Resistance gene cloning in wheat

Burkhard Steuernagel

2020-09-23

Wheat Pathogens

Hales et al.

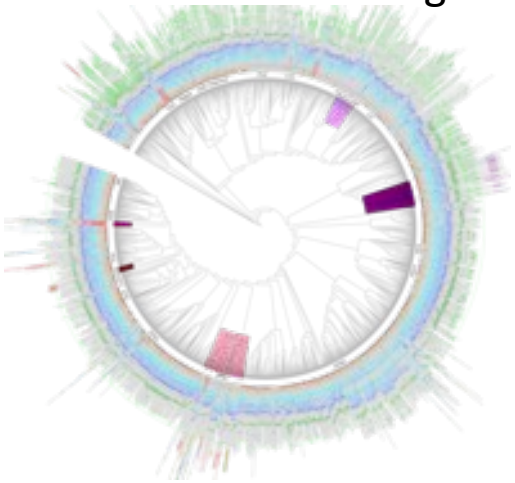
Amber Hafeez

www.rusttracker.org

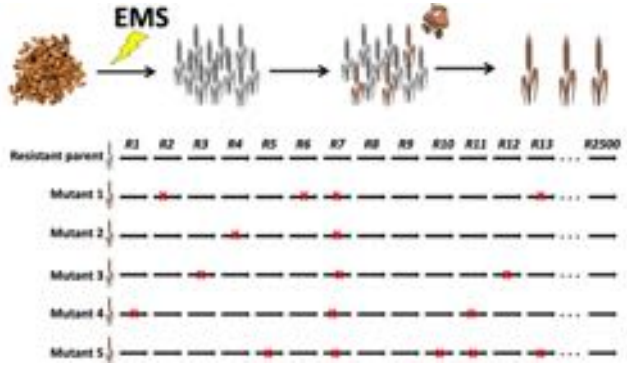
wikipedia

Outline

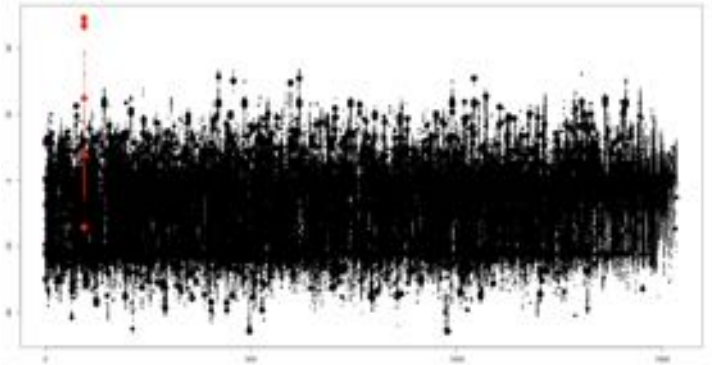
Annotation of NLR genes



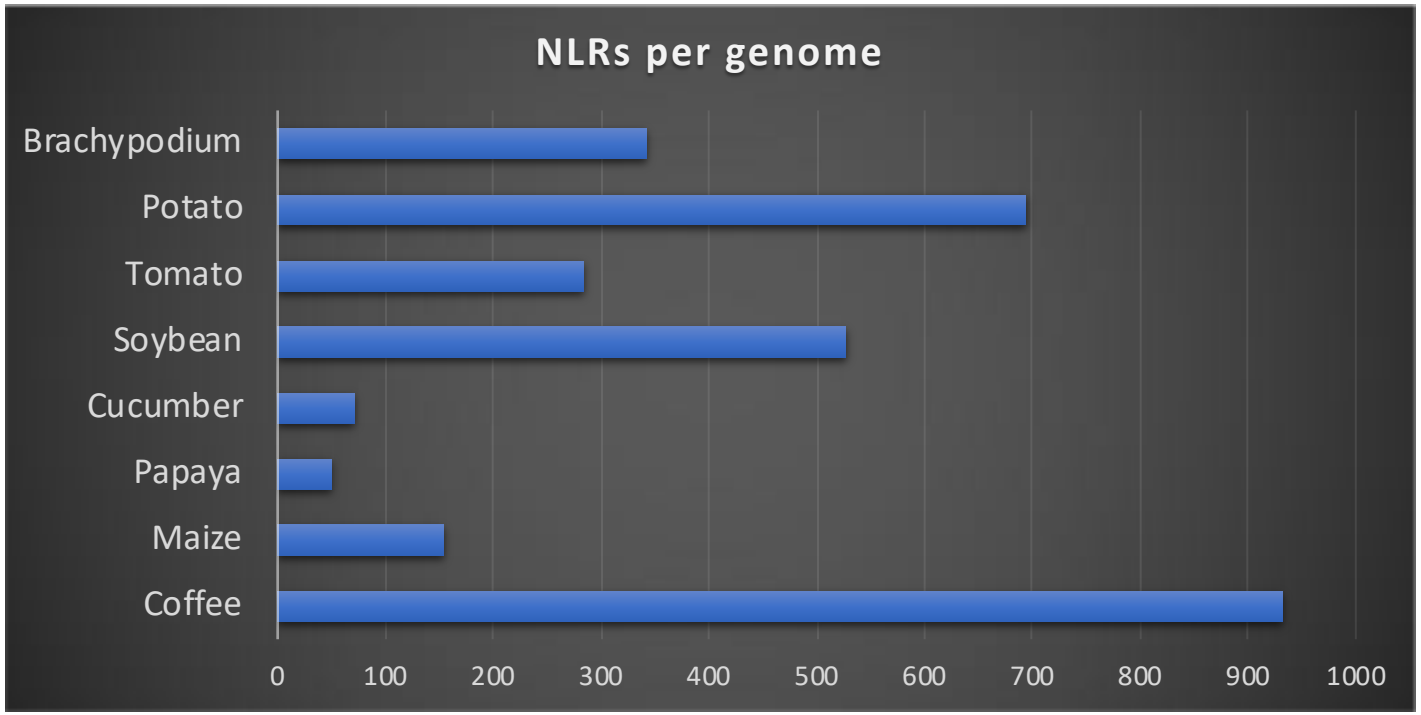
Mutational Genomics



