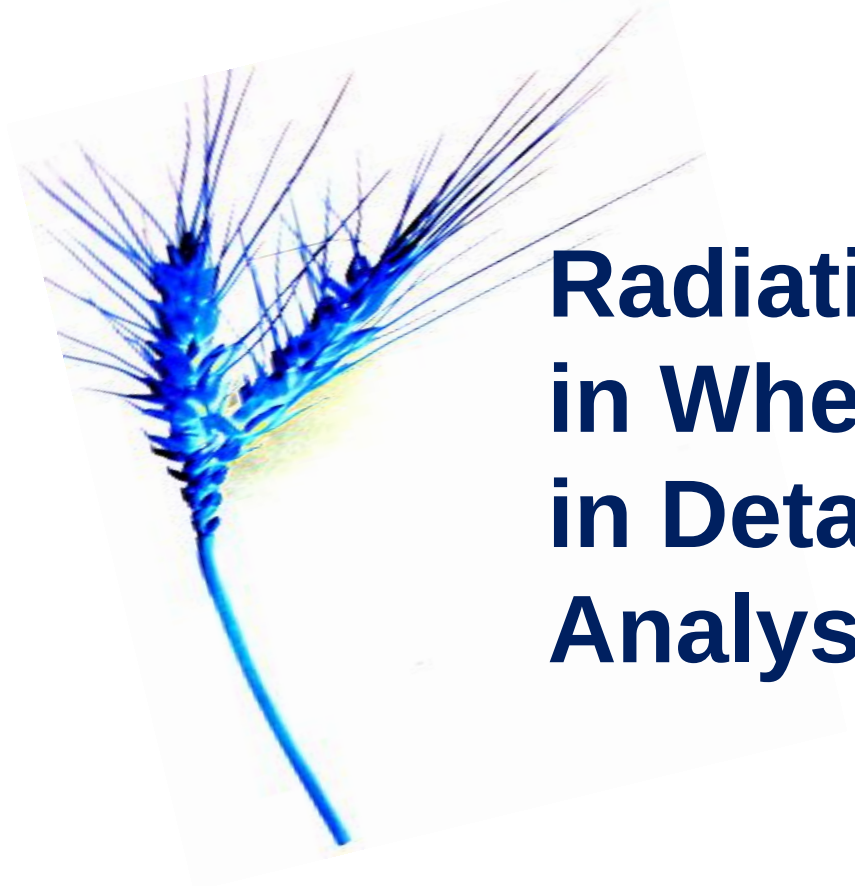
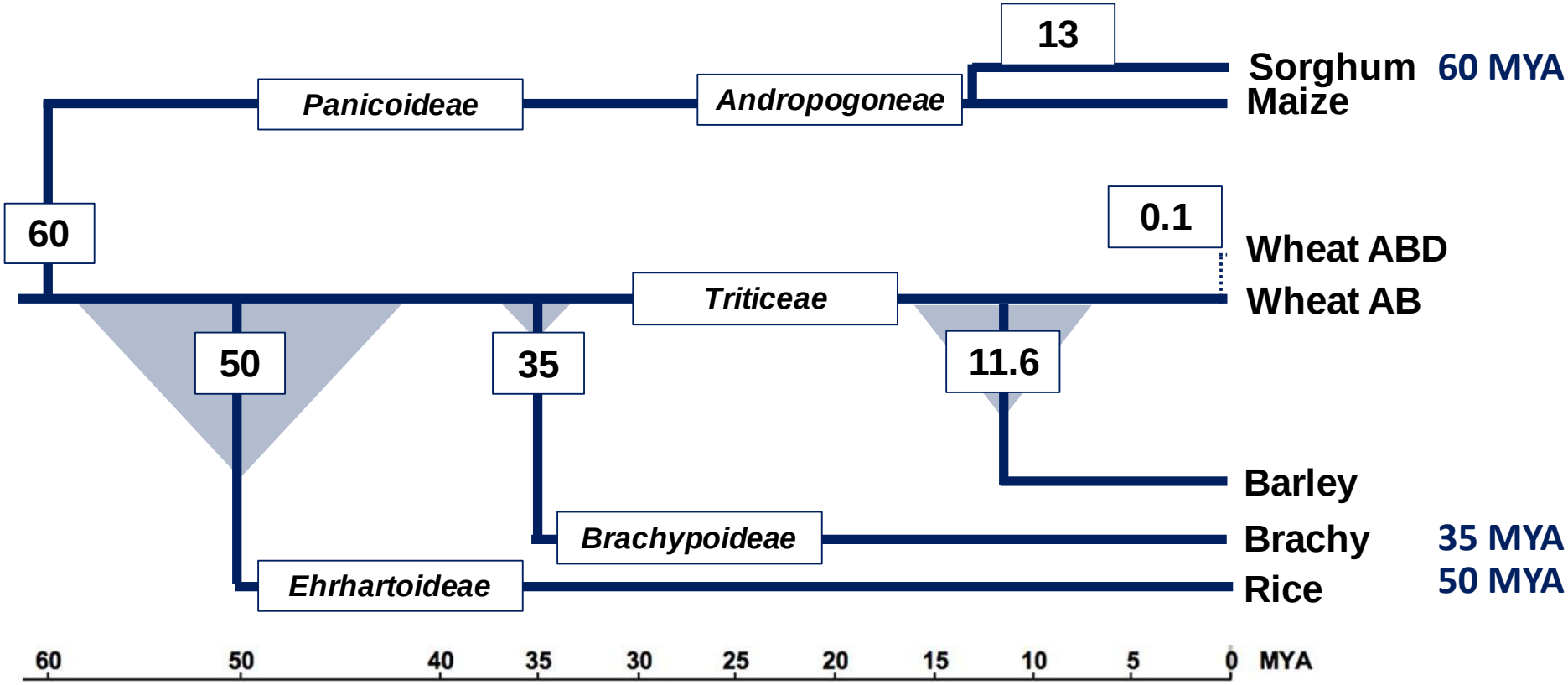
F.M. Bassi, M. Michalak de Jimenez, F. Ghavami, J. Zurn, H. Simkova, J. Dolezel, Y.Q. Gu, J. Dvorak, S.S. Maan, S.F. Kianian



Radiation Hybrid Mapping in Wheat and Its Use in Detailed Comparative Analysis of Grass Genomes

Simplified Time Line of Wheat Evolution

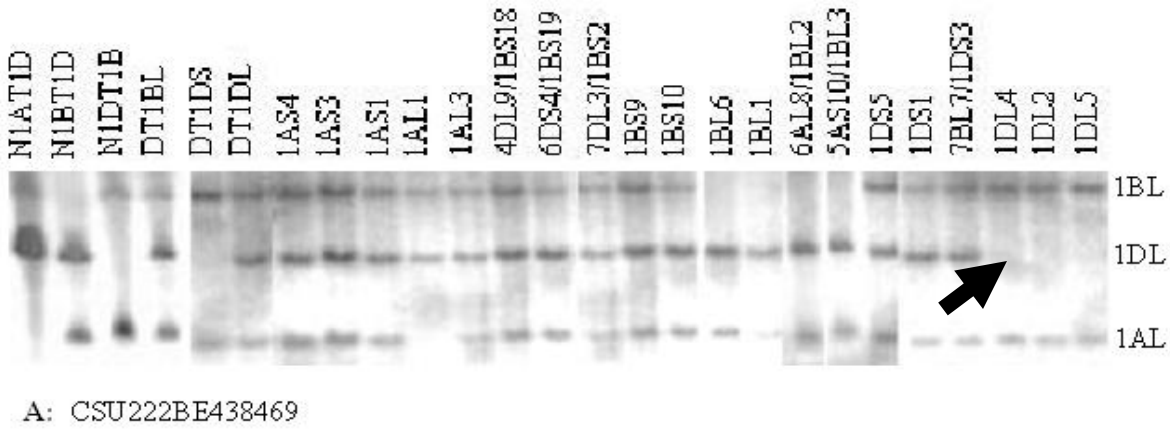
- **Based on sequence data for 10 genes**

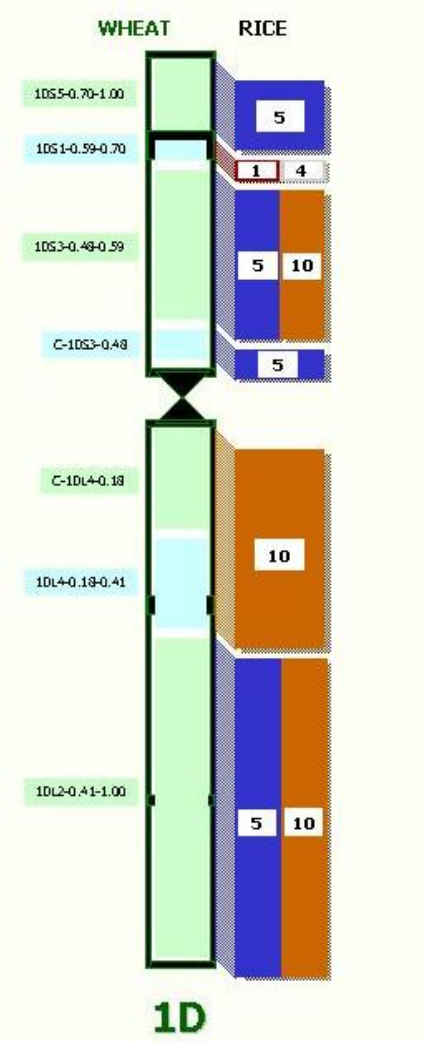


adapted from ***Chalupska et al. 2008*** *PNAS 105:9691-9696*

Synteny by Deletion Bin Mapping

- 8 Deletion Bins Chr. 1D
- Size 11% to 69% Chr. Arm
- 643 ESTs RFLP Map
- Synteny to Rice Chr: 5 and 10

