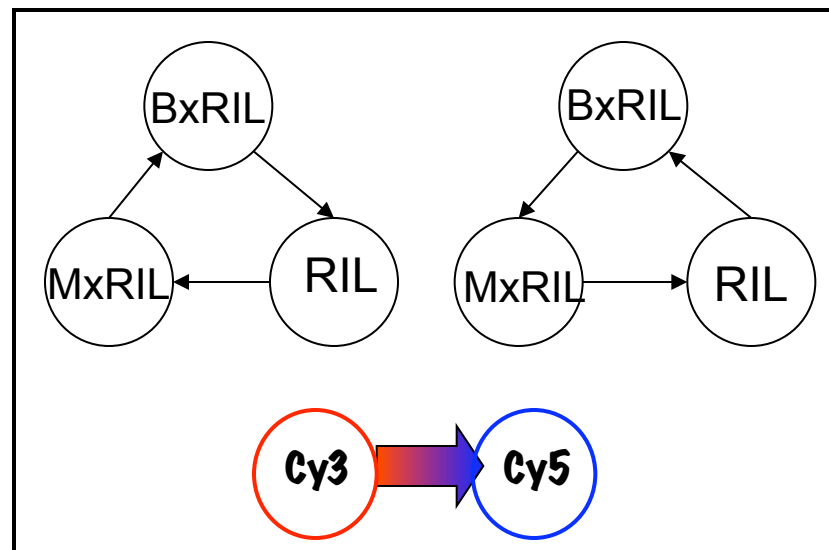
Radius =  $\log_2$  Fold Change

eQTL mapping: Determine the genetic mechanisms that regulate gene expression in hybrids versus inbreds

- Most eQTL experiments compare expression *across RILs* (two homozygous genotypes: e.g., B73 vs Mo17 allele)
- We additionally compared expression *in backcrosses of RILs to B73 and Mo17*
- Permits comparisons of heterozygous and homozygous genotypes

# Experimental Design

- 30 RILS from IBM population
- 3 genotypes: B73xRIL, Mo17xRIL, RIL
- 4 biological replications (14 DAP seedlings; highly controlled environment)
- Hybridize to SAM1.1 cDNA array (14,401 cDNAs): 360 arrays

