



Building an Oat Consensus Map – where we've been and where we are going

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Representing a very large collaborative group

Acknowledgments

❖ UNC-Charlotte

- ❖ **Shawn Chaffin**
- ❖ **Adriano de Bernardi S**
- ❖ Jaime Sheridan
- ❖ **Shannon Schlueter**



❖ General Mills

- ❖ **Eric Jackson**
- ❖ Ryan Brown
- ❖ Joe Lutz
- ❖ Lane Johnson

❖ Agriculture and Agri-Food Canada

- ❖ **Nick Tinker**
 - ❖ **Yung-Fen Huang**
 - ❖ Charlene Wight
-
- ❖ Jesse Poland (USDA-ARS)

❖ P2EP Project group

- ❖ Cory Brouwer (UNCC)
 - ❖ Robert Reid
 - ❖ Jeremy Jay
 - ❖ Stephen Blanchard
- ❖ Tzung-Fu (NCSU)
 - ❖ **Scott Smith**



❖ CORE

- ❖ Mike Bonman (USDA)
 - ❖ Ebrahiem Babiker
- ❖ Kathy Klos (USDA)
- ❖ Curt McCartney (AAFC)
 - ❖ Gnanesh Nanjappa
- ❖ Fred Kolb (U of IL)
 - ❖ Bradley Foresman
- ❖ Rick Jellen (BYU)
- ❖ Jennifer Mitchell Fetch (AAFC)
- ❖ Emir Islamovic
- ❖ Becky Oliver
- ❖ Shiaoman Chao

Summary

- Oat consensus map v. 1.0
 - Evaluation of the v. 1.0 map
 - Oat consensus map v. 2.0
 - Evaluation of the v. 2.0 map
 - Refining our final map so we can best represent the diversity of the oat genome
 - Where are we going now?
-

How do you put together a consensus?

- Start with individual populations, genotyping and mapping
 - Software: Multipoint (Mester et al. 2003)
 - Clean removing 10% missing data
 - Initial marker grouping recomb. freq. = 0.15
 - Ordering of grouped markers - sampling w/o replacement, 20 iterations
 - Manual curation to achieve stability
 - End to end joining by increasing recomb. freq by 0.05
 - Repeat curation and joining until final recomb. freq. of 0.30.

Individual component populations

Population	Abbr.	Pop. Size	Marker Type	Contributed by	Reference
94197A1-9-2-2-2-5 x Boyer	GB	76	Infinium	Bonman et al.	Abreham et al.
Provena x 94197A1-9-2-2-2-5	PGS	98	Infinium, GBS	Bonman et al.	Abreham et al.
Provena x Boyer	PB	139	Infinium	Bonman et al.	Abreham et al.
86-1156 x Clintland 64	IL4	112	Infinium	Kolb et al.	Foresman et al.
86-6404 x Clintlant 64	IL5	171	Infinium	Kolb et al.	Foresman et al.
Assinobia x MN841801	AM	161	Infinium	Mitchell-Fetch et al.	Nanjappa et al.
Otana x PI269616	OP	98	Infinium, GBS	Carson et al.	Oliver et al., 2013
CDC SoFi x Hi-Fi	SH	53	Infinium, GBS	Beattie et al	Oliver et al., 2013
Dal x Exeter	DE	145	Infinium,GBS	Tinker et al.	Hizbai et al., 2012
Hurdal x Z-597	HZ	53	Infinium,GBS	Bjørnstad et al.	Oliver et al., 2013
Ogle x TAMO 301	OT	53	Infinium, GBS	Jackson et al.	Portyanko et al., 1995
Kanota x Ogle	KO	52	Infinium, GBS	Tinker et al.	O'Donoghue et al., 1995

Population Map Construction – student driven heuristic approach



Individual population maps

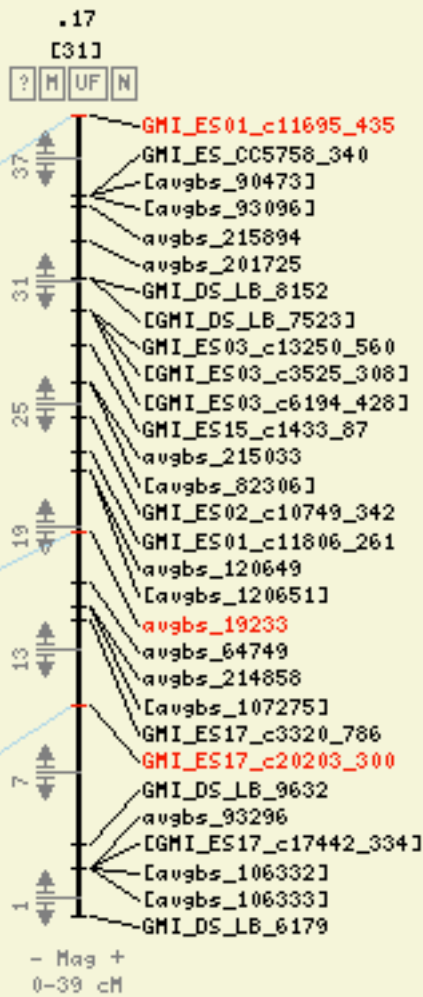
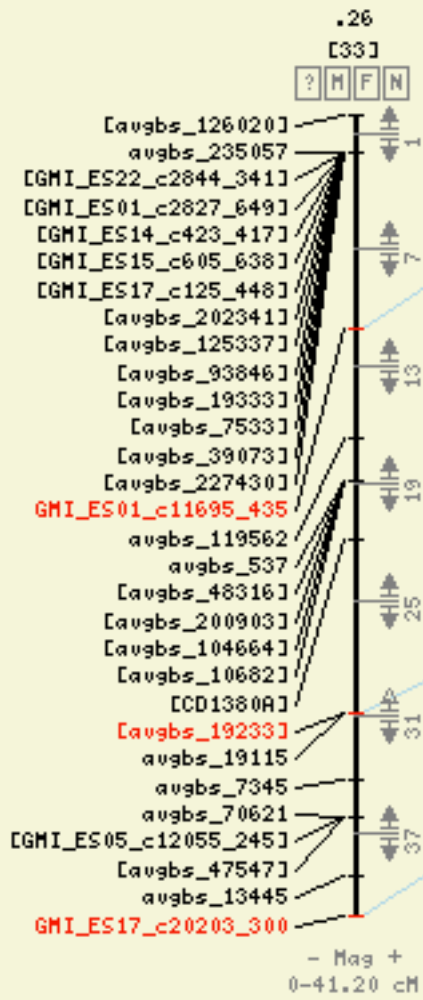
Pop	RILs	# of LGs	Total markers	Framework markers	cM Mapped	Marker Density
GB	76	24	1011	244	1462.6	5.99
PGS	98	36	3741	653	2510.8	3.84
PB	139	27	837	299	1613.1	5.39
IL4	112	23	972	244	10576	4.33
IL5	171	25	925	294	823.7	2.80
AM	161	34	1696	630	1652.7	2.62
OP	98	24	5372	618	2009.1	3.25
SH	53	33	2187	253	1195.4	4.72
DE	145	27	2206	329	1142	3.47
HZ	53	25	2450	243	1244.3	5.12
OT	53	29	6239	541	2369.3	4.37
KO	52	41	3390	381	2250.4	5.90
Average	100	29	2586	394	1610.9	4.32
Min	52 (KO)	23 (IL4)	837 (PB)	243 (HZ)	823.7 (IL5)	2.62 (AM)
Max	171 (IL5)	41 (KO)	6239 (OT)	653 (PGS)	2510.8 (PGS)	5.99 (GB)

How do you put together a consensus?

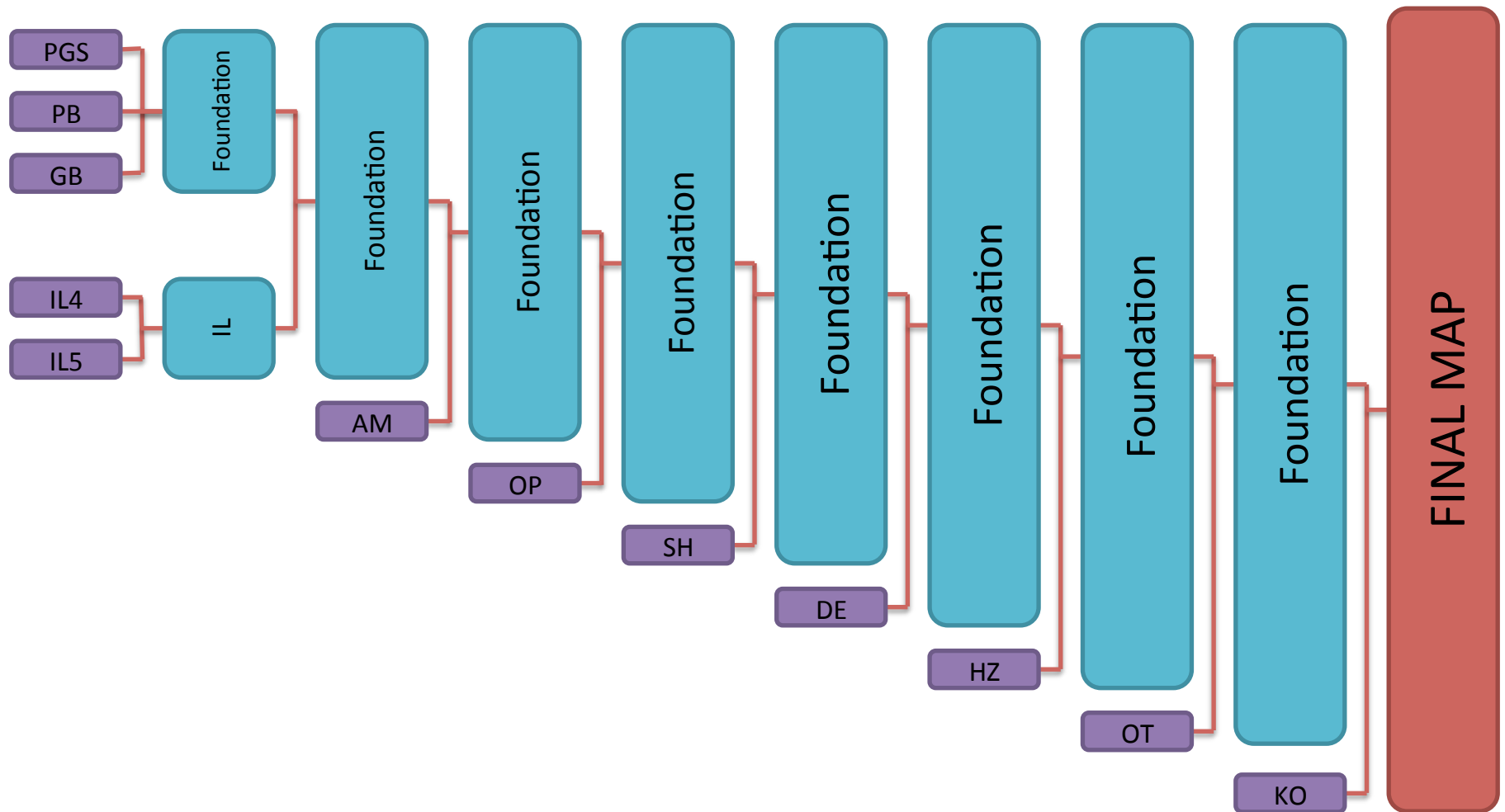
- Merge those individual maps together
 - Software: Merge Map (Wu et al. 2008)
 - V. 1.0 strategy
 - Split linkage groups with gaps of 30 cM or more
 - Continued the team-based consensus approach using C-map for visualization of syntenic relationships
 - Initial merge between 2 (or 3) populations first followed by comparison of the next population to current consensus
 - Merge decisions based upon team-driven decisions
 - Ordering of populations based on marker density, map size, and confidence.

Reference
Original
ot
i M

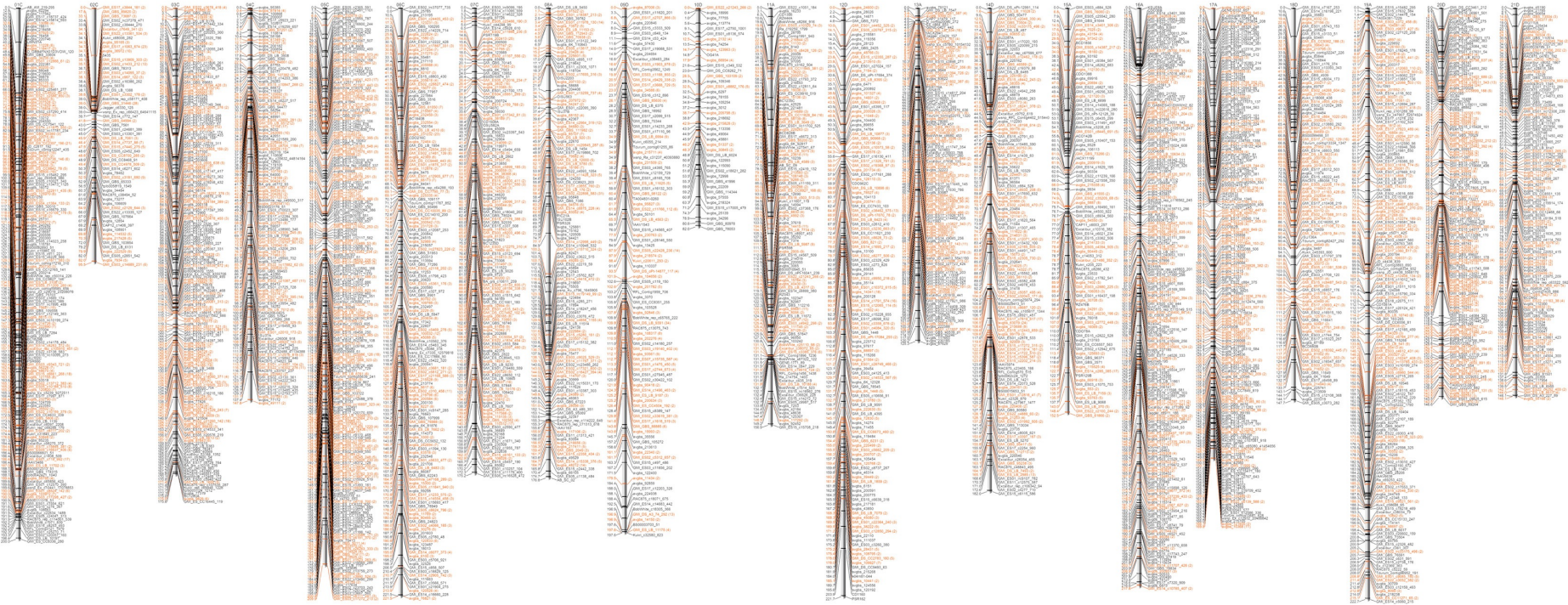
Comparative
Original
pgs
i M X



Consensus Map Construction

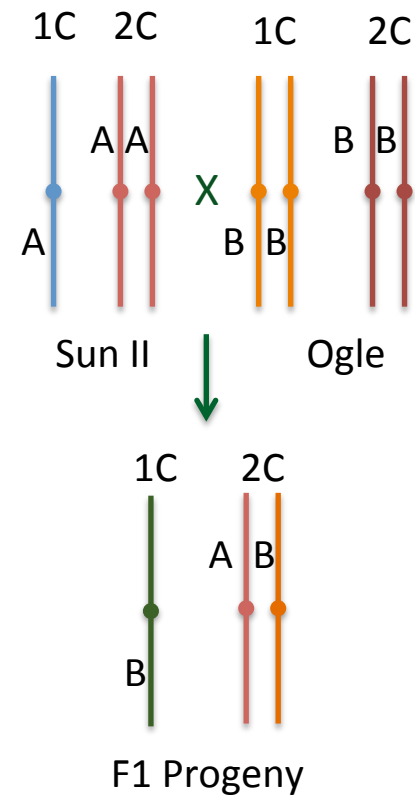
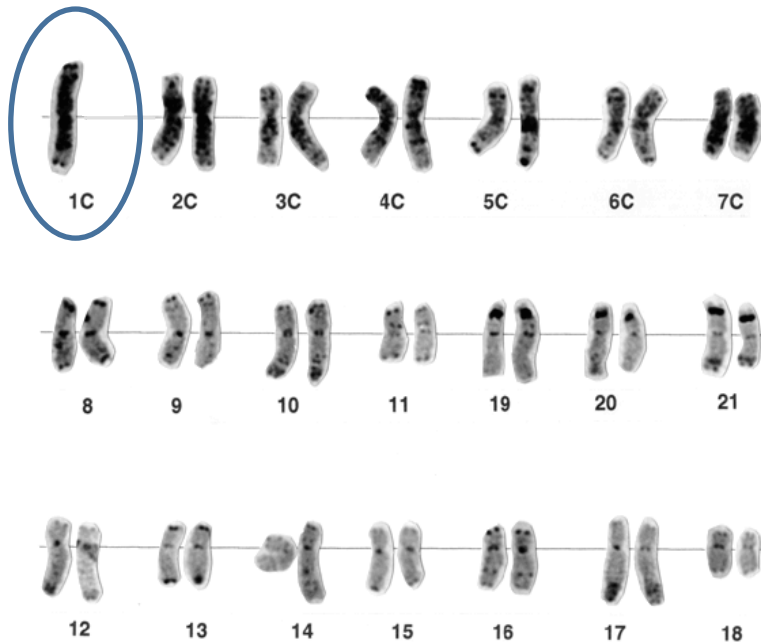


