Genetic anchoring of the chromosome shotgun assembly of bread wheat by population sequencing

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IPK Gatersleben

EUCARPIA ITMI meeting

June 29, 2014

Member of the Leibniz Leibniz Association

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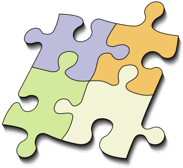
Shotgun assemblies and why we need to anchor them

- Whole genome or chromosome shotgun assemblies are a fast and easy way to generate genomic resources.

Shotgun assemblies and why we need to anchor them



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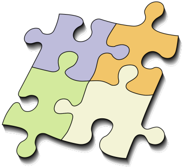

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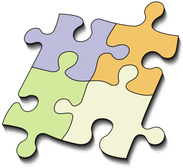

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- Shotgun assemblies are half-solved jigsaw puzzles.
- Genetic mapping to assign assembly contigs to chromosomal locations
- POPSEQ in barley and wheat

The idea of POPSEQ

- Only 25 % of the barley WGS assembly could be positioned in the physical framework.

no. of contigs	2.7 million
cumulative length	1.8 Gb
mean contig length	700 bp
no. contigs > 1kb	376,261
length of contigs > 1kb	1.1 Gb
N50	1,425 bp

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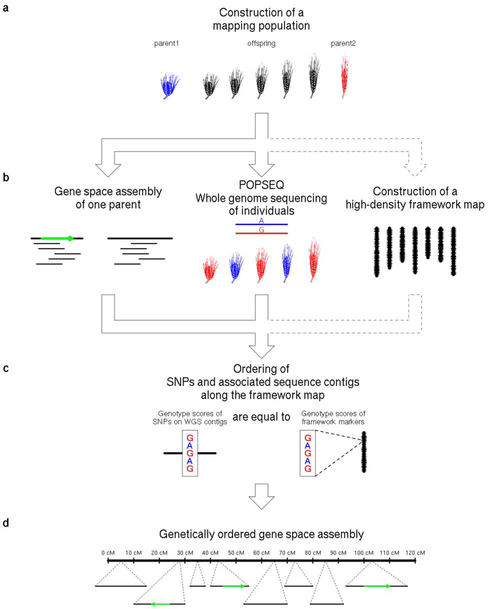
- Idea: use whole genome sequencing for genotyping to establish marker order from sequencing data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POPSEQ results in barley

- POPSEQ was done with one RIL (Morex × Barke) and one DH population (OWB).

	MxB + OWB WGS	IBSC
No. of SNPs used for anchoring	11,229,709	498,165
Framework map	iSelect/OWB GBS	iSelect
No. of anchored contigs	747,077	138,443
Size of anchored contigs	1,222 Mb (65%)	410 Mb (21%)
Median length of anchored contigs	891 bp	1,775 bp
No. of anchored HC genes	20,932 (80%)	14,923 (57%)

- Three times more anchored sequence compared to the physical and genetic framework

Chromosome shotgun sequencing (CSS) in wheat

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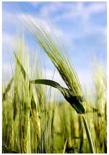
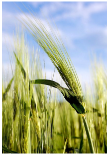
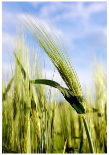
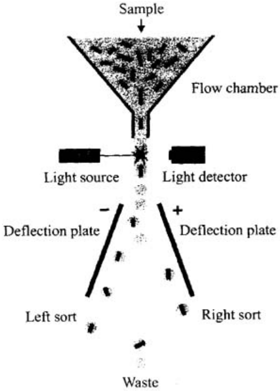


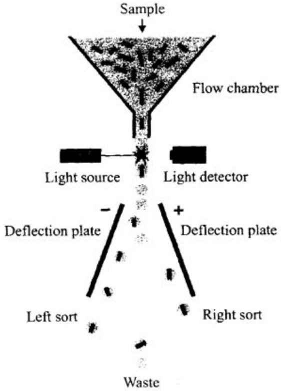






Chromosome shotgun sequencing (CSS) in wheat

- IWGSC has created shotgun sequence assemblies of all 40 wheat chromosome arms + 3B
- Single chromosome arms were isolated from cytogenetic stocks using flow cytometry



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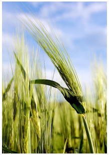
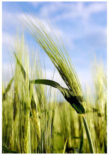
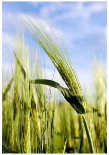
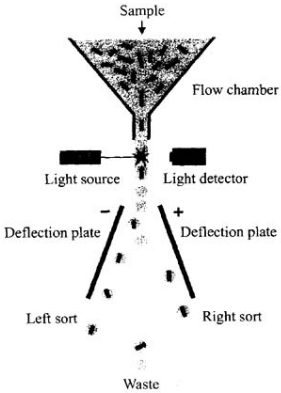