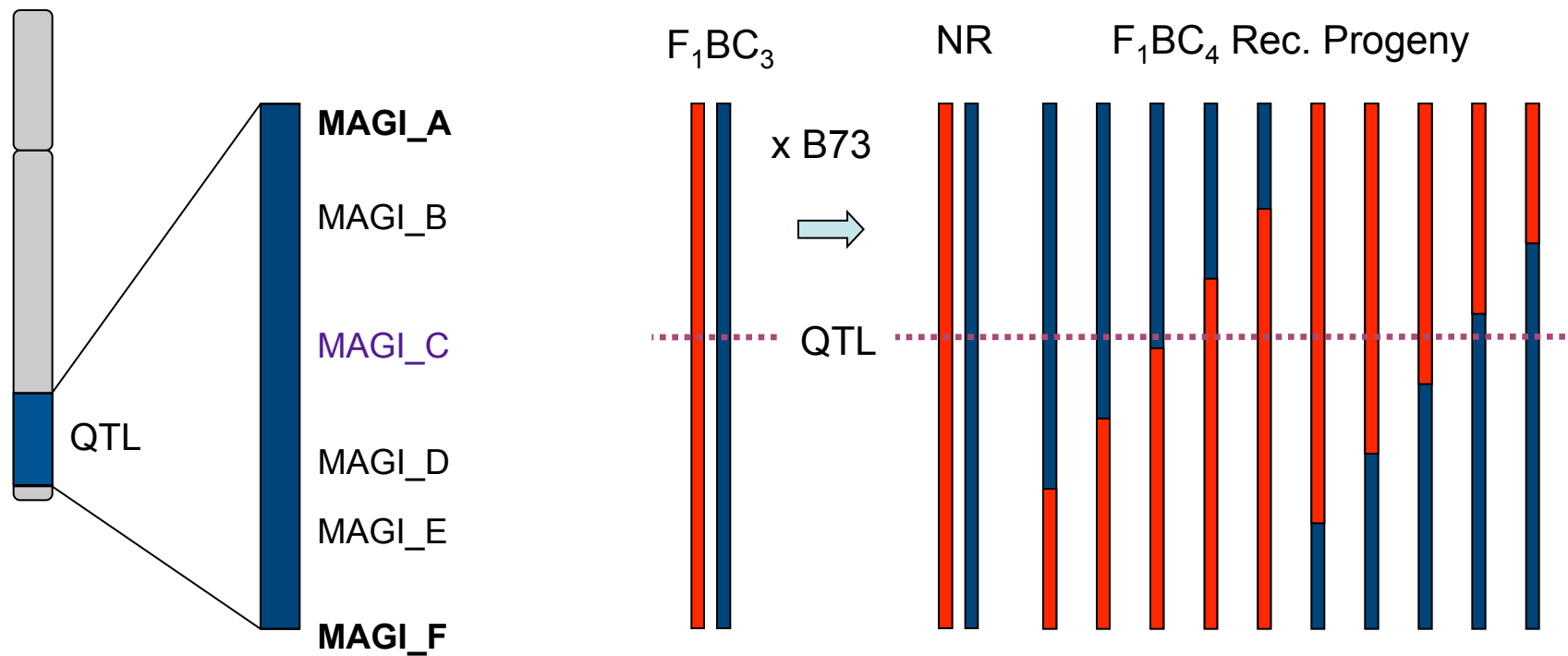


# Identification of *Cis* and *Trans* eQTLs



Group	No. Significant eQTLs	FDR (%)	No. Mapped Genes	No. Cis	No. Trans	No. Other	No. Not Mapped	% Cis (of mapped)	% Trans (of mapped)	% Other (of mapped)
<b>B73xRIL</b>	530	9.8	163	11	140	12	367	<b>7</b>	<b>86</b>	7
<b>RIL</b>	711	1.3	242	79	145	18	469	<b>33</b>	<b>60</b>	7
<b>Mo17xRIL</b>	536	5.1	169	21	131	17	367	<b>12</b>	<b>78</b>	10
<b>Merged (Non-redundant)</b>	1531	--	486	81	362	43	1,045	<b>17</b>	<b>74</b>	9

# Fine-Mapping of *trans*-eQTL



*Trans*-eQTL (2-5 cM resolution) have been Mendelized; positional cloning is underway. Screened 10,000  $F_1BC_4$  w/ PCR-based markers that flank eQTL interval.