Oat Consensus Map v 1.0



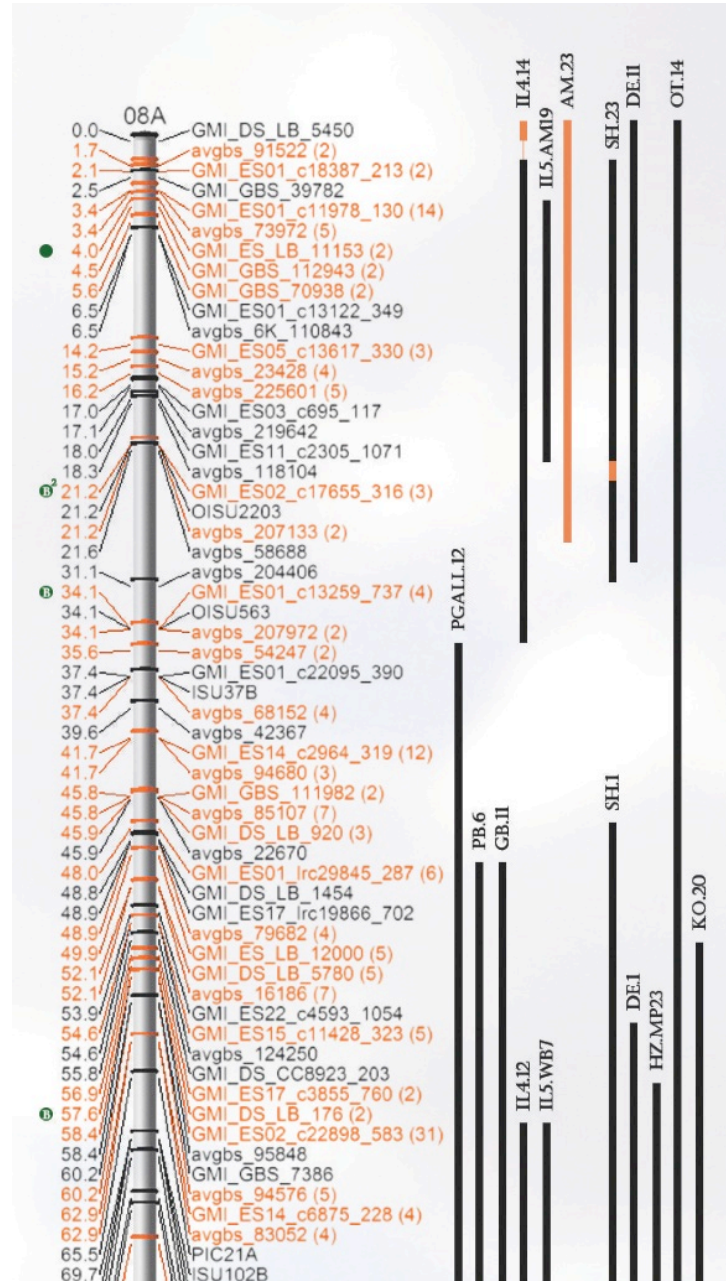
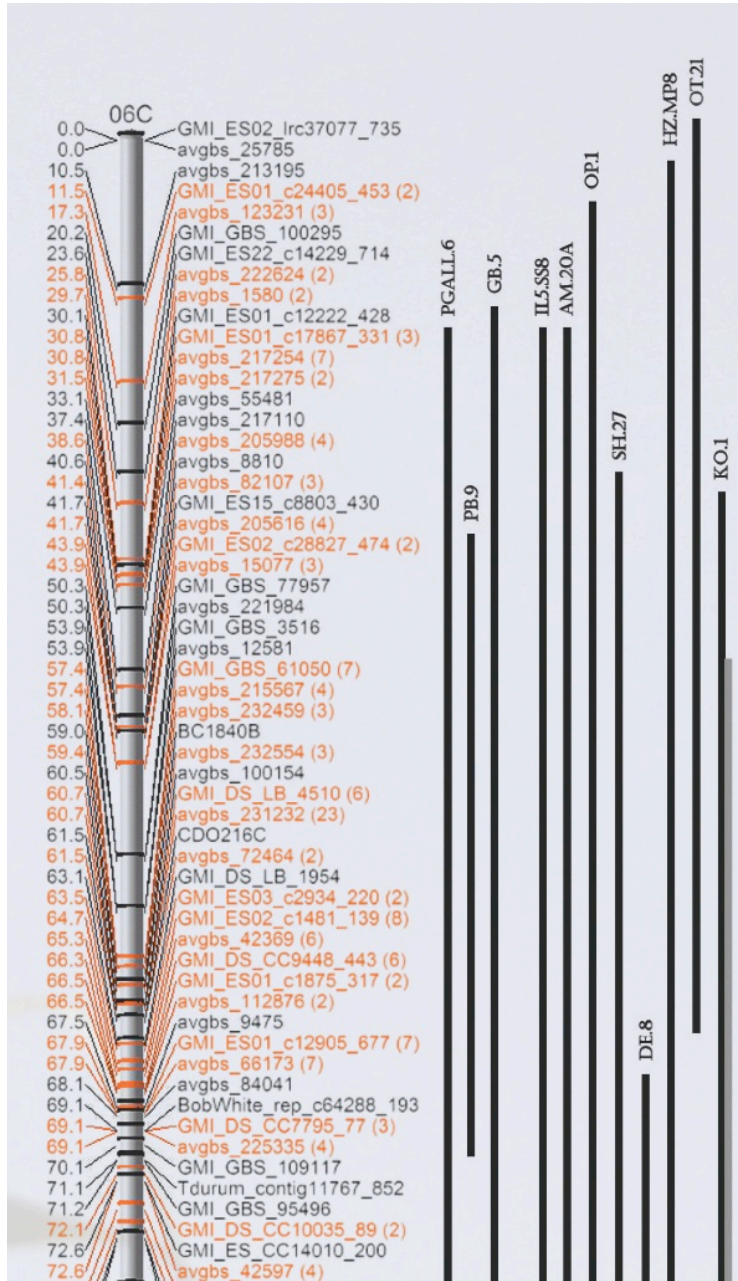
Physical Anchoring

Monosomic Hybrid Approach



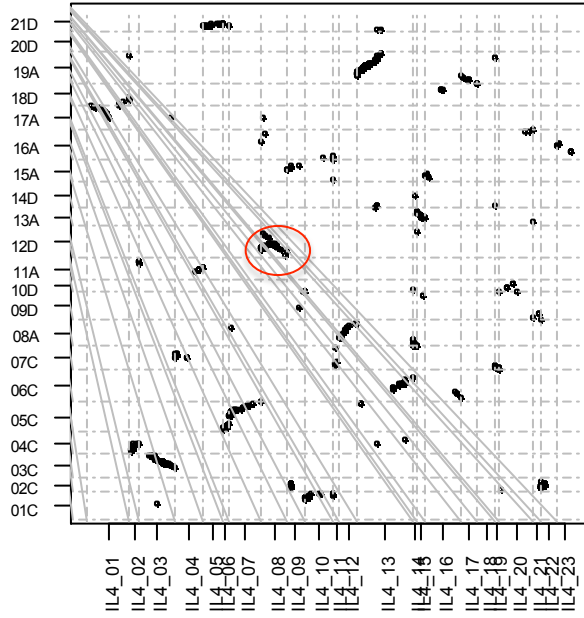
Oliver RE, Tinker N a, Lazo GR, Chao S, Jellen EN, et al. (2013) SNP discovery and chromosome anchoring provide the first physically-anchored hexaploid oat map and reveal synteny with model species. PLoS One 8: e58068.

Chr	cM Mapped	Total Markers	Framework Markers	Marker Density	Min Gap	Max Gap
1 C	200	485	130	1.54	0.1	12.8
2 C	95.47	191	50	1.91	0.1	17.8
3 C	175.6	500	137	1.28	0.1	7.3
4 C	147.76	328	102	1.45	0.1	10.4
5 C	209.89	525	172	1.22	0.05	9.5
6 C	221.5	409	117	1.89	0.1	10.5
7 C	175.2	363	106	1.65	0.1	8.3
8 A	176.84	323	100	1.77	0.1	15.3
9 D	197.6	273	81	2.44	0.1	17.9
10 D	82	65	41	2.00	0.1	7.9
11 A	156.9	260	82	1.91	0.1	10.2
12 D	221.7	321	110	2.02	0.1	17.8
13 A	125.3	345	80	1.57	0.1	6.2
14 D	181.97	314	97	1.88	0.1	9.4
15 A	152.46	191	60	2.54	0.18	10
16 A	217.76	422	155	1.40	0.03	11.2
17 A	169.8	357	142	1.20	0.1	7.8
18 D	147.7	292	74	2.00	0.1	10.6
19 A	222.71	408	123	1.81	0.1	9.6
20 D	146.55	411	108	1.36	0.1	9
21 D	146.05	237	102	1.43	0.1	8.1
Average	170.04	334.29	103.29	1.73	0.10	10.84
Sum	3570.76	7020	2169	-	-	-
Min	82 (10 D)	65 (10 D)	41 (10 D)	1.20 (17 A)	0.03 (16A)	6.20
Max	222.71 (19 A)	525 (5 C)	172 (5 C)	2.54 (15A)	0.18	17.90 (9D)

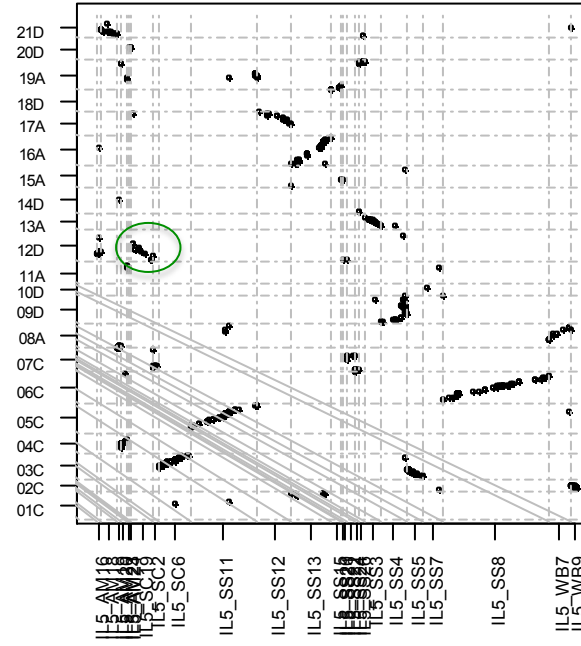


How well do individual component maps match v1.0?

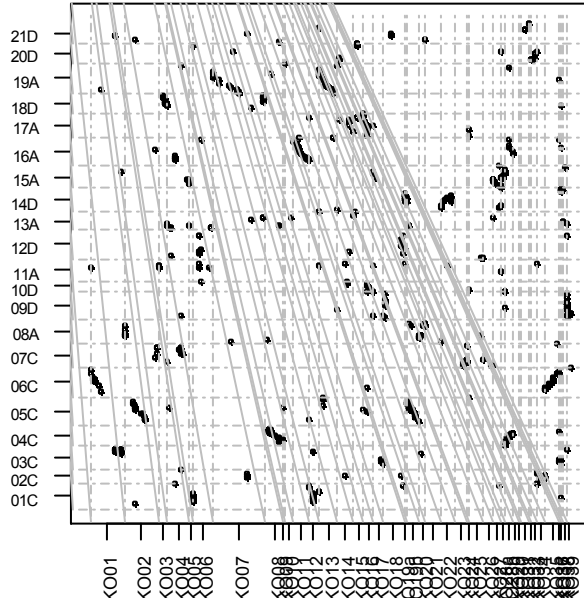
IL4



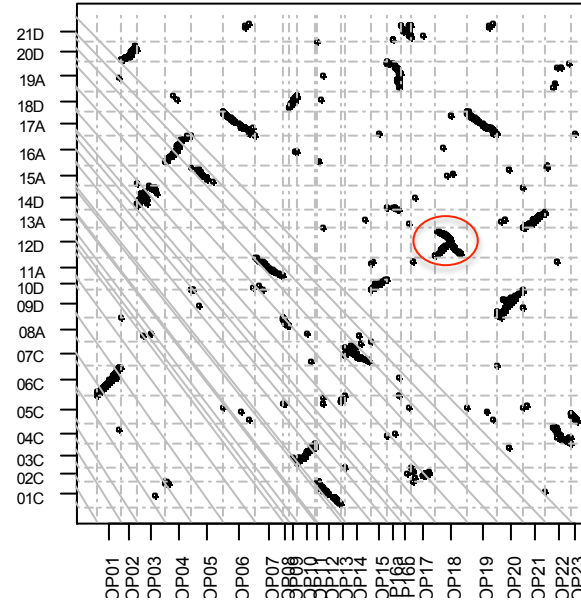
IL5



KO

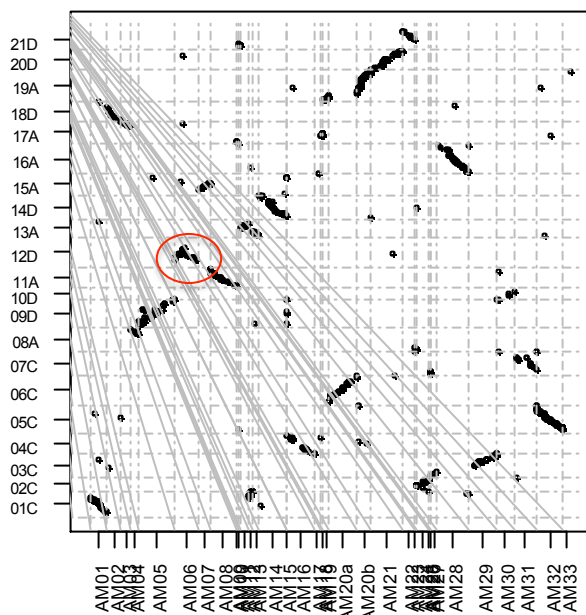


OP

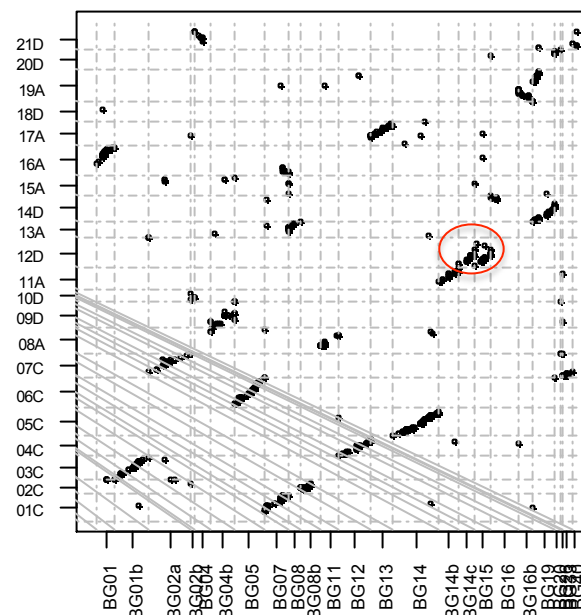


How well do individual component maps match v1.0?

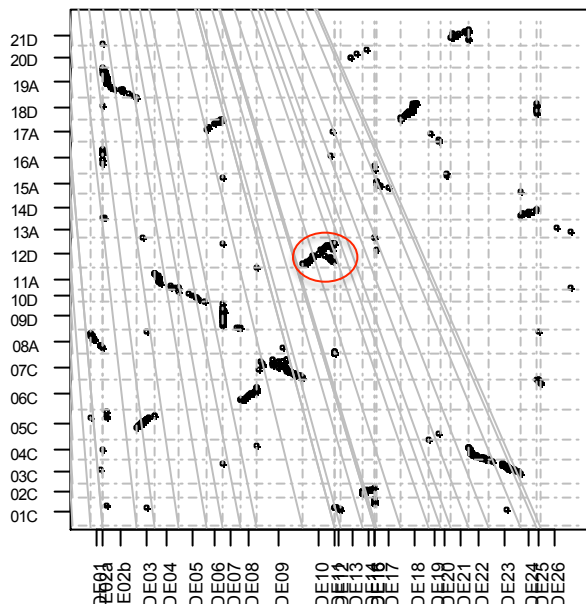
AM



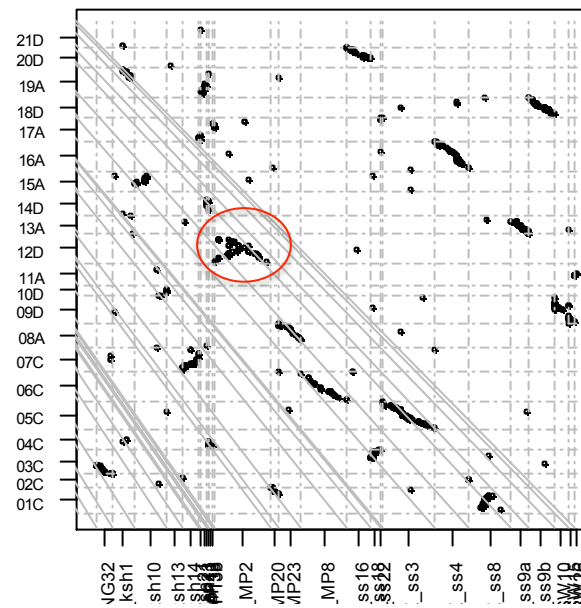
BG



DE

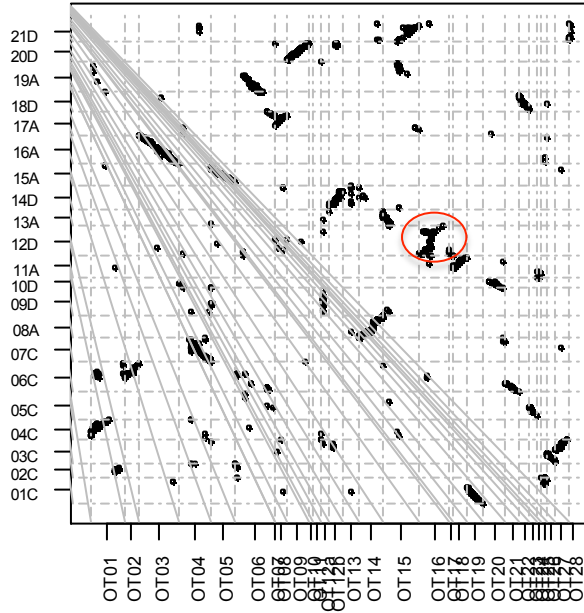


HZ

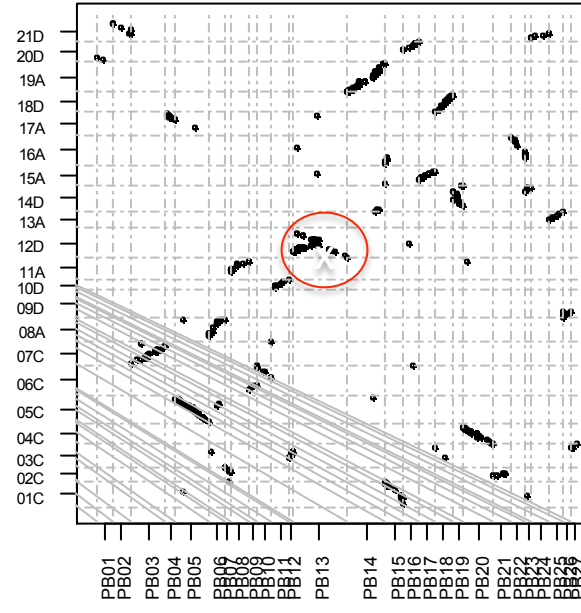


How well do individual component maps match v1.0?

