Exploitation of Wheat Genomic Resources and Collaborations to Rapidly Clone Multiple Disease Susceptibility Genes

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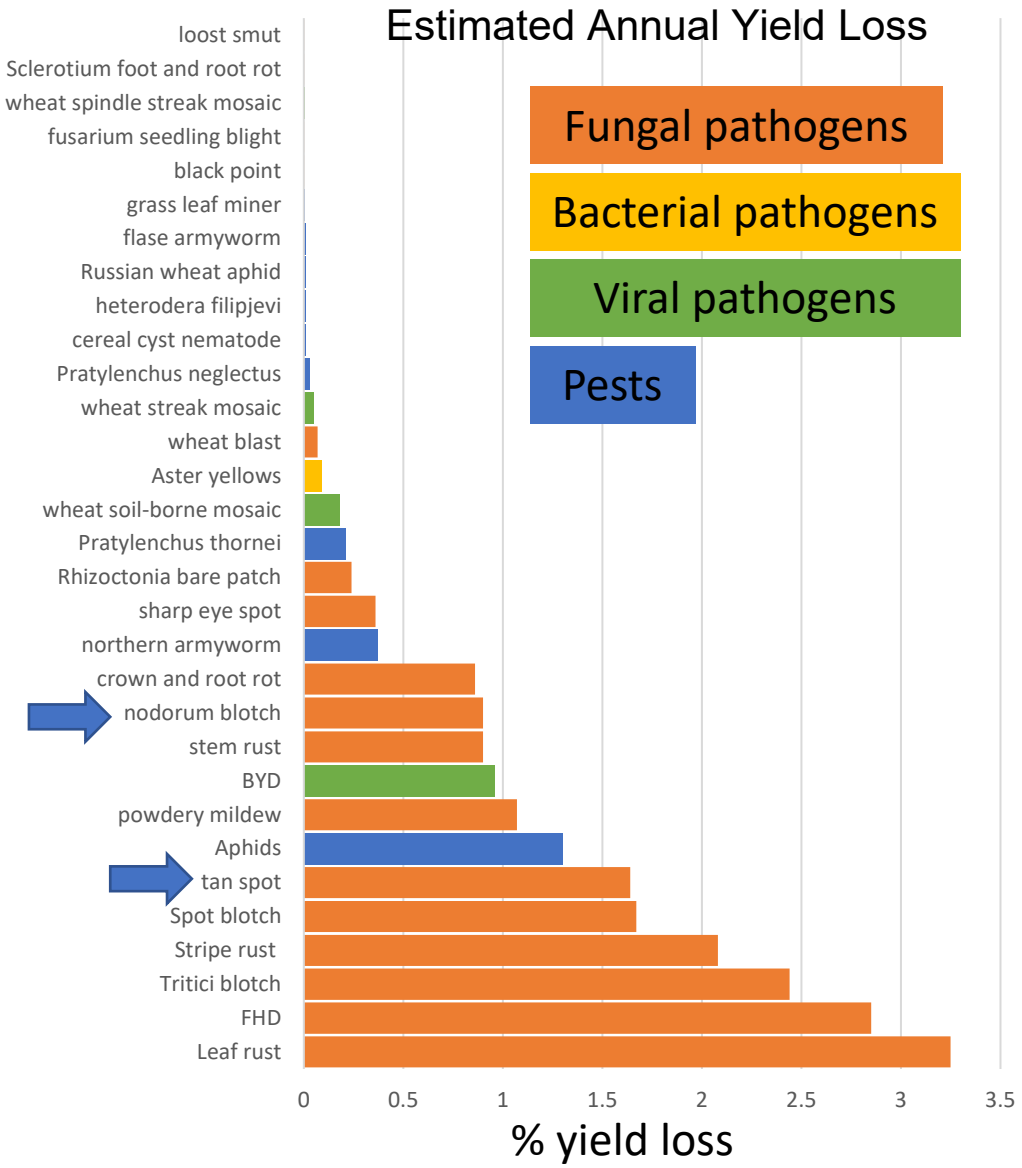
Katherine Running and Justin Faris