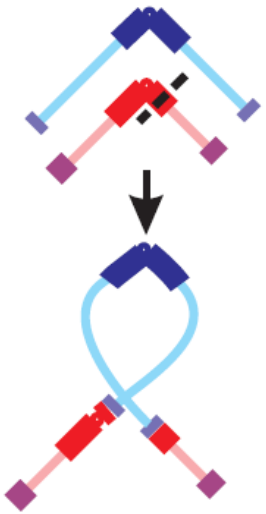


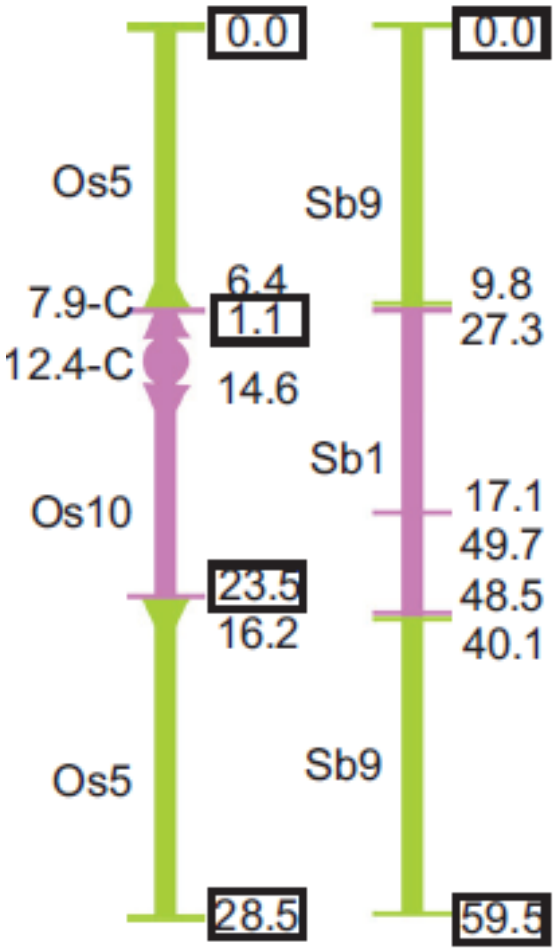


Peng et al., 2004 Genetics 168:609-623

Synteny by Geneting Mapping

- **572 F_2 *Ae. tauschii* (AS75 x AL8/78)**





Luo et al., 2009 PNAS 106:15780-15785

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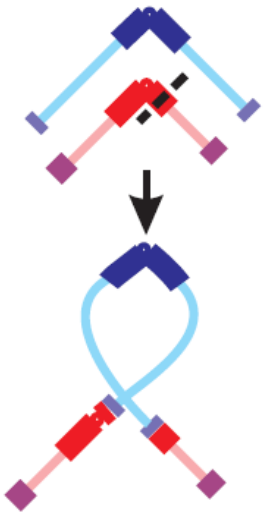

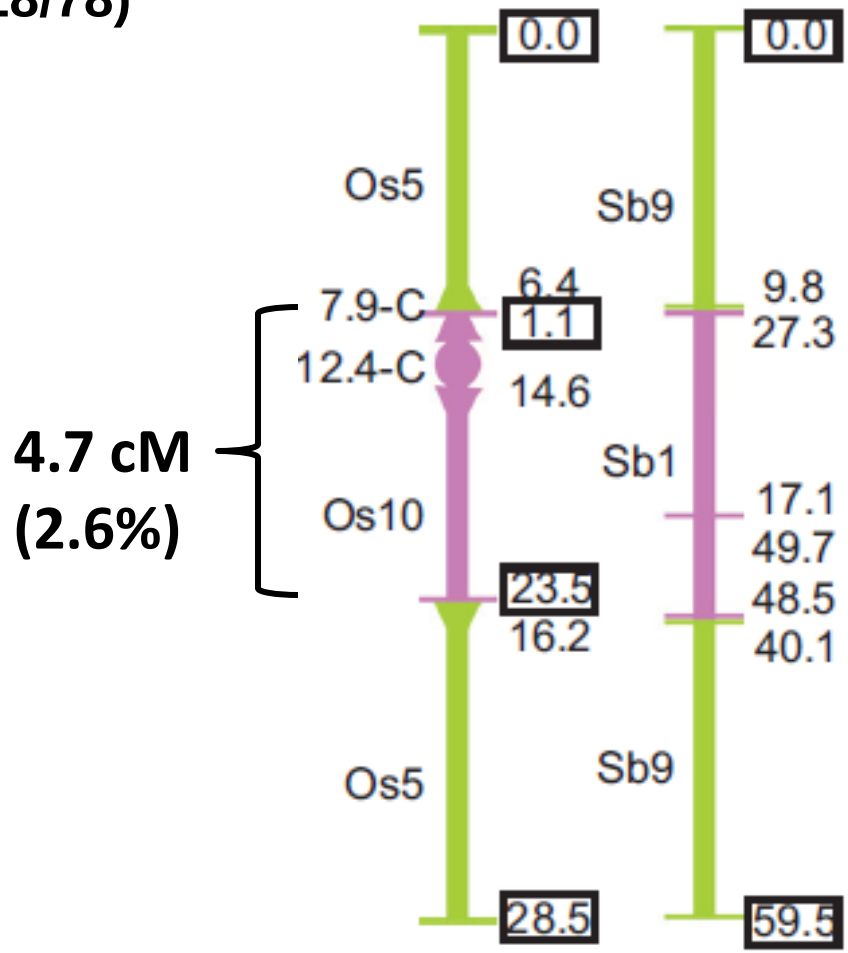

Luo et al., 2009 PNAS 106:15780-15785

Radiation Hybrids as Small Deletion Bins

- Independent from recombination
- High map resolution
- Fast production mapping population (6 months)
- Monomorphic markers

- RH[1] used for phenotyping (K.O.)

RH Population Development

- **Gamma Ray causes deletions of random size in the genome**

LDN (durum wheat)	LDN (aneuploid)
1A1A	--
2A2A	2A2A
3A3A	3A3A
4A4A	4A4A
5A5A	5A5A
6A6A	6A6A
7A7A	7A7A
1B1B	1B1B
2B2B	2B2B
3B3B	3B3B
4B4B	4B4B
5B5B	5B5B
6B6B	6B6B
7B7B	7B7B
	1D1D

RH Population Development

- **Gamma Ray causes deletions of random size in the genome**

LDN (durum wheat)

1A1A
2A2A
3A3A
4A4A
5A5A
6A6A
7A7A
1B1B
2B2B
3B3B
4B4B
5B5B
6B6B
7B7B

35 Krad Irradiation

LDN (aneuploid)

--
2A
3A
A
A
A
1
2B
3B
4B4
5B5B
6B6B
7B7B
1D1D

RH Population Development

- **Gamma Ray causes deletions of random size in the genome**

LDN (durum wheat)	LDN (aneuploid)
1A1A	--
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6A6A	6A6A
7A7A	7A7A
1B1B	1B1B
2B2B	2B2B
3B3B	3B3B
4B4B	4B4B
5B5B	5B5B
6B6B	6B6B
7B7B	7B7B
	1D1D

RH Population Development

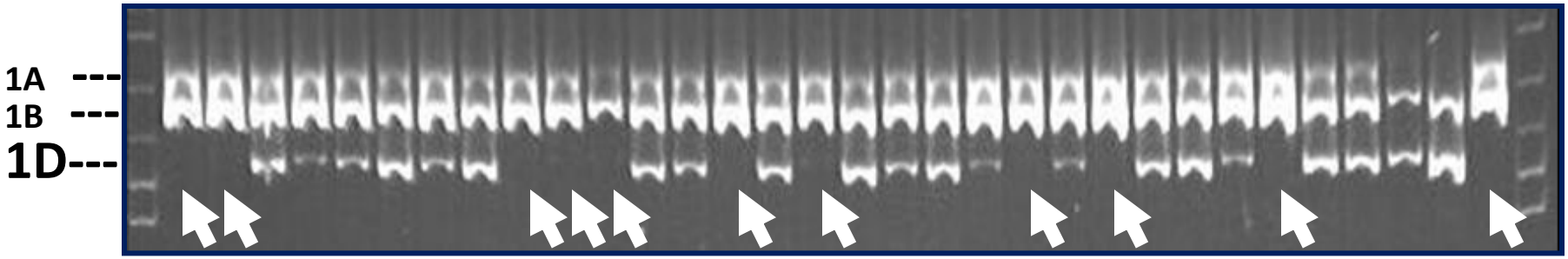
- **Gamma Ray causes deletions of random size in the genome**

	RH[1]	
1A		-
2A		2A
3A		3A
4A		4A
5A		5A
6A		6A
7A		7A
1B		1B
2B		2B
3B		3B
4B		4B
5B		5B
6B		6B
7B		7B
		1D

RFLP to Polyacrylamide Mapping

- **Primers designed on EST bin mapped (RFLP)**
- **42 markers chr. 1D specific on Polyacrylamide gel (NDSU)**
- **Populations:**