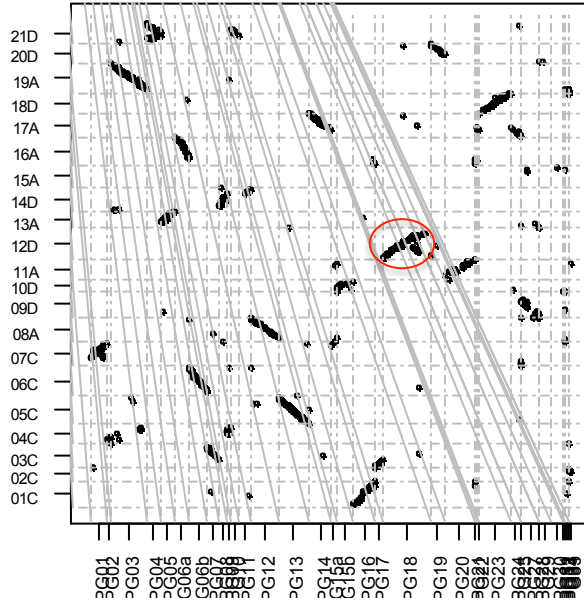
OT



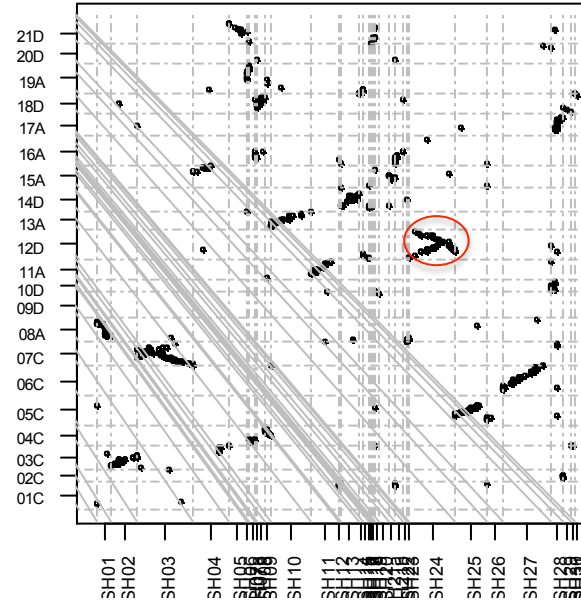
PB



PG



SH



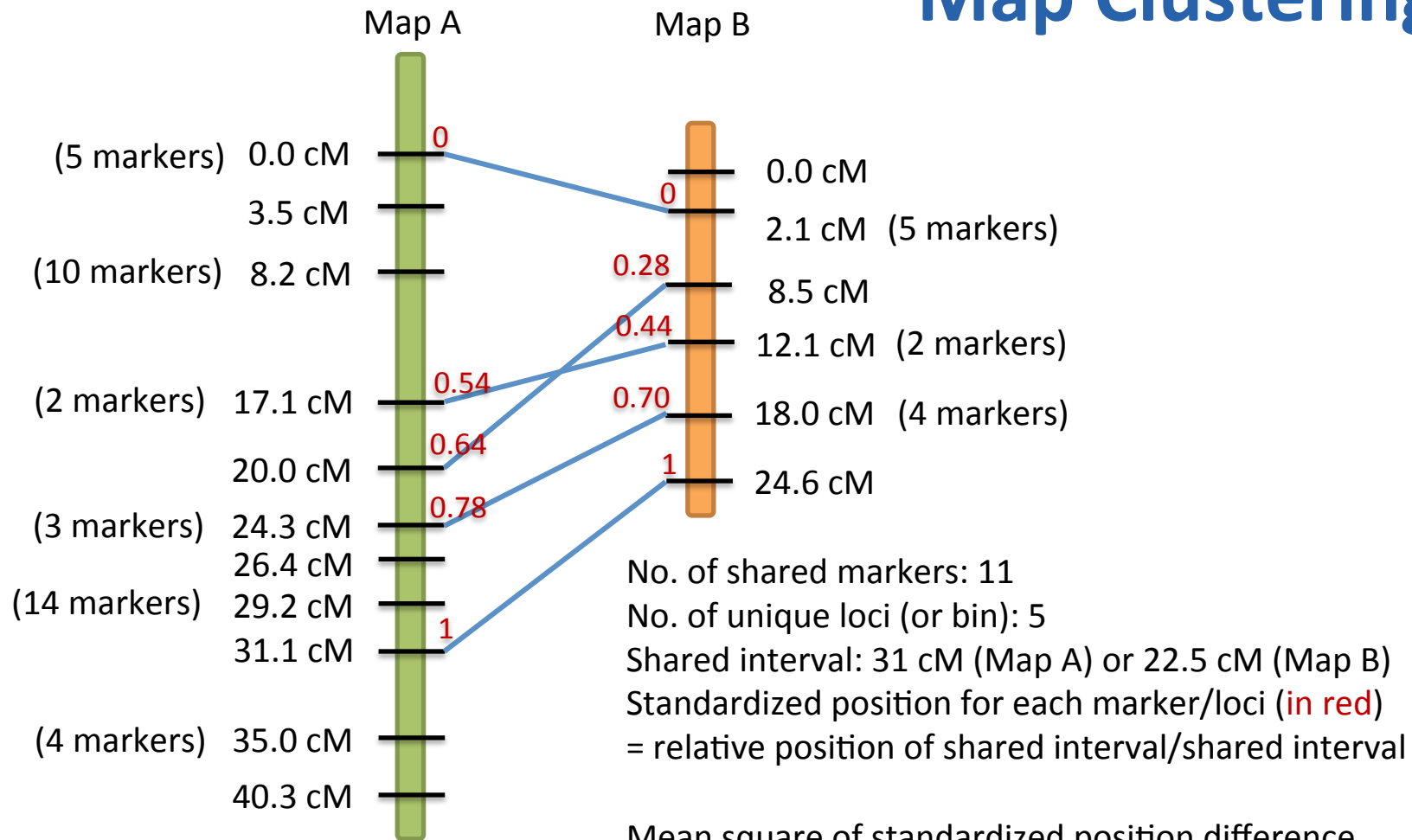
Evaluation of the v. 1.0 map

- Most linkage groups support the individual population maps
- Some merged groups do not support all the populations (12D, 9D, 7C, 21D)
 - Bias in the initial merge with which translocation the population carried, ex. IL5
- Inclusion of duplicate markers in the merge
- Documentation of the merging process was not sufficient to recreate maps

Putting together v 2.0 consensus

- Script the process so that it is as automated as possible (can be repeated with parameter changes) and it is easily documented
- Clustering-based approach to determining which linkage groups to merge
- Rely on MergeMap to batch merge all clustered linkage groups together
- Refining of the membership of each cluster group (Yung-Fen and Shawn) using dot-plots against component maps
- Evaluation of both weighted and un-weighted merging
- Overall – this process went through 4 iterations to refine inclusion of particular linkage groups in the consensus
- Used a new KO population map

Map Clustering



No. of shared markers: 11

No. of unique loci (or bin): 5

Shared interval: 31 cM (Map A) or 22.5 cM (Map B)

Standardized position for each marker/loci (in red)
= relative position of shared interval/shared interval

Mean square of standardized position difference

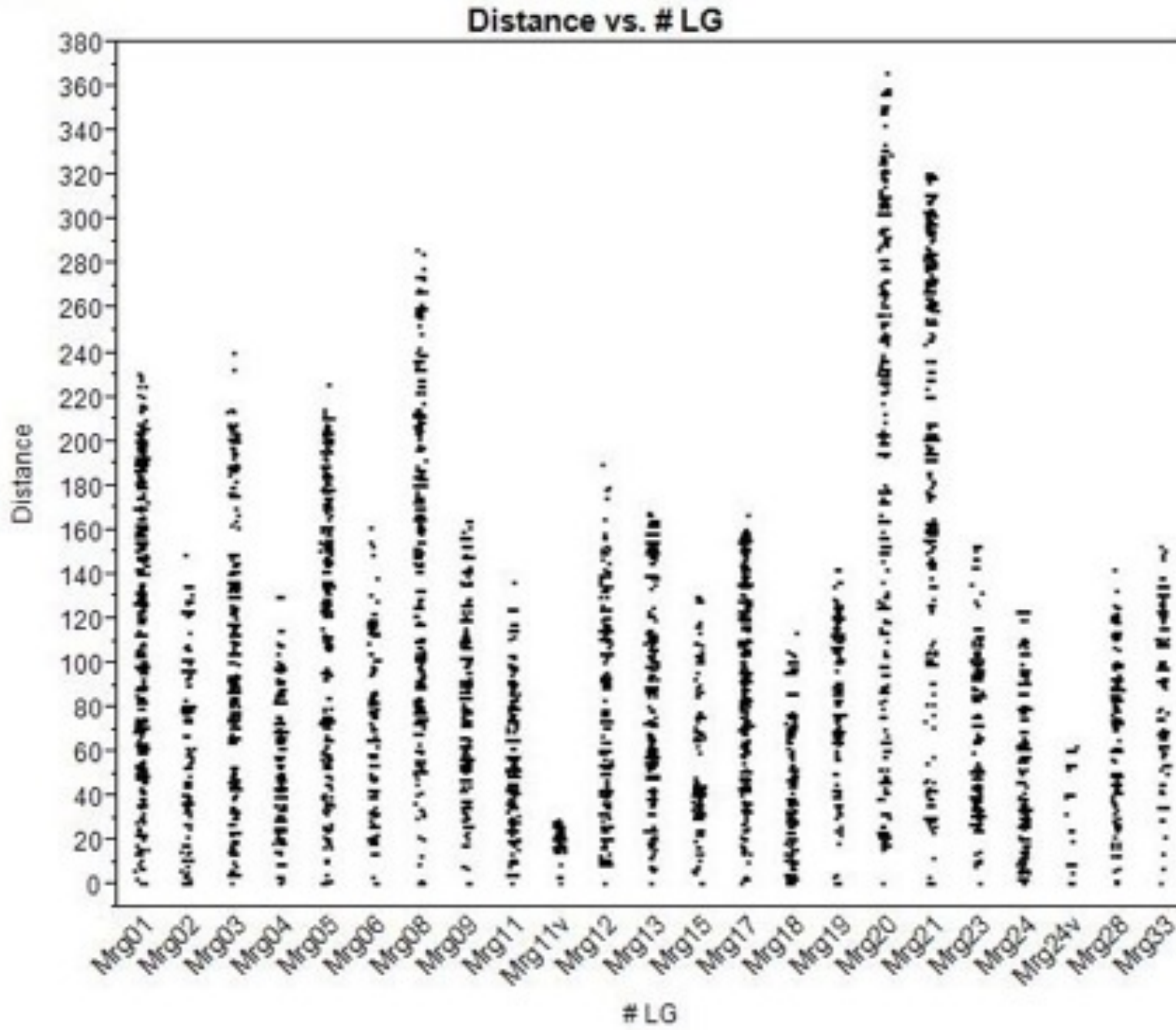
=

n : number of shared marker or loci
(based on custom setup)

