FM Bassi, Y Wang, M Michalak de J, K Simons, K Forrest, S Kong, R Saetan, L Alnilmer, R Dizon, H Simkova, J Dolezel, F Ghavami, A Denton, J Dvorack, MC Luo, Y Gu, M Hayden, SF Kianian

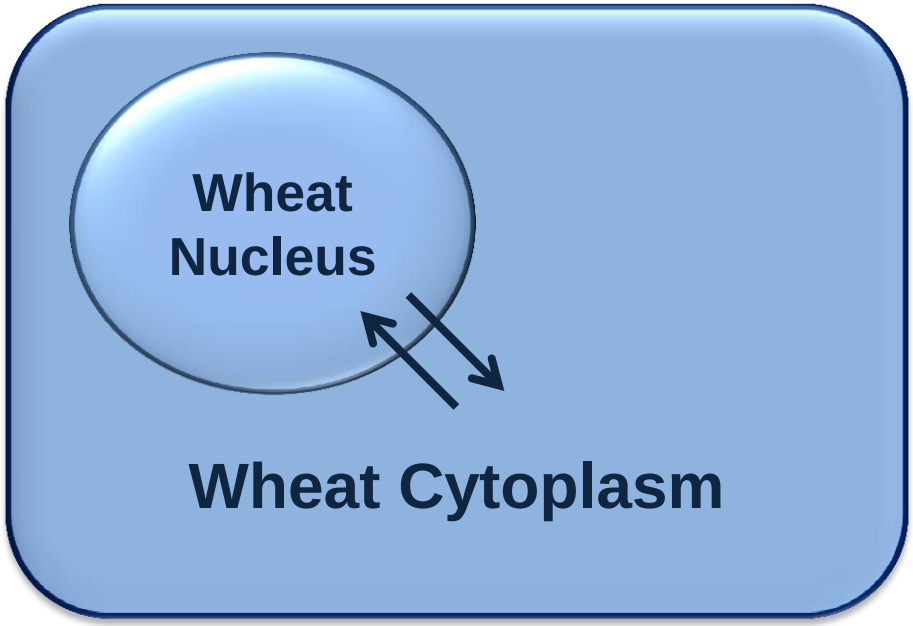
Wheat Zapper, Illumina BSA, and radiation hybrids for synteny analysis of the *scs* region

Outline

- ***Species cytoplasm specific*** **function**
- **Radiation hybrid for fine mapping**
- **Wheat Zapper a versatile on-line application for synteny**
- **RH bulk segregant analysis by Illumina sequencing**
- **Paleofusion in proximity of the *scs* region**
- **A candidate for the *scs* locus**

Nuclear-Cytoplasm Interaction

- Energy production
- Disease resistance
- Fertility
- Plant vigor
- Maturity
- Yield

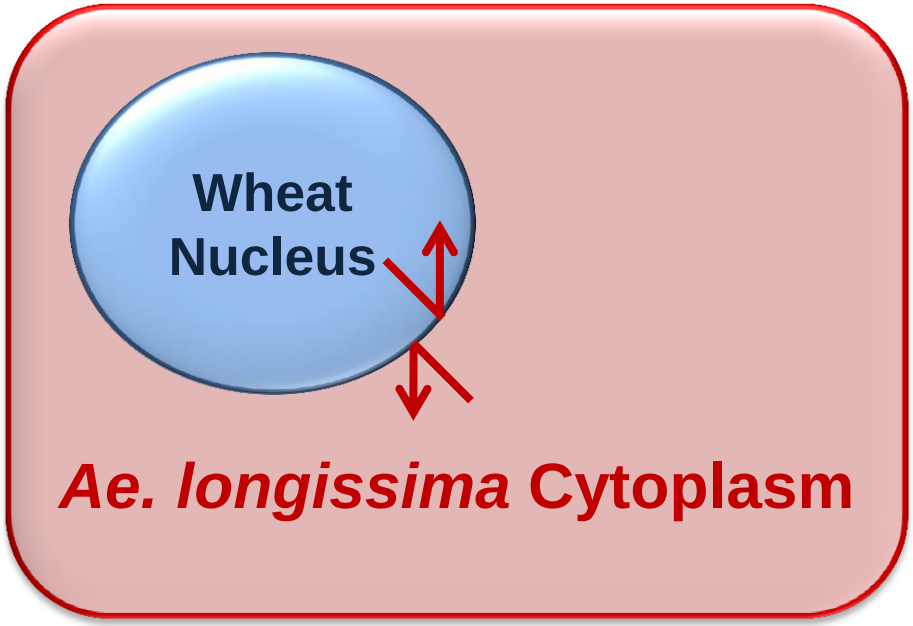


Euplasmic

Maan 1991 *Proc Int Symp Nuclear Organellar Genetics of Wheat*

Nuclear-Cytoplasm Interaction

- ✗ Energy production
- ✗ Disease resistance
- ✗ Fertility
- ✗ Plant vigor
- ✗ Maturity
- ✗ Yield



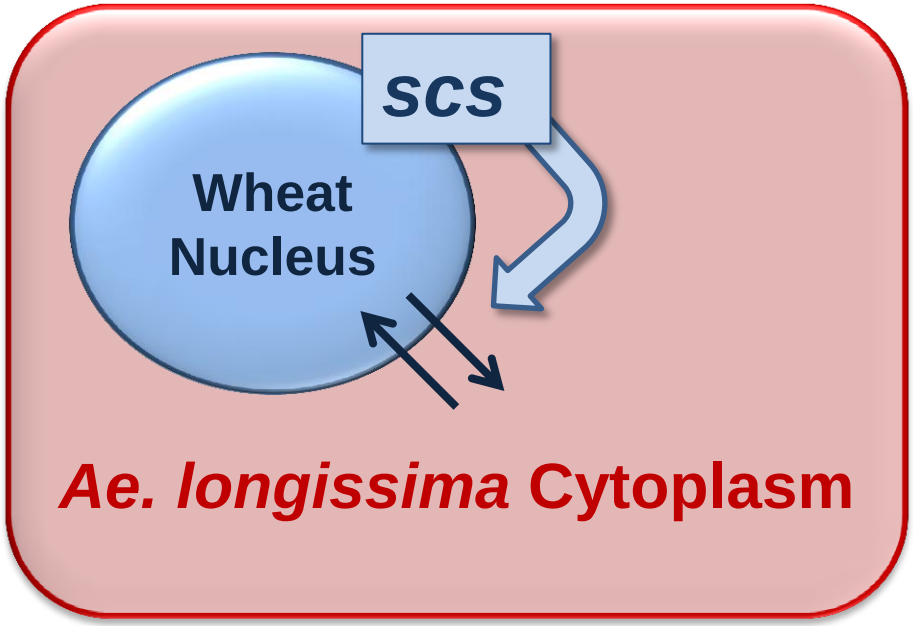
Alloplasmic

Maan 1991 *Proc Int Symp Nuclear Organellar Genetics of Wheat*

Species Cytoplasm Specific

- **Restore interaction**
- **3 characterized versions:**

Scs ^{ae}	**1D**
Scs ^{ti}	**1A**^{timophevii}
Scs ^{spt}	**?**^{speltoides}



Alloplasmic

Species Cytoplasm Specific

- **Restore interaction**
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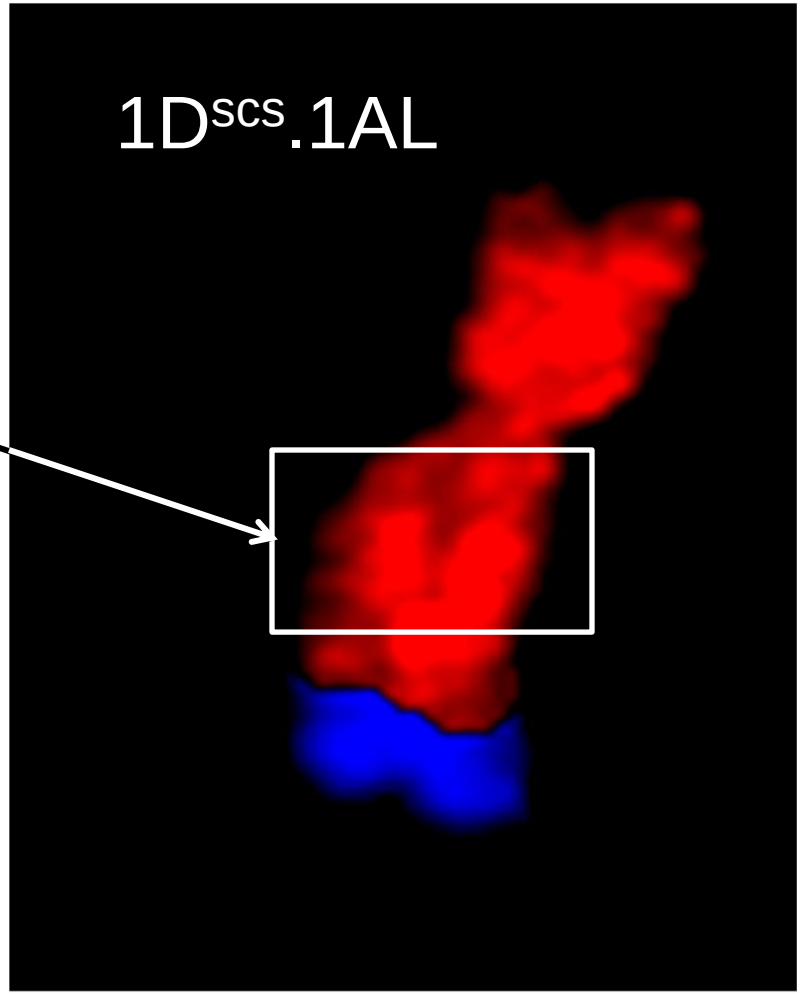
Scs	***ae***	**1D**
Scs	***ti***	**1A** **timophevii**
Scs	***spt***	**?** **speltoides**