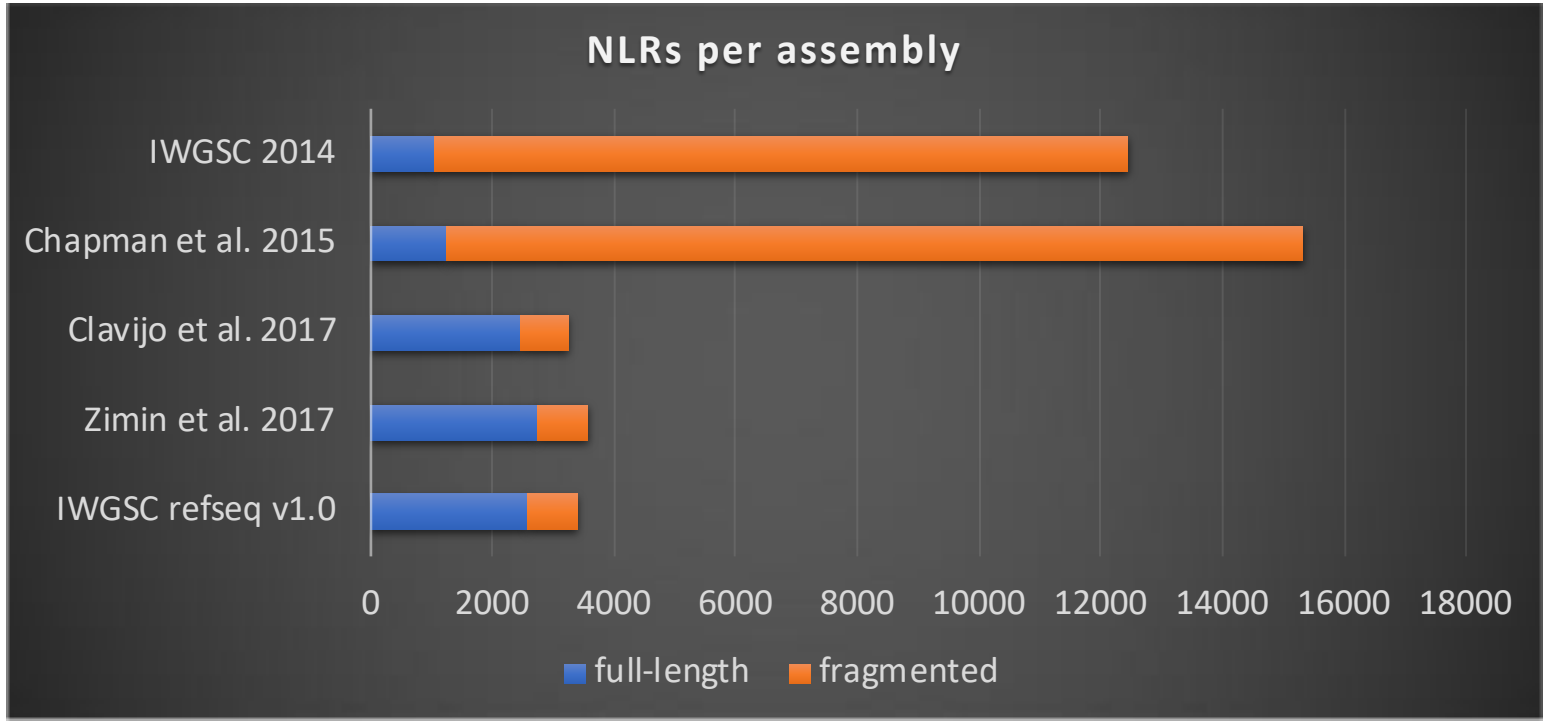
AgRenSeq



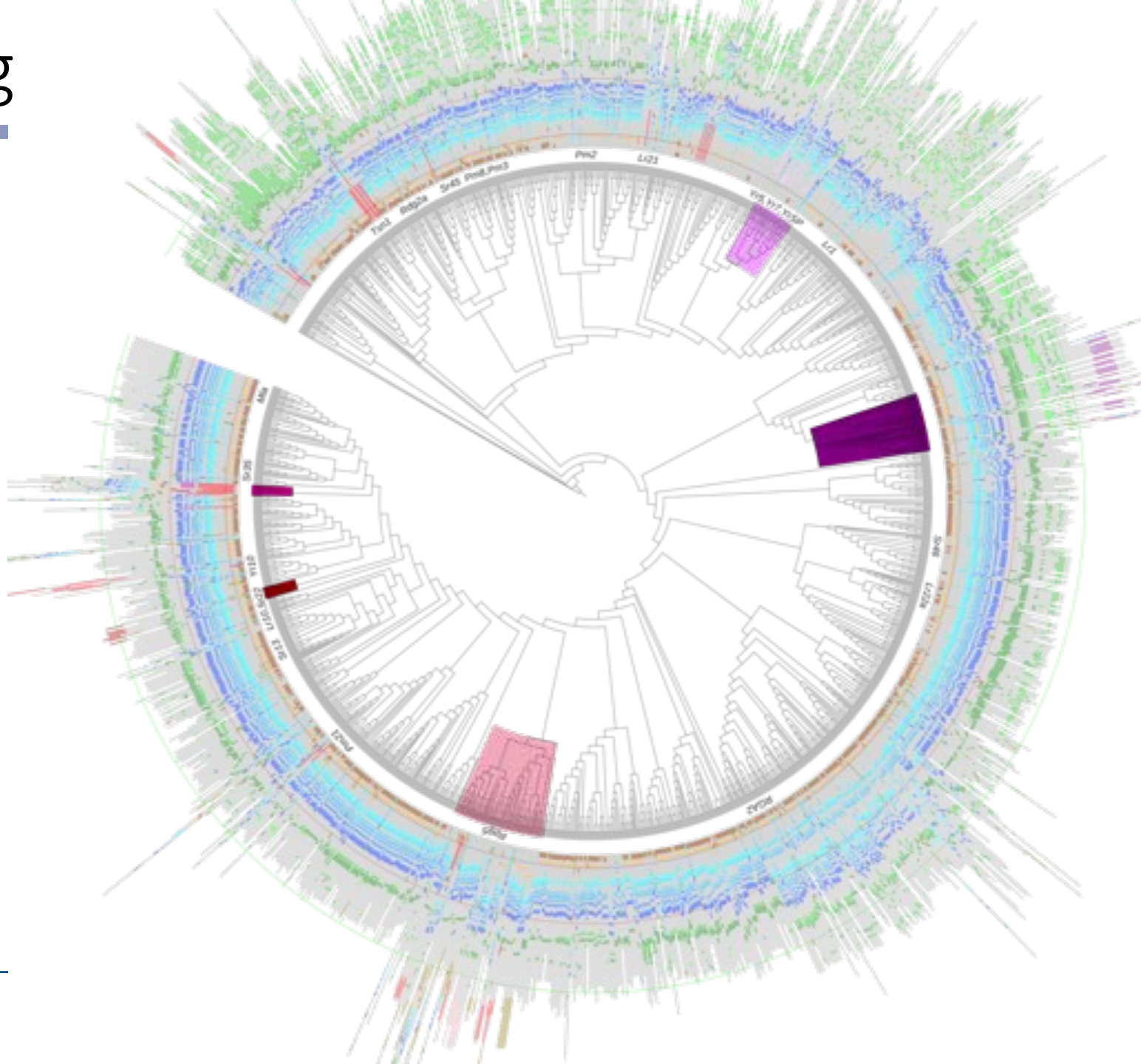
The NLR Gene Family



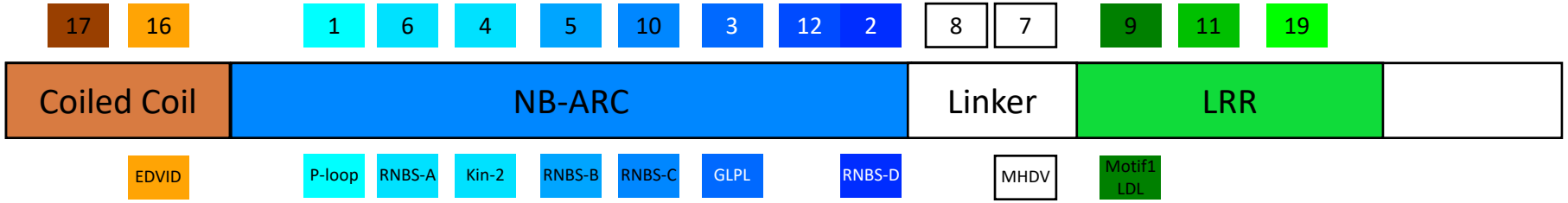
NLRs of Chinese Spring

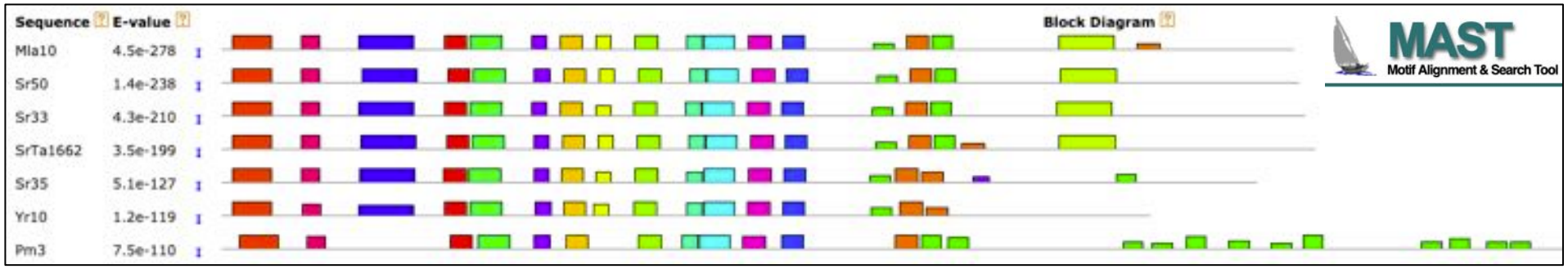


NLRs of Chinese Spring



NLR Annotation





2015

Bioinformatics, 31(10), 2015, 1665–1667
doi: 10.1093/bioinformatics/btv005
Advance Access Publication Date: 12 January 2015