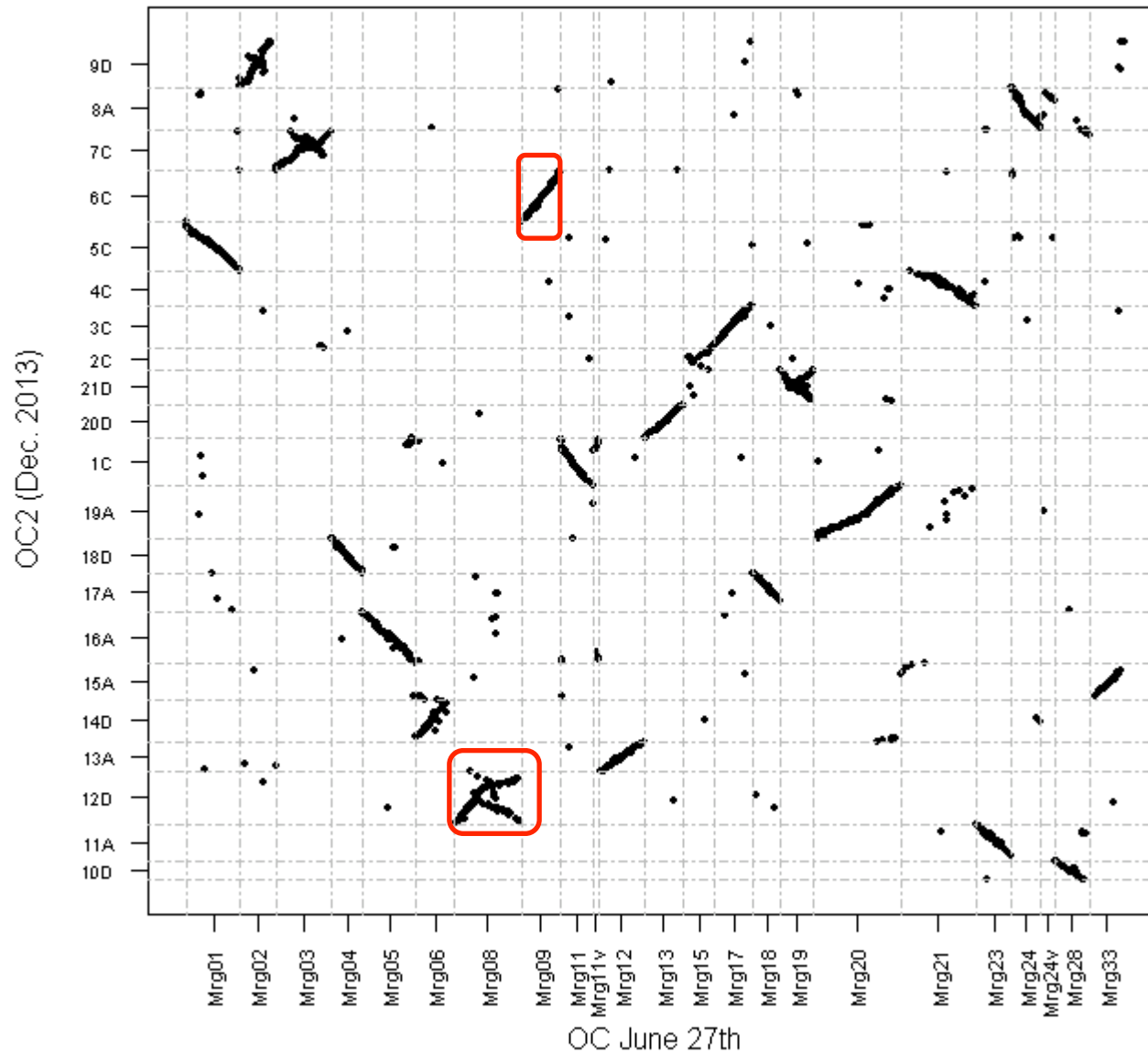
i : the i th shared marker

L_{iA} : the relative position of i th shared
marker or loci on map A

Consensus v. 2.0 map



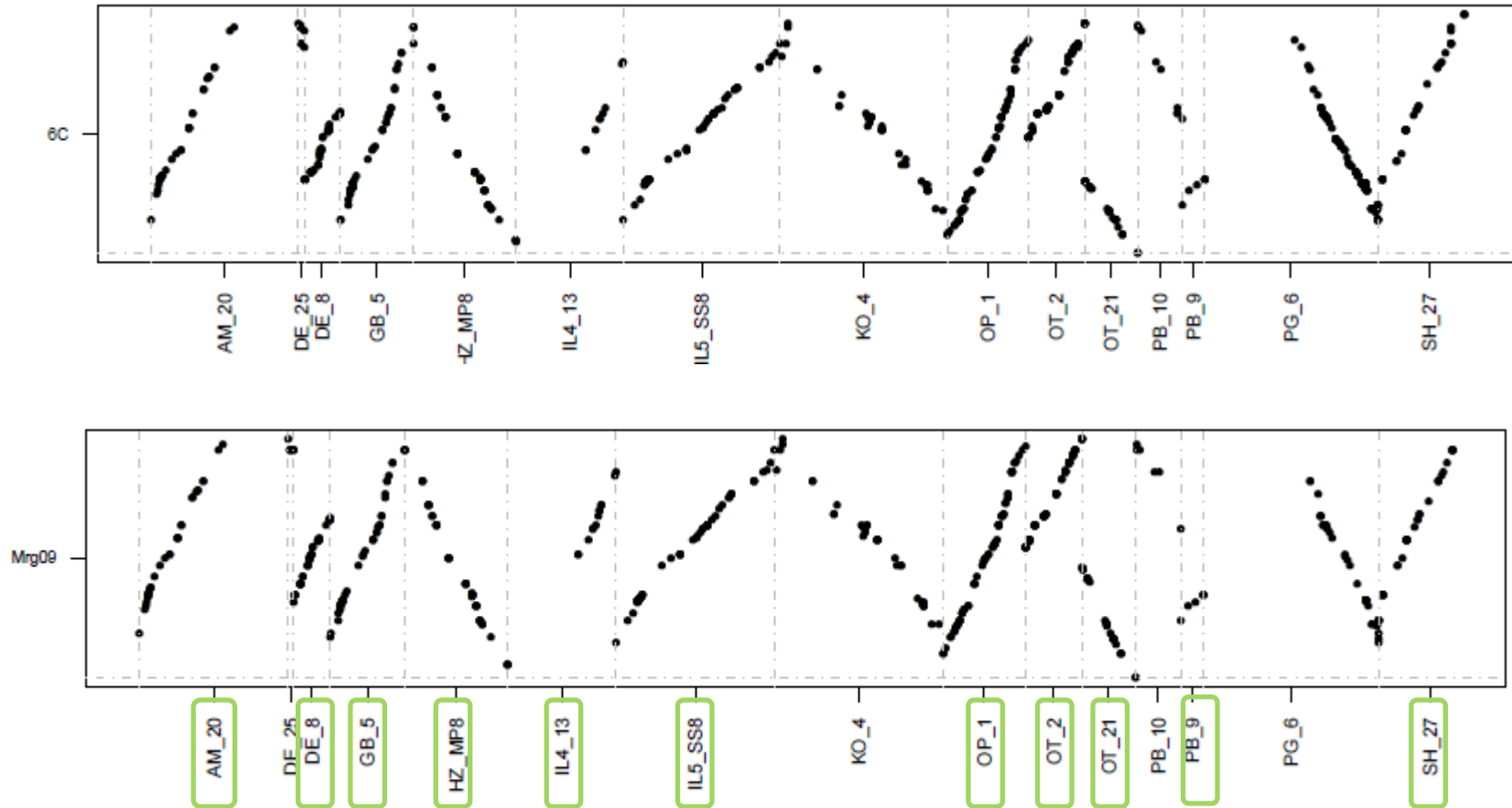
Comparing the v. 1.0 to v. 2.0



Consistent match (v.
1.0 6C vs. Mrg09)

Not-so consistent
match
(v. 1.0 12D vs. Mrg08)

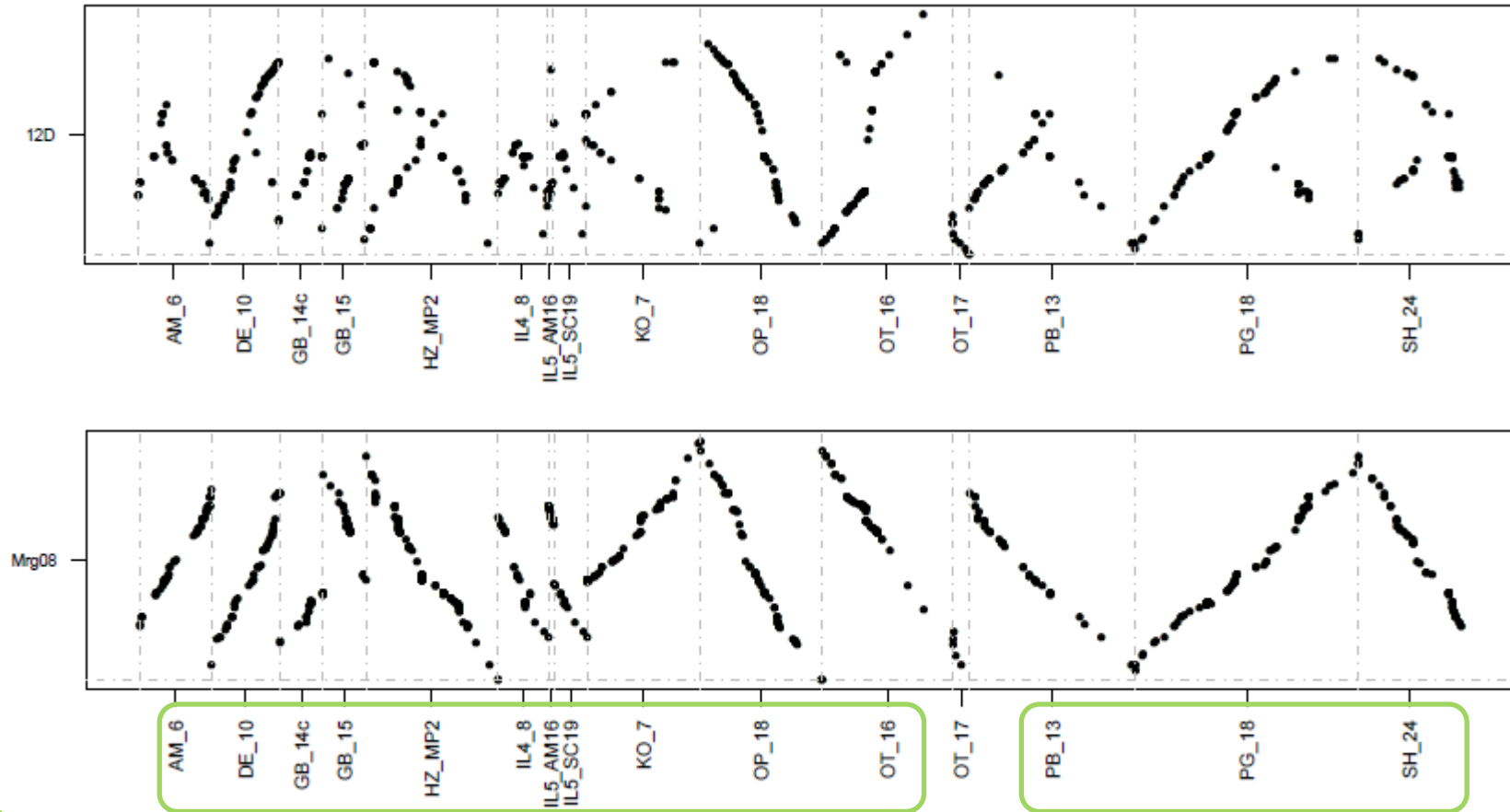
Example of a consistent re-merge 6C vs Mrg09




: used for merging.

For AM_20 and IL4_13, only the part of LG matching to this region was used for merging (the other part matches to Mrg20)

Example of a not so consistent re-merge 12D vs Mrg08



 : used for merging.

Evaluation of the v. 2.0 map

- More of the merged groups support the individual consensus groups
- Data into the merge has been cleaned of duplicate markers
- Excellent documentation of the process
- Two variant groups
- Map lengths (overall cM distance) are larger than the v. 1.0 merge

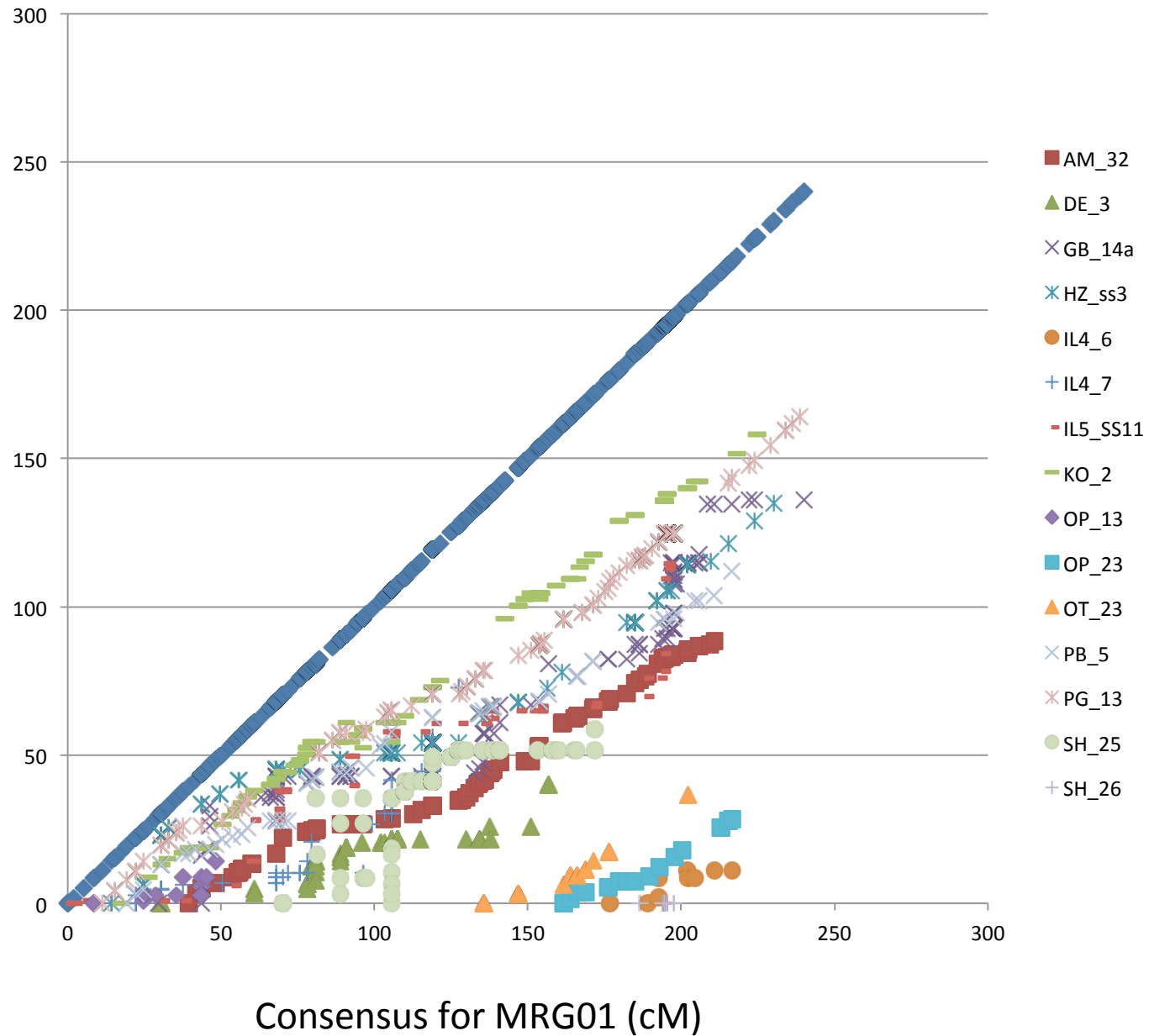
So how do we address the map length inflation??

- MergeMap is known to inflate map distances, part of the way the algorithm works.
- Initial approach was to replicate what had been done in barley
 - Arithmetic mean of genetic distances between consecutive markers in component population maps divided by the same distance in the consensus map. Close et al (2009) and Munoz et al (2011)
 - This approach was marginally successful – overall lengths were not greatly reduced
- Next approach was SMUSH – Smooth Map Unitization by Slope Heuristics
 - referentially rescale regions of a consensus map to match the average scale of matching component maps in each region.
 - Hypothesis:
 - This can be done by scaling each consensus interval by a factor equal to the average slope of the component-vs-consensus, across regressions in a moving window (e.g. 30 cM) centered around the interval.

Components vs consensus - flipped adjustment

Components
For MRG01
(cM)

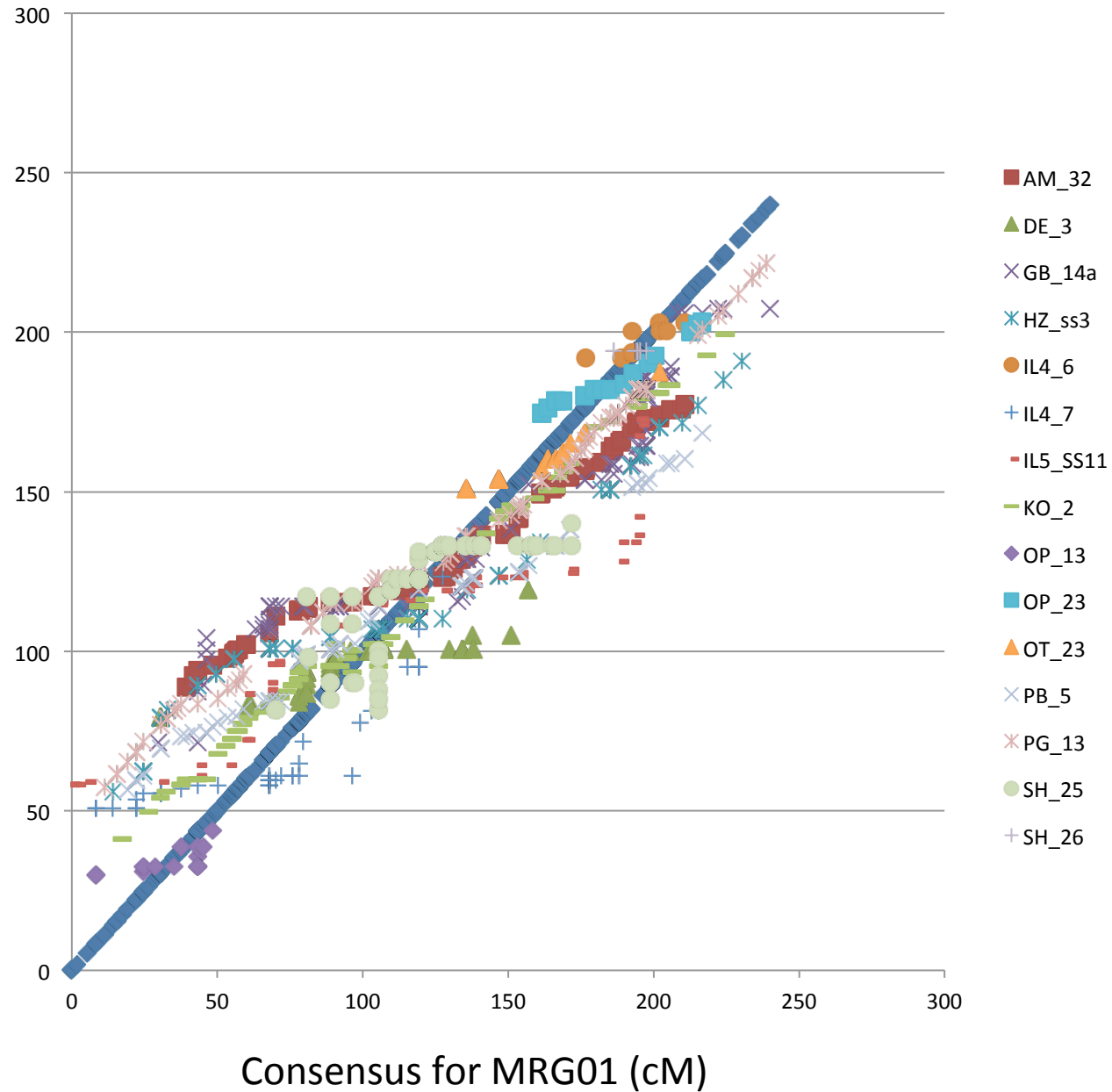
Components
have been
Flipped to
consistent
direction



Components (flipped/centered) vs consensus

Components
For MRG01
(cM)