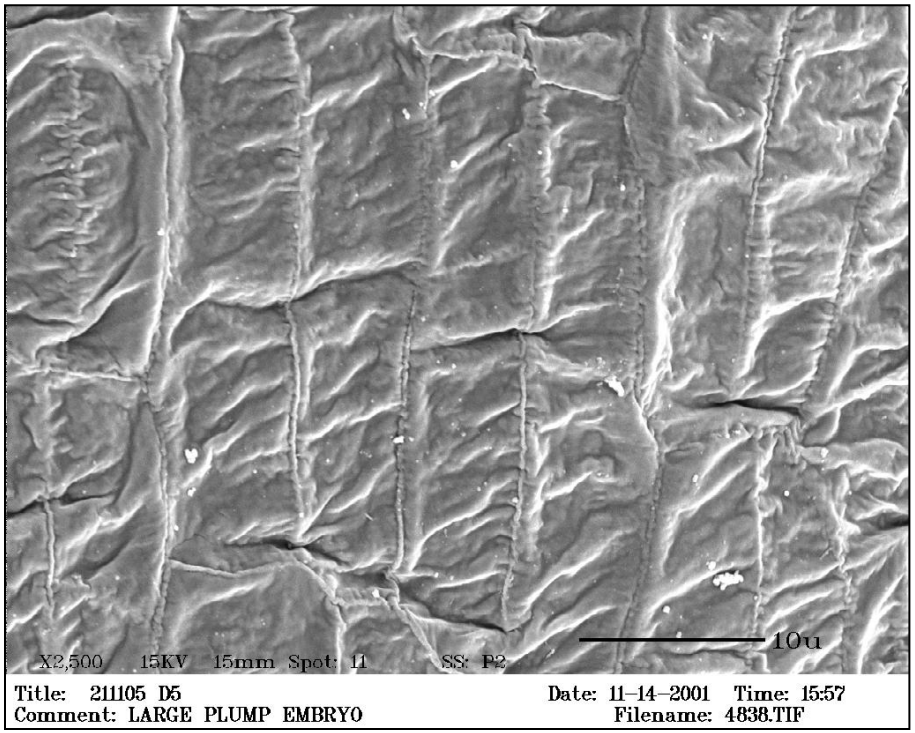
Michalak et al. *submitted*

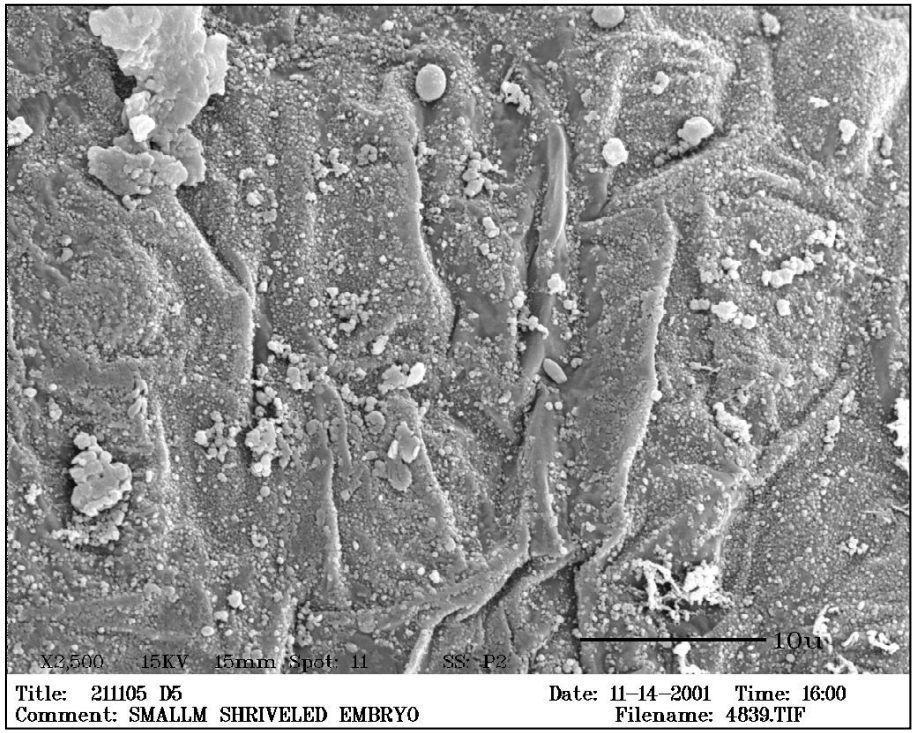
Species Cytoplasm Specific

scs **Δscs**

Michalak et al. *submitted*

Species Cytoplasm Specific





scs **Δscs**

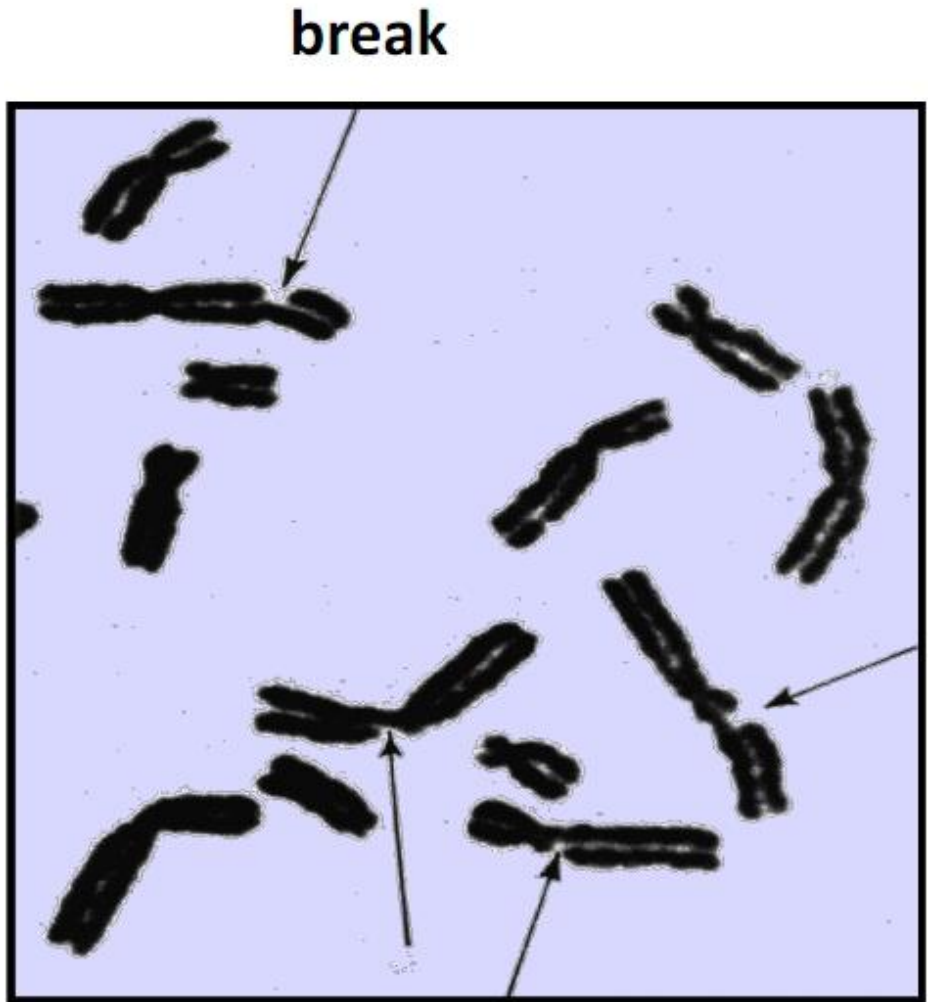
Michalak et al. submitted

Species Cytoplasm Specific

scs **Δscs**

Radiation Hybrids for Fine Mapping

- **Radiation-mediated breaks**
- ***In vivo* RH**



http://www.ratical.org/radiation/CNR/PP/chp3.html

Radiation Hybrids for Fine Mapping

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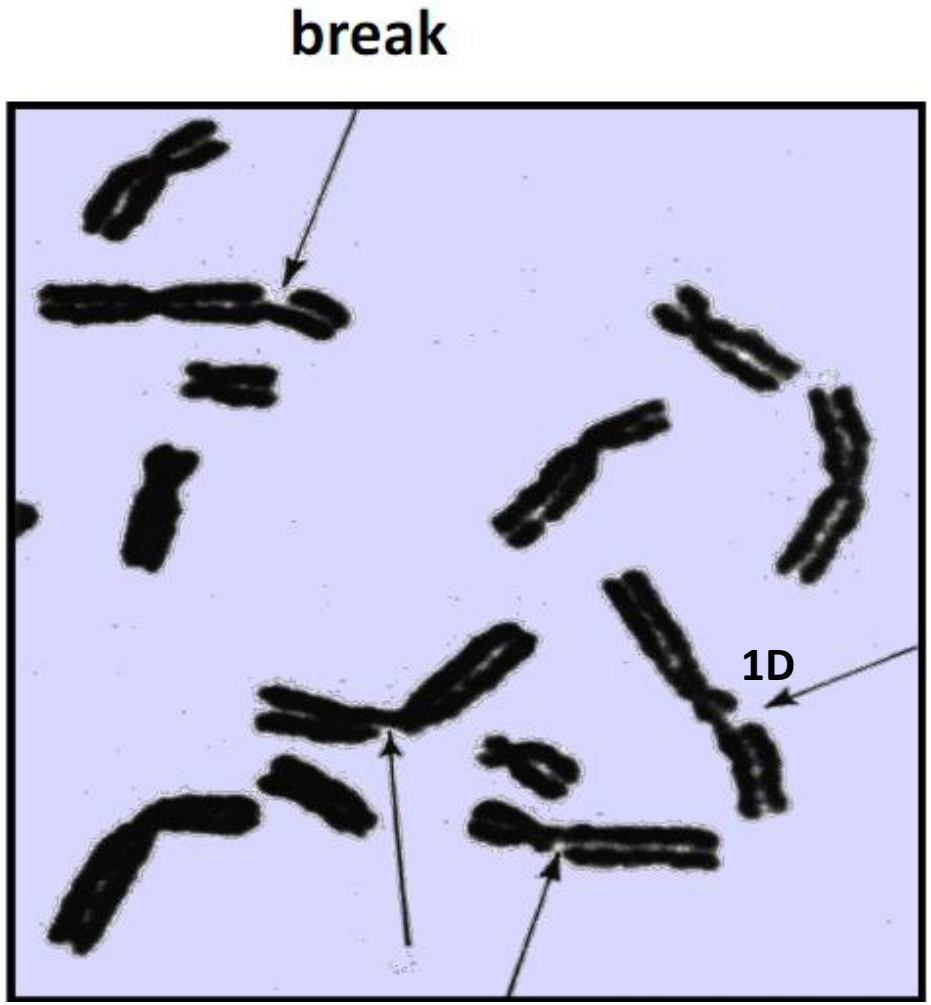

http://www.ratical.org/radiation/CNR/PP/chp3.html

Radiation Hybrids for Fine Mapping

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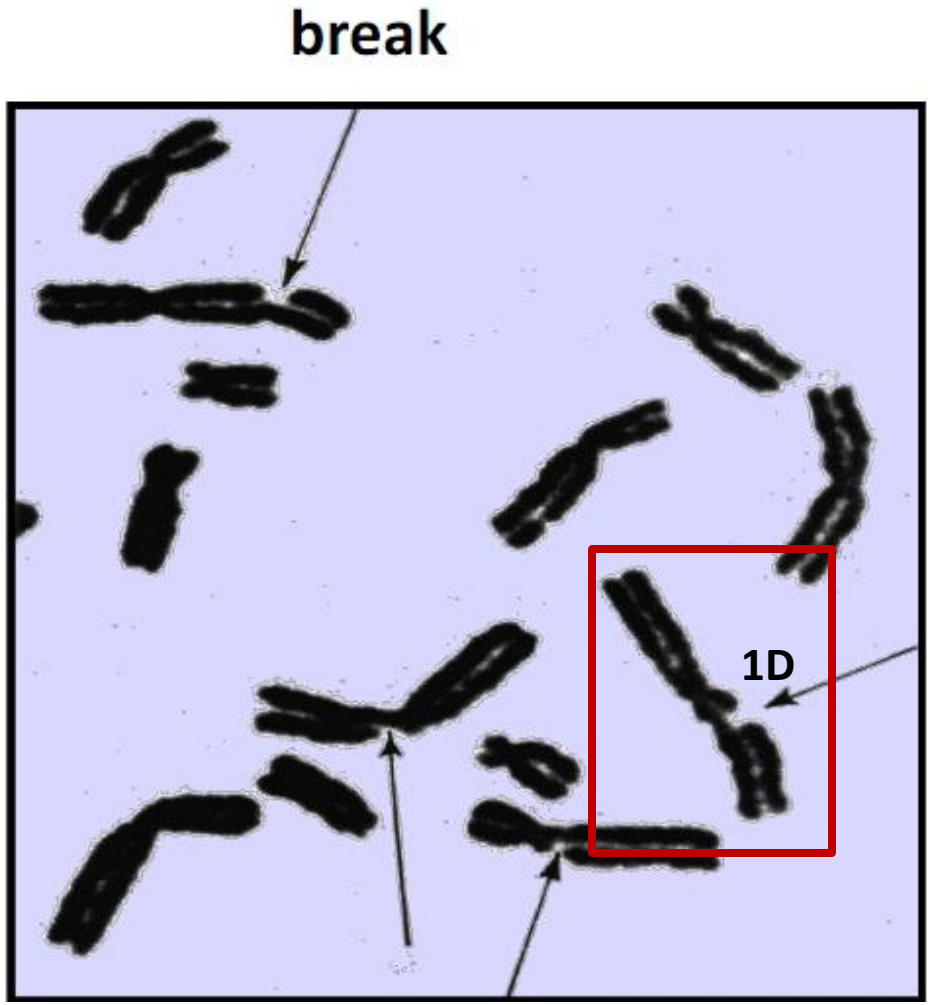