- IWGSC has created shotgun sequence assemblies of all 40 wheat chromosome arms + 3B
- Single chromosome arms were isolated from cytogenetic stocks using flow cytometry
- DNA libraries of sorted chromosomes were sequenced to high coverage on the HiSeq2000 and assembled by TGAC
- Total assembly size: 10.1 Gb



Sequencing the Synthetic W7984 × Opata M85 population

- POPSEQ anchoring of the CSS assembly by sequencing the SynOp doubled haploid population
- Synthetic wheat: artificial hybridization of a tetraploid durum wheat with *Ae. tauschii*.

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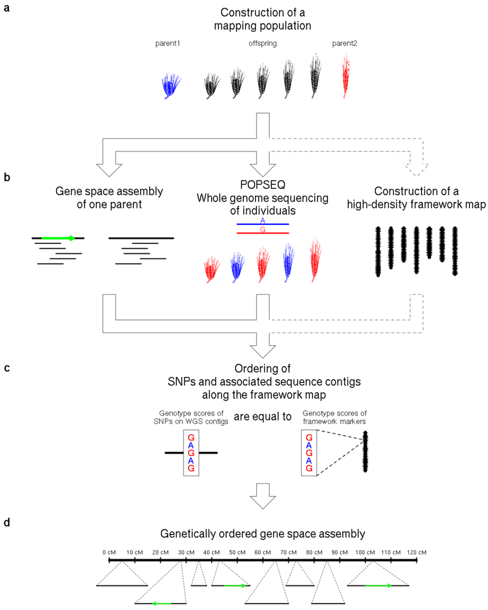
- Synthetic wheat: artificial hybridization of a tetraploid durum wheat with *Ae. tauschii*.
- JGI sequenced 90 doubled haploid lines to 1x coverage.
- Read mapping and SNP calling were done with BWA and SAMTools.

POPSEQ: putting together the pieces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- Annotated sequence contigs of the wheat CSS assembly (IWGSC)

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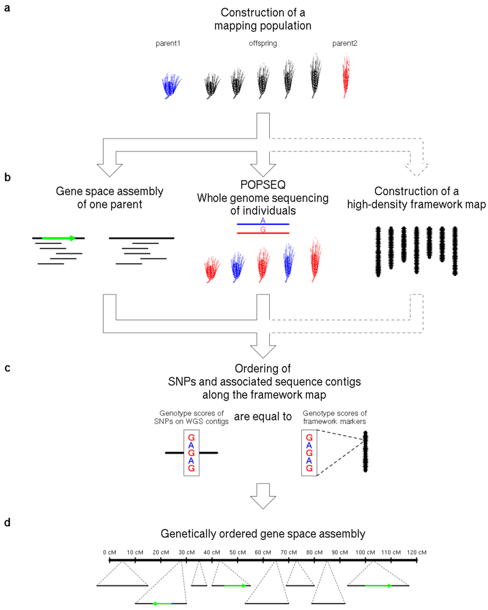

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- Annotated sequence contigs of the wheat CSS assembly (IWGSC)
- A high-density genetic map was constructed through GBS of the Synthetic x Opata population (Poland, 2012).

Placing the SNPs into a framework map

RIL #	1	2	3	4	5	6	7	8	9	10
SNP on WGS contig	A	G	A	A	G	G	A	A	G	G

- WGS SNPs and framework markers are represented as binary genotype vectors.

Placing the SNPs into a framework map

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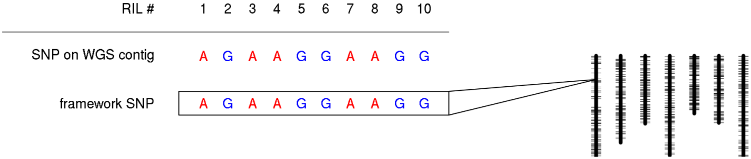

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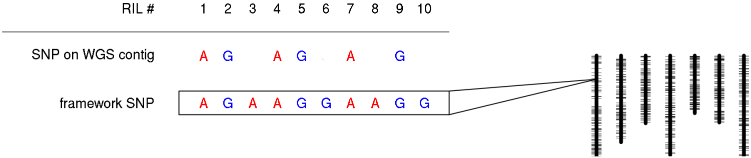

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- Consistency criteria for multiple nearest neighbors: framework are required to be within 5 cM.