Yield loss due to pathogens and pests

775 million tonnes

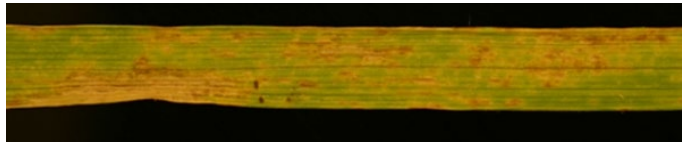
21.5% yield loss



Savary et al. 2019

septoria nodorum blotch and tan spot

Parastagonospora nodorum → Septoria nodorum blotch

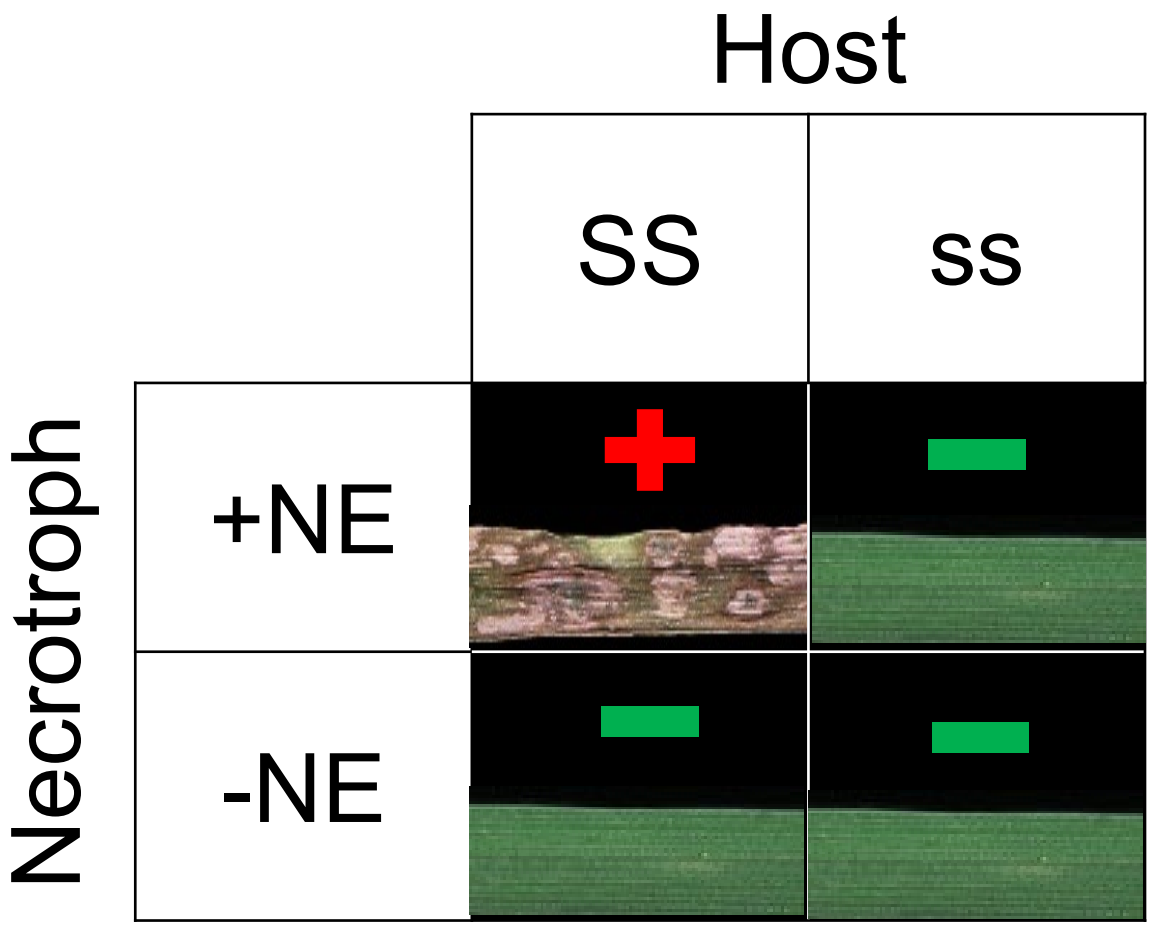


Pyrenophora tritici repentis → Tan spot

infection → Cell death → ↓ photosynthesis → ↓ yield

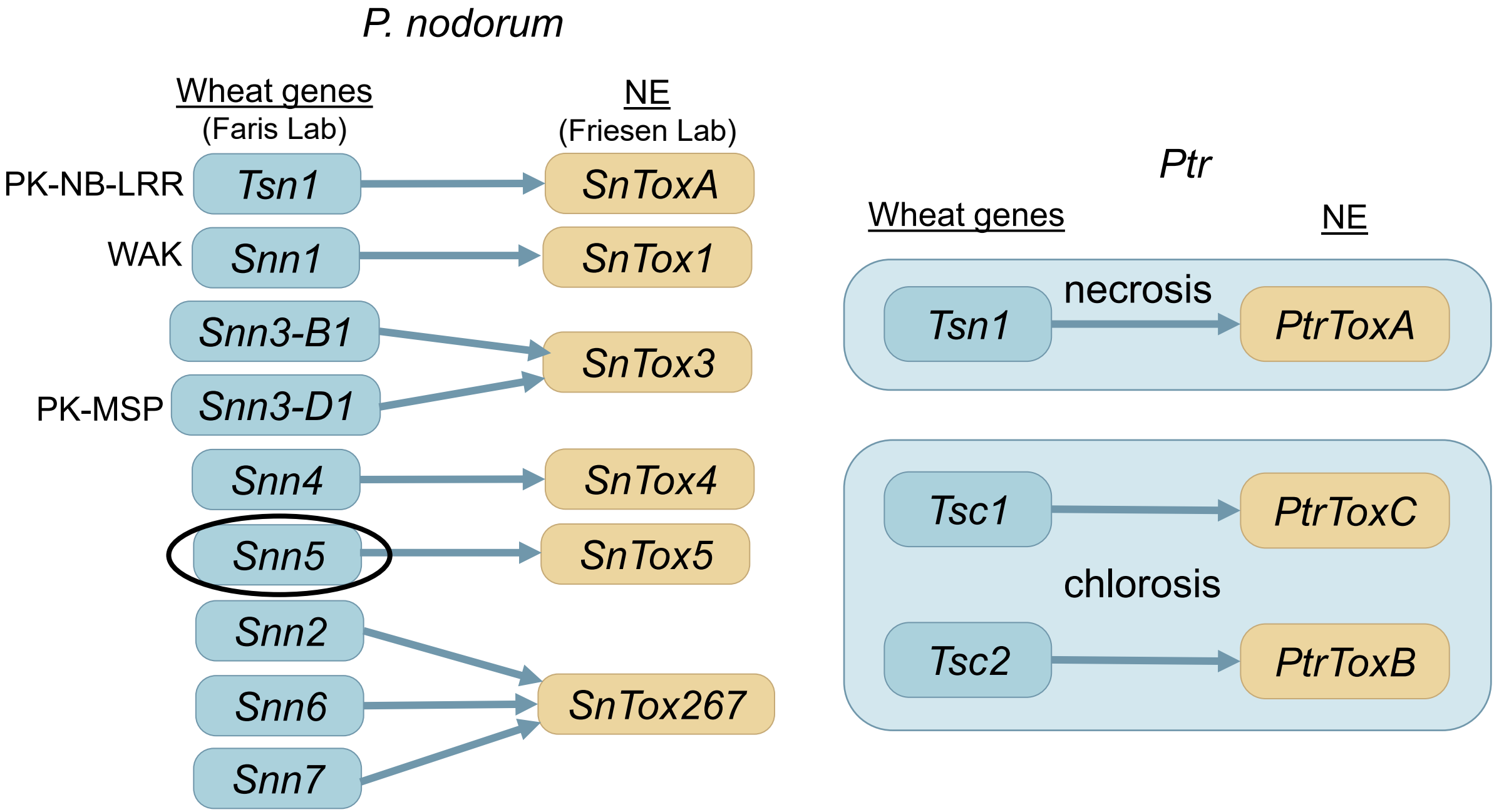
necrotrophic effector + dominant host susceptibility gene → susceptibility

Inverse gene-for-gene interaction



Clone susceptibility genes, design markers to <u>eliminate susceptibility genes</u>

genetically resistant wheat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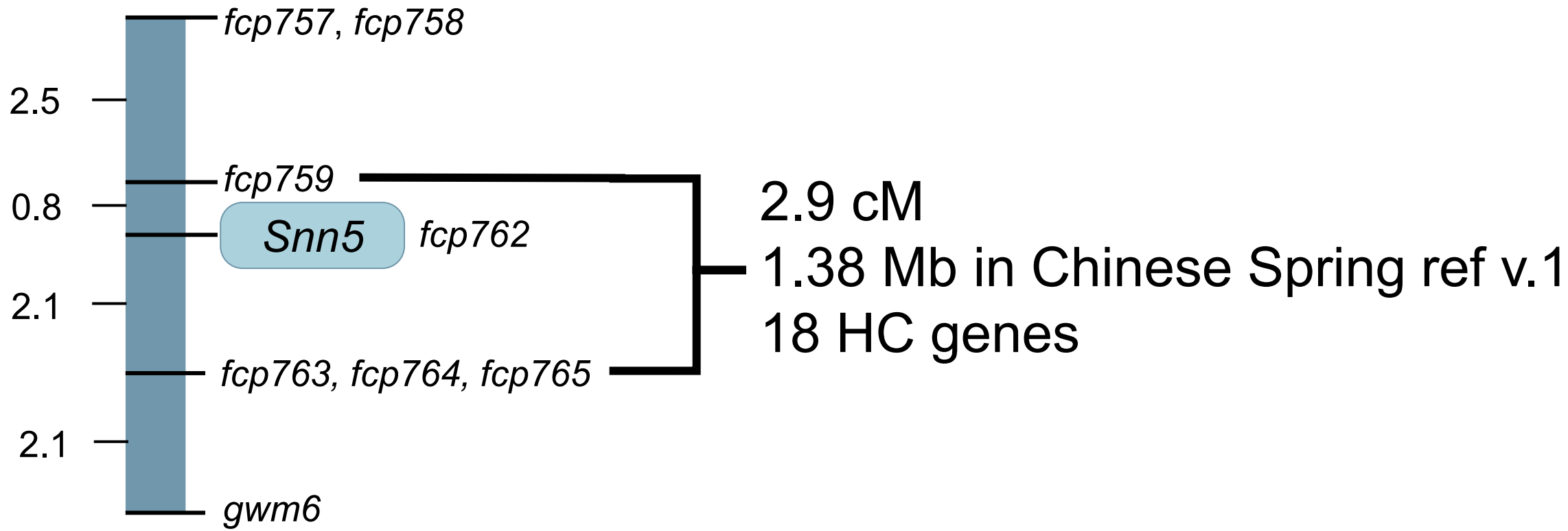
Genetic mapping of *Snn5*

Chromosome 4B



Sapna Sharma

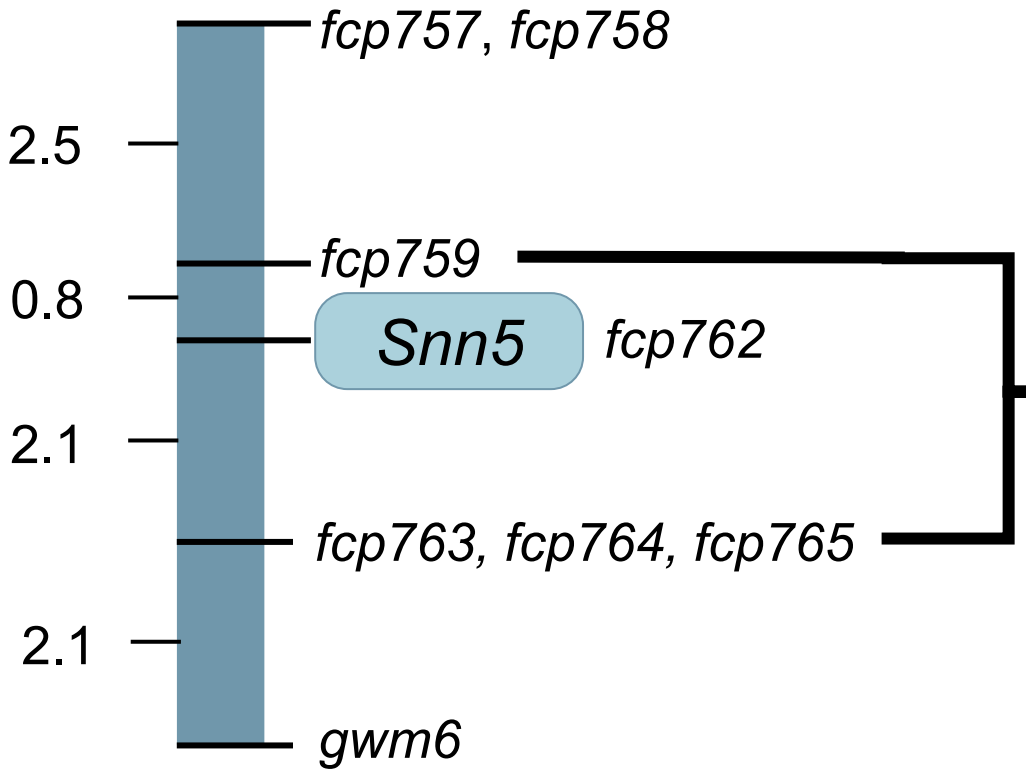
Genetic mapping of *Snn5*

Jonathan Richards

SnTox5 structurally similar to SnTox3

Are *Snn3-D1* and *Snn5* similar?