Radiation Hybrids for Fine Mapping

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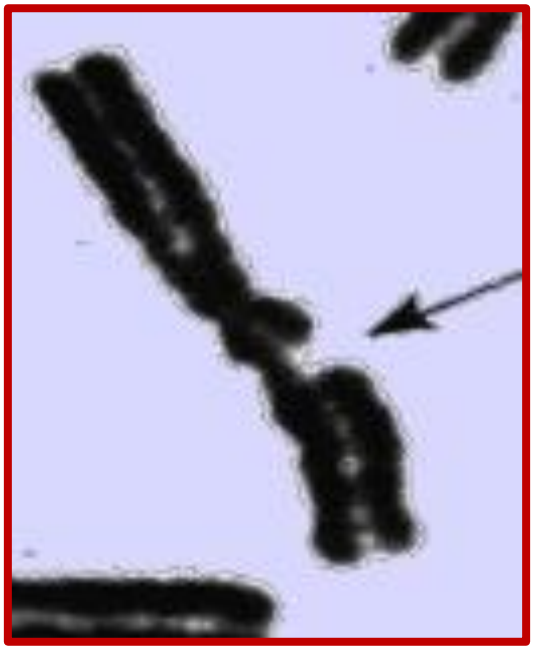

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Radiation Hybrids for Fine Mapping

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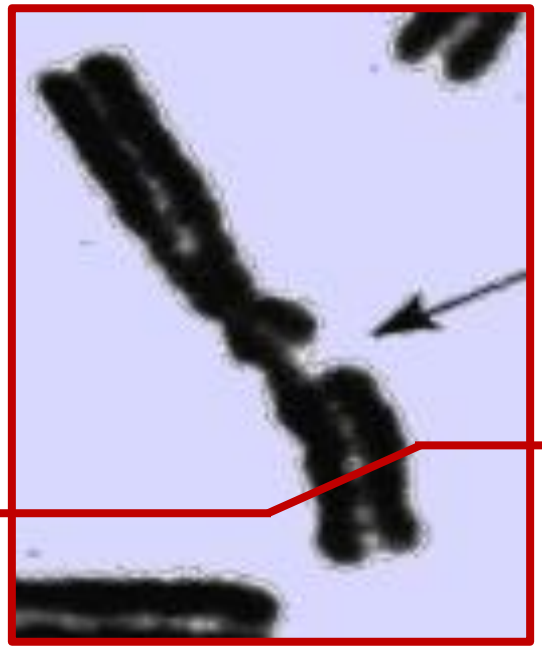

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Radiation Hybrids for Fine Mapping

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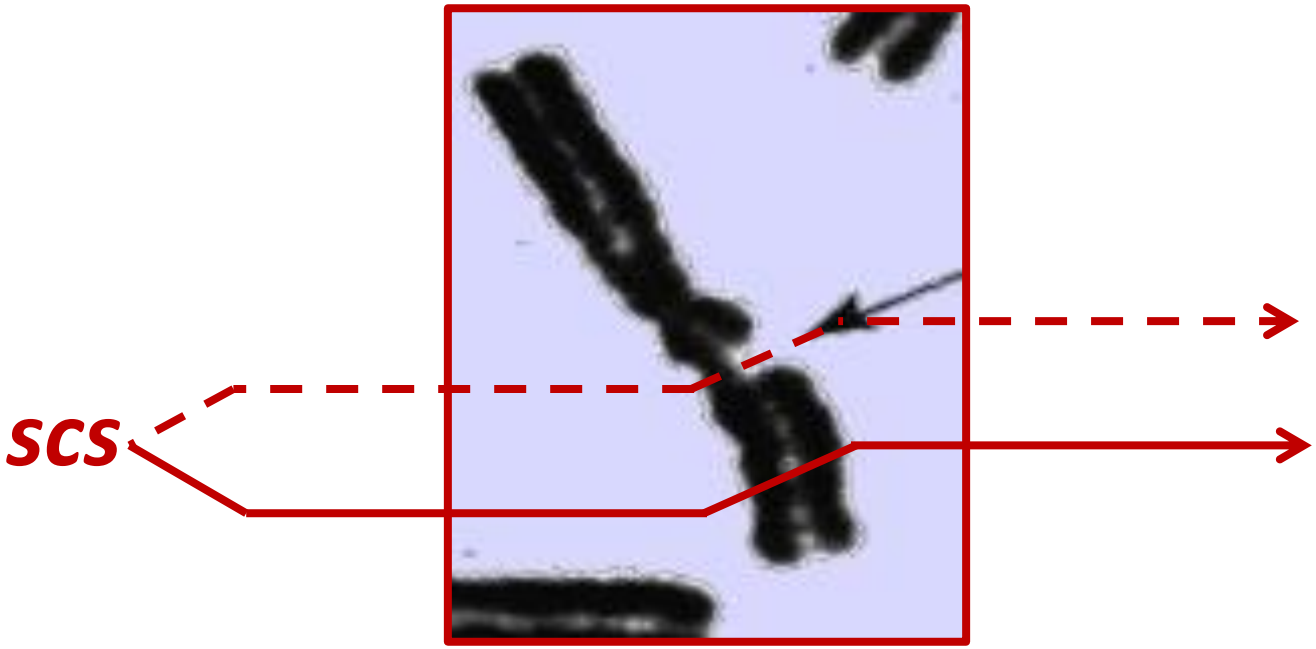

http://www.ratical.org/radiation/CNR/PP/chp3.html

Radiation Hybrids for Fine Mapping

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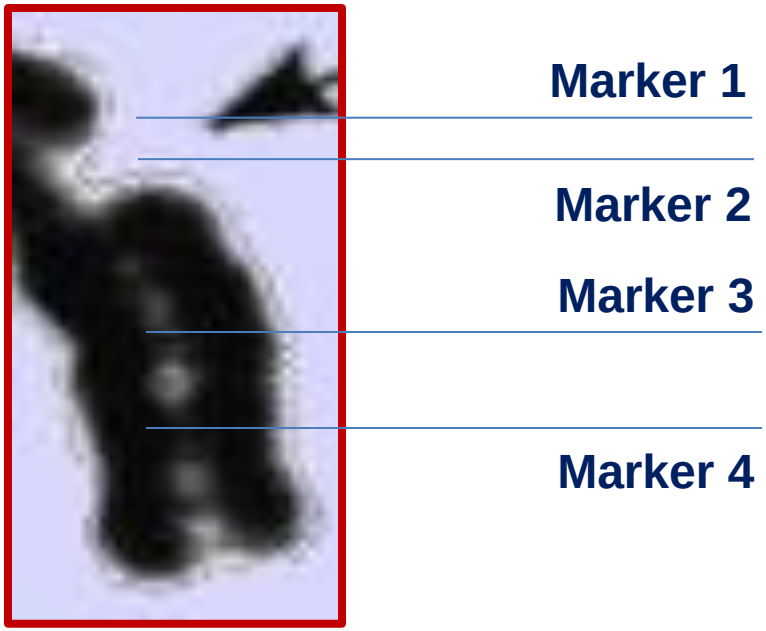
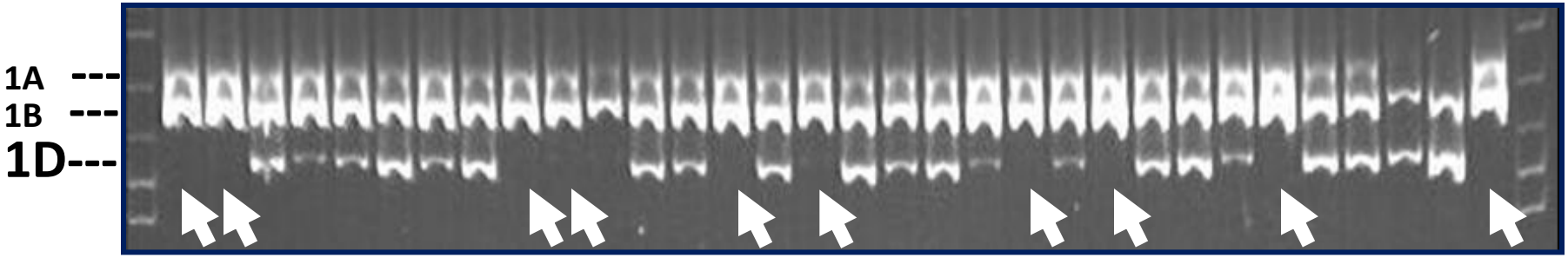