Wheat POPSEQ results

	wheat	barley
population	SynOp DH	OWB DH
assembly size	10.1 Gb (63 %)	1.8 Gb (38 %)
N50	2,308 bp	1,425 bp
size in contigs $\geq$ 1 kb	7.0 Gb	1.1Gb
size in contigs $\geq$ 5 kb	3.1 Gb	382 Mb
anchored length	4.4 Gb	1.0 Gb
anchored length $\geq$ 1 kb	4.2 Gb	811 Mb
anchored length $\geq$ 5 kb	2.3 Gb	279 Mb

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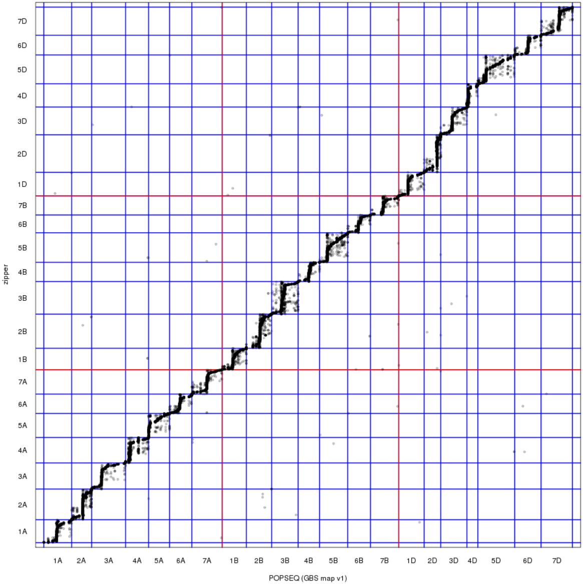
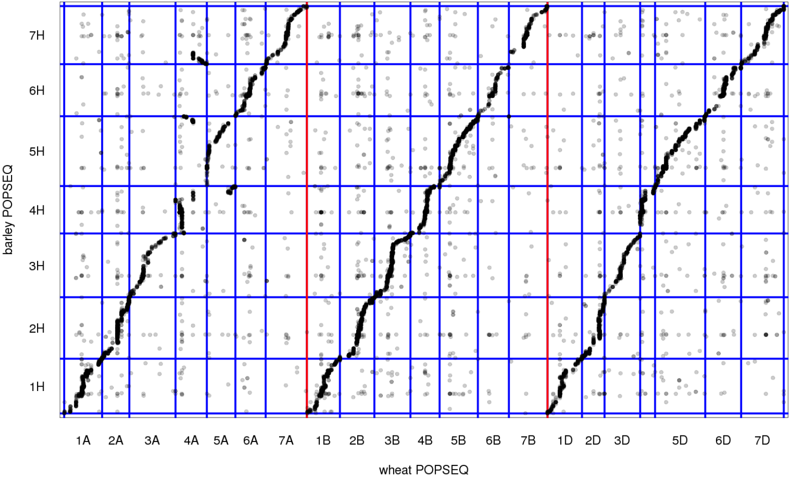
- 99.4 % agreement between POPSEQ and flow sorting

Collinearity with the GenomeZipper



- 99.8 % agreement of chromsome assignments
- 85 % correlation within linkage groups
- 75,183 genes anchored by POPSEQ and/or GenomeZipper

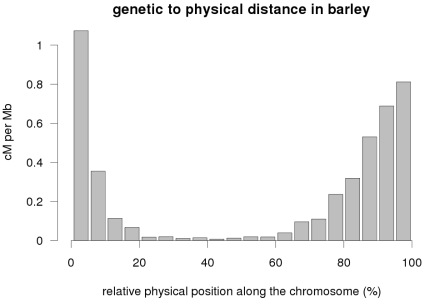
Collinearity with barley



- 93 % agreement of group assignments; 91 % collinearity within groups

Challenges and limitations of POPSEQ

- Biology: POPSEQ relies on recombination.



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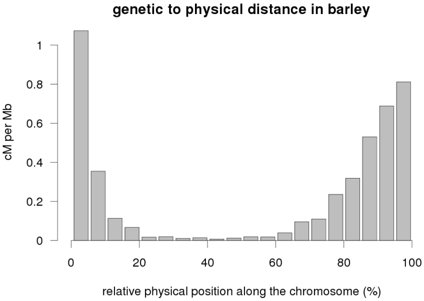
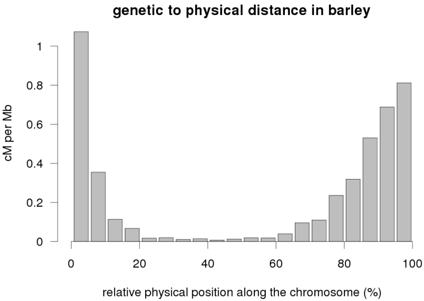
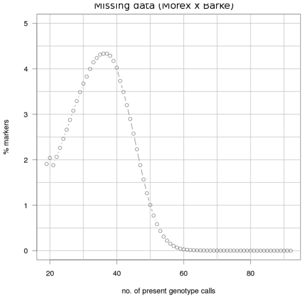


Challenges and limitations of POPSEQ

- Biology: POPSEQ relies on recombination.
- Algorithms: assembly quality (contig size and number)
- Technology/money: sequencing costs limit sequencing depth, population size and mapping resolution.