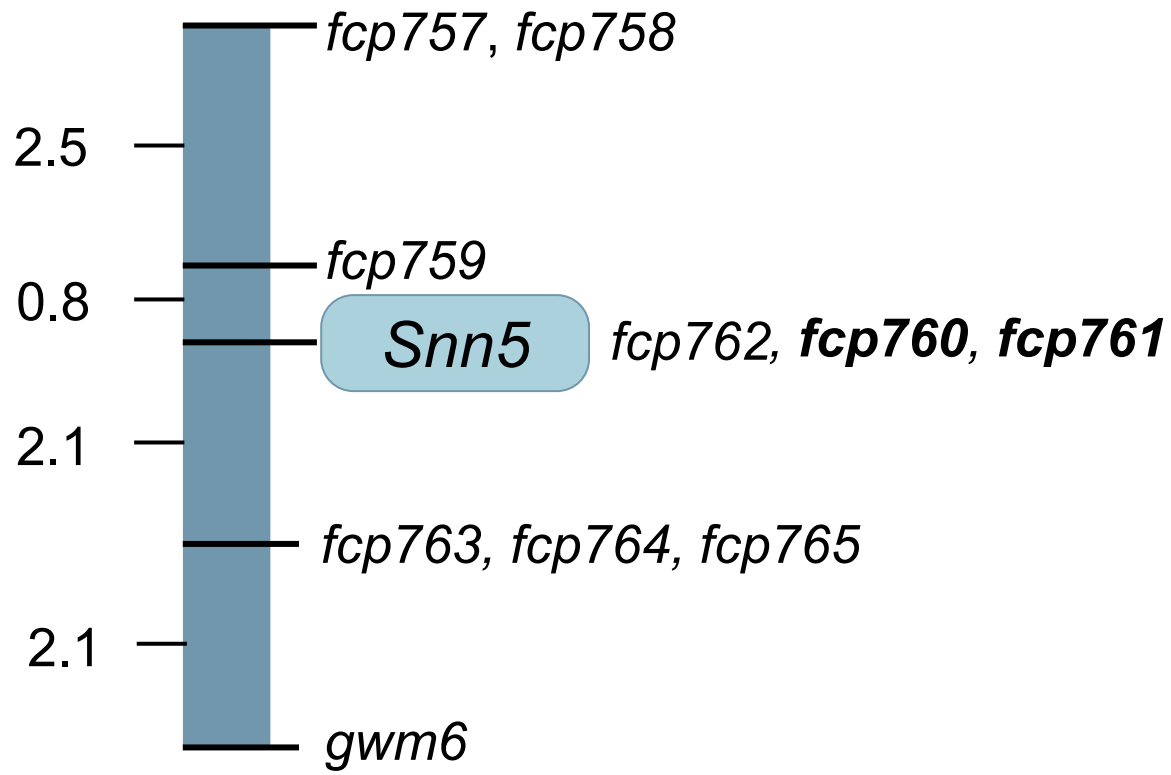
Chromosome 4B



Sapna Sharma

Genetic mapping of *Snn5*

Chromosome 4B



Sapna Sharma

Are *Snn3-D1* and *Snn5* similar?

- Blast search of *Snn3-D1* against *Snn5* candidate region
- Found two genes with PK and MSP domains

Validating *Snn5* using Cadenza TILLING

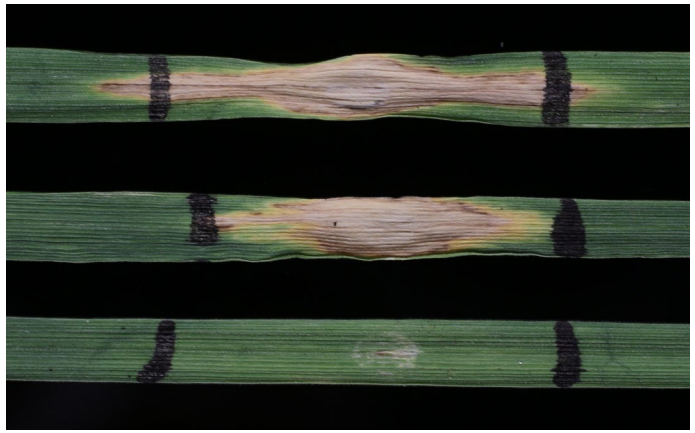
Uncovering hidden variation in polyploid wheat

Krasileva et al. 2017

Chinese Spring (*Snn5*)

Cadenza (*Snn5*)

PI 94749 (*snn5*)