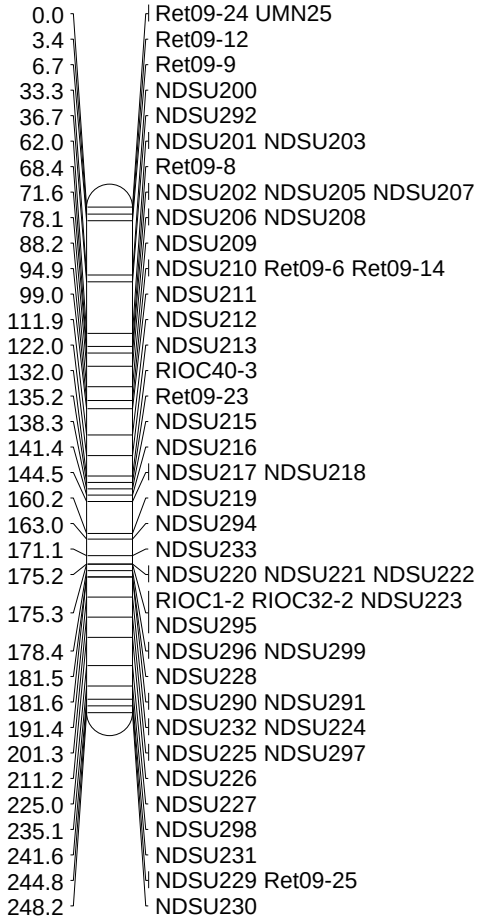
94 RH1	**LDN 1D**	**15 Krad**
94 RH1	**LDN 1D**	**35 Krad**
72 RH2	**(lo)scs**	**35 Krad**



Michalak et al., 2009 *Maydica 54:471-483*

RFLP to Polyacrylamide Mapping

- **94 RH[1] 15 Krad**



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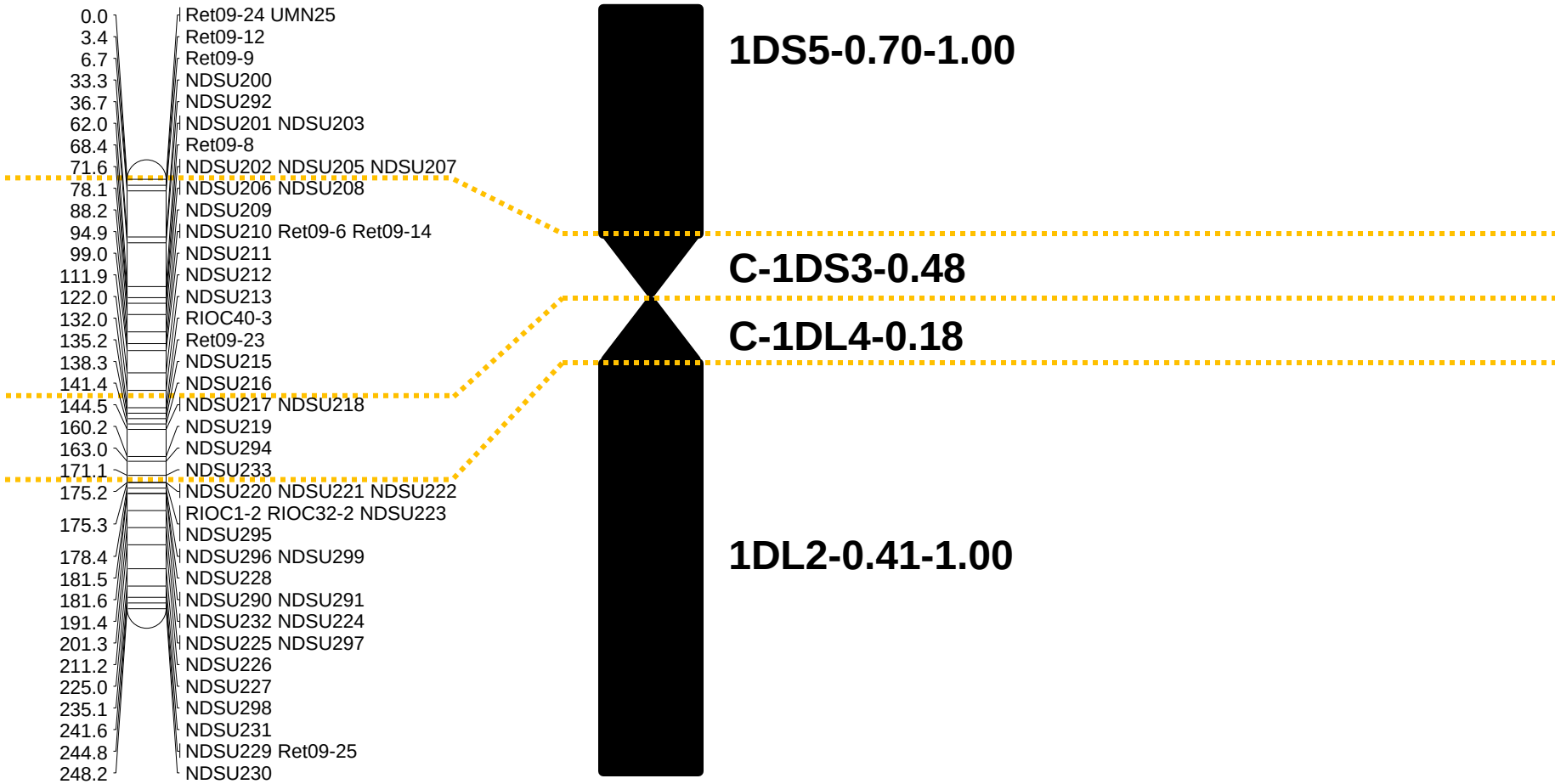

RFLP to Polyacrylamide Mapping

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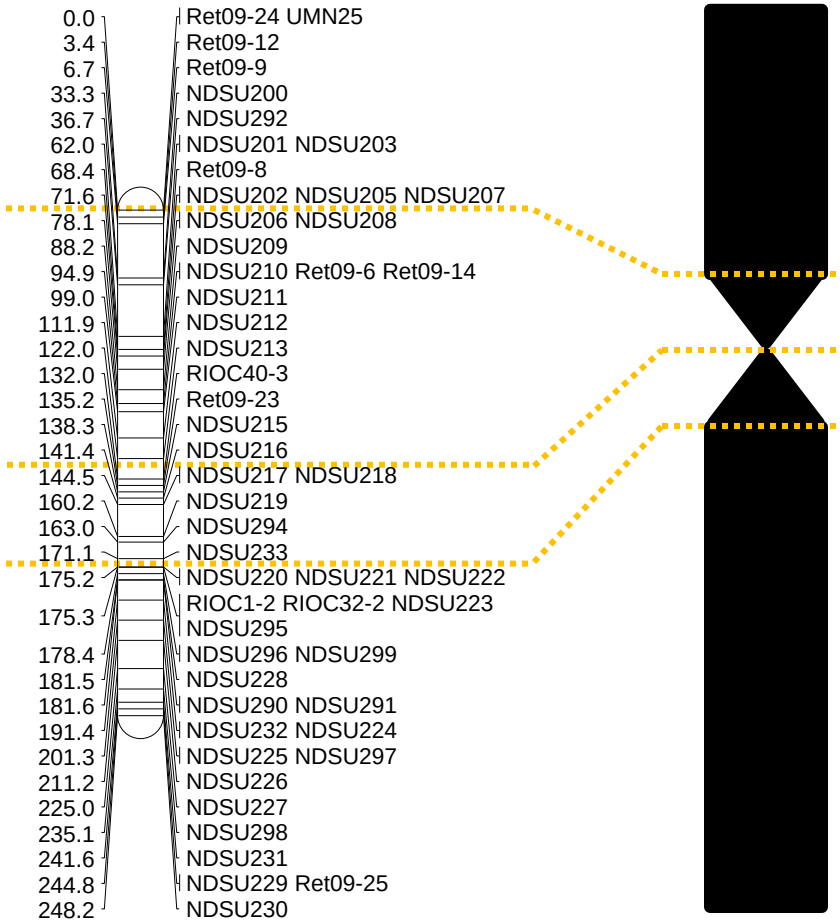
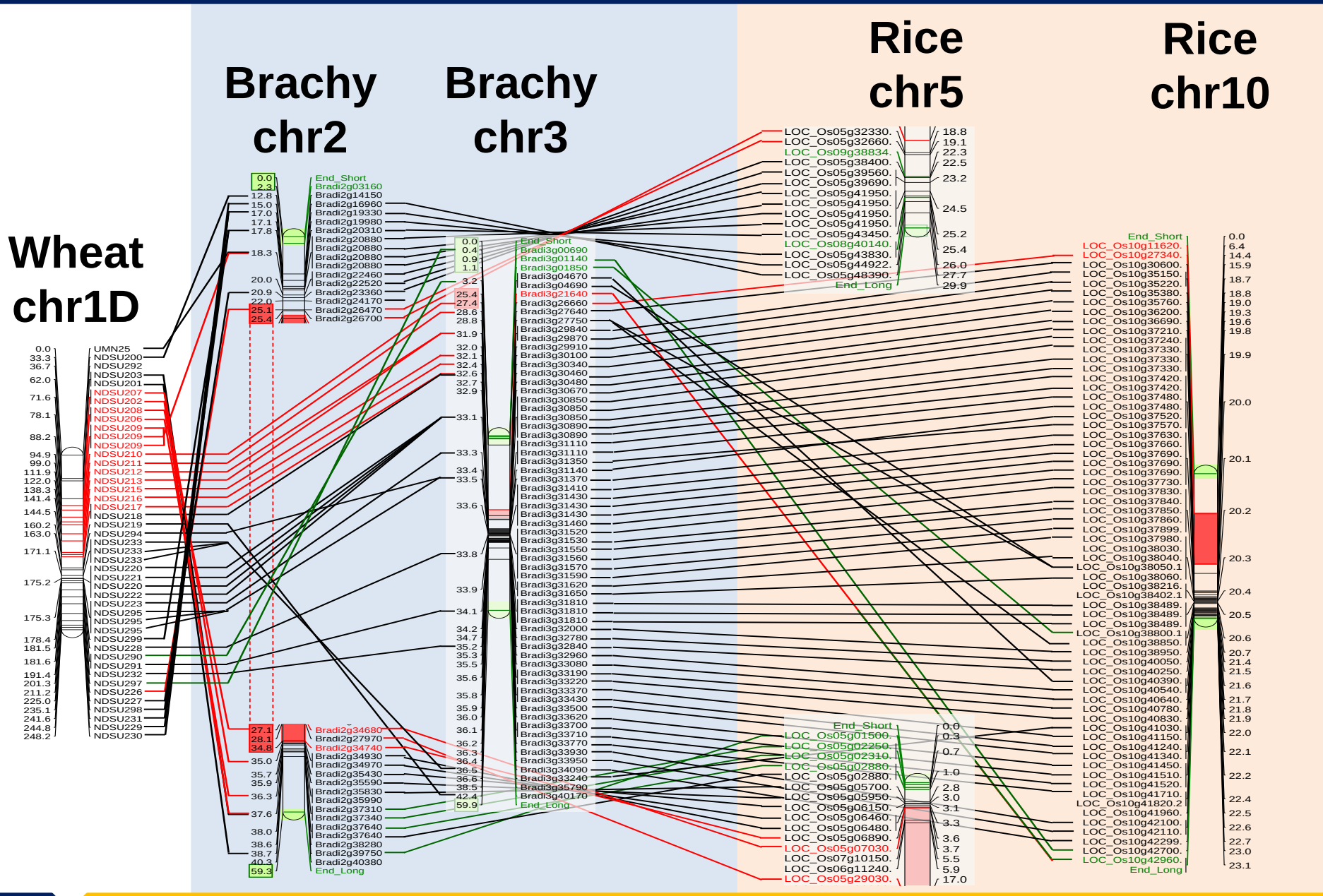