# Array-based Comparative Genome Hybridizations (CGH)

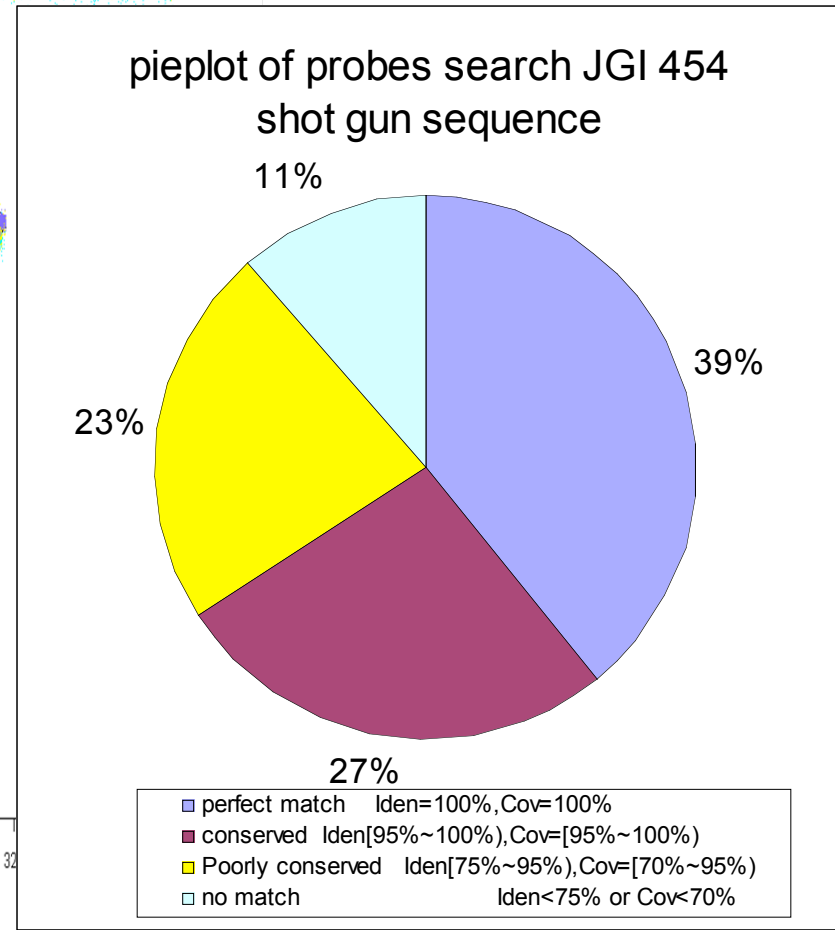
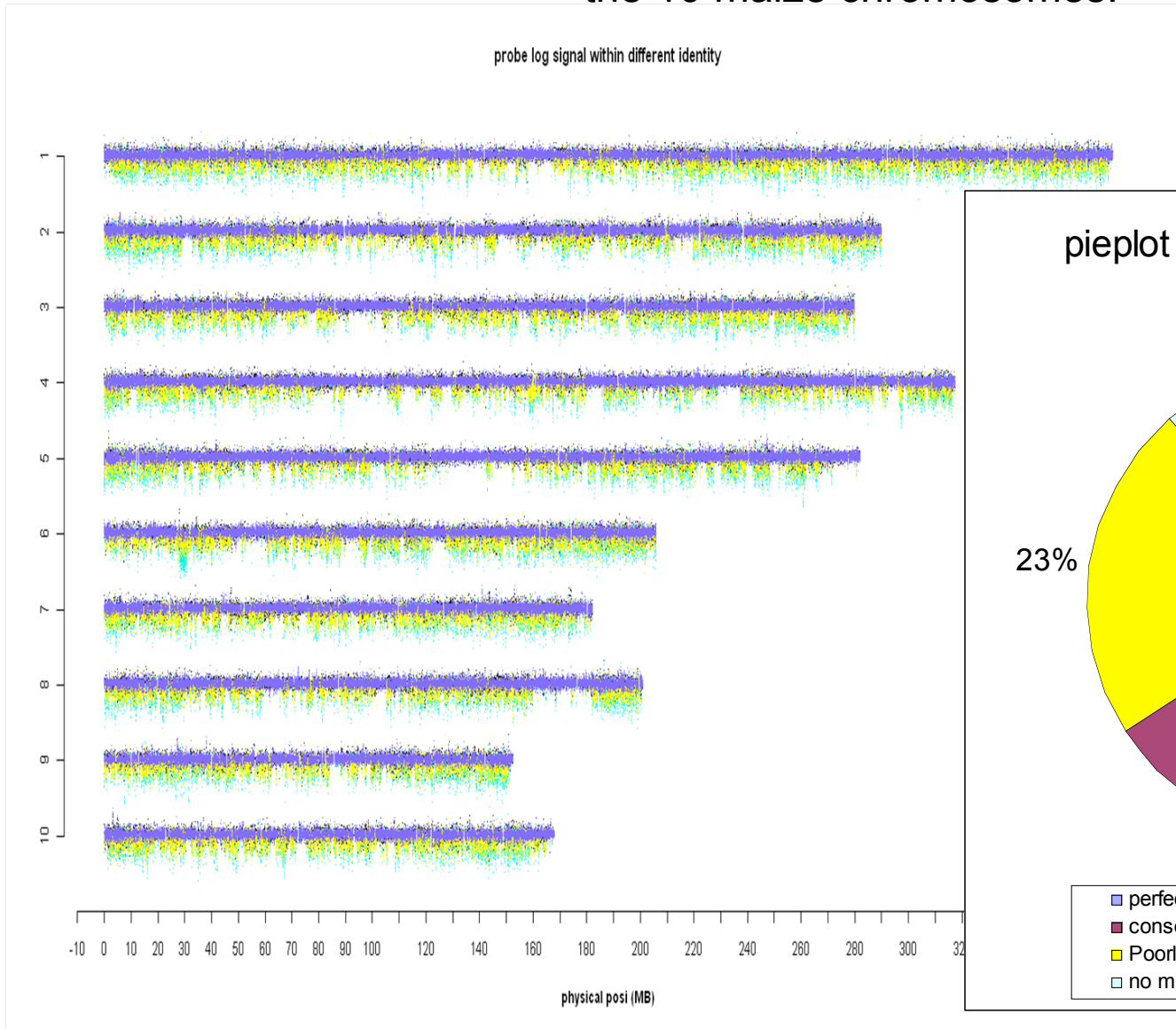
- Nimblegen's HD2 Array (~2.1M probes)
- Probes designed using a "frequency masked" 200 bp tile-path through the *draft* B73 genome sequence
- Genotypes: B73, Mo17, and two IBM (B73 x Mo17) RILs