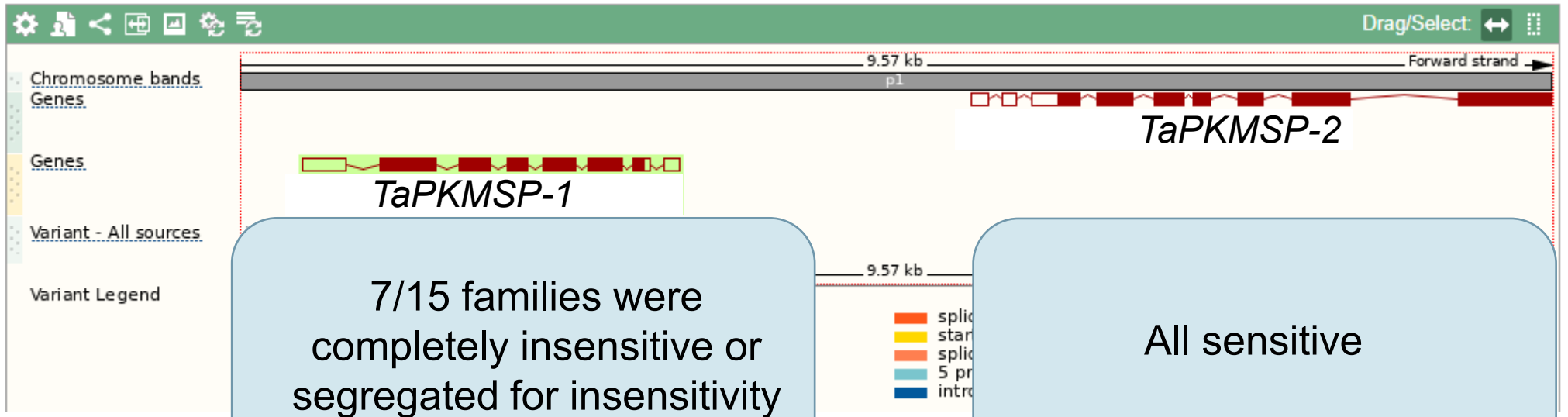


Bread wheat ‘Cadenza’ – 1200 EMS mutants

Durum wheat ‘Kronos’ -1535 EMS mutants

Can browse TILLING mutants on Ensembl and order from seedstor websites

Validating *Snn5*



7/15 families were completely insensitive or segregated for insensitivity

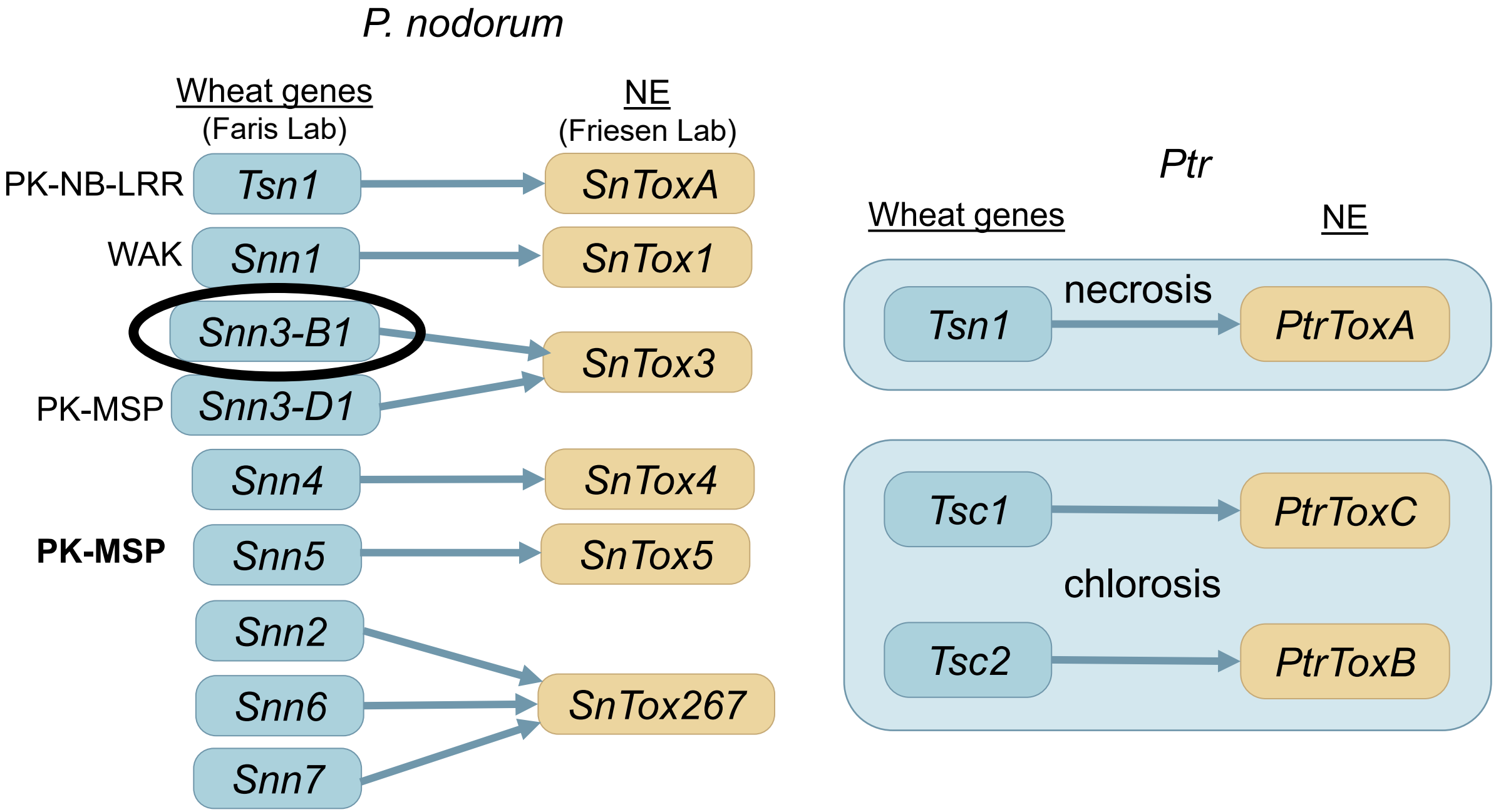
All sensitive

Additional validation

5 independent EMS mutants in ‘LP29’

Snigdha Poddar

5 independent Cas9-RNP mediated gene knockouts in ‘Fielder’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Wheat genes
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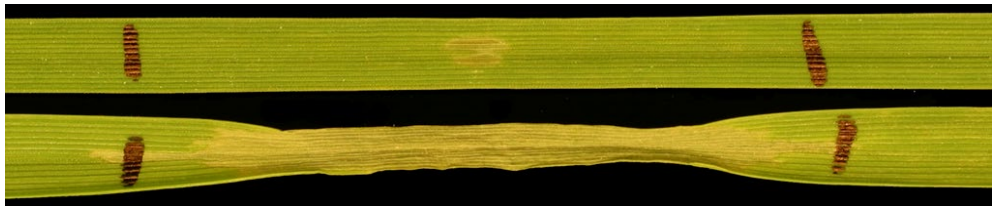
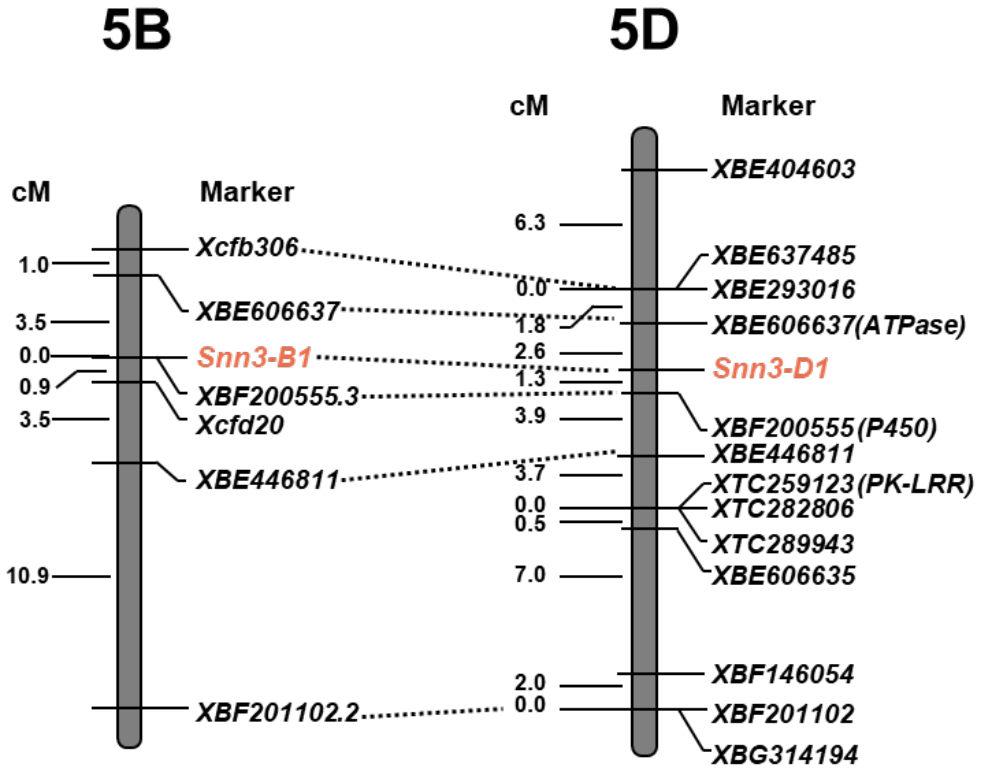
Snn3-B1

Kronos

Cadenza





- We can use Cadenza TILLING for *Snn3-B1*
- Have genetic map of *Snn3-B1* region, know where to look for candidates
- Suspect *Snn3-B1* is homoeologous to *Snn3-D1*

Zengcui Zhang

Validating *Snn3-B1*

- 9/13 Cadenza TILLING families insensitive or segregating
  - *Snn3-D1* homoeolog on chromosome 5B = *Snn3-B1*
  - 3 months from finding out Cadenza was SnTox3 sensitive to cloning *Snn3-B1*
- Sumai3 EMS population
  - Sequenced *Snn3-B1*, NONE had mutations
  - Does Sumai3 have a different SnTox3 sensitivity gene?

Gongjun Shi

Zengcui Zhang

Allelism test

Sumai3 (*Snn3 ?*) × BG220 (*Snn3-B1*)

Screened 176 F_2
174 sensitive : 2 insensitive

Sumai3 has a different SnTox3 sensitivity gene

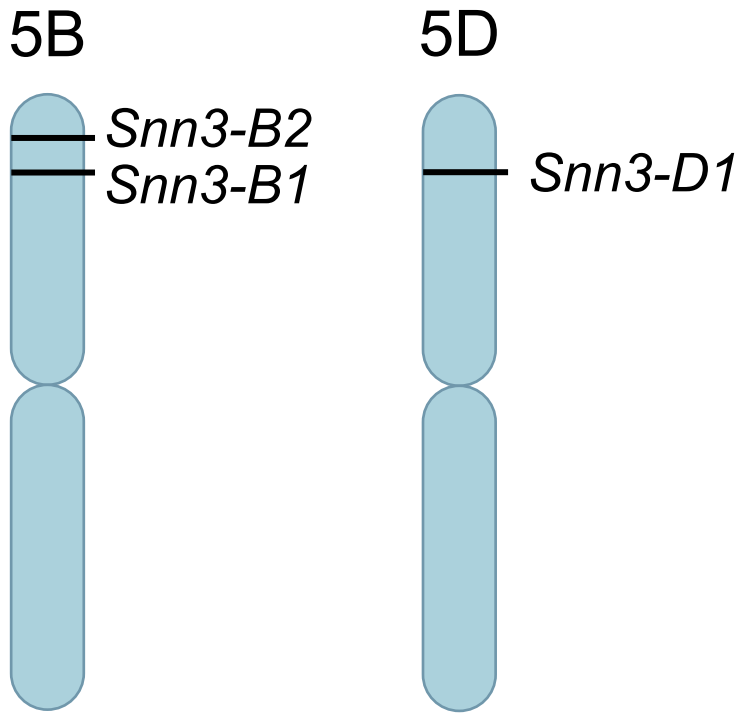
Which gene?