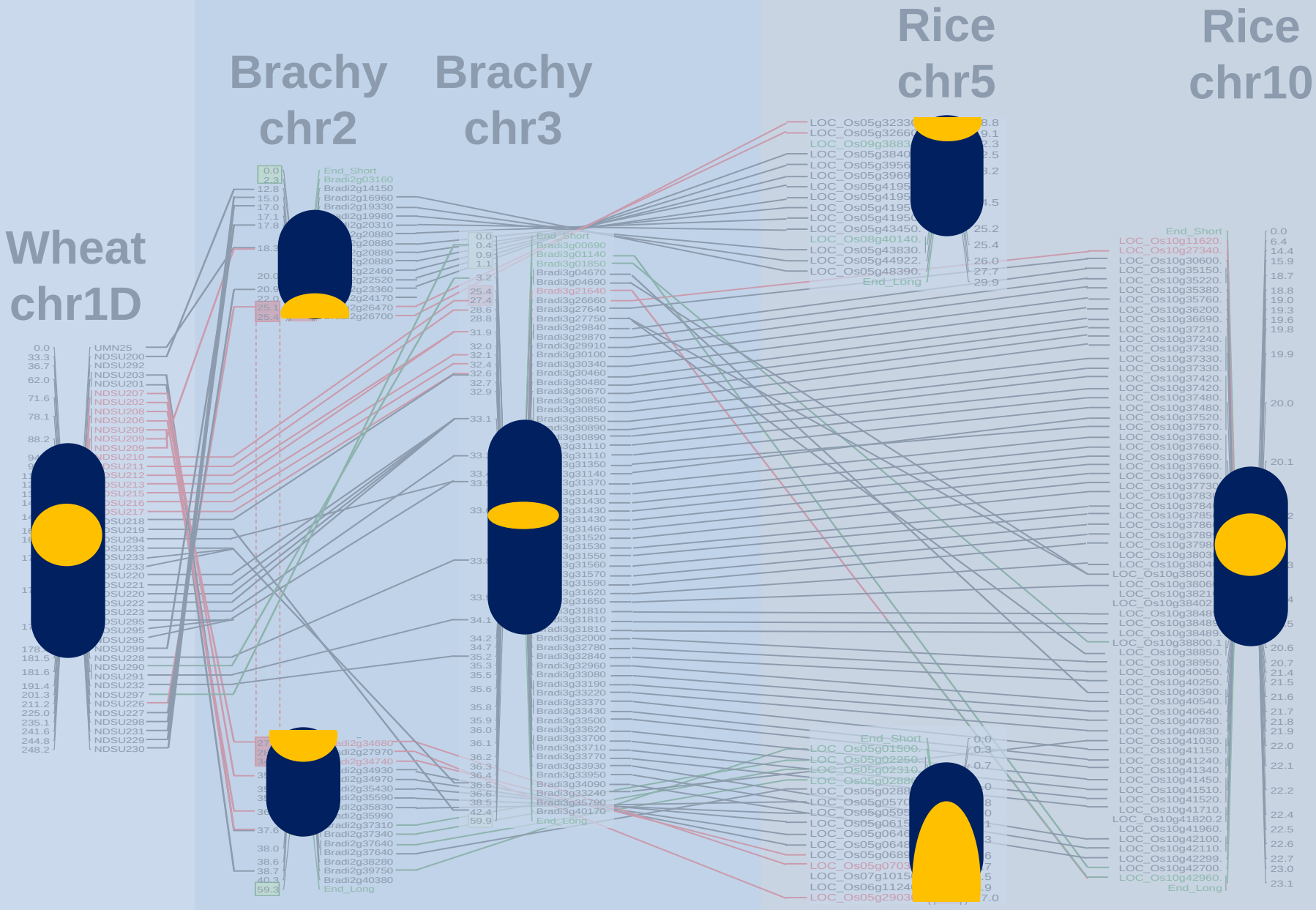
1DS5-0.70-1.00	**71.6 cR**	56.85 cM
C-1DS3-0.48	**69.8 cR**	0.7 cM
C-1DL4-0.18	**26.6 cR**	0.1 cM
1DL2-0.41-1.00	**77.1 cR**	121 cM