Acknowledgements

- Nils Stein
- Uwe Scholz
- IWGSC
- Dan Rokhsar
- Jarrod Chapman
- Kerrie Barry
- Robbie Waugh
- Jesse Poland
- Gary Muehlbauer

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GEFÖRDERT VOM

Bundesministerium
für Bildung
und Forschung

International
Wheat Genome
Sequencing
Consortium

JGI

KANSAS STATE
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Hutton
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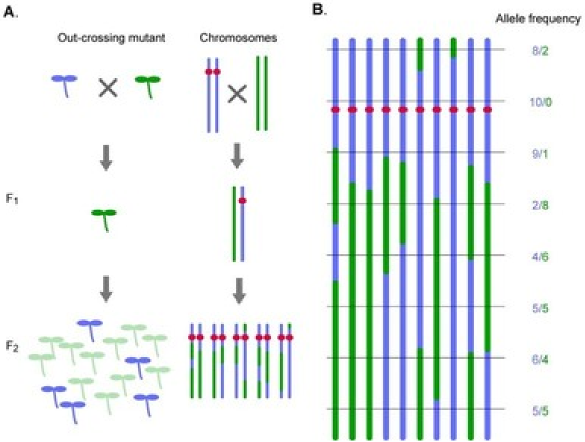
OF MINNESOTA
Driven to Discover℠

Mapping-by-sequencing

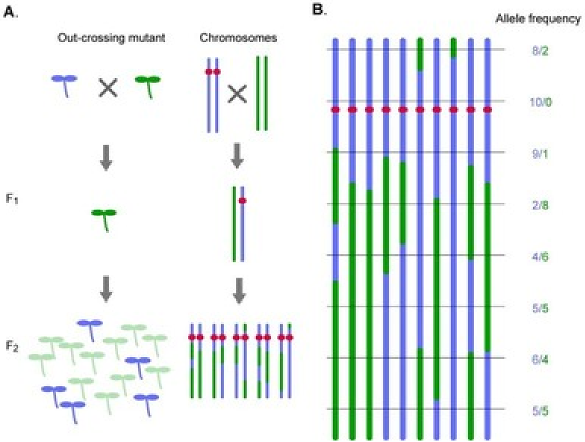
- Identification of causal genes by sequencing phenotypic pools



Schneeberger *et al.*, TIPS 2010

Mapping-by-sequencing

- Identification of causal genes by sequencing phenotypic pools
- Requires an ordered reference sequence



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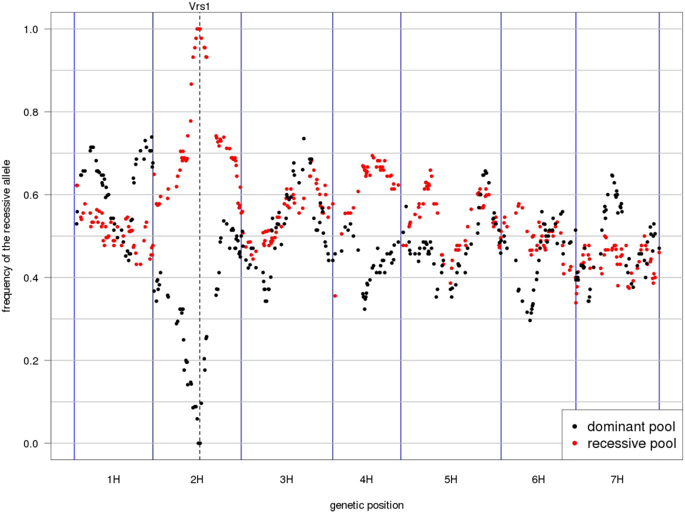
Mapping-by-sequencing

- Mapping-by-sequencing of the sixed-row spike gene (vrs1) in OWB



POPSEQ anchoring of the barley physical map

	BAC contigs	sequenced clones
POPseq data	MxB + OWB	MxB + OWB
# all contigs	9,265	6,278
# with WGS contigs	5,872	6,243
# with anc. WGS contigs	5,720	6,189
# anchored	5,193	5,591
length	3.95 Gb	703 Mb

- POPSEQ can assign additional physical contigs to chromosomes to assist MTP sequencing