Zengcui Zhang

MutChromSeq

Jaroslav Doležel's Lab at The Institute of Experimental Botany

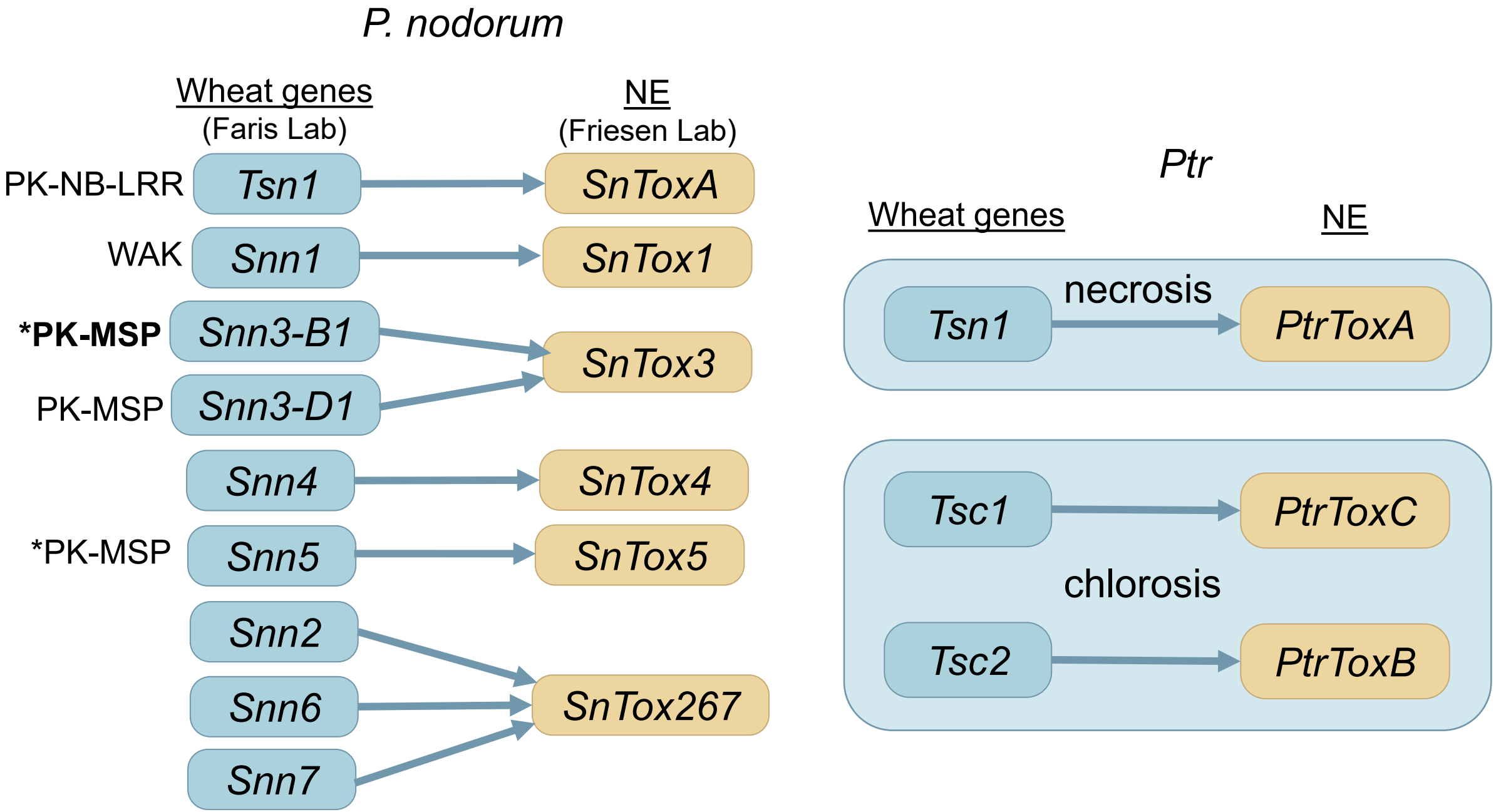
- Grow mutants, extract DNA
- Flow sort chromosome 5B
- Sequence wild type and mutants
- Assemble WT 5B scaffolds
- Map mutant reads to WT 5B scaffolds