Luo et al., 2009 *PNAS 106:15780-15785*

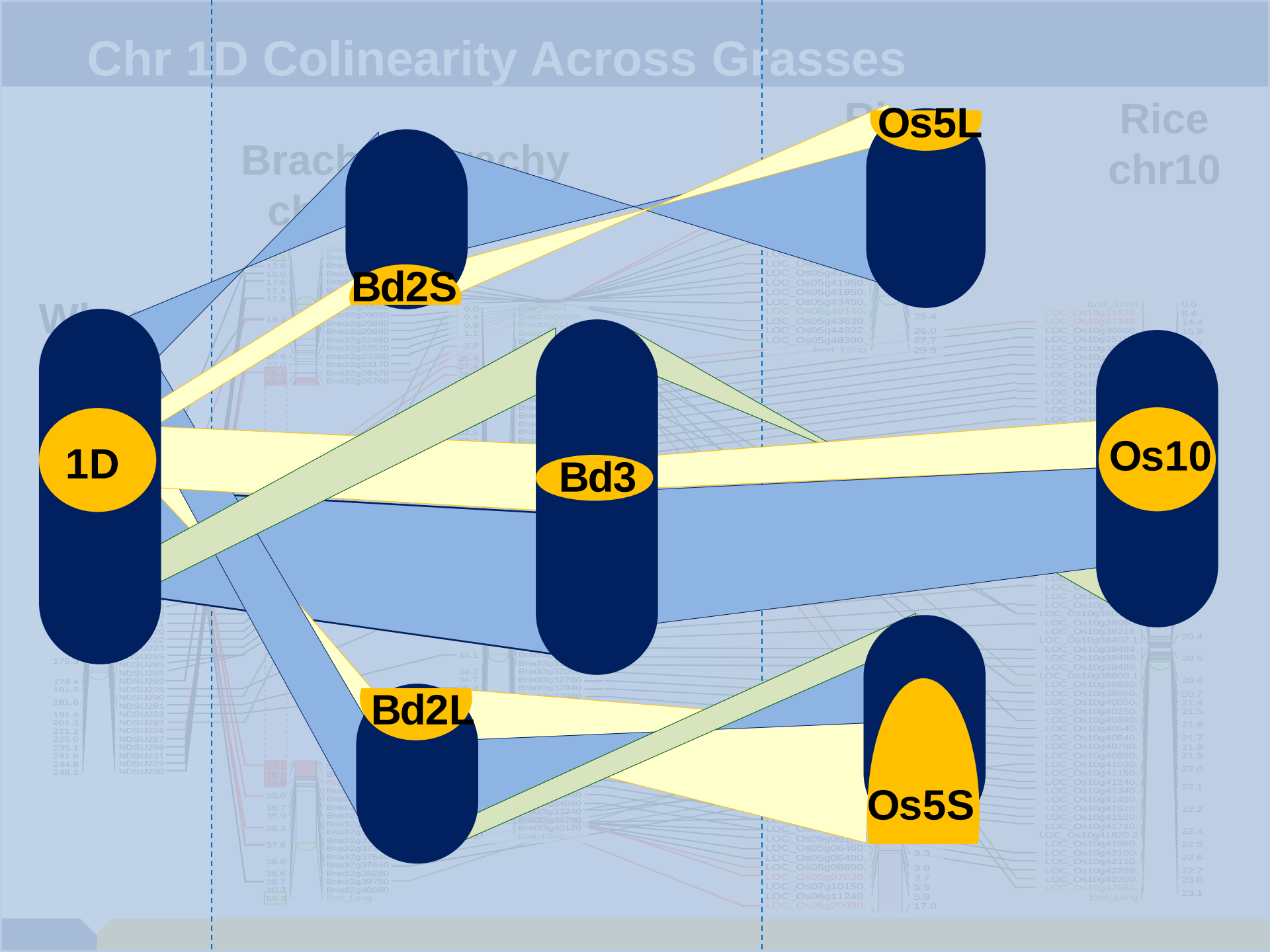
Chr 1D Colinearity Across Grasses

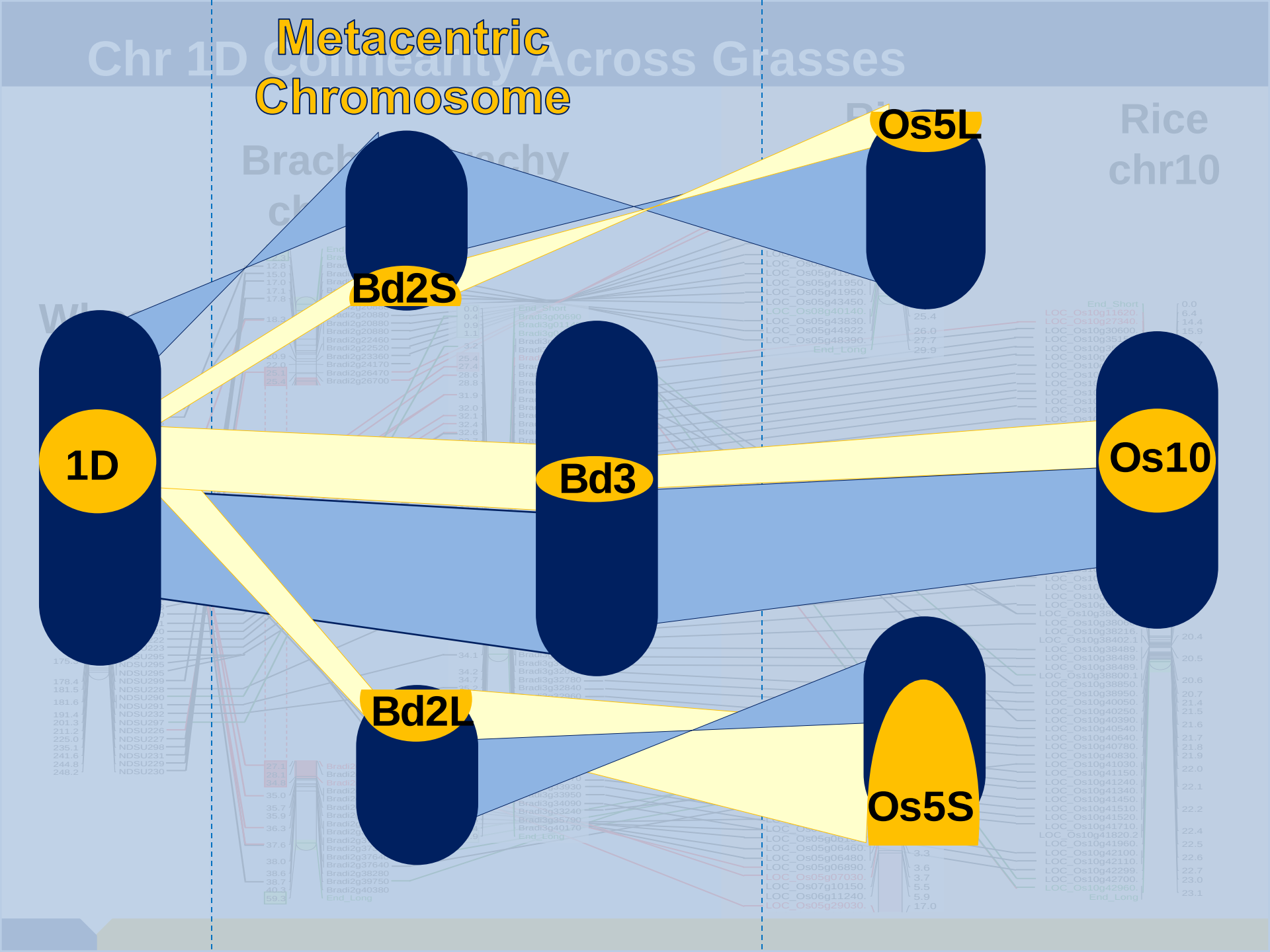


Chr 1D Colinearity Across Grasses

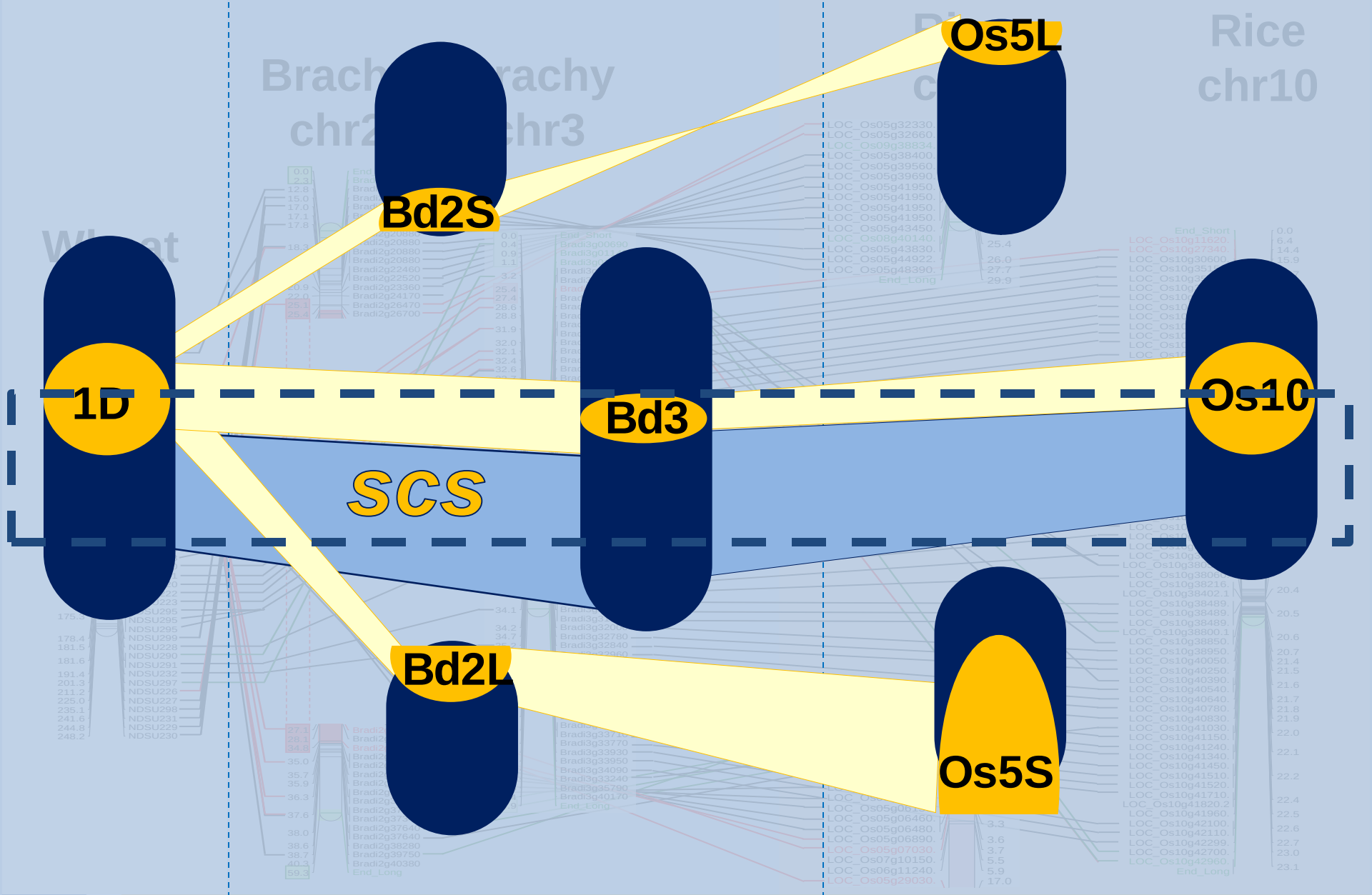


Chr 1D Colinearity Across Grasses




Chr 1D Colinearity Across Grasses
Metacentric Chromosome
Brachypodium chr1
Rice chr10
1D
Bd2S
Bd3
Bd2L
Os5L
Os10
Os5S