SID	Cy3	Cy5
1	B73	Mo17
2	Mo17	B73
3	Mo17	B73
4	Mo17	B73
5	RIL1 (M0023)	B73
6	RIL2 (M0022)	B73
7	Mo17	RIL1 (M0023)
8	Mo17	RIL2 (M0022)
9	RIL1 (M0023)	B73

Kai Ying (应开)   Yan Fu (傅延)   Wei Wu (吴薇)



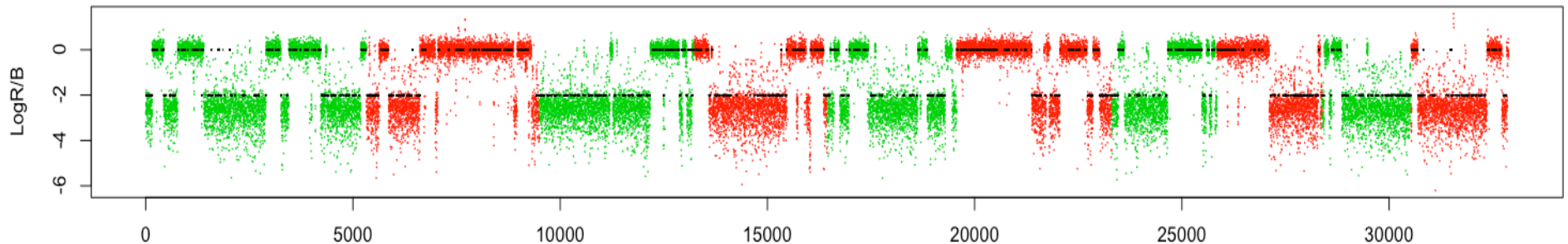
**Genome-wide analysis of log ratios of signal strengths between Mo17 and B73 as a function of the degree of probe sequence conservation.**  
 Log ratios of probe signals are plotted relative to their physical positions on the 10 maize chromosomes.