All Sumai3 mutants had mutations in the same PK-MSP gene

Snn3-B2

Zengcui Zhang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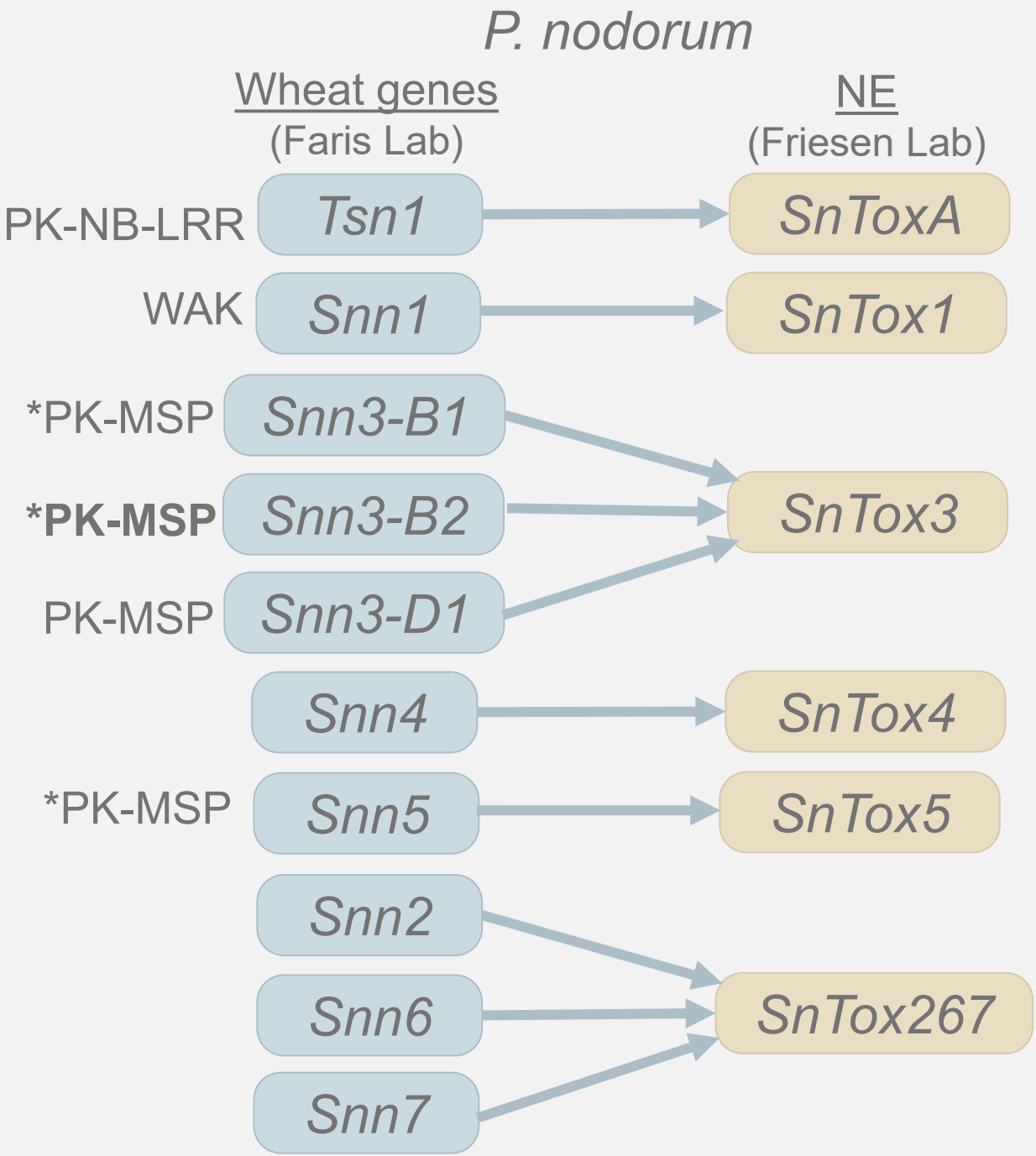
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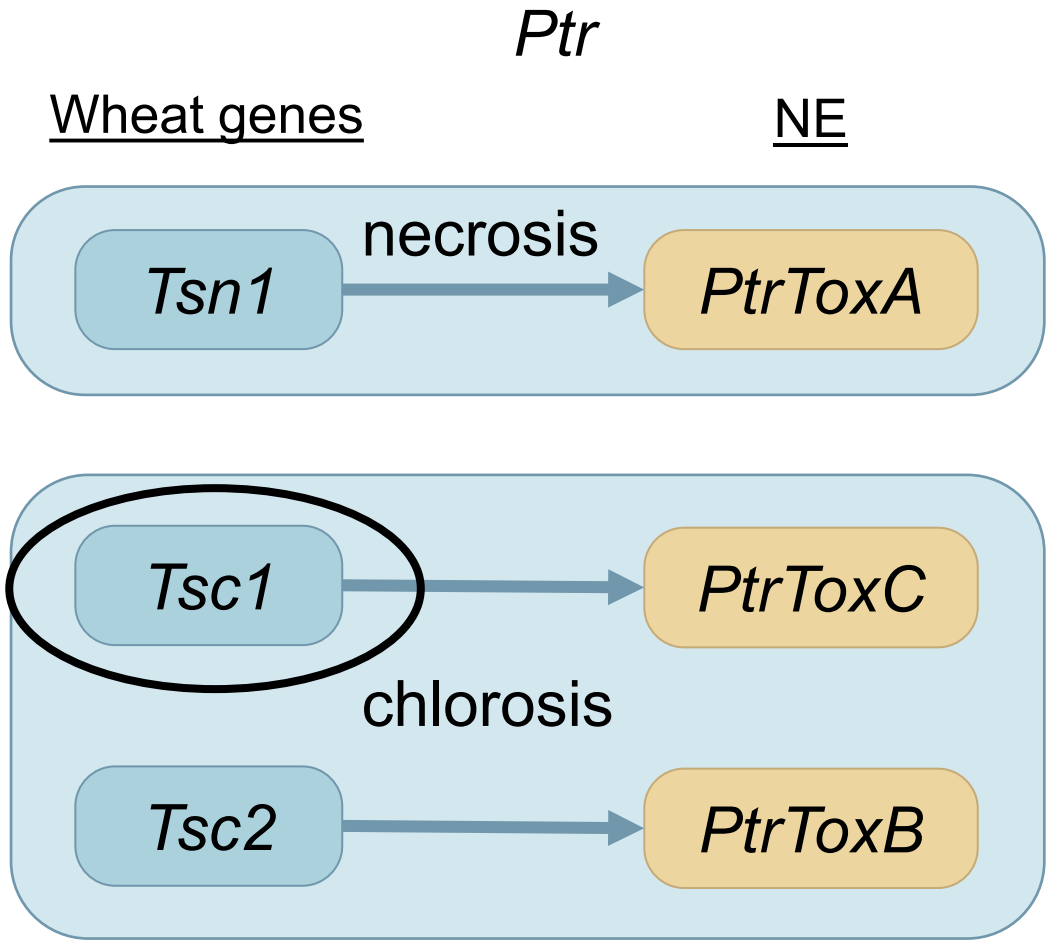
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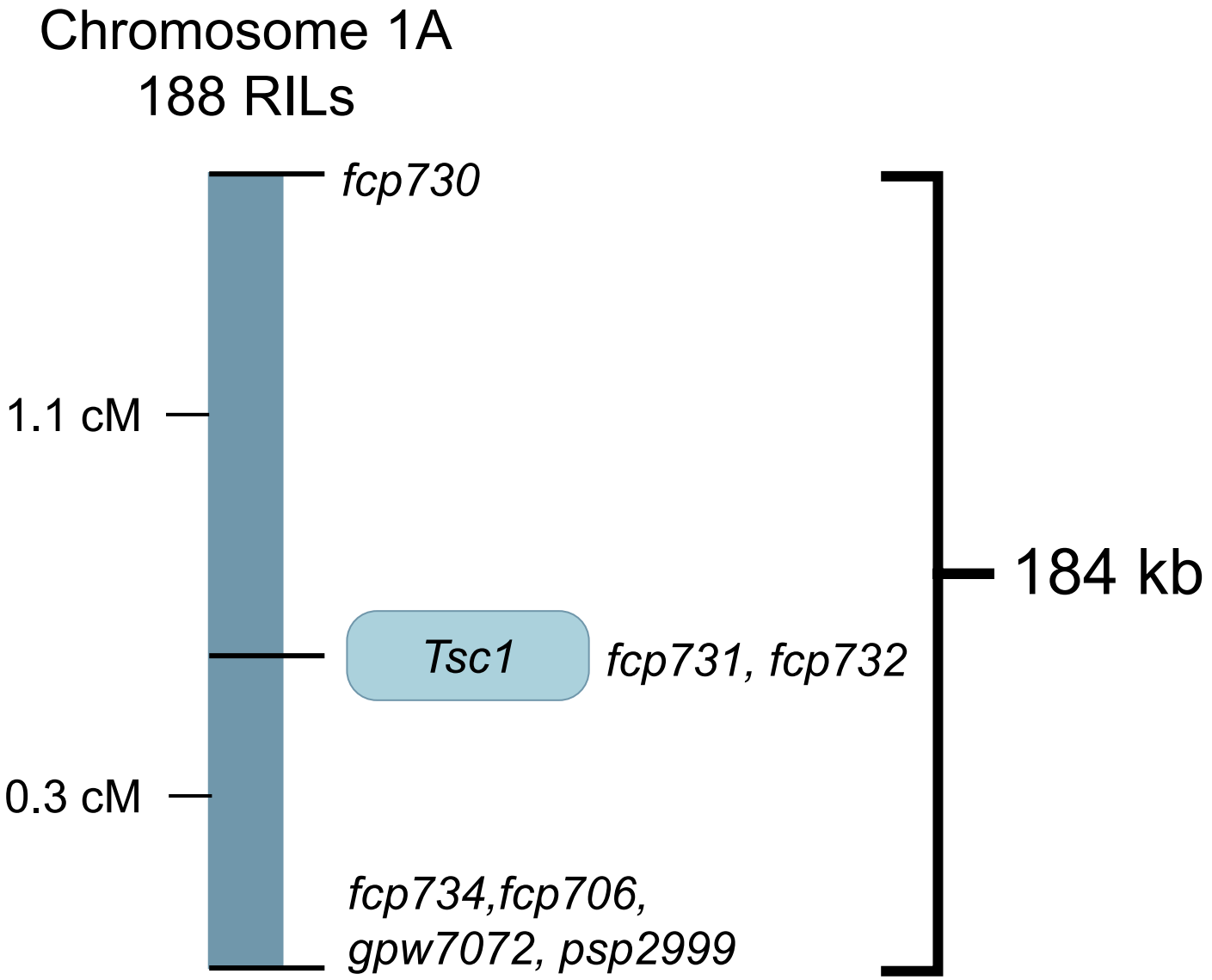
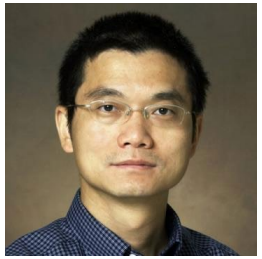
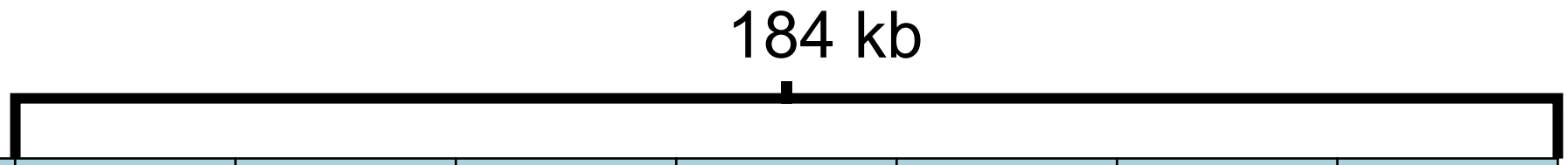