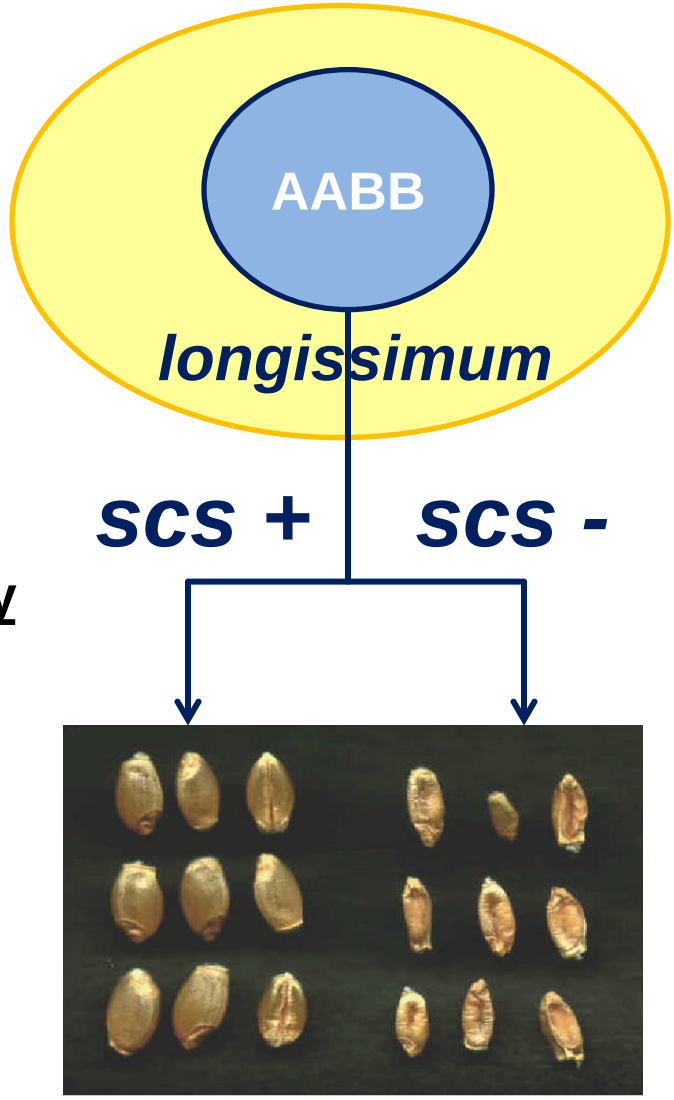
Chr 1D Colinearity Across Grasses



The *scs* Phenotype

- ***Species Cytoplasm Specific***

from *T. aestivum*	***1D***
from *T. timophevii*	***1A***



<u>Restores Nuclear-Cytoplasm compatibility</u>

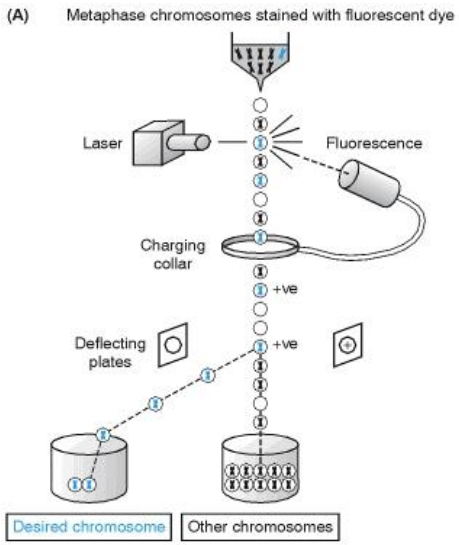
Hossain et al., 2004 *Genome 47:206-214*

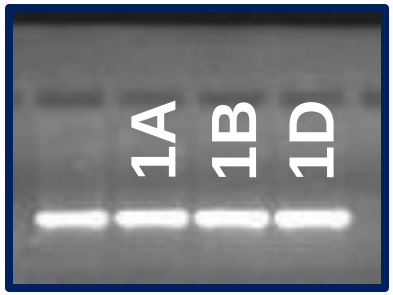
Flow Sorted Saturation

- ***scs^{ae}*** **interval 350 rice genes (develop 1 marker ~ 10 genes)**

Flow Sorted Saturation

- ***scs^{ae}*** **interval 350 rice genes (1 marker ~ 10 genes)**





Strachan and Read, 1999 *NBK7580*

Flow Sorted Saturation

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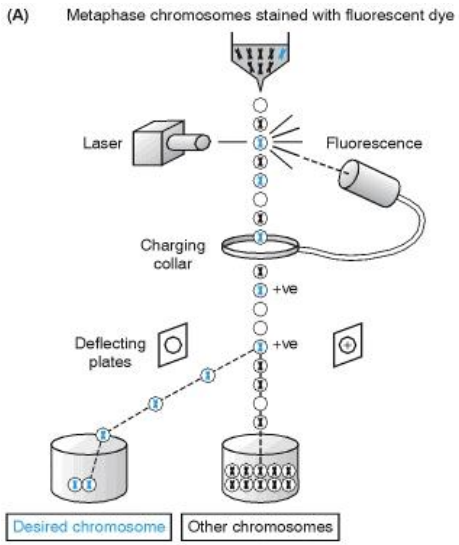
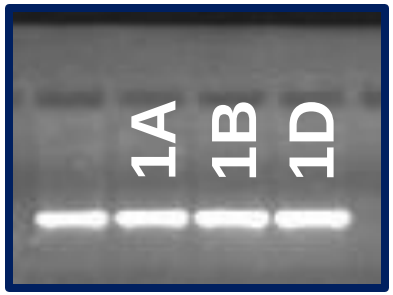
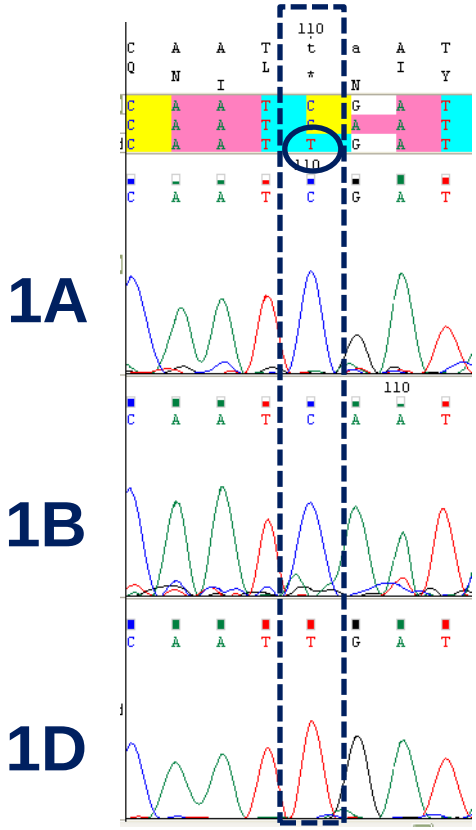




Band sequencing



Strachan and Read, 1999 NBK7580

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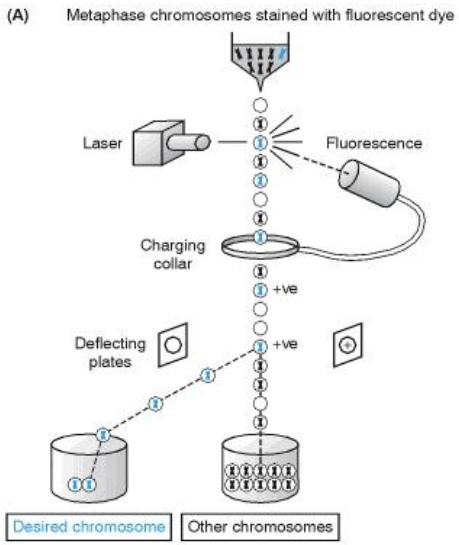

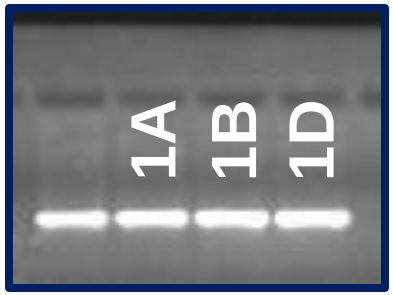

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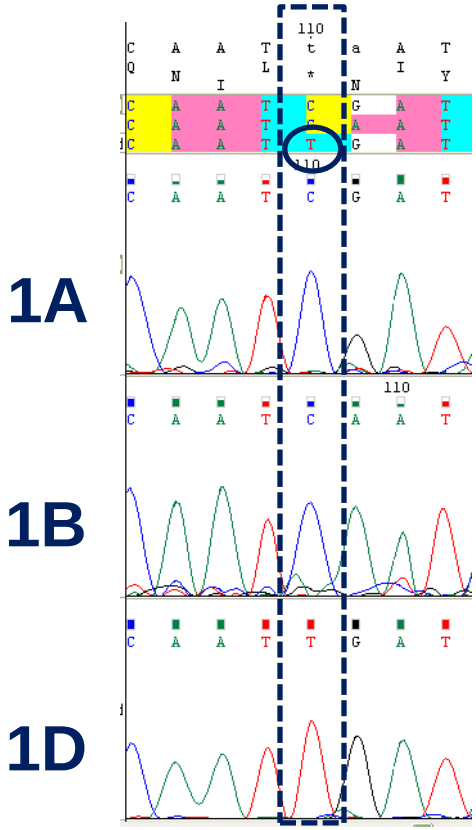
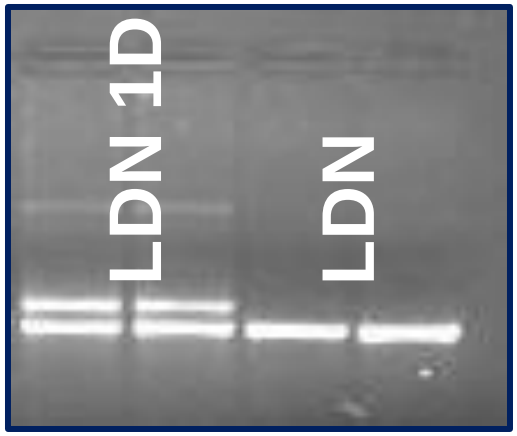
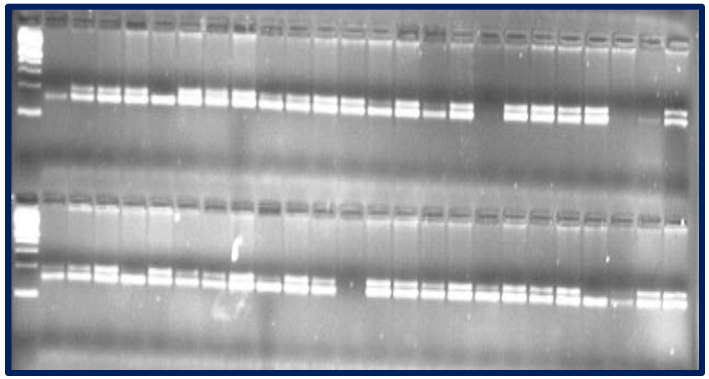