http://www.ratical.org/radiation/CNR/PP/chp3.html

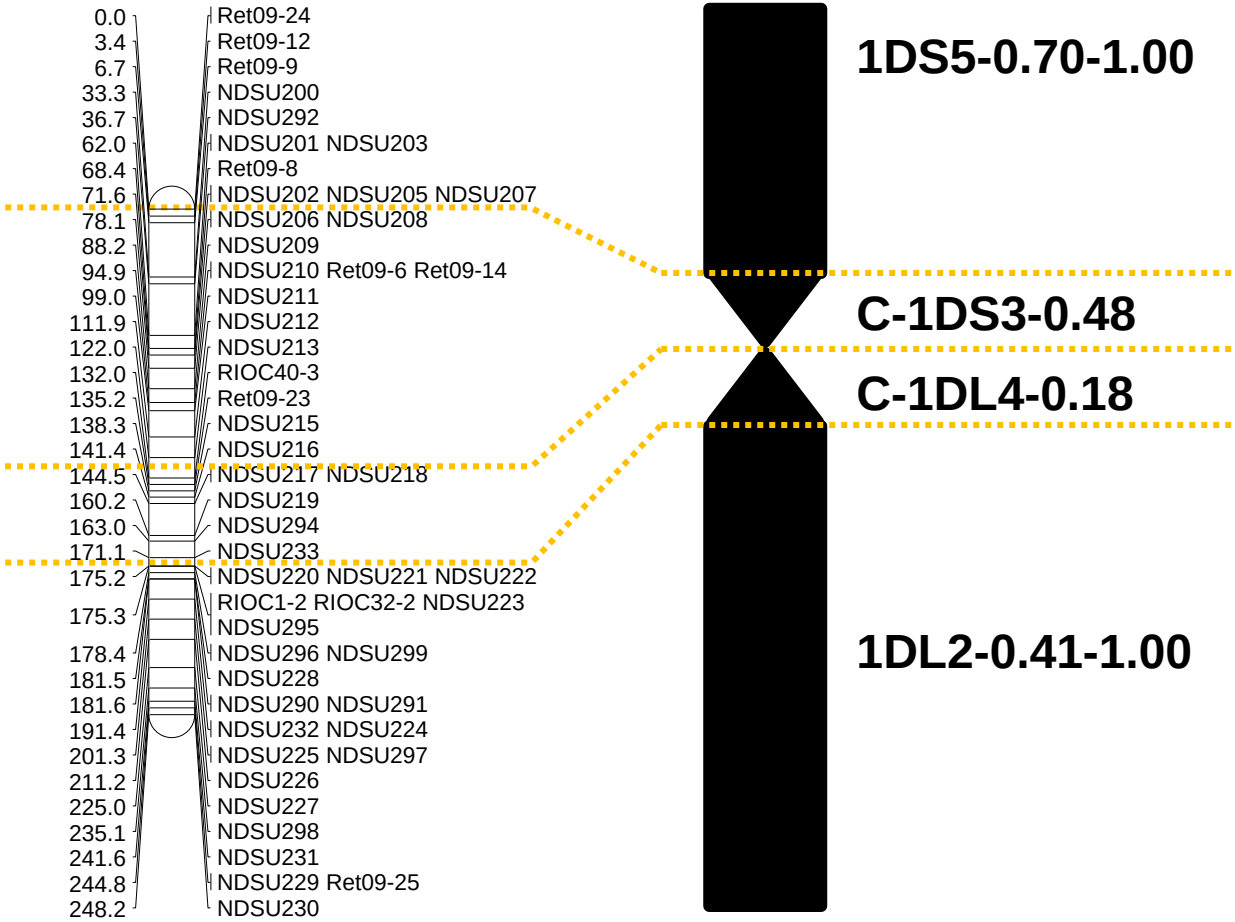
Radiation Hybrids for Fine Mapping

- **EST RH Mapping**



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

Radiation Hybrids for Fine Mapping



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

http://wge.ndsu.nodak.edu/wheatzapper/

Wheat Germplasm Enhancement NDSU

Alnilmer et al. submitted

http://wge.ndsu.nodak.edu/wheatzapper/

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Job ID: 911

Get Table | Download EST Sequences | Find Exon/Intron | Design Primers | Pretty Picture

	Query	Wheat EST	Rice Gene	Rice Start	Rice End	Brachy Gene	Brachy Start	Brachy End	Sorghum Gene	Sorghum Start	Sorghum End	Pfam Description
☐	**TC388800**	**TC453202**	**LOC_Os10g37480**	**19989644**	**19998510**	**Bradi3g31110**	**33263613**	**33272546**	**Sb01g016990**	**17179375**	**17194939**	**STE_PAK_Ste20_STLK.6 - STE kinases include homologs to sterile 7, sterile 11 and sterile 20 from yeast, expressed**
☐	-	TC450049	-	-	-	Bradi3g31110	33263613	33272546	Sb01g016990	17179375	17194939	Protein kinase domain
☐	-	CD894600	LOC_Os10g37430	19960244	19965341	Bradi3g31090	33239683	33246697	Sb01g017010	17230294	17235698	expressed protein
☐	-	TC403732	LOC_Os10g37420	19957959	19957417	Bradi3g30890	33137031	33139616	Sb01g017020	17236581	17239126	cytochrome b5-like Heme/Steroid binding domain containing protein, expressed
☐	**TC406578**	**TC406578**	**LOC_Os10g37410**	**19953587**	**19956560**	**Bradi3g30880**	**33133705**	**33136872**	**Sb01g017030**	**17239543**	**17242458**	**TBC domain containing protein, expressed**

Predicts “non-COS”

Pfam description

Alnilmer et al. *submitted*

http://wge.ndsu.nodak.edu/wheatzapper/

Wheat Germplasm Enhancement NDSU

Downstream applications

CDS prediction

Markers development

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	Query	Wheat EST	Rice Gene	Rice Start	Rice End	Brachy Gene	Brachy Start	Brachy End	Sorghum Gene	Sorghum Start	Sorghum End	Pfam Description
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Alnilmer et al. *submitted*

http://wge.ndsu.nodak.edu/wheatzapper/

Wheat Germplasm Enhancement NDSU

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Job ID: 911

Get Table | Download EST Sequences | Find Exon/Intron | Design Primers | Pretty Picture

	Query	Wheat EST	Rice Gene	Rice Start	Rice End	Brachy Gene	Brachy Start	Brachy End	Sorghum Gene	Sorghum Start	Sorghum End	Pfam Description
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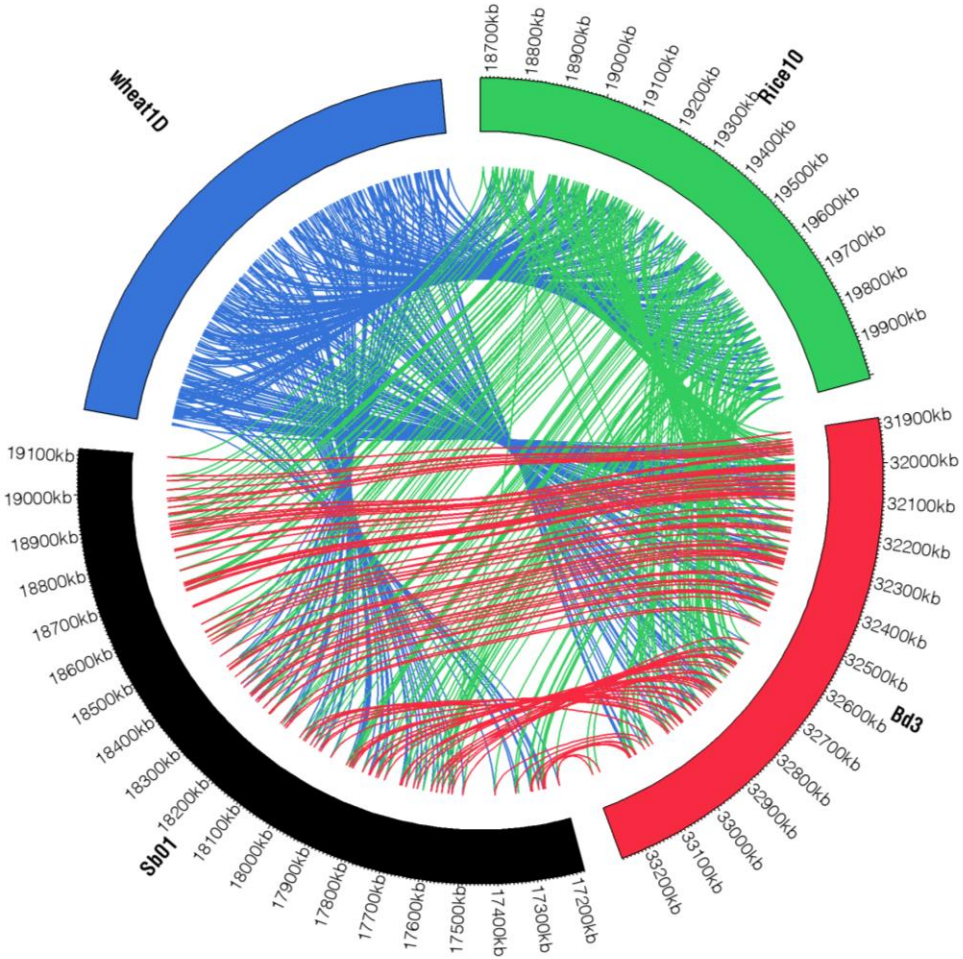
Alnilmer et al. *submitted*

Wheat Zapper + Circos 0.53



Krzywinski et al., 2009. *Genome Res 18:doi:10.1101;* ***Alnilmer et al.*** *submitted*