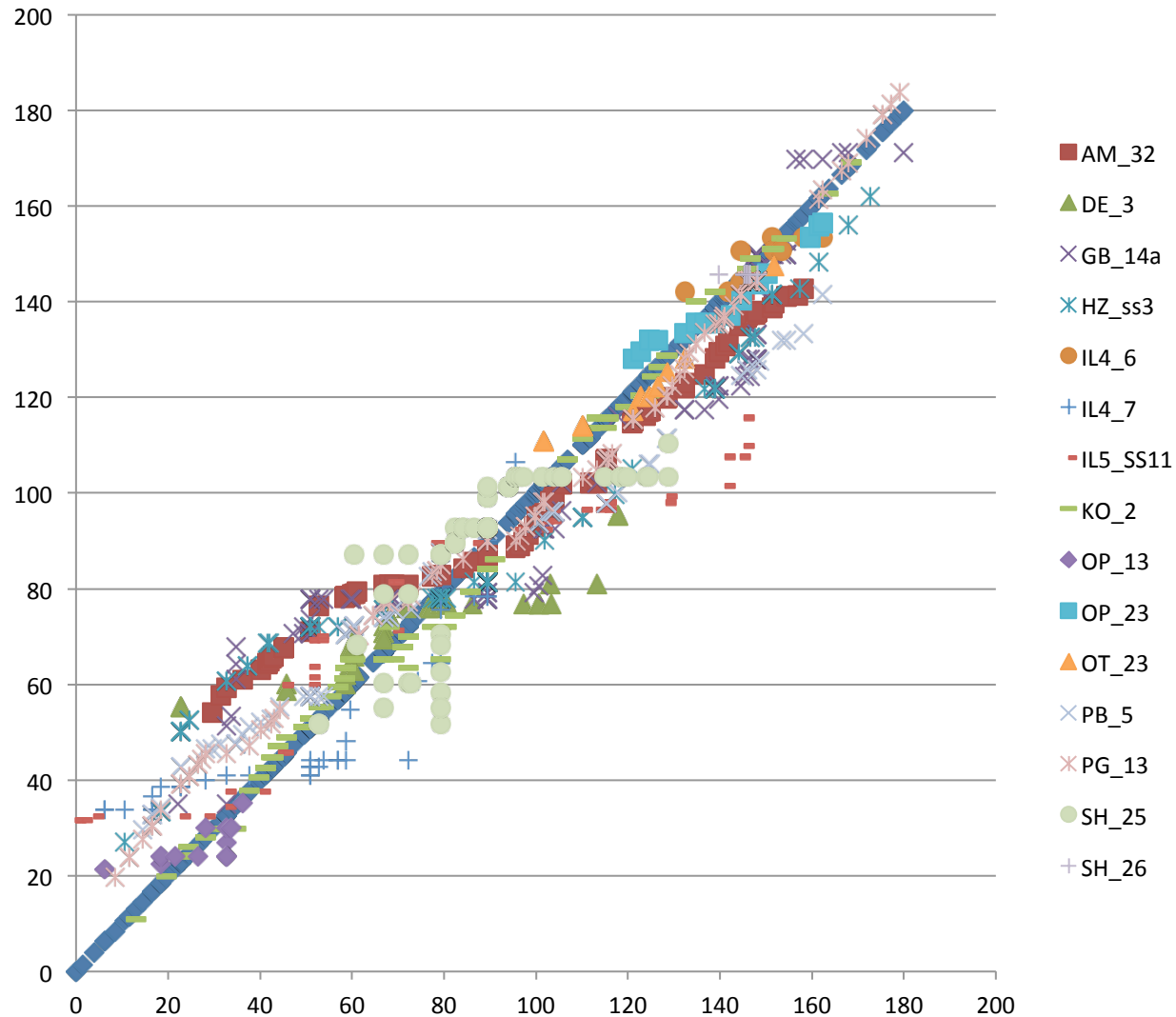
Components
have been
flipped
and centered



Components vs naïve adjusted consensus

Components
For MRG01
(cM)

Components
have been
flipped
and centered

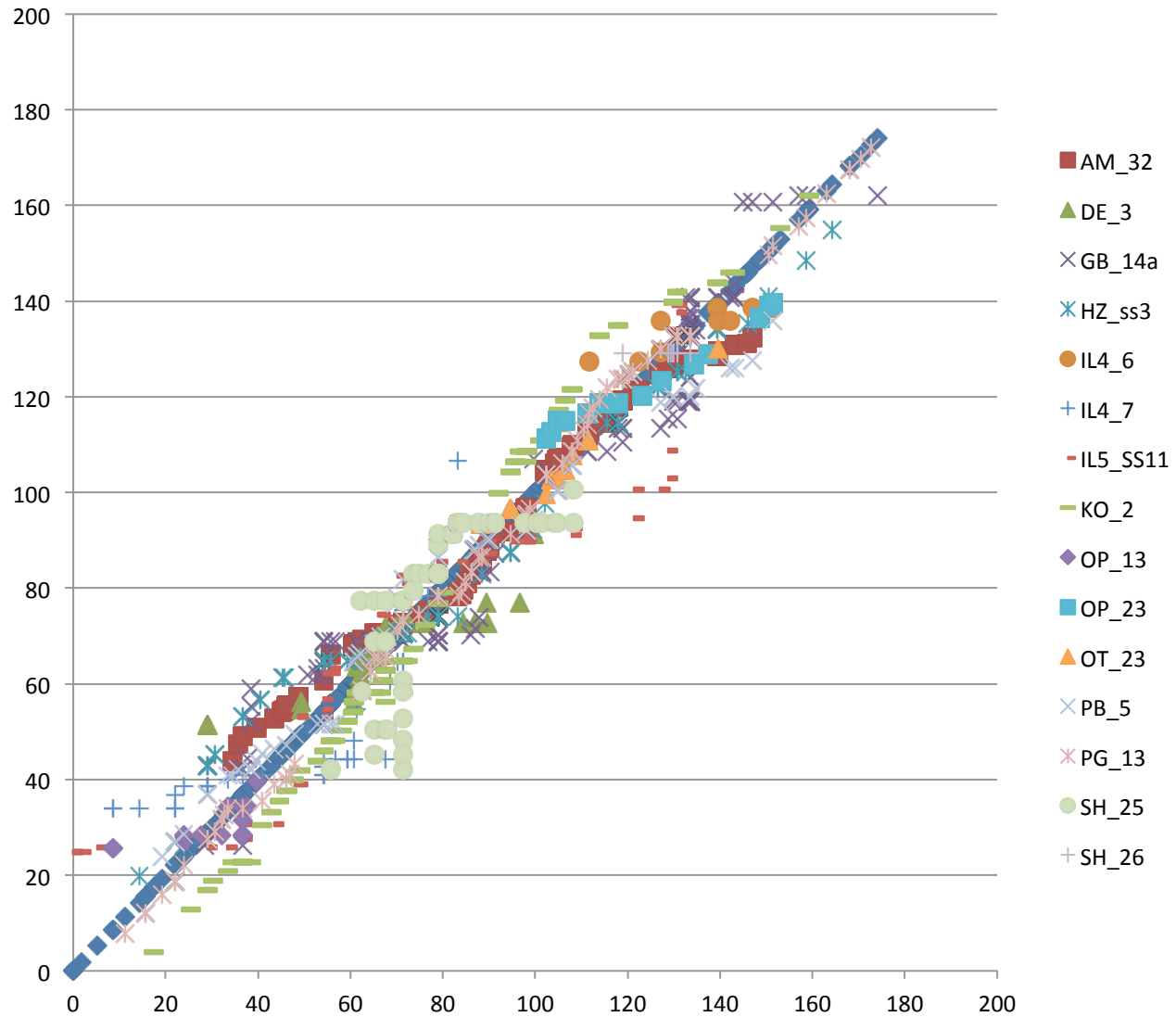


Consensus for MRG01 (cM) – naïve adjustment (scaled evenly by 0.75 across chromosome)

Components vs SMUSHED consensus

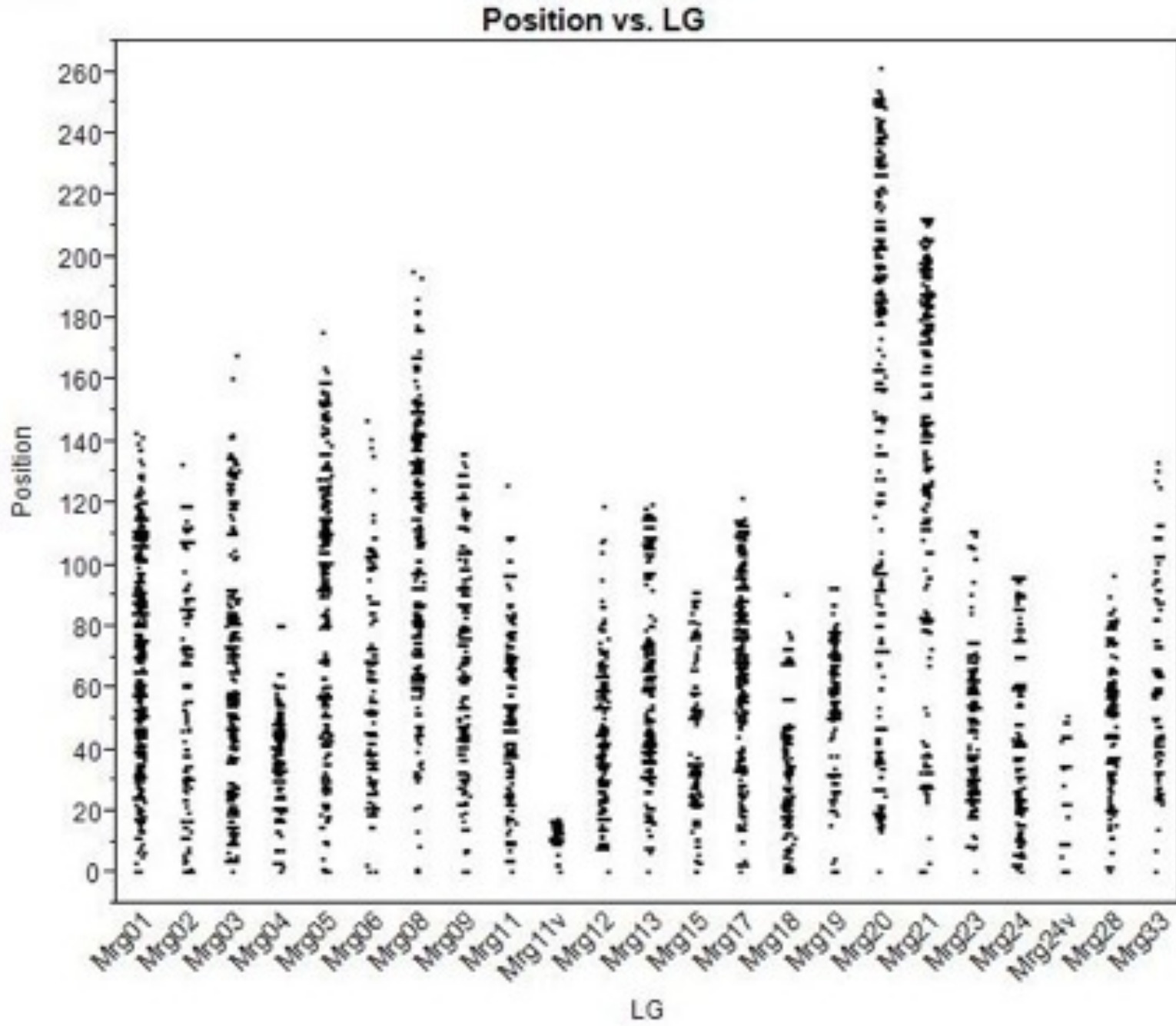
Components
For MRG01
(cM)

Components
have been
flipped
and centered



SMUSHED Consensus for MRG01 (cM)

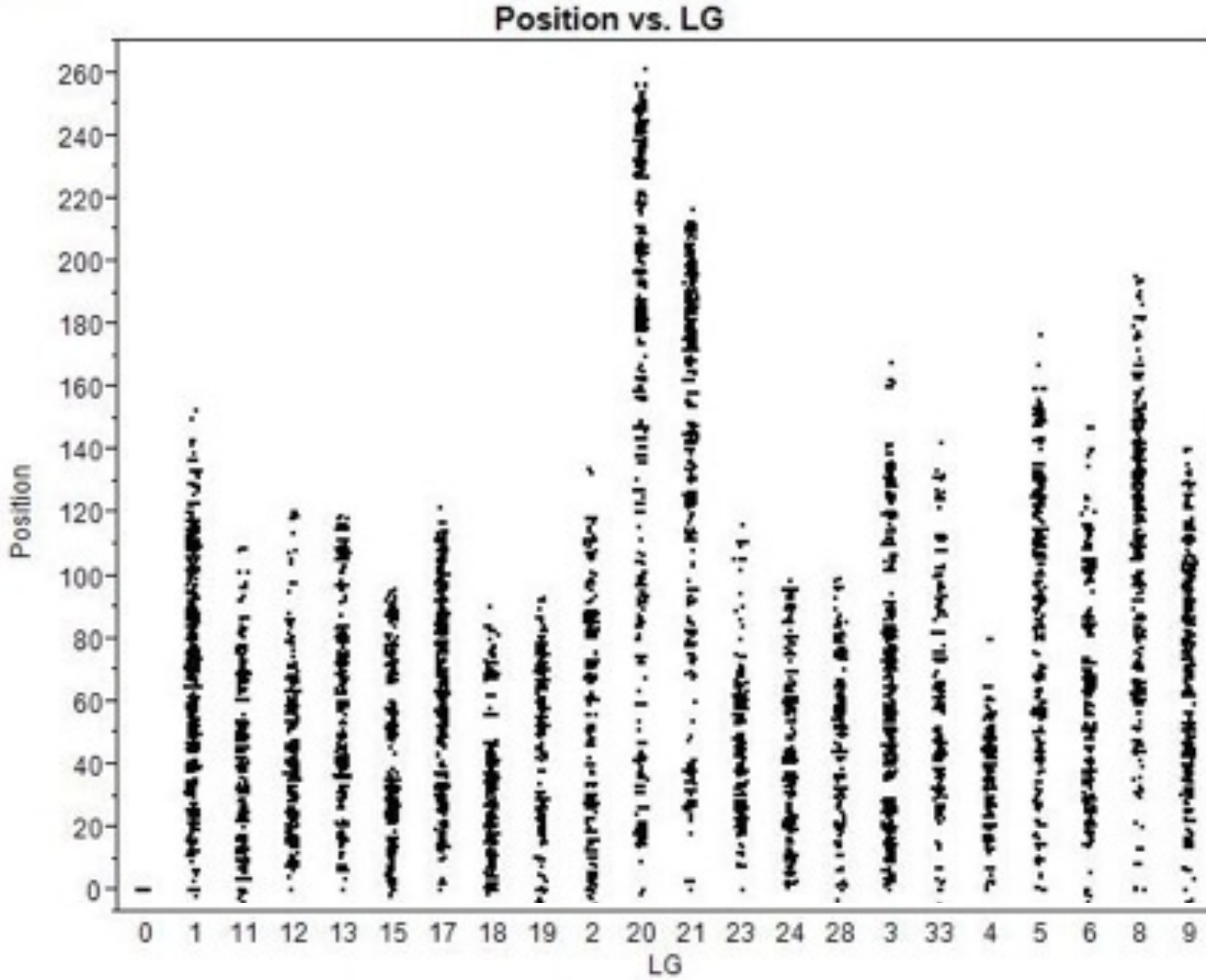
Consensus v. 2.0 SMUSHed map



Ahoy placement of markers

- all remaining markers placed at the location that minimizes the recombination fraction within the component map where the fraction involving that marker is smallest. Distance is interpolated when 2 r values are available in that map.

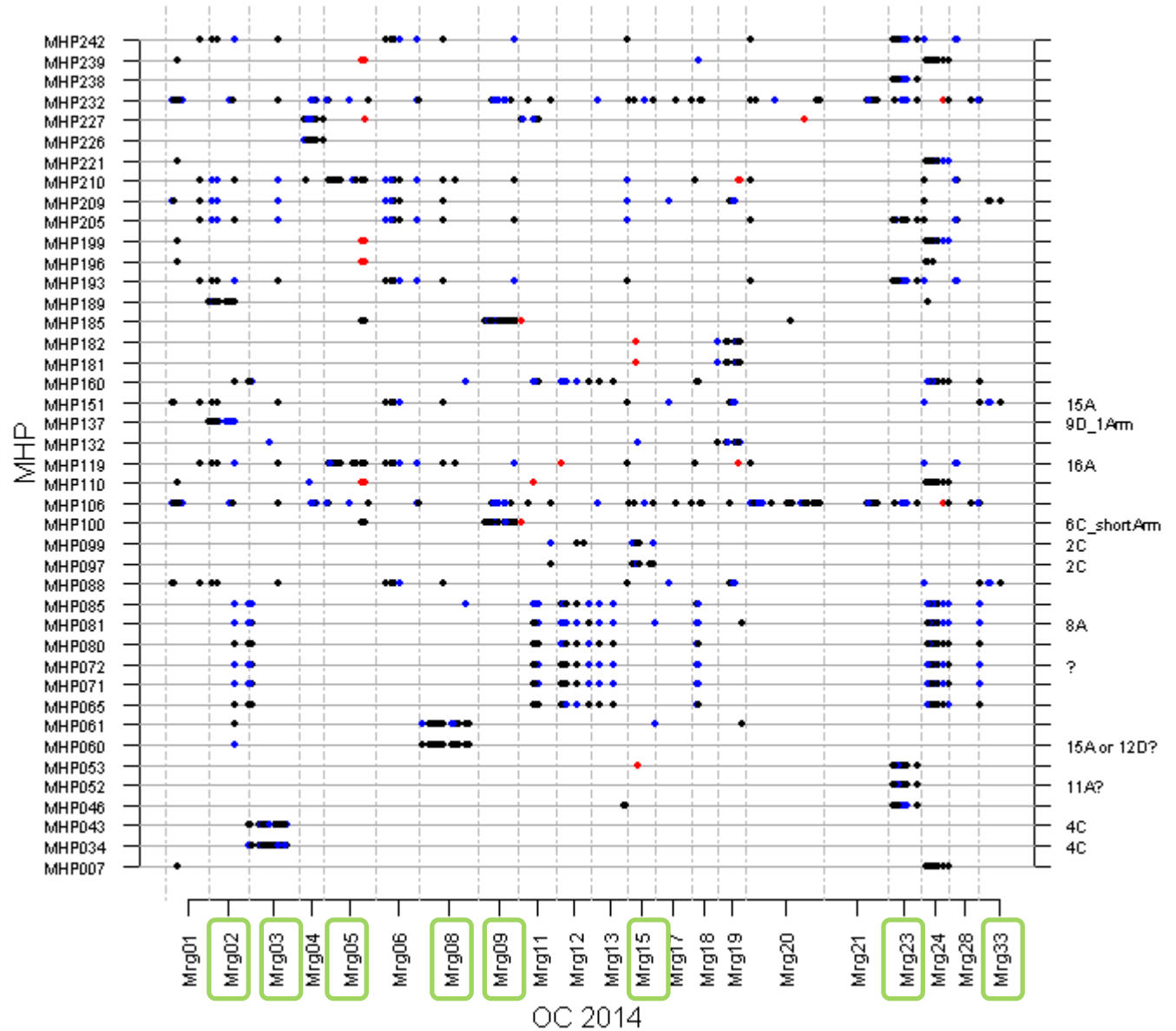
Consensus v. 2.0 SMUSHed and Ahoy Placement Map



We can confidently assign 8 chromosomes combining marker and latest karyotype data!