SNP primers





Strachan and Read, 1999 *NBK7580*

SNP Survey

- **14 genes sequenced for 1A, 1B, and 1D**

	SNP	SNP Intron	SNP Exon
	---------- SNP/bp ----------		
1A vs 1B-1D	32.0	18.5	50.4
1D vs 1A-1B	58.9	26.4	107.2

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1A vs 1B-1D	32.0	18.5	50.4
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	Brachy chr3			Brachy chr2		
	SNP	SNP Intron	SNP Exon	SNP	SNP Intron	SNP Exon
	---------- SNP / bp ----------					
1A vs 1B-1D	79.5	33.0	172.6	16.9	12.9	21.8
1D vs 1A-1B	58.1	16.9	126.3	116.3	162.7	88.4

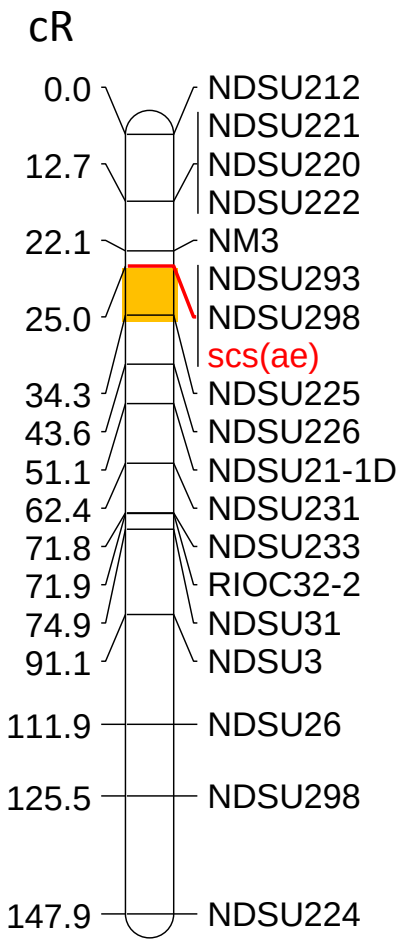
Intron Evolution

- **Comparison of length of 24 wheat Introns:**

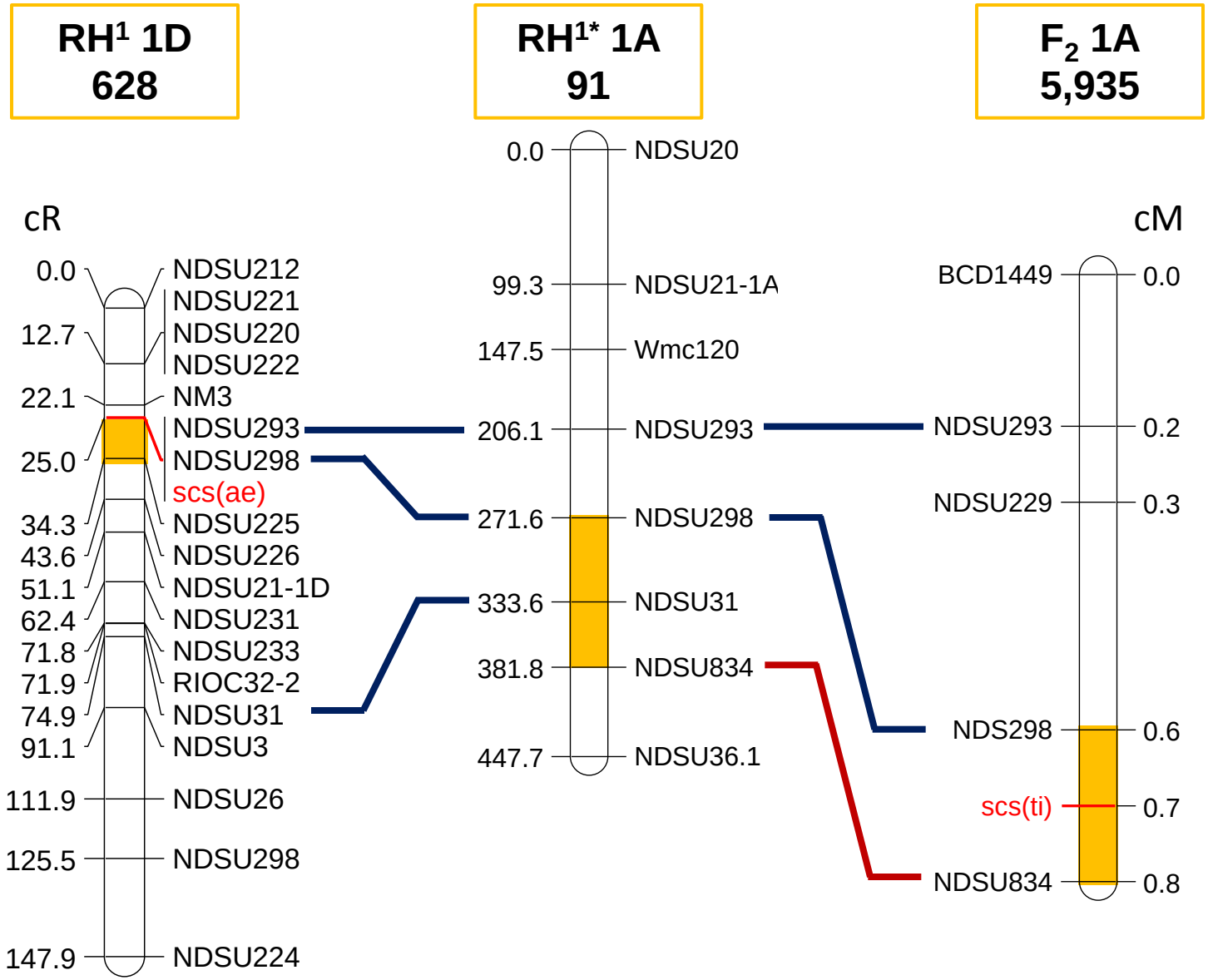
	Rice	Brachy
Average bp Δ	39%	19%

1A to 1D Conservation

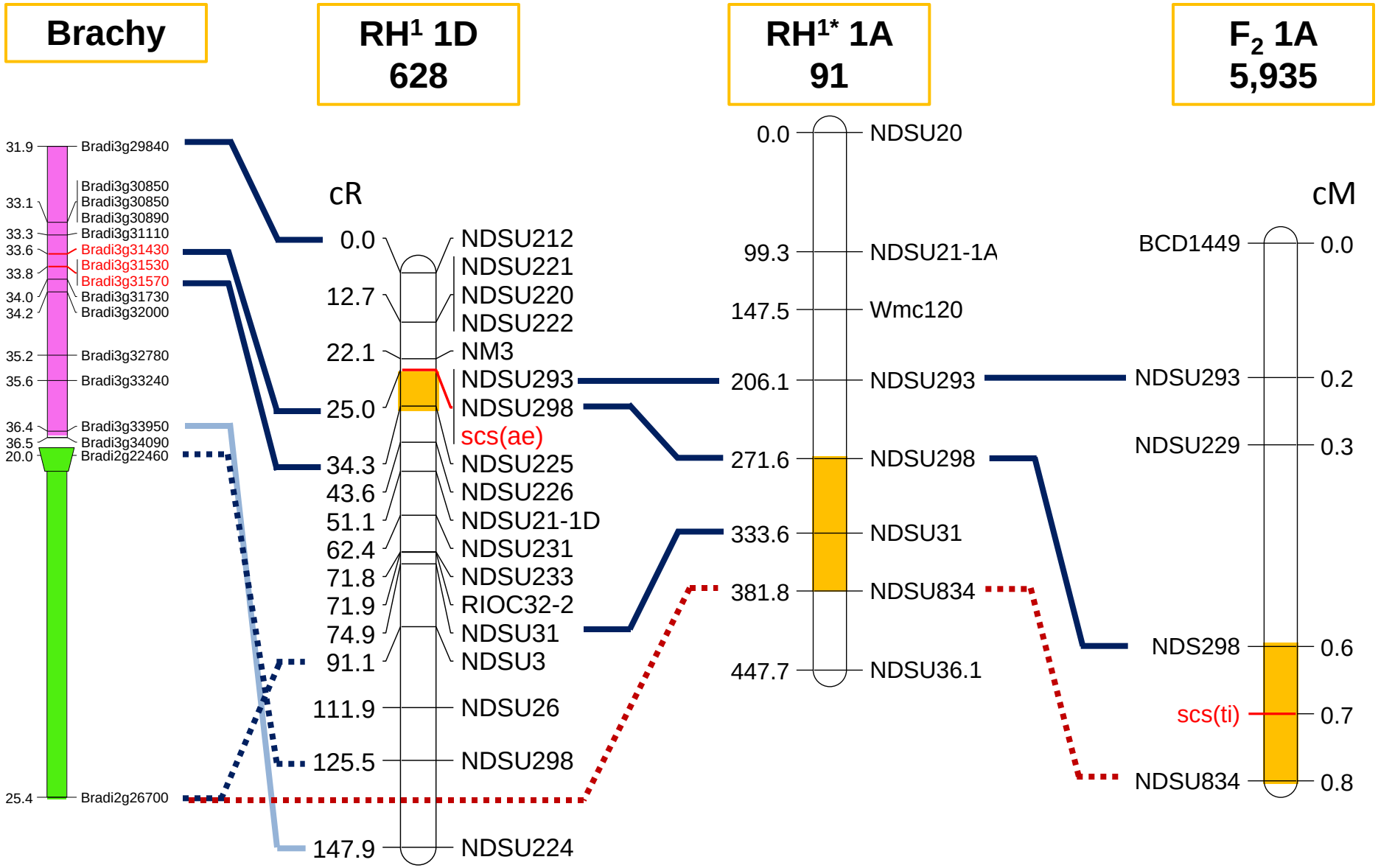
RH[1] 1D
628