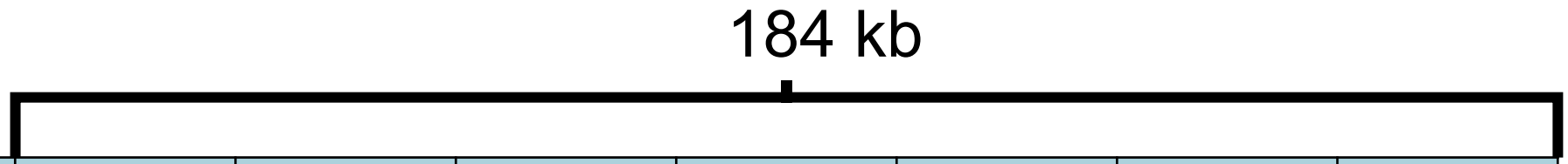


Zhaohui Liu

Aliya Momotaz



Genome	*Tsc1* allele	Gene 1	Gene 2	Gene 3	Gene 4	Gene 5	Gene 6	Gene 7
Chinese Spring	*Tsc1* -	+	+	+	+	+	+	+
CDC Landmark	*Tsc1* +	+	+	-	-	-	-	-



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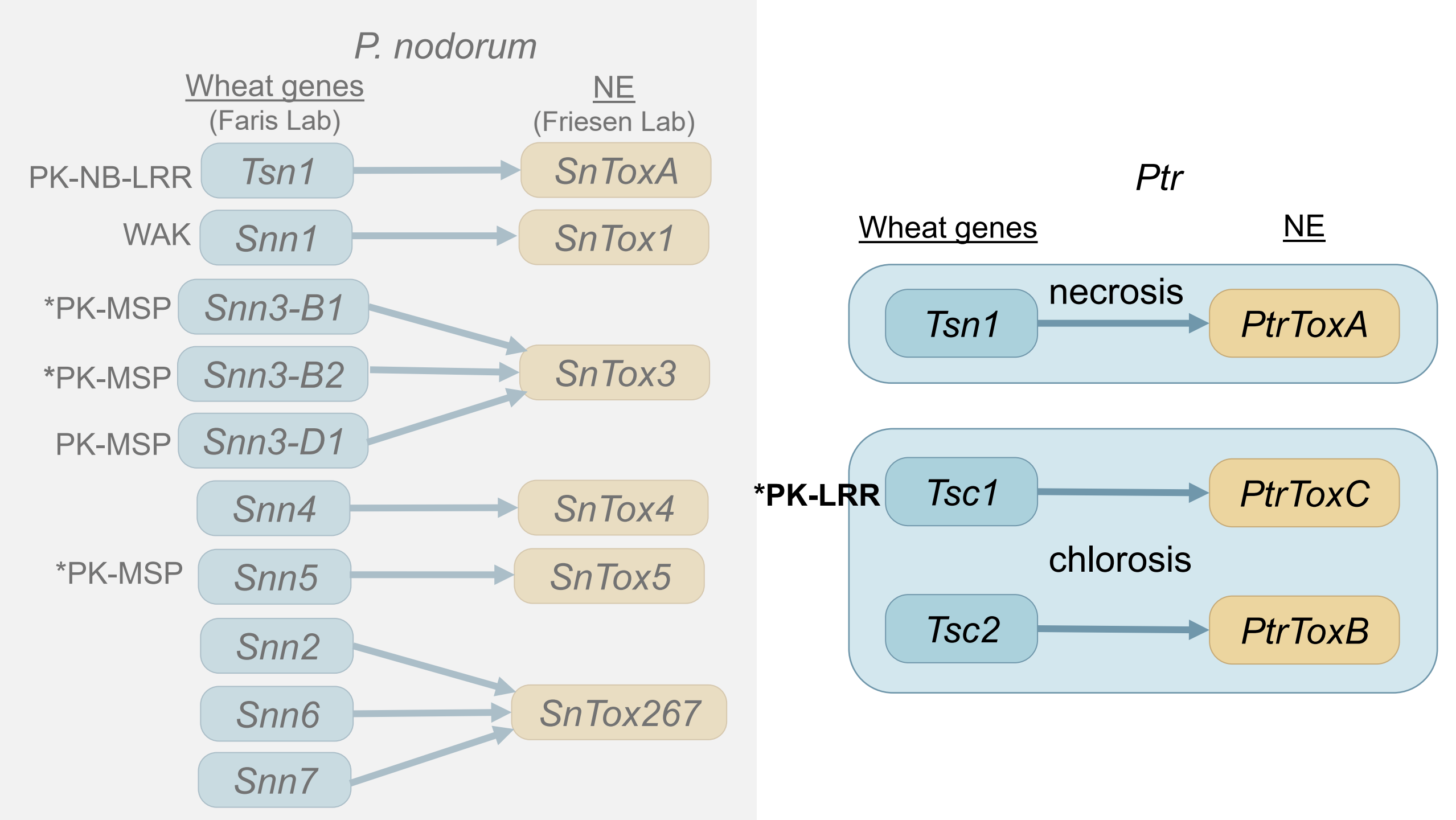
Sequenced Gene 1 and 2 from five independent mutants in two EMS populations

Gene 2 = *Tsc1*

PK-LRR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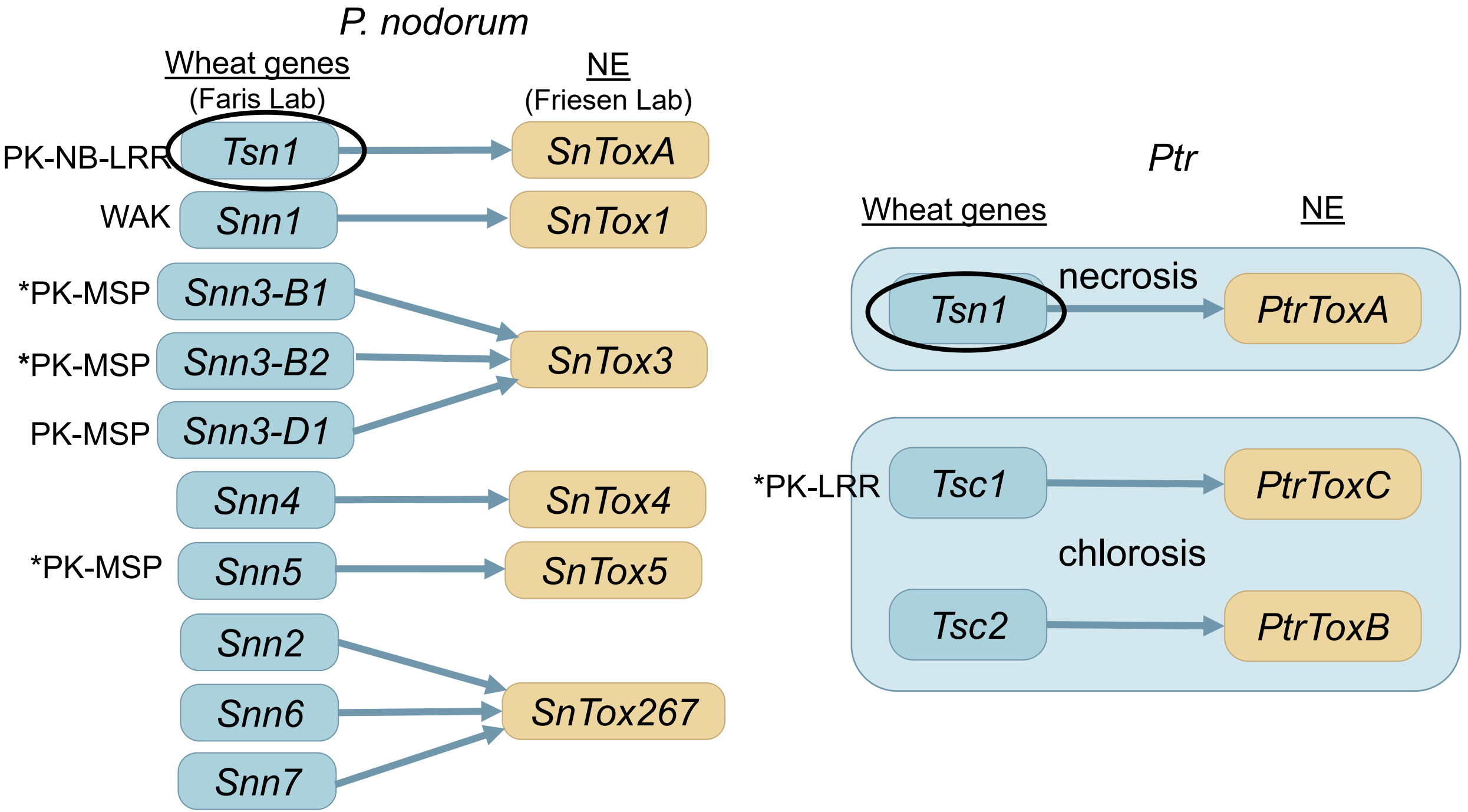
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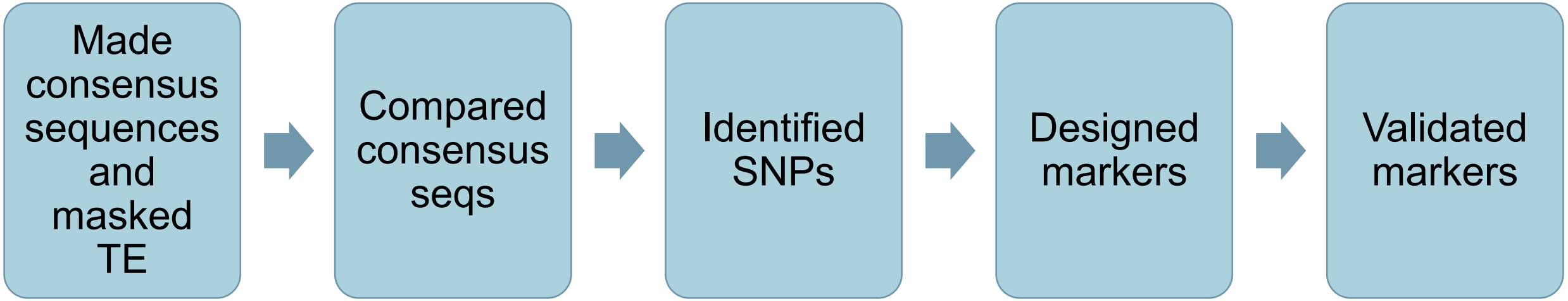
Tsn1