Paleofusion

Ta1D

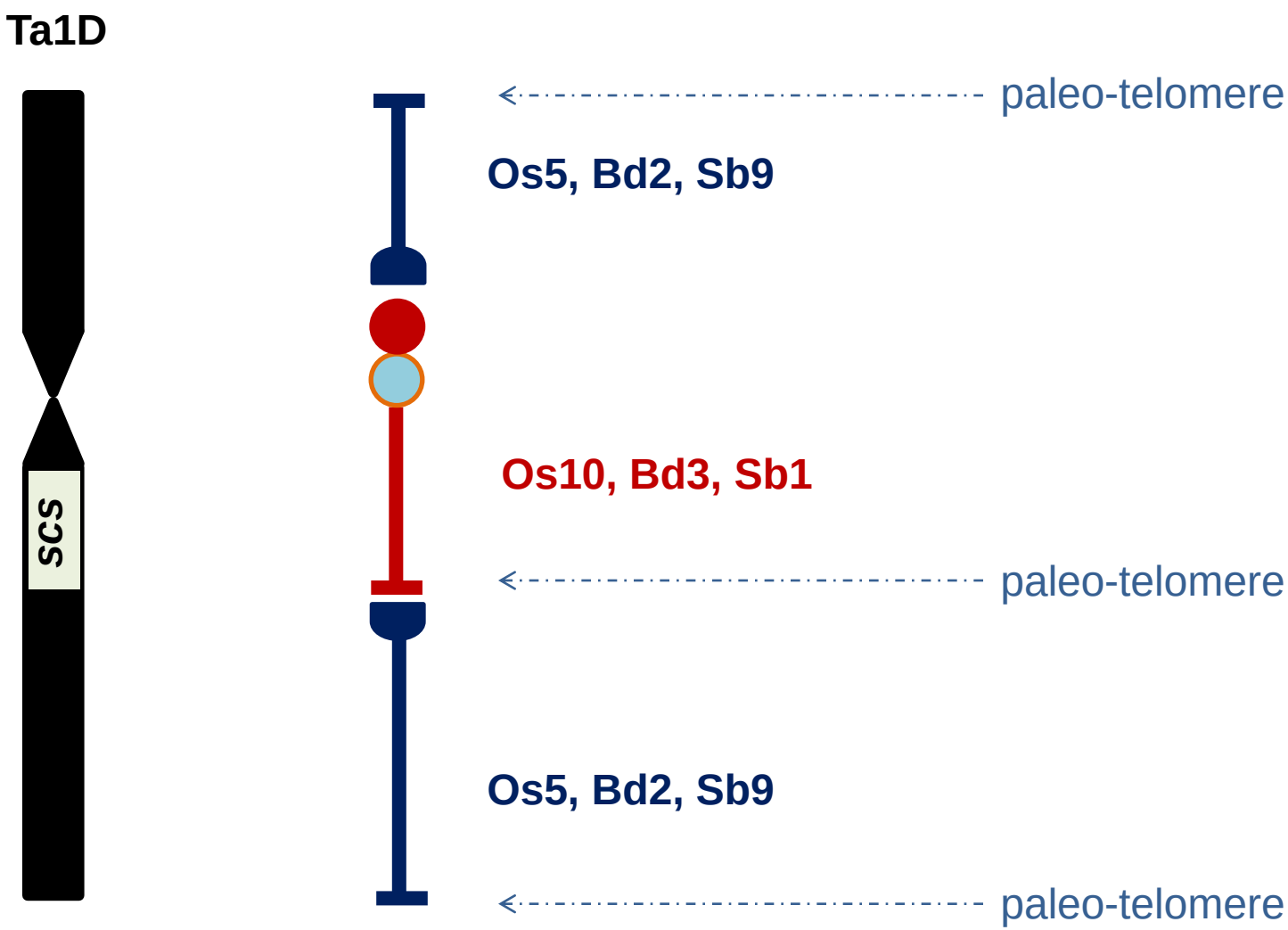
Os5, Bd2, Sb9

Os10, Bd3, Sb1

Os5, Bd2, Sb9

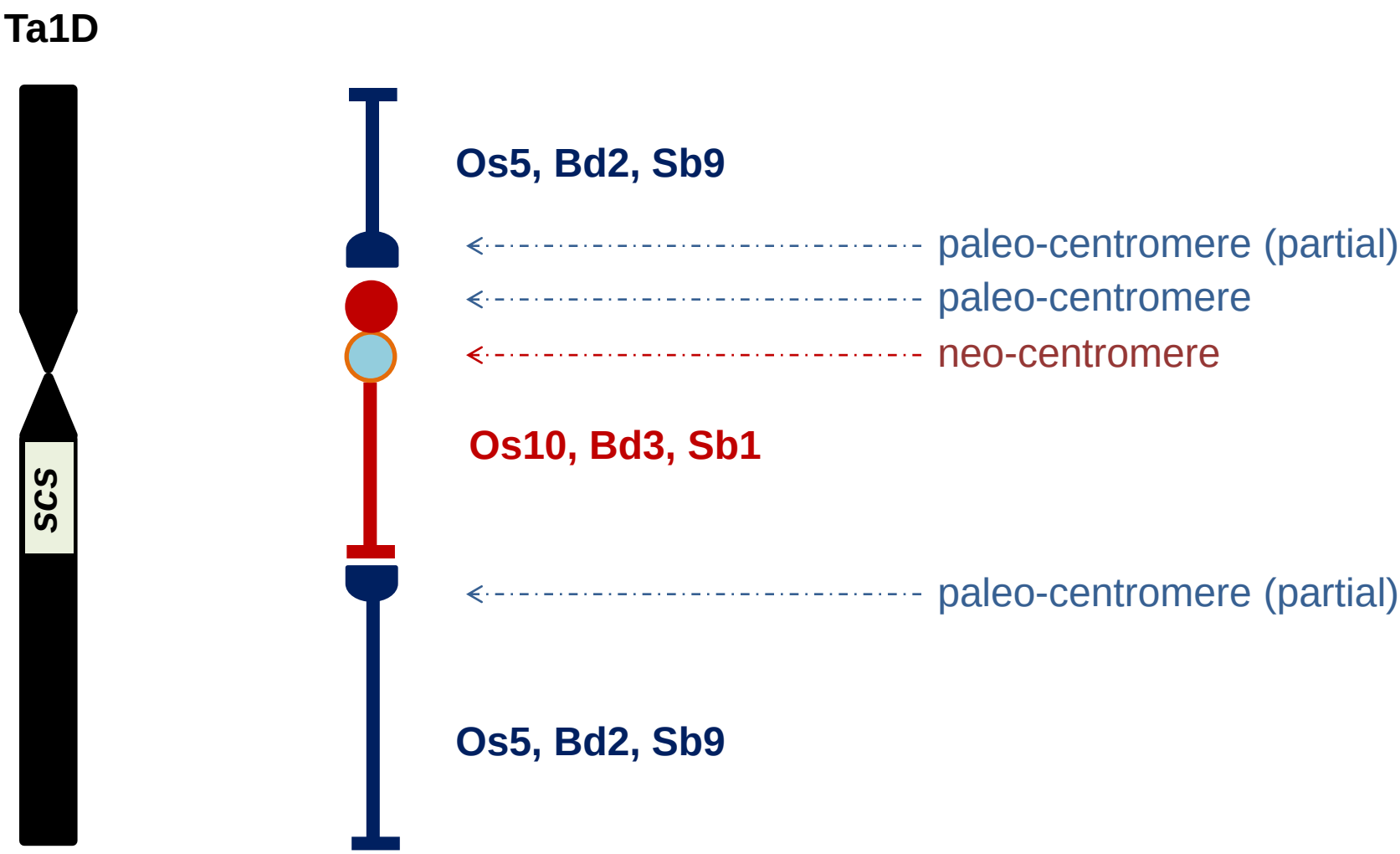
Michalack et al. *submitted;* ***Luo et al., 2009*** *PNAS 106:15780-15785*

Paleofusion



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

Paleofusion



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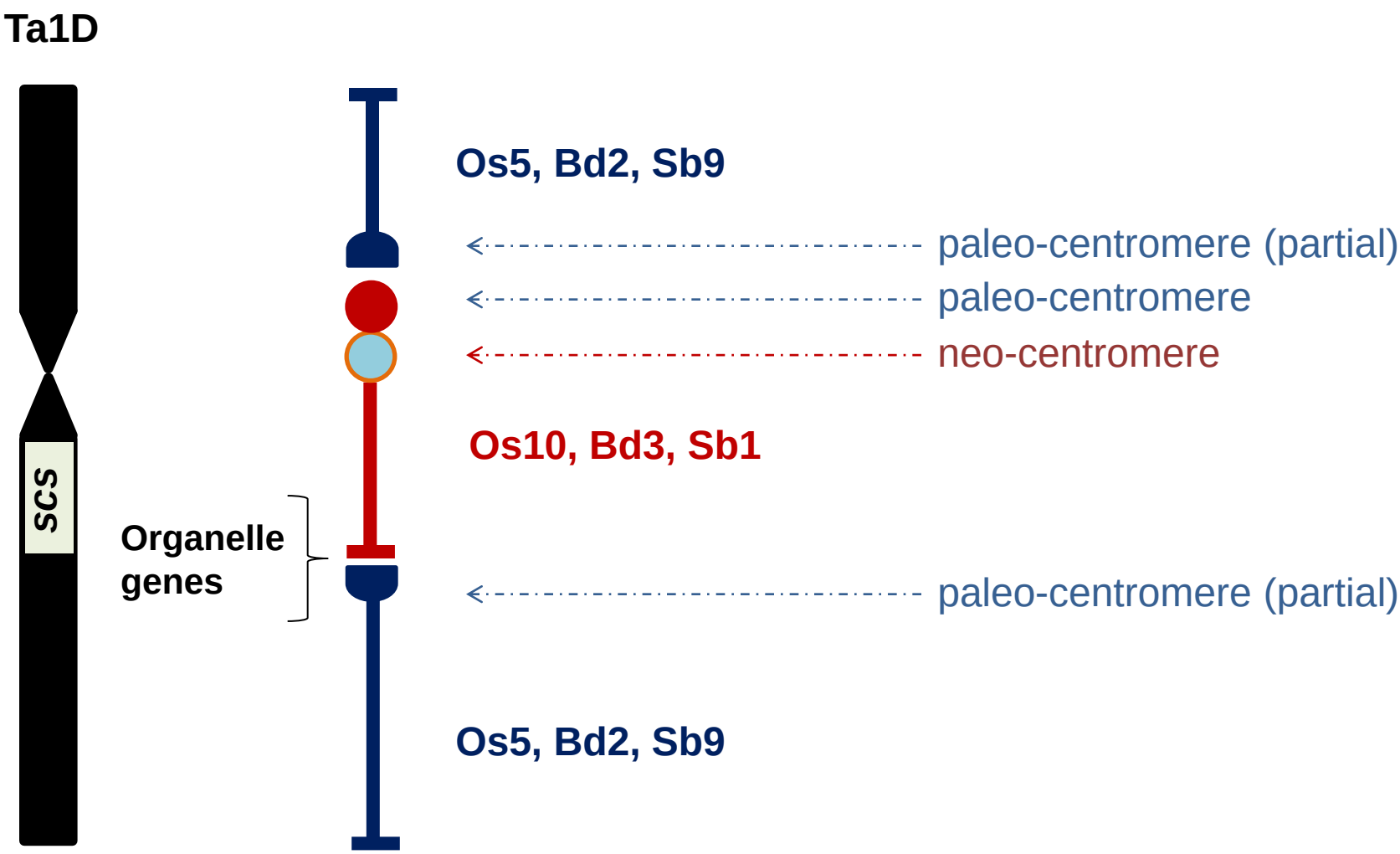
Paleofusion



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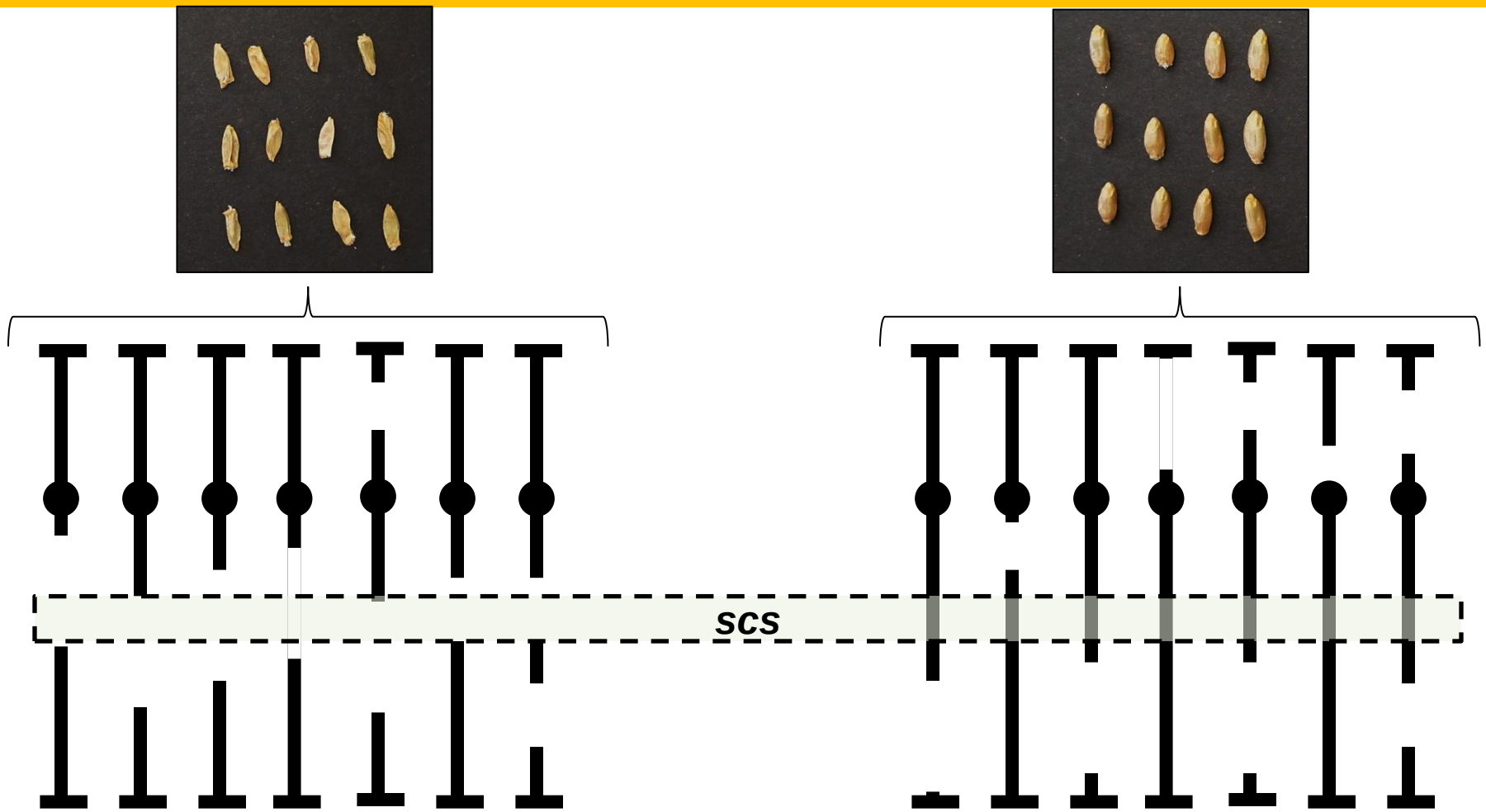
Illumina Bulk Segregant Analysis