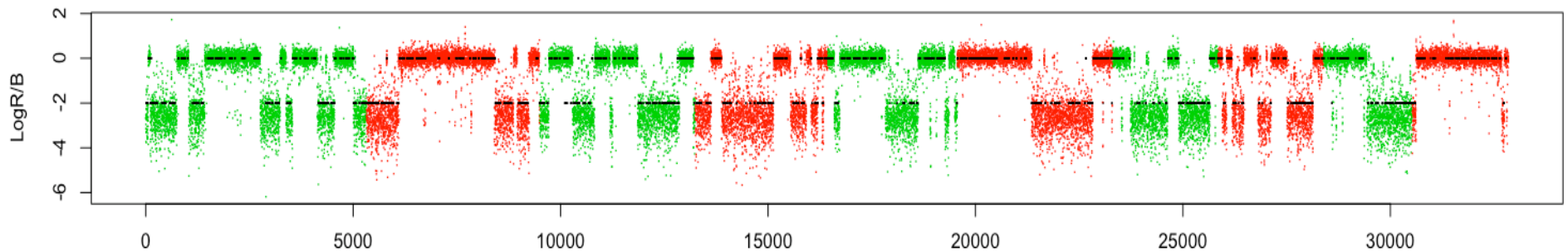
# CGH-Based High-Resolution Genotyping of IBM RILs

RIL and B73 DNA hybridized to Nimblegen's HD2 array. Log ratios of signals from ~34k genic probes with B-M log ratios >2 are plotted relative to their physical positions. CGH probes mapping to odd and even chromosomes are indicated in green and red, respectively. Note high level of consistency with PCR-based genotyping results (black spots\*).