- Multi-pathogen susceptibility gene
  - Sensitivity to ToxA produced by *P. nodorum*, *Ptr*, and *Bipolaris sorokiniana*
- Our old flanking markers were over 300kb apart, not always linked to *Tsn1*
- Need better markers to eliminate *Tsn1*

Designing accurate *Tsn1* markers

Hexaploid pseudomolecule level assemblies

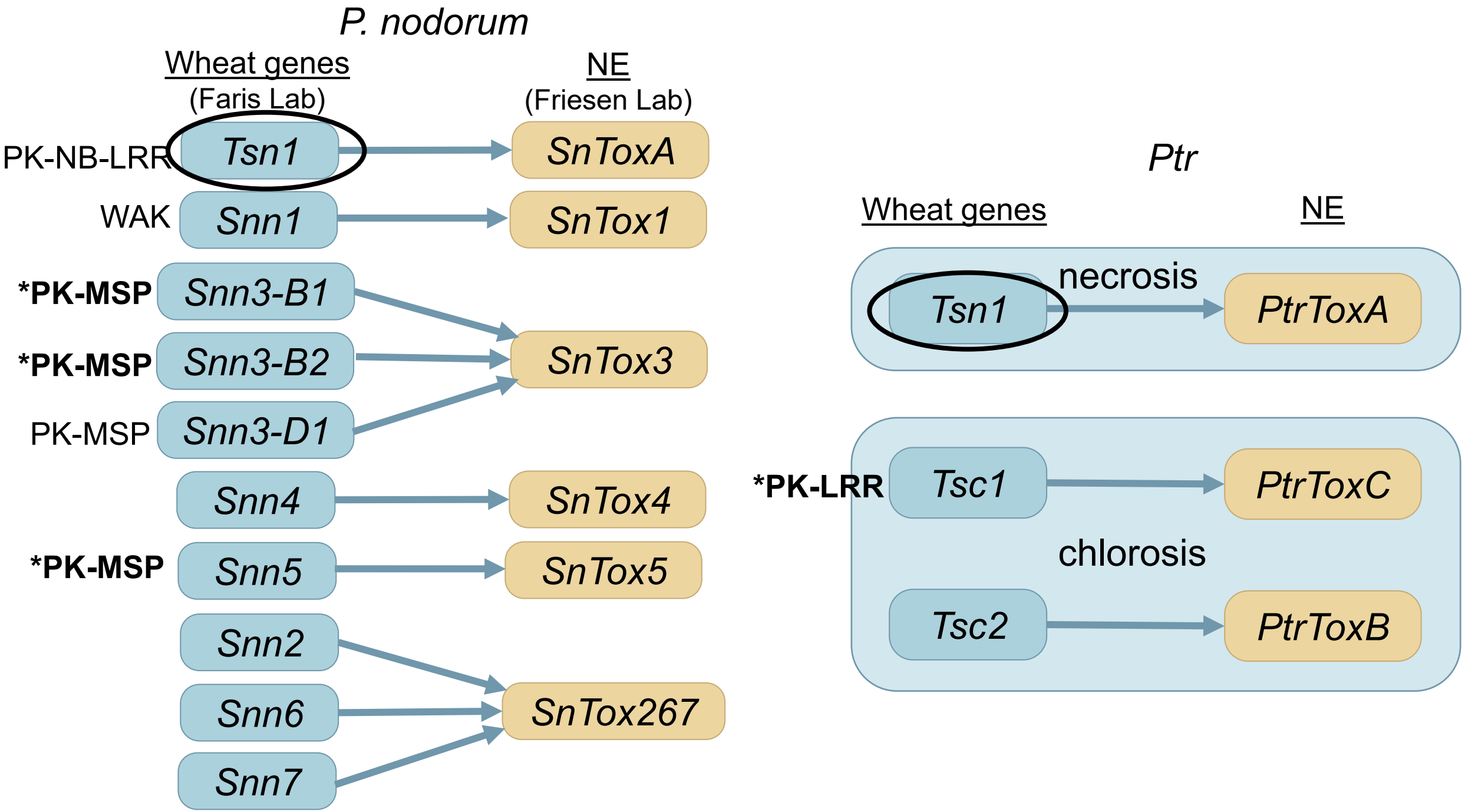
- 6 *Tsn1* - : Chinese Spring, Julius, SY Mace, Mattis, ArinaLrForno, CDC Stanley
- 4 *Tsn1* + : Jagger, CDC Landmark, LongReach Lancer, Norin61



Validating *Tsn1* markers

	Amanda Peters Haugrud	**Agnes Szabo-Hever**	**Gurminder Singh**
	Winter Wheat (n= 263)	Global Durum Panel (n = 512)	Hard Red Spring Wheat (n= 812)
% ToxA insensitive	61%	72.3%	48.3%
Tsn1-1Ka	100%	100%	99.4%
Tsn1-2Ka	99.3%	99.7%	99.4%

Please contact Dr. Faris for marker sequences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Four susceptibility genes cloned in four years

- Eliminated need for high resolution mapping
- Identified candidates using sequenced wheat genomes
- Reduced number of candidate genes based on presence/absence variation
- Validate with Cadenza TILLING
- Cloned a gene not in our reference genome using MutChromSeq

Acknowledgments

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Faris Lab

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- Aliya Momotaz
- Megan Overlander
- Amanda Peters Haugrud
- Sudeshi Seneviratne
- Sapna Sharma
- Agnes Szabo-Hever
- Cayley Steen
- Stephanie McCoy
- Gurminder Singh
- Gongjun Shi
- Numerous undergraduate students

Friesen Lab

- Danielle Holmes
- Jon Richards
- Nathan Wyatt
- Gayan Kariyawasam

United States Department of Agriculture
National Institute of Food and Agriculture

North Central Small Grains Genotyping Lab

Jason Fiedler
Mary Osenga

Steven Xu Lab

IWGSC

Earlham Institute

URGI

North Dakota State University

Philip McClean

Liu Lab

- Zhaohui Liu
- Gongjun Shi

University of California-Berkeley

Brian Staskawicz Lab

University of Saskatchewan

Curtis Pozniak

Institute of Experimental Botanv

Jaroslav Dole

Institute of Experimental Botany of the Czech Academy of Sciences
We are Discovering the Plant World

John Innes Centre

Cristobal Uauy

Washington State University

Aaron Carter

Questions?

katherine.running@ndsu.edu