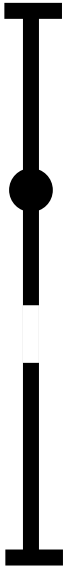


Illumina Bulk Segregant Analysis

Genome complexity reduction libraries:

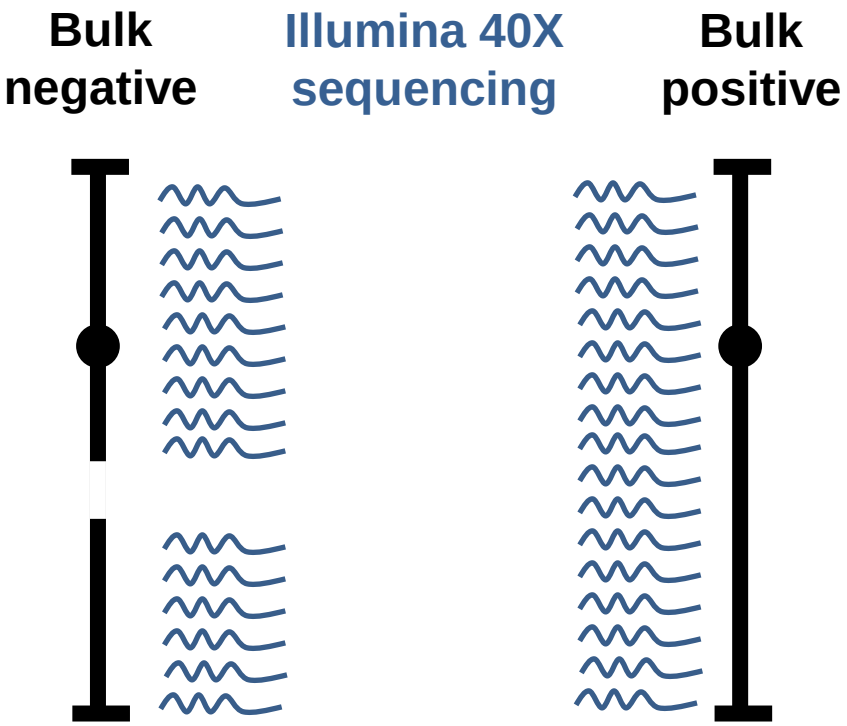
- ***PstI* + sonication**
- ***AatII* + sonication**

Bulk negative



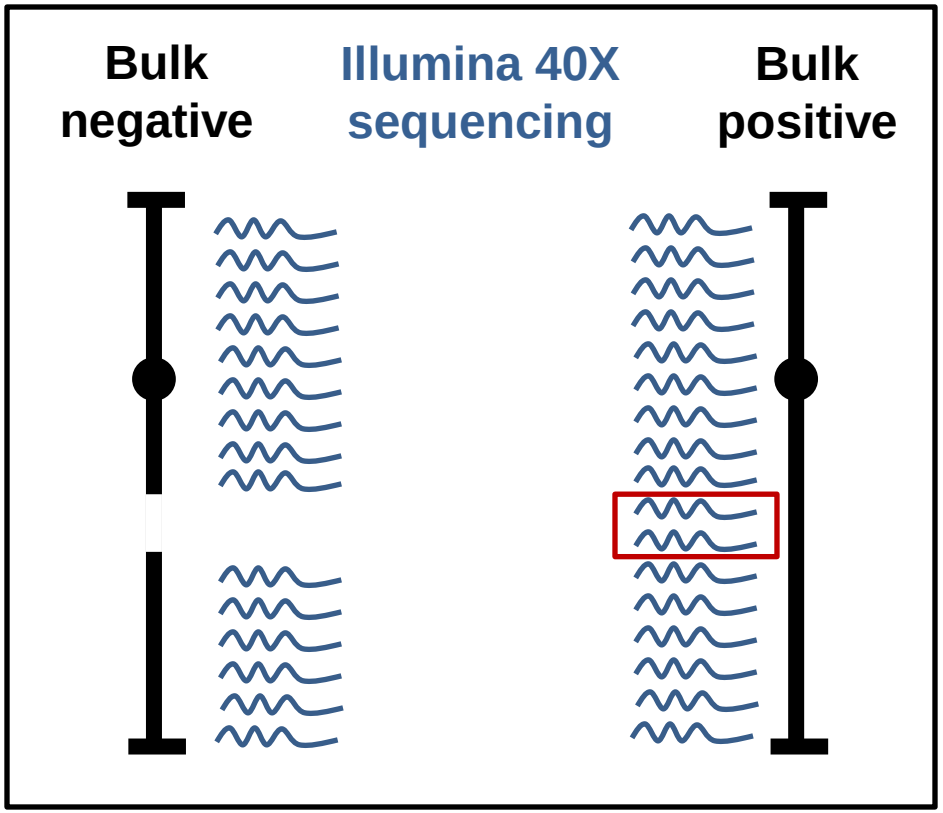
Bulk positive

Illumina Bulk Segregant Analysis

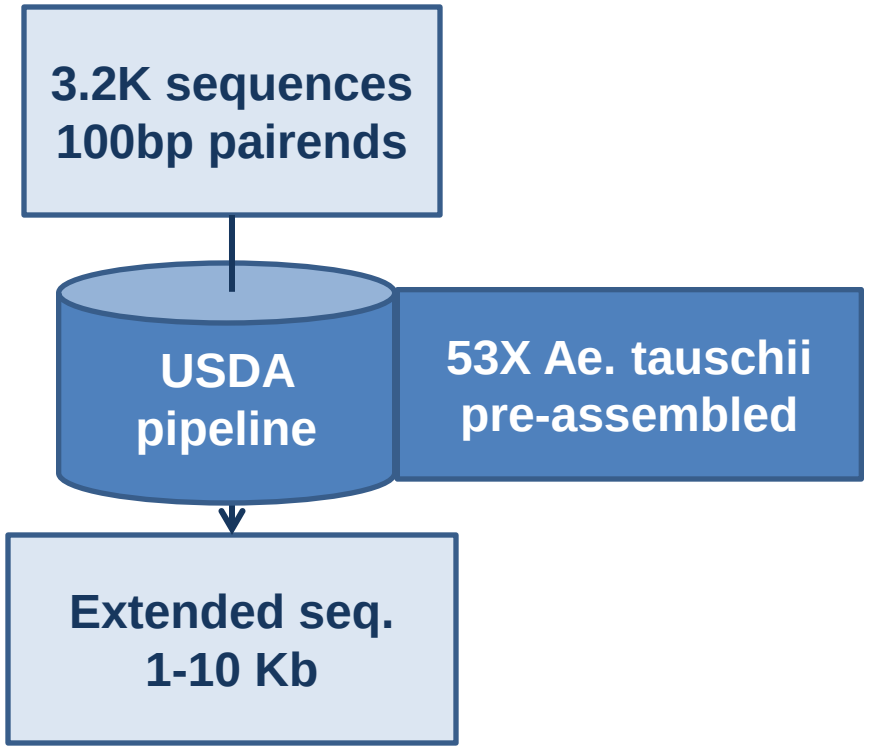


Illumina Bulk Segregant Analysis

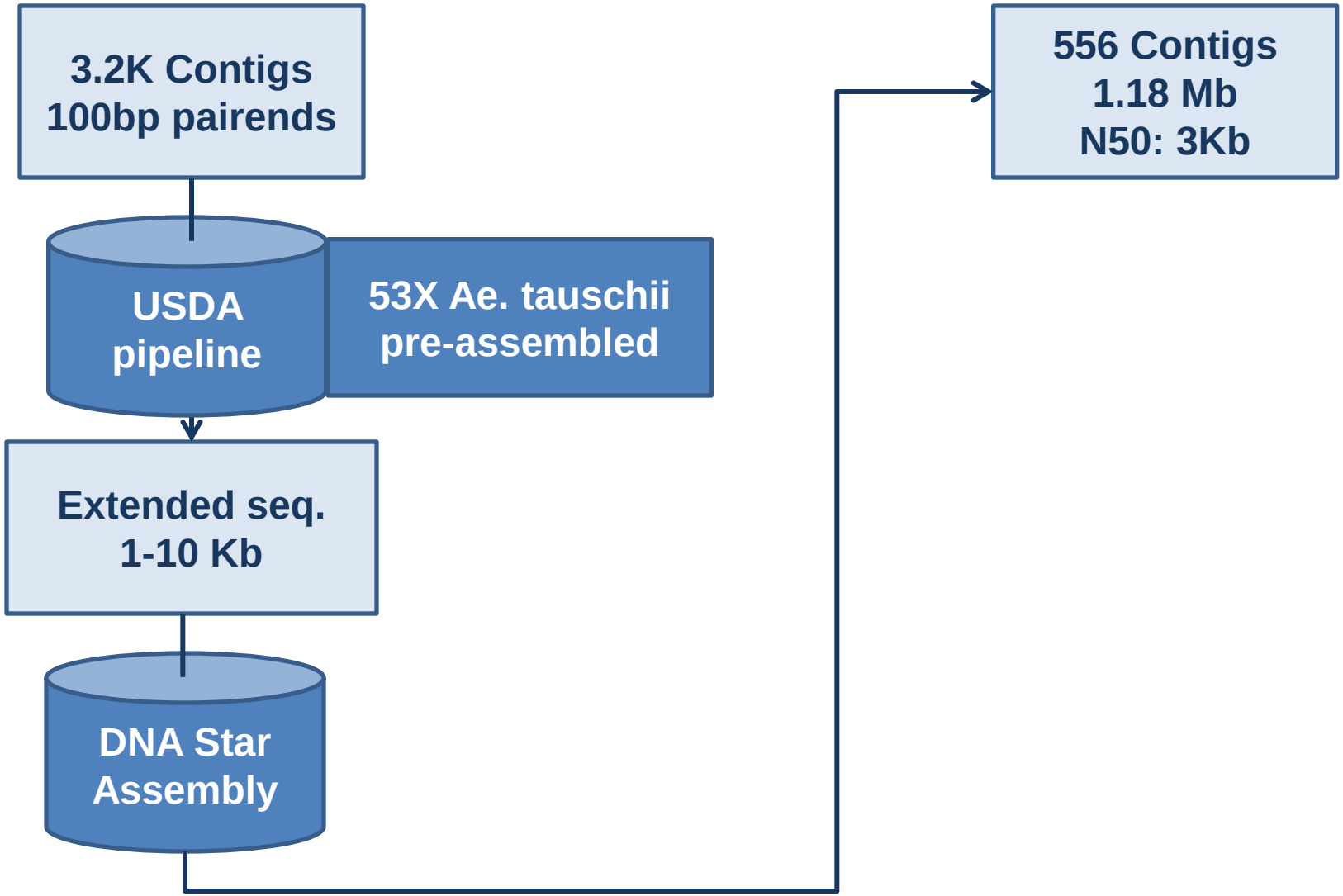
- 100 M good quality reads
- 20 Gb pair-end sequence
- 1 M digestion sites
- 3.2 K unique sequences



Assembly


3.2K sequences
100bp pairends
USDA
pipeline
53X Ae. tauschii
pre-assembled
Extended seq.
1-10 Kb

Assembly


3.2K Contigs
100bp pairends
USDA pipeline
53X Ae. tauschii pre-assembled
Extended seq.
1-10 Kb
DNA Star Assembly
556 Contigs
1.18 Mb
N50: 3Kb