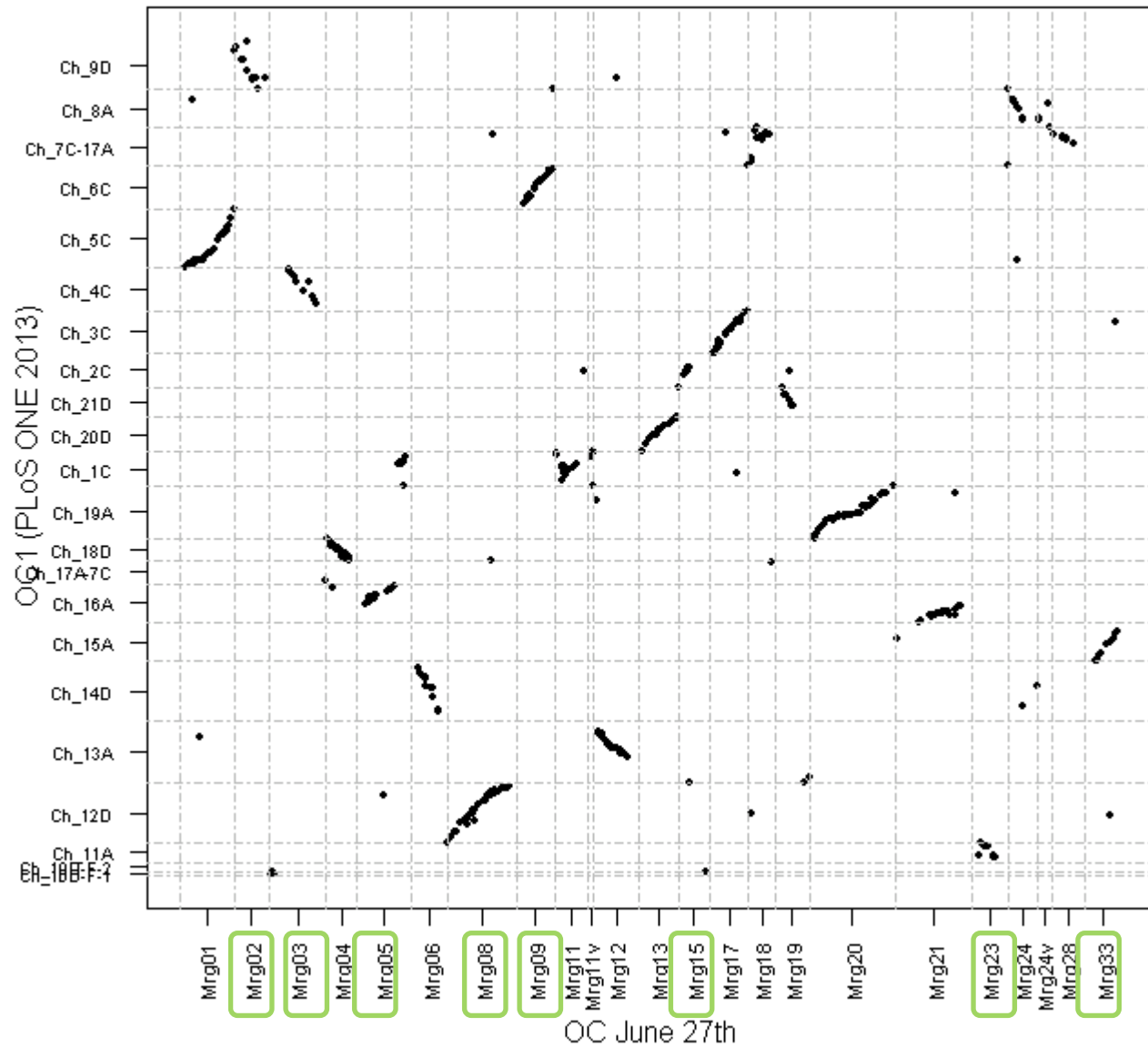
Mrg	chr
Mrg02	9D
Mrg03	4C
Mrg05	16A
Mrg08	12D
Mrg09	6C
Mrg15	2C
Mrg23	11A
Mrg33	15A

OC2014 vs. MHP

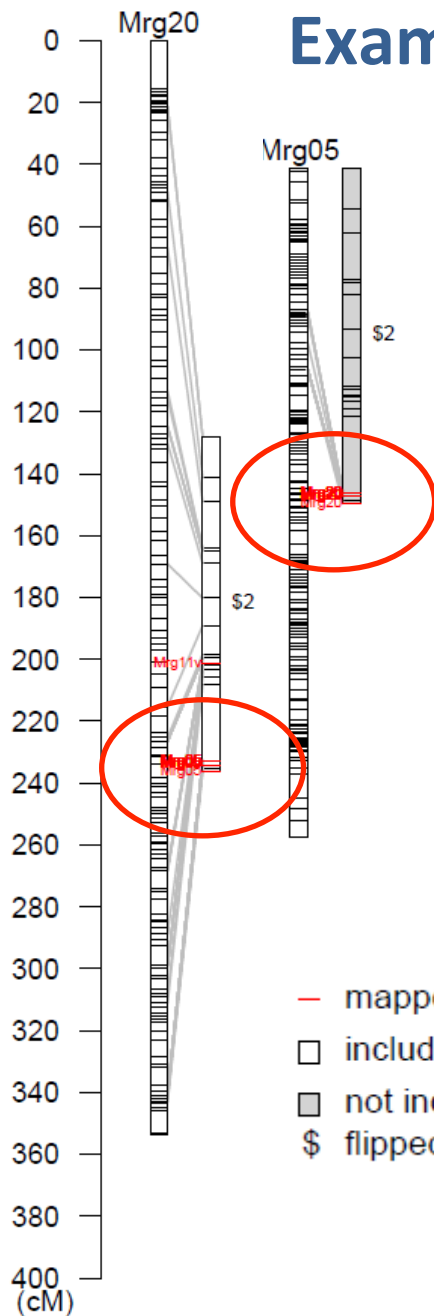


This assignment is pretty consistent with that of OC1

Mrg	chr
Mrg02	9D
Mrg03	4C
Mrg05	16A
Mrg08	12D
Mrg09	6C
Mrg15	2C
Mrg23	11A
Mrg33	15A



Example of population specific-rearrangement



DE_2:

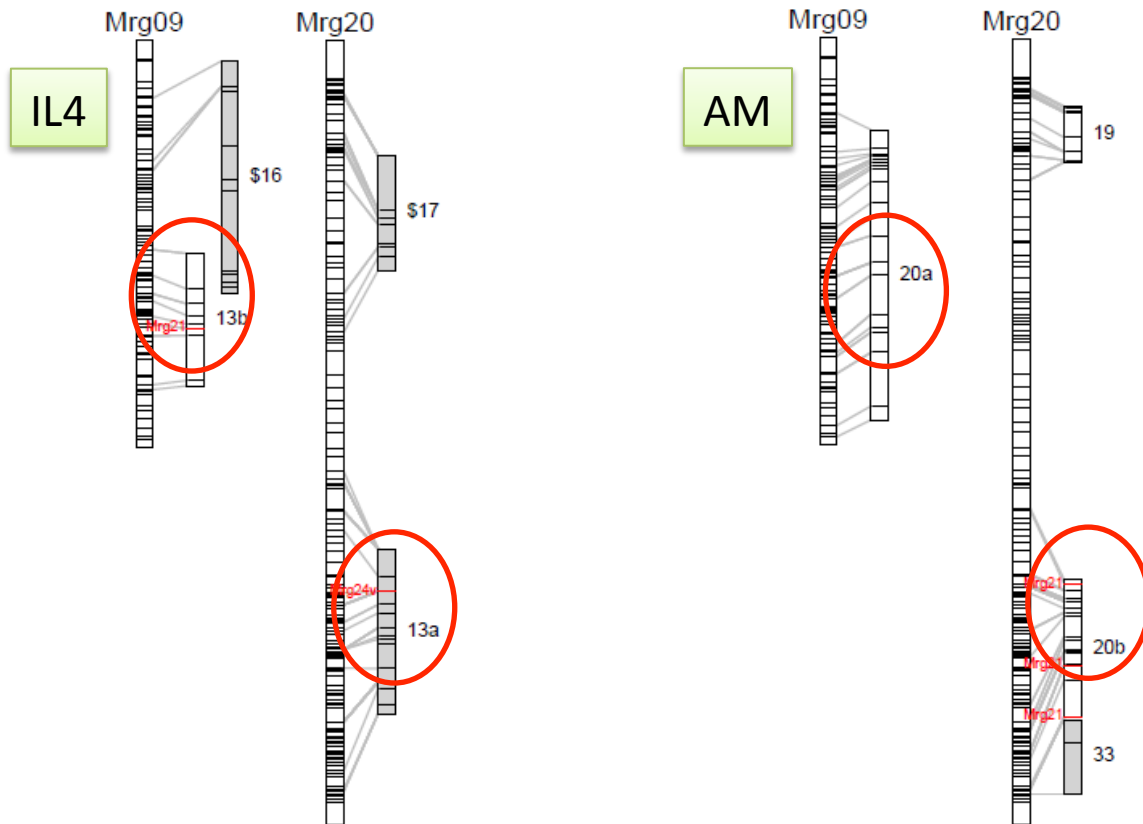
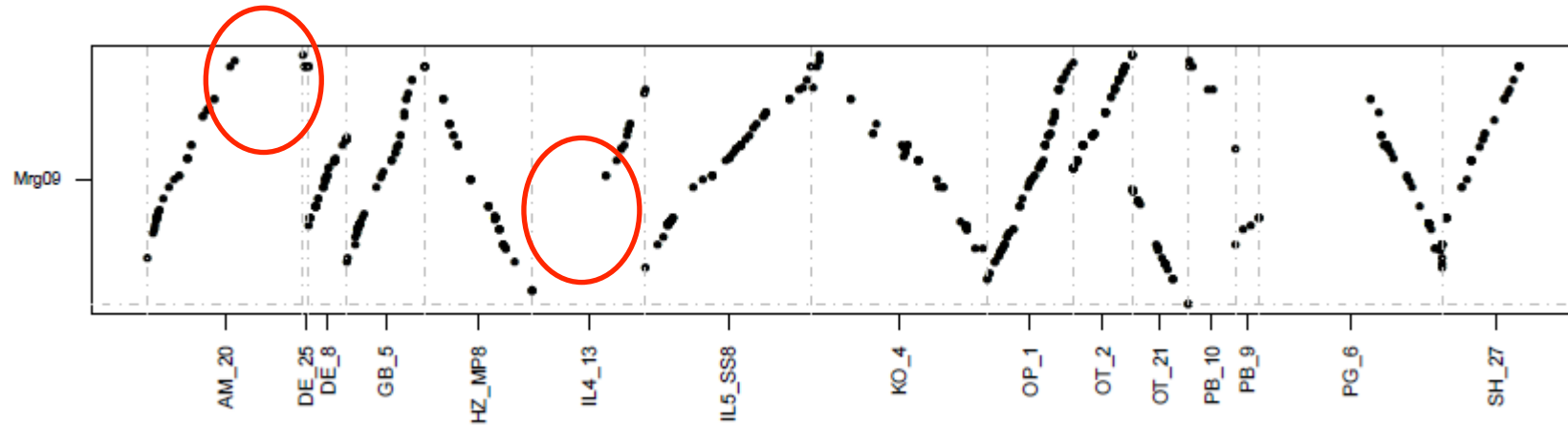
- Included in the merging of Mrg20 (transparent box).
- One end of DE_2 matches to Mrg05.

→ In DxE, a piece of Mrg05 is attached to Mrg20, most probably a result of chromosome rearrangement (or a false linkage due to artifact).

→ For this particular case, it is necessary to discard the part of DE_2 matching to Mrg05 prior to merging. Otherwise, these markers from Mrg05 will also be positioned on Mrg20 and will further lead to erroneous merged map.

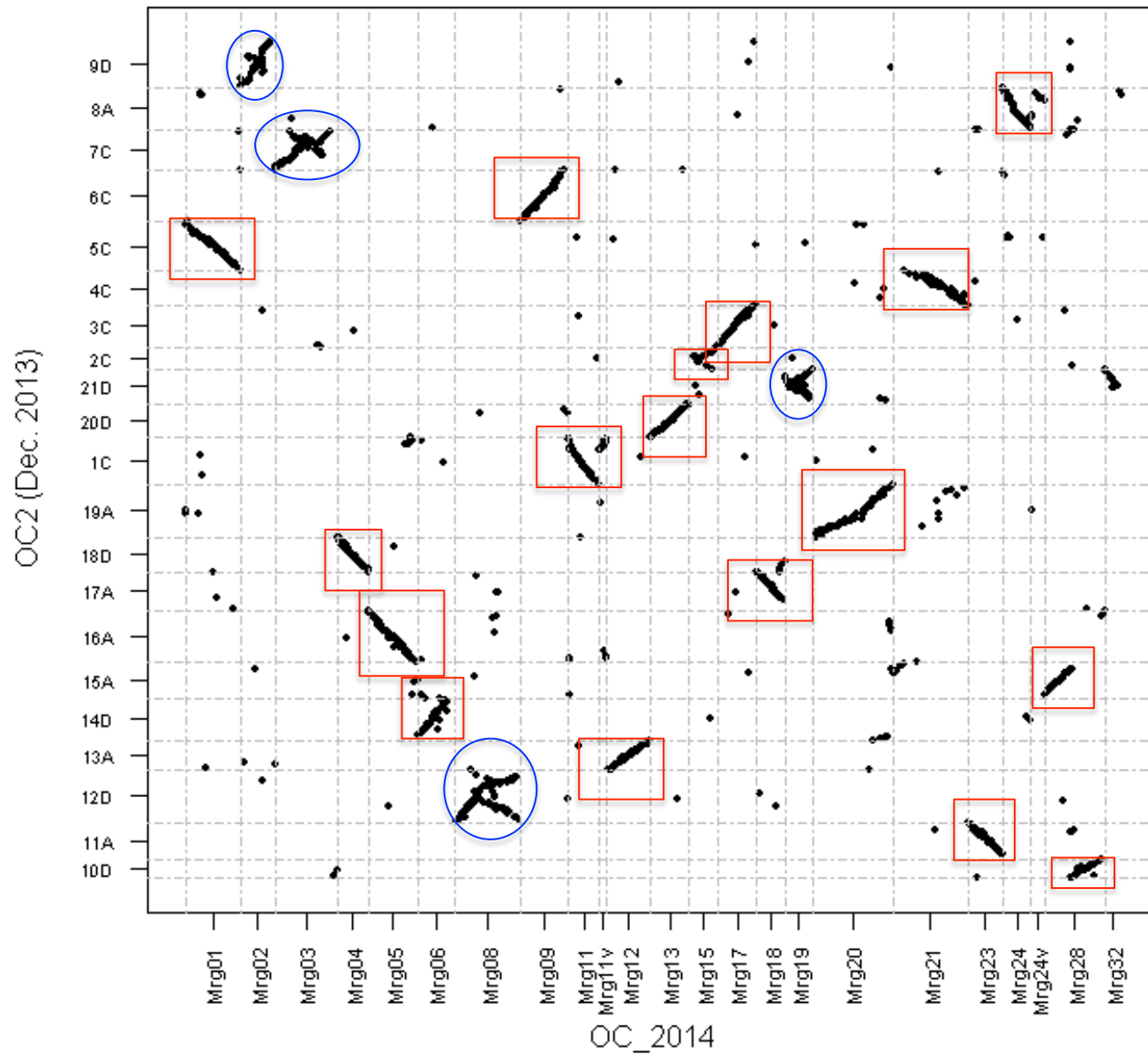
- mapped to multiple locations
- included in merging
- not included in merging
- \$ flipped

Example of population specific-rearrangement

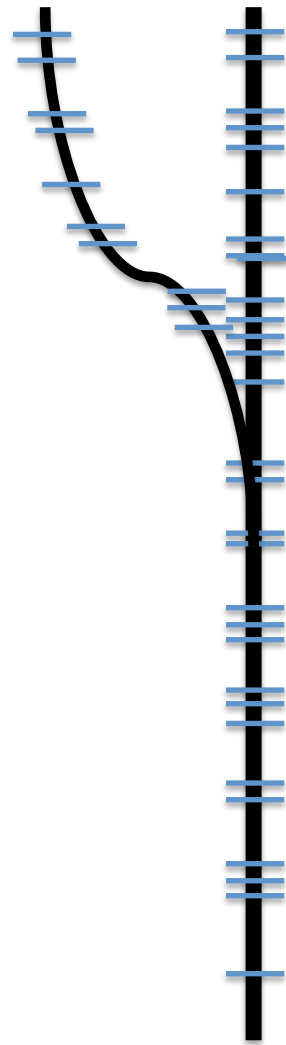


A part of AM_20 and IL4_13 match to Mrg09. For both LGs, their other half match to Mrg20. This may be a specific genome rearrangement for the parental lines. (we split these LGs prior to merging. There was an interval > 20 cM).

Ok, oat has a complex genome, but is the consensus map v. 2.0 better?



So how can we improve our map???



Traditional linear way of thinking of a linkage group

This linear view in our consensus currently represents most of the populations

But, what about those translocations???