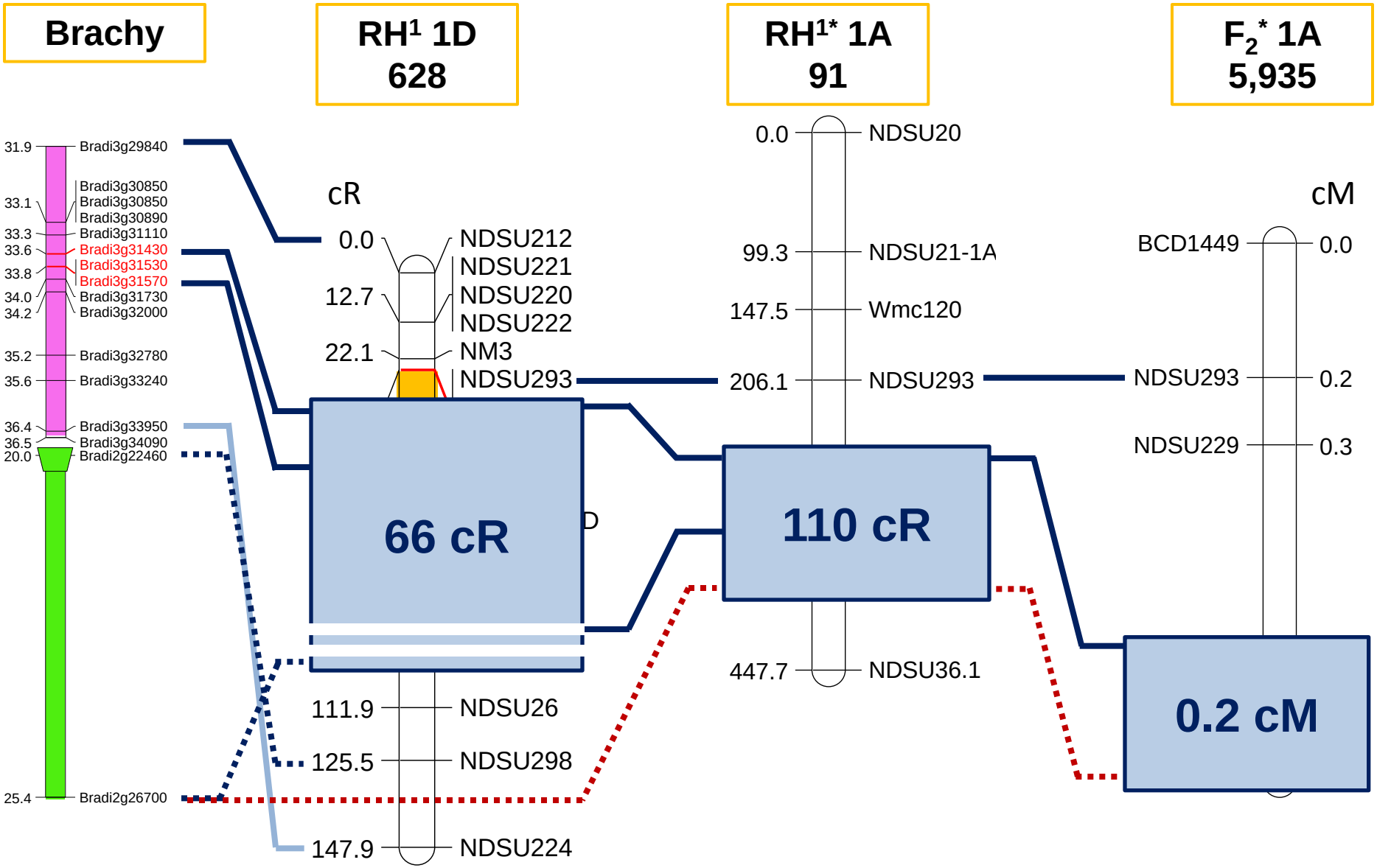
1A to 1D Conservation



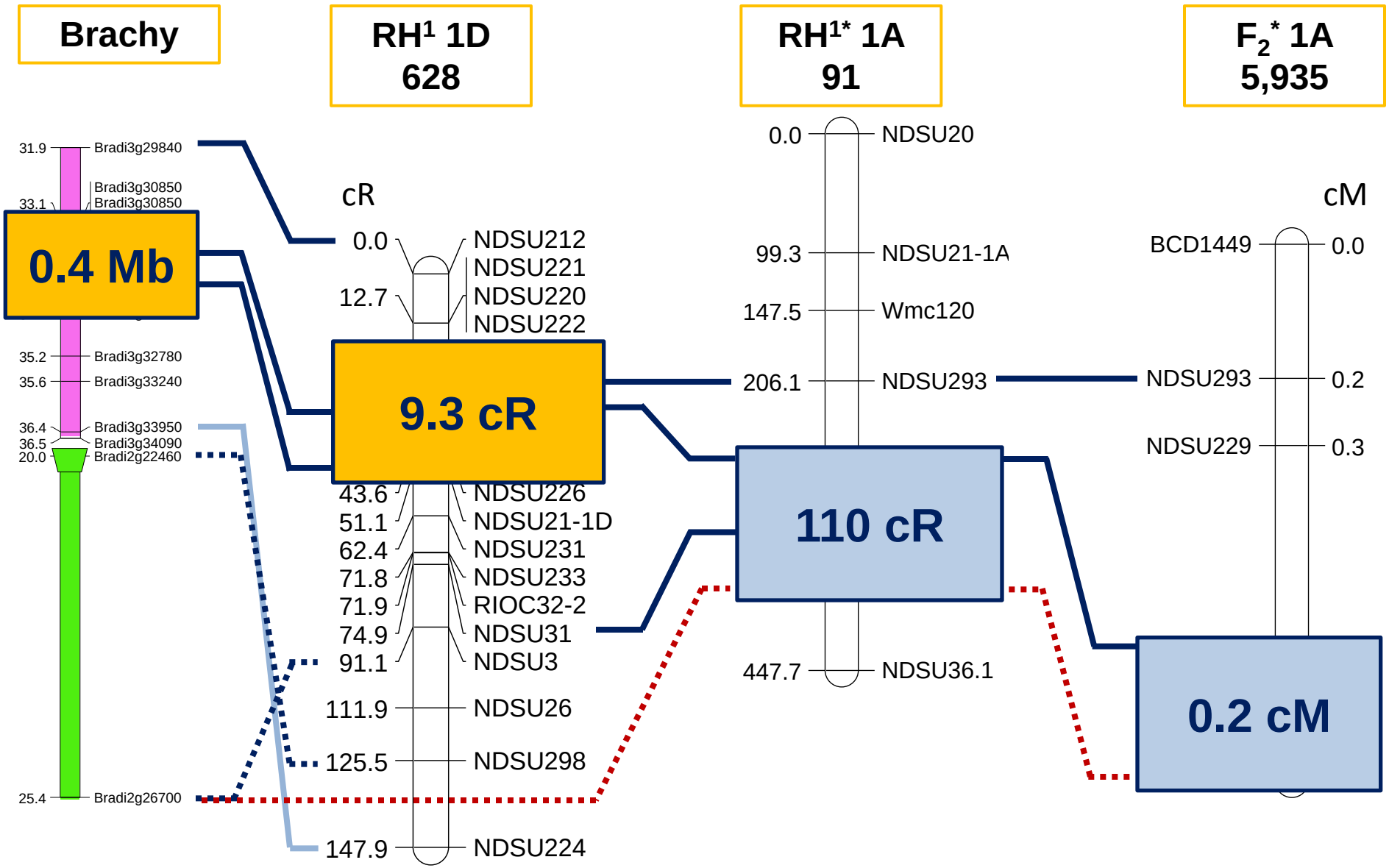
1A to 1D Conservation



1A to 1D Conservation



1A to 1D Conservation



Conclusions

- Brachy supports chr. fusion to originate wheat homeologus 1
  Bd 2-3-2 Os 5-10-5 Sb 9-1-9
- Synteny eroded at telomeric ends but not in the (peri)centromere
- 1A is evolving faster in syntenic breaks (*14 genes*)
- Brachy better gene model for wheat (*24 introns*)
- RH great system for synteny studies
  - Resolution (10-100X)
  - Monomorphic EST mapping
- Keep up “this” generation wet lab with “next” generation seq.

Acknowledgments

Wheat Germplasm Enhancement NDSU

http://avena.pw.usda.gov/RHmapping/

Poster #275

Monsanto's Beachell-Borlaug International Scholars Program

MASTER and BACK
REGIONE AUTONOMA DELLA SARDEGNA

… Brain Storming !!??

THANK YOU