Assembly

DNA Star

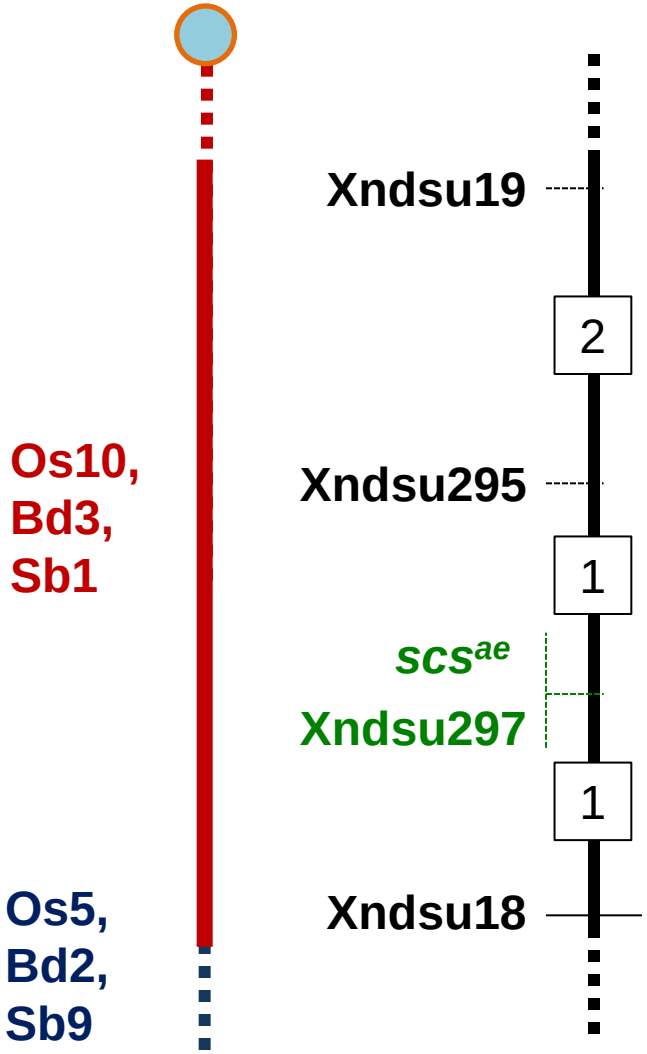
556 Contigs
1.18 Mb
N50: 3Kb

Wheat Zapper

18 contigs
30 genes
Synteny sorted

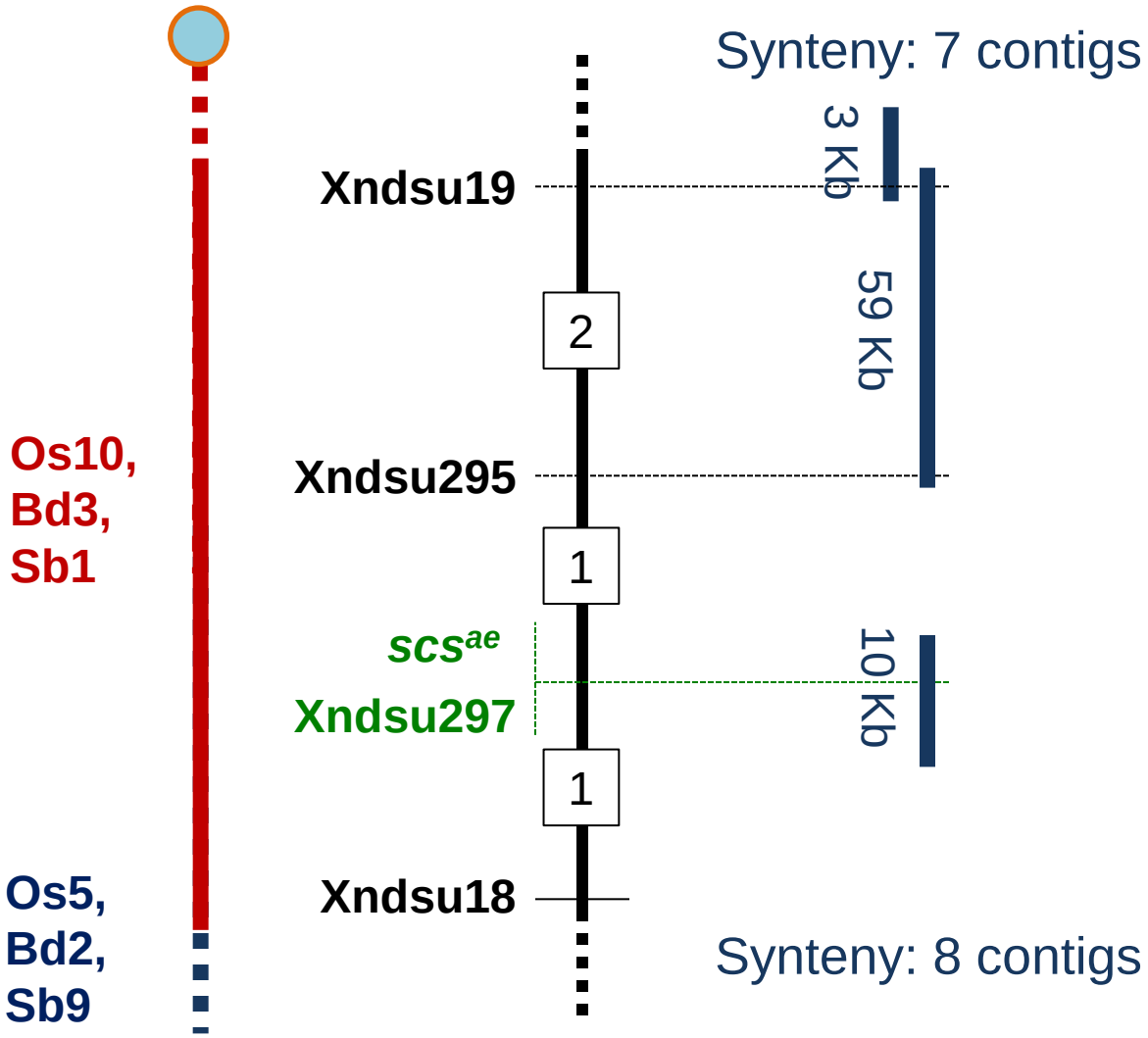
Map contigs on RH

The *scs* region

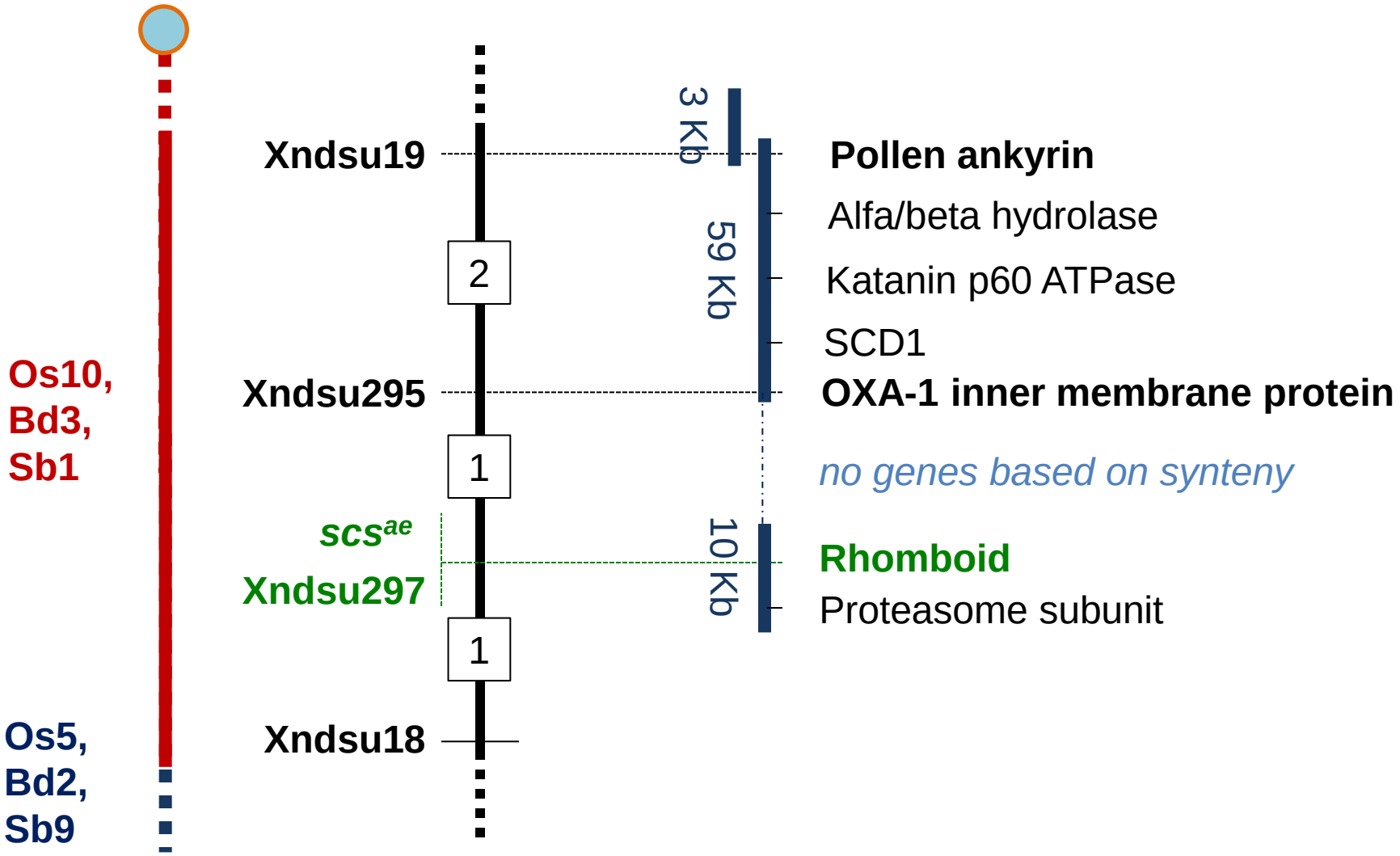


- 450 RH-1D searched

The *scs* region



The *scs* region



A candidate for *scs*

Wheat Germplasm Enhancement NDSU

Rhomboid proteins: conserved membrane proteases with divergent biological functions

Sinisa Urban

Genes Dev. 2006 20: 3054-3068
Access the most recent version at doi:10.1101/gad.1488606

tein ring. A thorough consideration of all known examples of rhomboid function suggests that, despite biochemical similarity in mechanism and specificity, rhomboid proteins function in diverse processes including quorum sensing in bacteria, mitochondrial membrane fusion, apoptosis, and stem cell differentiation in eukaryotes; rhomboid proteins are also now starting to be linked to human disease, including early-onset blindness, diabetes, and parasitic diseases. Regulating cell signaling is at the heart of rhomboid protein function in many, but not all, of these processes. Further study of

animal kingdom but are not found in other kingdoms, including plants, perhaps because plants and animals evolved multicellular development after splitting from a common ancestor (Meyerowitz 2002). But in recent years a small number of factors have been discovered that are conserved across kingdoms, raising the exciting possibility that they are key regulators that evolved very early. One such class of regulatory proteins are intramembrane proteases.