A phylogenetic approach

- Maximal pairwise alignment between linkage groups using dynamic programming

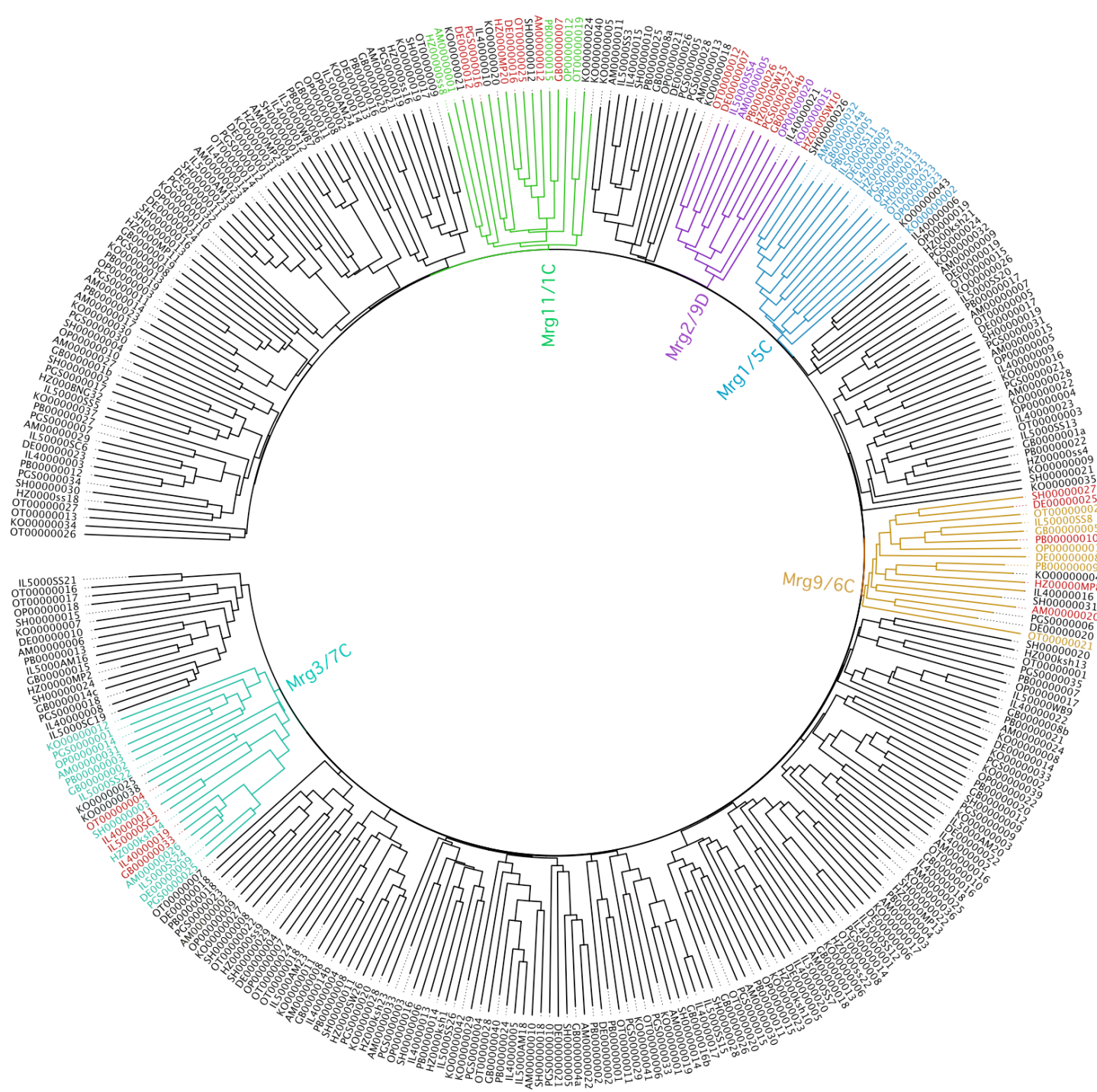
- Rec distance with
- 5
- Ma maximal
- For -5,

C	Cys	12																				
E	Ser	0	2																			
T	Thr	-2	1	3																		
P	Pro	-3	1	0	6																	
A	Ala	-2	1	1	1	2																
Q	Gly	-3	1	0	-1	1	5															
N	Asn	-4	1	0	-1	0	0	2														
D	Asp	-5	0	0	-1	0	1	2	4													
E	Glu	-5	0	0	-1	0	0	1	3	4												
Q	Gln	-5	-1	-1	0	0	-1	1	2	2	4											
H	His	-3	-1	-1	0	-1	-2	2	1	1	3	6										
R	Arg	-4	0	-1	0	-2	-3	0	-1	-1	1	2	6									
K	Lys	-5	0	0	-1	-1	-2	1	0	0	1	0	3	5								
M	Met	-5	-2	-1	-2	-1	-3	-2	-3	-2	-1	-2	0	0	6							
I	Ile	-2	-1	0	-2	-1	-3	-2	-2	-2	-2	-2	-2	-2	2	3						
L	Leu	-6	-3	-2	-3	-2	-4	-3	-4	-3	-2	-2	-3	-3	4	2	6					
V	Val	-2	-1	0	-1	0	-1	-2	-2	-2	-2	-2	-2	-2	2	4	2	4				
F	Phe	-4	-3	-1	-3	-3	-3	-4	-6	-5	-3	-2	-4	-3	0	1	2	-1	9			
Y	Tyr	0	3	-3	-3	-3	-3	-2	-4	-4	-4	0	-4	-4	-2	-1	1	-3	7	10		
W	Trp	-6	-3	-5	-6	-6	-7	-4	-7	-7	-5	-1	3	-1	-4	-5	-2	-6	0	0	17	
			C	S	T	P	A	G	N	D	E	Q	H	R	K	M	I	L	V	F	Y	W

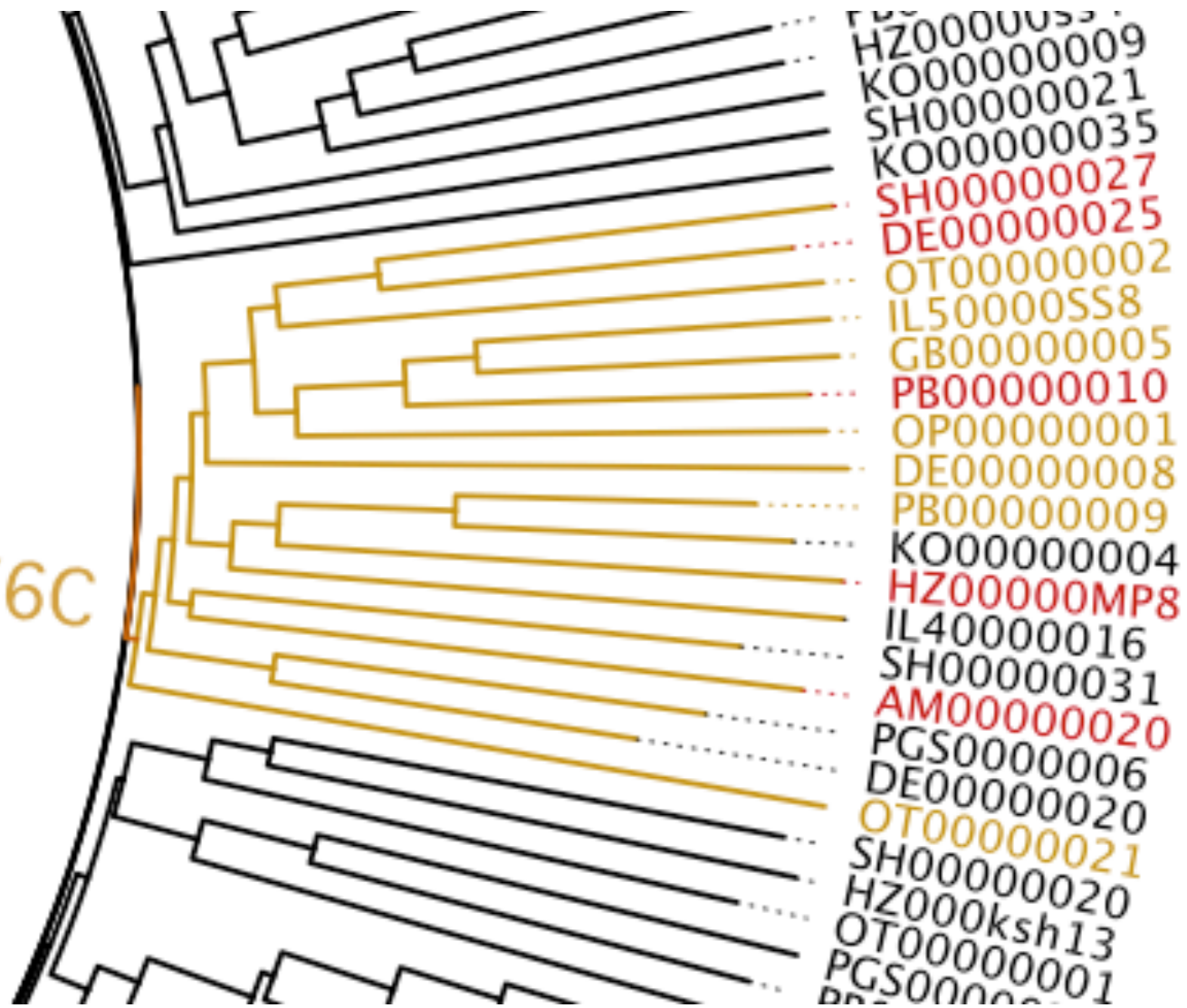
maps

ind
matrix

=



Mrg9/6C



So where can we go from here?

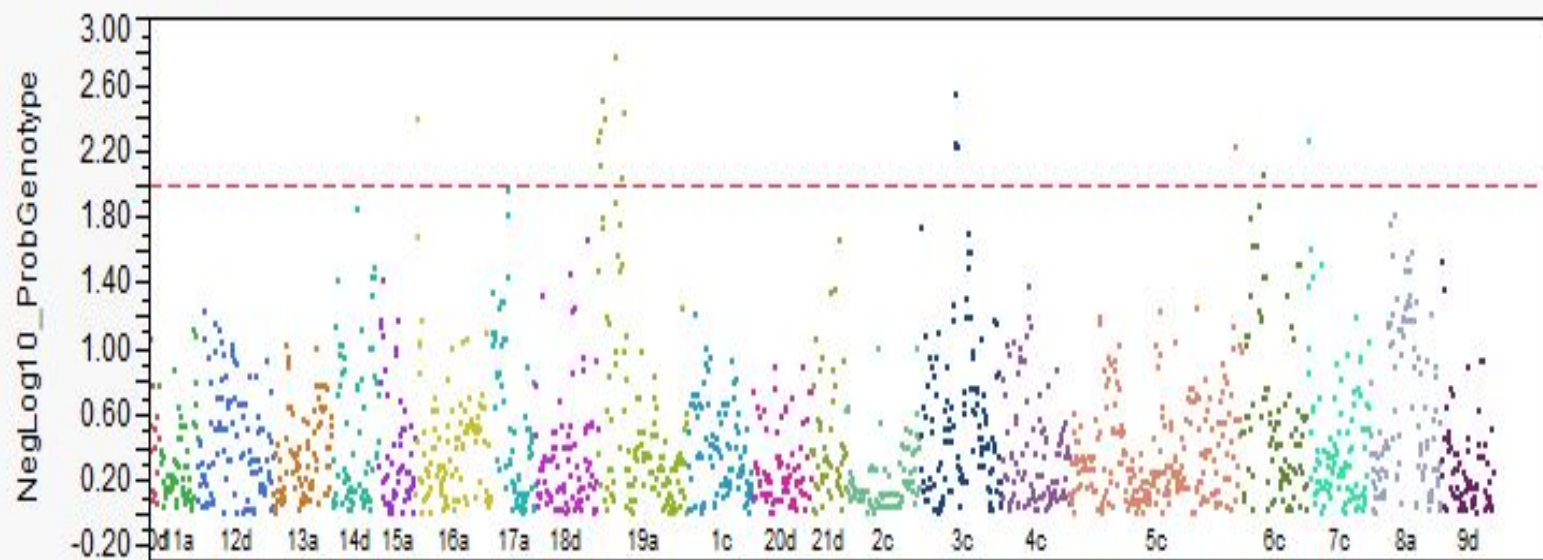
- Is the map ready to use?
 - Yes, pretty much for most of it
- Are we done yet?
 - Almost....
- Can I get a copy and begin using it?
 - Yes, with the caveat that there may be slight changes.
- How does this map look for association studies
 - Defer to Kathy!

Summary Chart

Manhattan Plot

Frame Size for Graphs Below

Overlay Plot

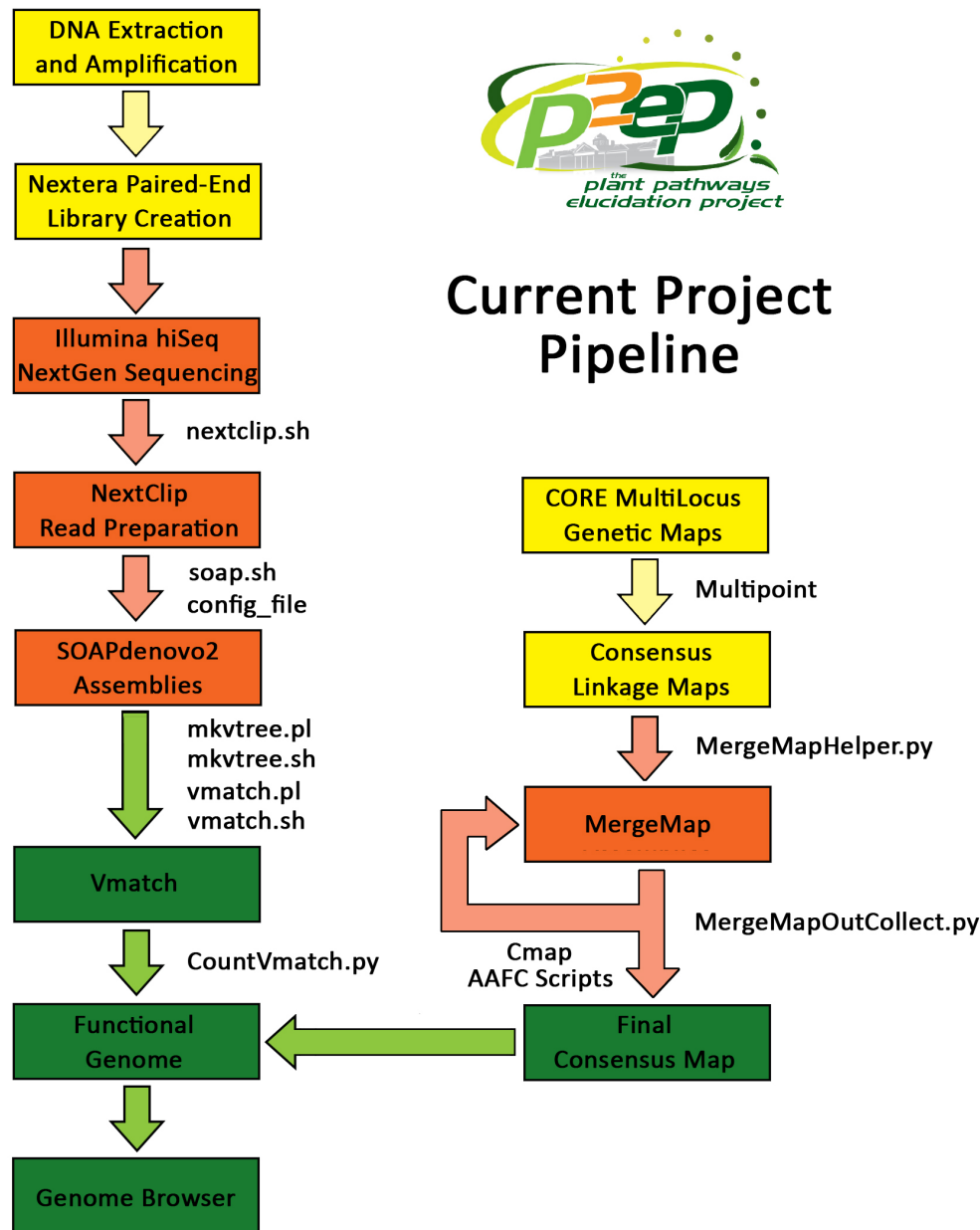


LG

● 10D



Current Project Pipeline



What else can the consensus map do for oats? (or at least what we are doing)