RIL:M0023

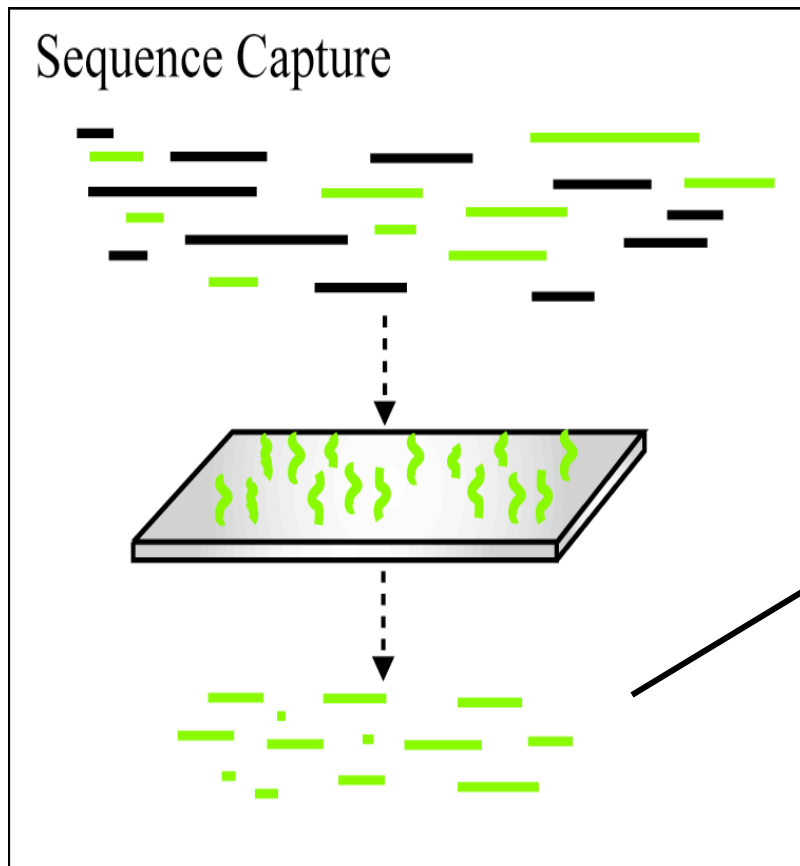


RIL:M0022



\*PCR-based mapping scores from Fu et al., 2006 Genetics

# Region-Specific SNP Discovery Using Nimblegen's Sequence Capture\*



QTL Interval

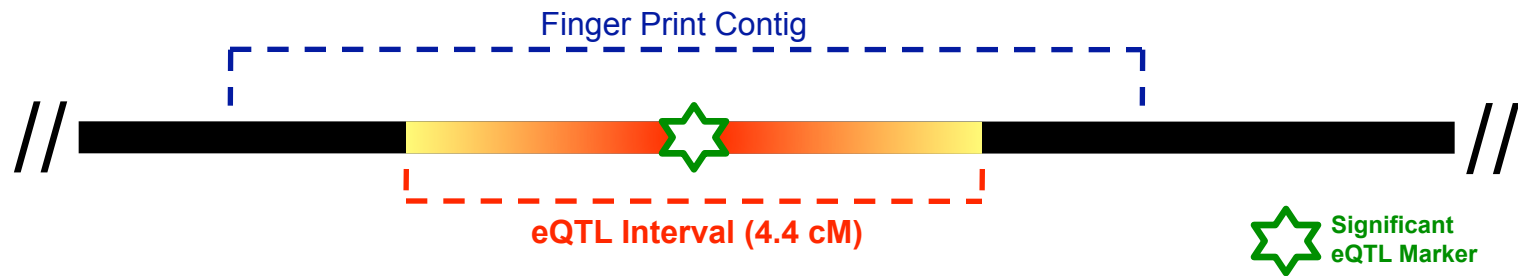
**Next Generation  
Sequencing of  
captured Mo17**

**Alignment to B73 ->  
SNP discovery**

\*Hodges et al. (2007) Nature Genetics  
\*Albert et al. (2007) Nature Methods

Emrich et al., 2007 Genome Research\*  
Barbazuk et al., 2007, Plant Journal\*  
*\*open access articles*

# Sequence Capture from eQTL Interval



- **1.5 Mb** eQTL interval contains:
  - **350 non-TE genes** predicted using FGENESH and repeat filtered w/ ISU Cereal Repeat DB:
  - 806,653 bp repeat masked
  - 738,611 bp unmasked
- 60,000 454 reads (~10% “on-target” *wo/ considering repeats*)
  - **525 SNPs** discovered in non-repetitive sequences
- Validation necessary (NIPs, Emrich et al., Genetics 2006)

# Validated and Mapped ~1,000/1,359 (~74%) Putative 454-SNPs via Sequenom MassARRAY



- Sequenom® software used to design PCR & extend primers for 1,359 putative SNPs
- 1,359 SNPs formed 48 “plexes”
- (ave. 28 SNPs/plex, now 36)

Throughput and cost:

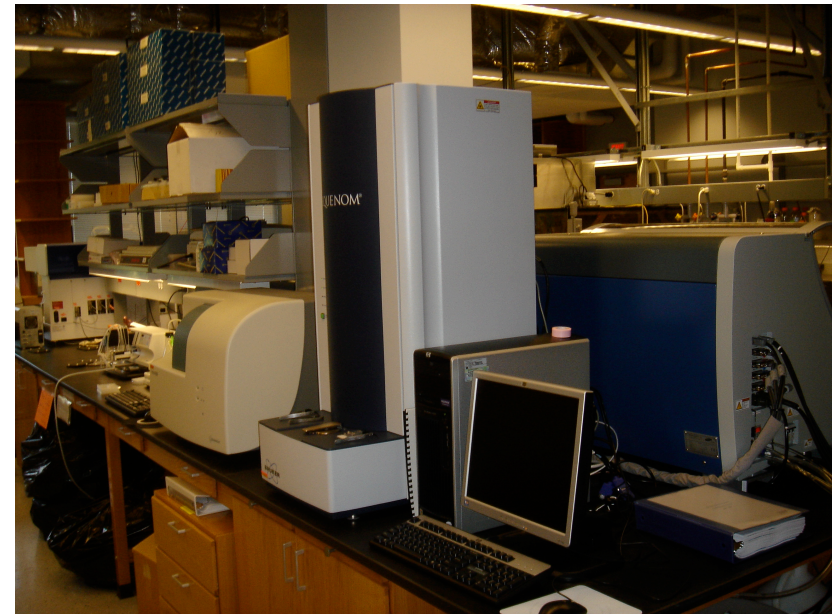
36,864 data points possible per day at a supply cost of \$0.05-0.10 per data point.