Intramembrane proteolysis, the cleavage of membrane proteins within their membrane spanning segments, has

Genomic sequencing of Rhomboid

- **Primers designed on Illumina contig**

  220 bp upstream
  390 bp downstream

  total: 2,048 bp

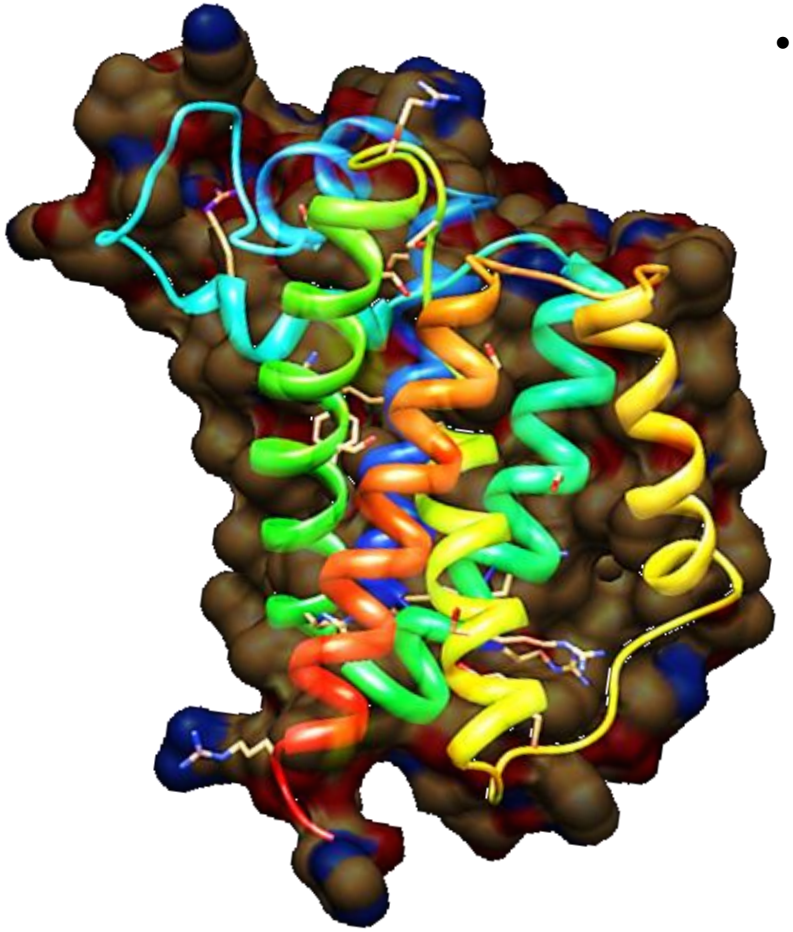
  TriAnnot: 984 bp CDS, 5 exons

Genomic sequencing of Rhomboid

- **Functional** **1 SNP** **Lys to Arg**

Ae. Tauschii	TTCATCA**G**AG
"Scs ^{ae}"	TTCATCA**G**AG
"Scs ^{ti}"	TTCATCA**G**AG
"Scs ^{ti}"	TTCATCA**G**AG
Chromosome 1A	TTCATCA**A**AG
Chromosome 1B	TTCATCA**A**AG
"Δ Scs ^{ti}"	TTCATCA**A**AG
"Δ Scs ^{ti}"	TTCATCA**A**AG

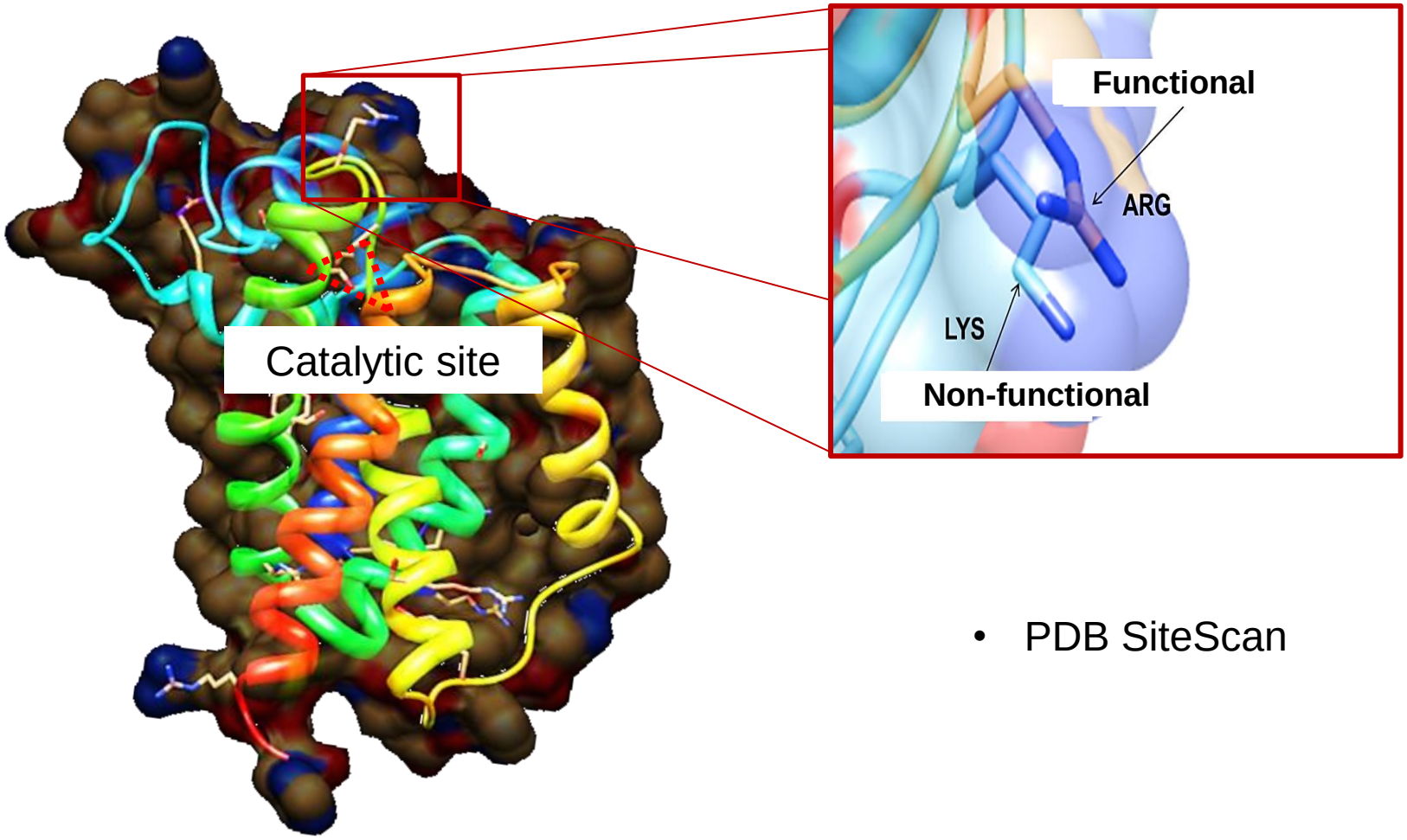
Protein model for Rhomboid



- ESyPred3D on 2NR9 crystal structure

  6 transmembrane helixes

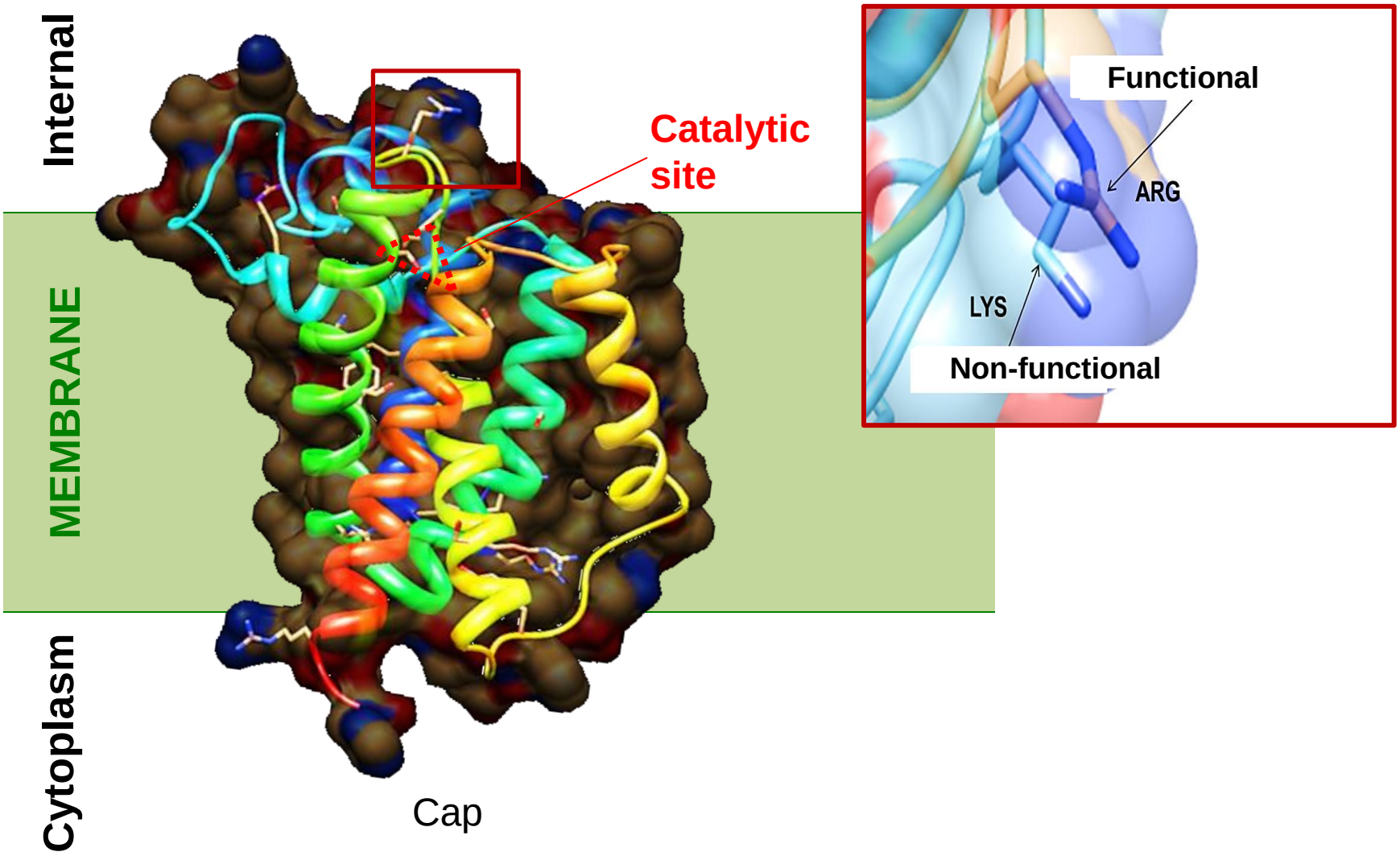
Protein model for Rhomboid



Cap

- PDB SiteScan

Protein model for Rhomboid



Conclusions: re-discovery of hot water

- **Developing the best biological material for the task**

  Combination of Illumina BSA and RH very effective

- **WheatZapper is valuable for fine mapping initiatives**

- **Very important to work with bio-informaticians**

- **Cloning of the *scs* gene:**

  70 Kb region
  6 (2) genes
  Unable to DIS-proof the Rhomboid as candidate

Acknowledgments

Wheat Germplasm Enhancement NDSU

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

Monsanto's Beachell-Borlaug International Scholars Program

MASTER and BACK
REGIONE AUTONOMA DELLA SARDEGNA

… come visit again

THANK YOU

avena.pw.usda.gov/RHmapping/