- Provide a framework for assembling a hexaploid oat zipper

Acknowledgments

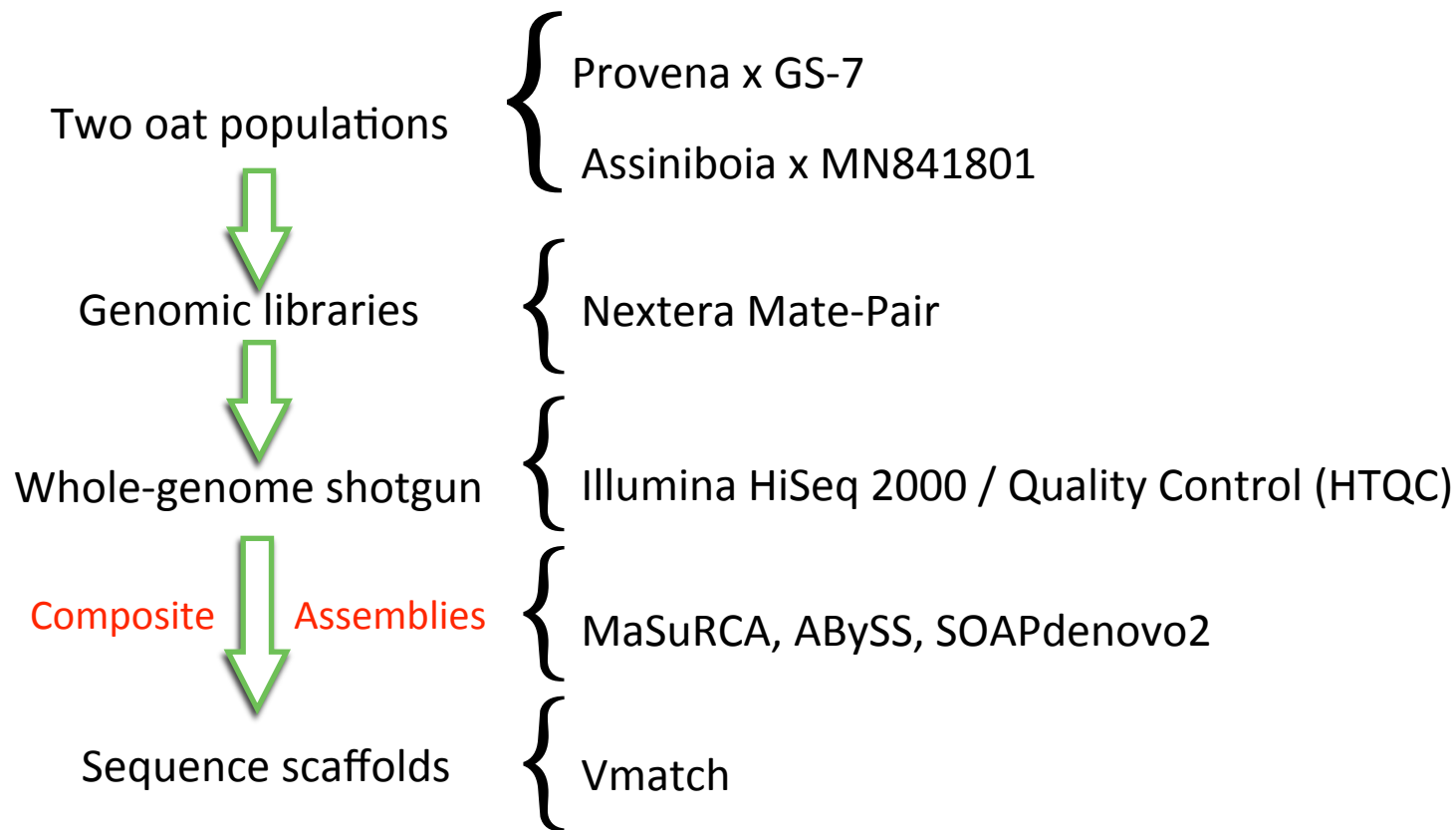


P2EP Support



Questions??

The Oat Zipper



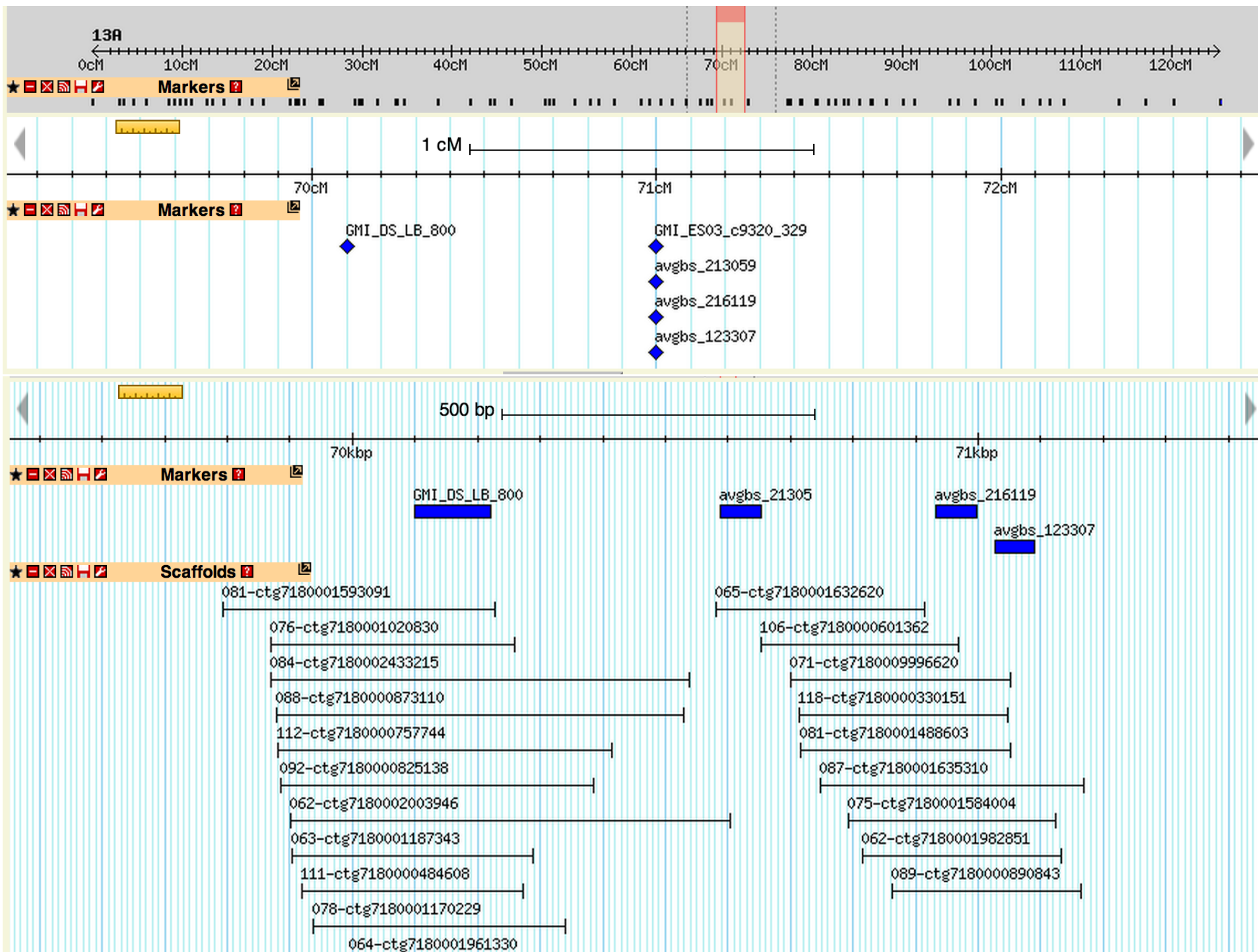
Current Sequencing Statistics



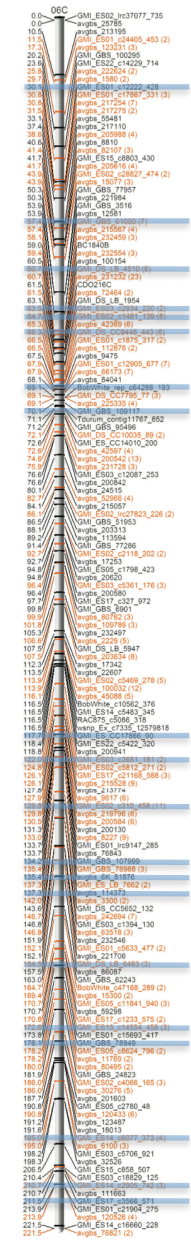
- ❖ Full lane sequence of each population parents (Assiniboia, MN841801, Provena and GS-7)
- ❖ 43 AxMN progeny sequenced (32 currently sequencing @ Illumina)
- ❖ 42 PxGS-7 progeny sequenced (33 ready for sequencing)
- ❖ Averaging 44,205,038 reads per progeny sequenced
- ❖ Currently have ~ 37x coverage of the oat genome across available sequences

- ❖ Calculated an effective N50 of ~2000 bp based on a composite assembly (only Provena x GS-7 sequences)
- ❖ Assembly methodology based upon this being a hexaploid genome forces us to split these into progeny assemblies (less than 1x coverage each) and use the maker map as a framework for initial contig anchoring giving a reduced N50

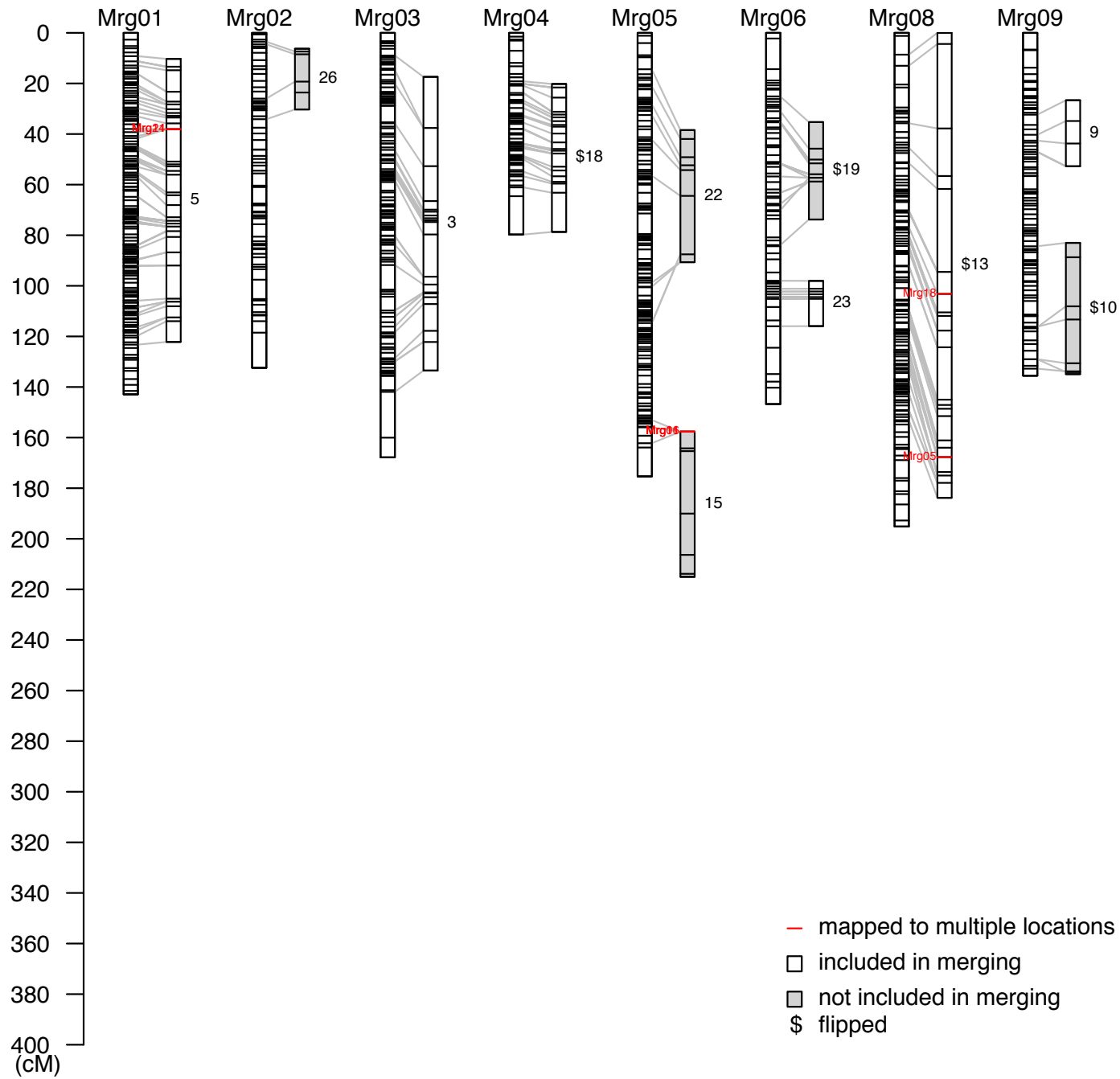
- ❖ Individual contig assemblies have been mapped to ~10% of the markers



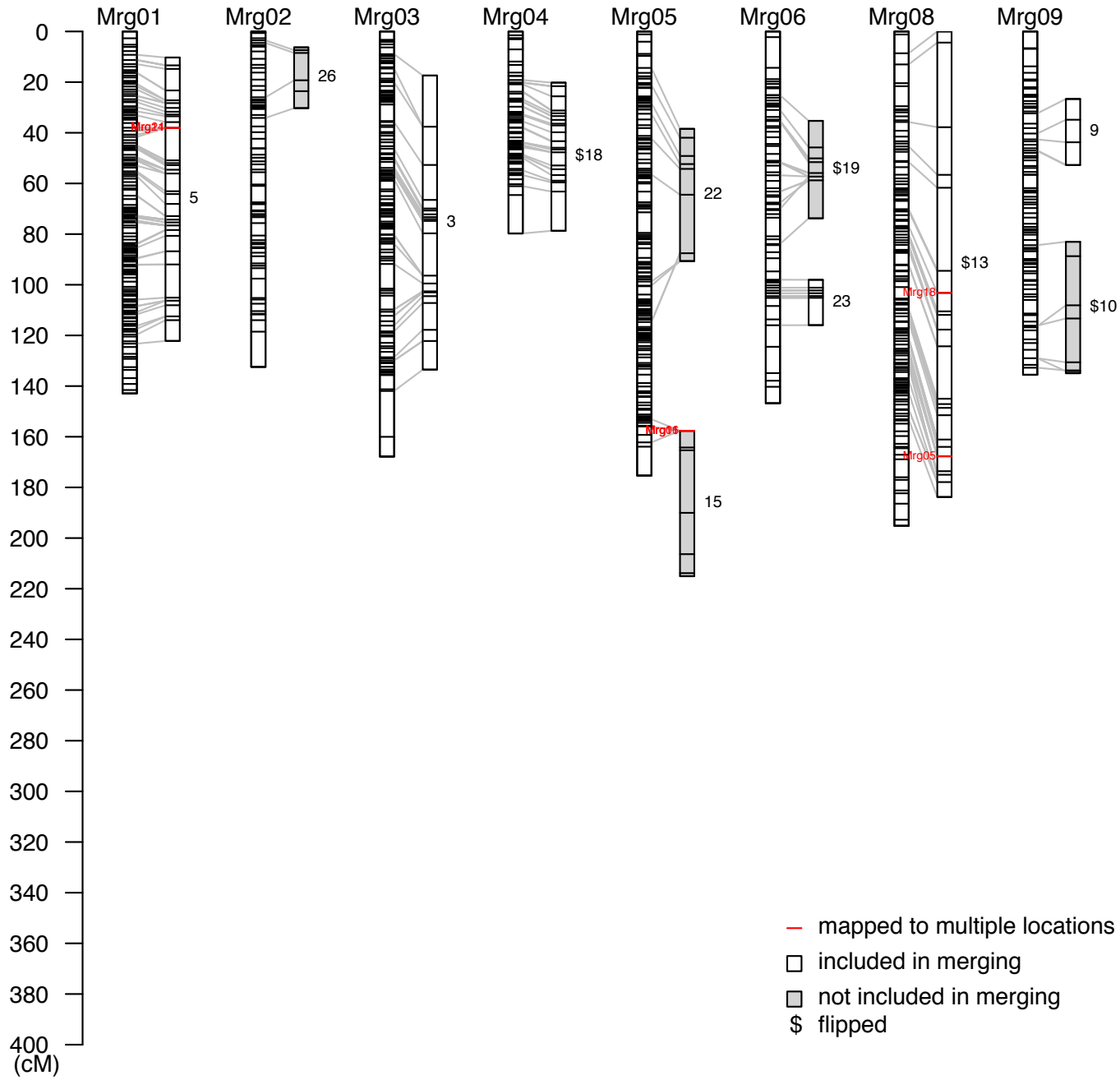
					MHP_99	MHP_38	MHP_52	MHP_185
Marker	SNP	Sun II	Ogle	1C	2C	4C	5C	6C
GMI_ES01_c12222_428	[T/C]	BB	AA	AB	AB	AB	AB	AA
GMI_ES15_c10866_209	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_DS_oPt-14552_101	[T/C]	BB	AA	AB	AB	AB	AB	AA
GMI_DS_CC9093_95	[T/C]	BB	AA	AB	AB	AB	AB	AA
GMI_ES03_lrc23007_427	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_GBS_18001	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES17_c5666_258	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES05_c6476_469	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_GBS_720	[T/C]	BB	AA	AB	AB	AB	AB	AA
GMI_DS_CC9448_443	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_GBS_6566	[T/C]	BB	AA	AB	AB	AB	AB	AA
GMI_GBS_109117	[T/C]	BB	AA	AB	AB	AB	AB	AA
GMI_ES_CC17866_90	[T/C]	BB	AA	AB	AB	AB	AB	AA
GMI_ES03_c3681_181	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES05_c9758_147	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_GBS_29683	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES01_lrc29198_650	[T/C]	BB	AA	AB	AB	AB	AB	AA
GMI_GBS_107999	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_GBS_81876	[T/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES_LB_7662	[A/C]	BB	AA	AB	AB	AB	AB	AA
GMI_DS_LB_6483	[T/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES22_c3591_416	[A/C]	BB	AA	AB	AB	AB	AB	AA
GMI_GBS_78949	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES14_c18825_425	[T/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES14_c2905_742	[A/G]	BB	AA	AB	AB	AB	AB	AA
GMI_ES17_c3566_571	[A/G]	BB	AA	AB	AB	AB	AB	AA



Consensus vs. PB



Consensus vs. PB



Consensus vs. PB