Sarah Hargreaves



Debbie Chen



# Take home-messages

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- Sequence capture works in complex crop plant genomes
  - Efficiency depends on multiple factors, some of which are still under investigation
- CGH can help to optimize capture design in species with high levels of genomic variation

# Collaborators

- Brad Barbazuk (Univ of FL)
- Rhonda DeCook (Statistics, Univ of IA)
- Jeff Jeddelloh et al. (Roche/Nimblegen)
- Abraham Korol (Univ Haifa)
- Dan Nettleton (Statistics, ISU)
- Nathan Springer (Univ of MN)
- Qixin Sun & Zhiyong Liu (China Agriculture Univ)



**The Maize Genome  
Sequencing  
Project, Rick  
Wilson, PI**





# Schnable Laboratory



An-Ping Hsia



For more details and discussion, please visit Poster 327



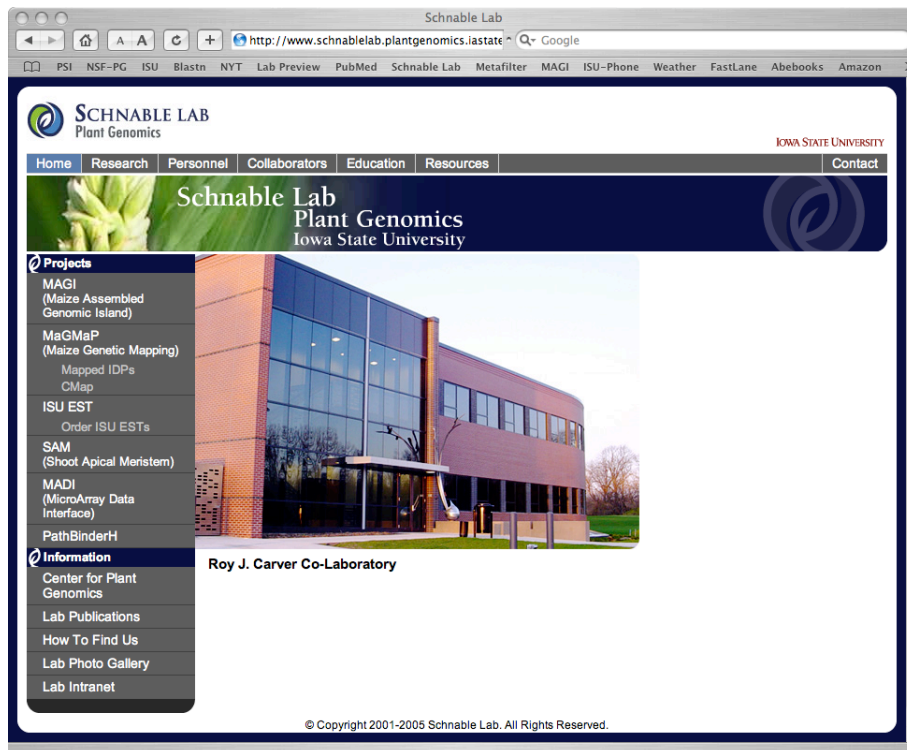
Kai Ying  
(应开)



**Nathan Springer**

*"Application of NimbleGen Sequence Capture to Complex Plant Genomes"*

Roche Nimblegen Workshop:  
Tuesday, 6:10 pm - 8:20 pm



[www.schnablelab.plantgenomics.edu](http://www.schnablelab.plantgenomics.edu)