Applications Note

Genome analysis

NLR-parser: rapid annotation of plant NLR complements

Burkhard Steuernagel[1,*,†], Florian Jupe[2,*,†,‡], Kamil Witek[2], Jonathan D.G. Jones[2] and Brande B.H. Wulff[1]

2020

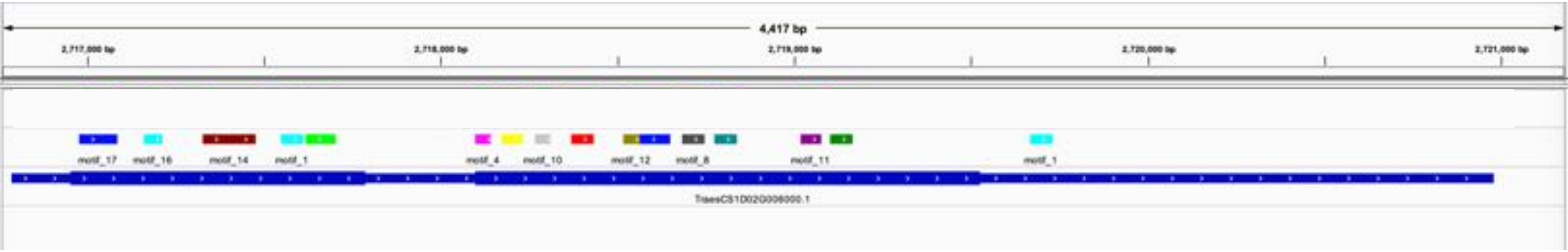
Plant Physiology

Breakthrough Technologies

The NLR-Annotator Tool Enables Annotation of the Intracellular Immune Receptor Repertoire[1][OPEN]

Burkhard Steuernagel,[a] Kamil Witek,[b] Simon G. Krattinger,[c,d] Ricardo H. Ramirez-Gonzalez,[a] Henk-jan Schoonbeek,[a] Guotai Yu,[a] Erin Baggs,[e] Agnieszka I. Witek,[b] Inderjit Yadav,[f] Ksenia V. Krasileva,[b,e] Jonathan D.G. Jones,[b] Cristobal Uauy,[a] Beat Keller,[c] Christopher J. Ridout,[a] and Brande B.H. Wulff[a,2,3]

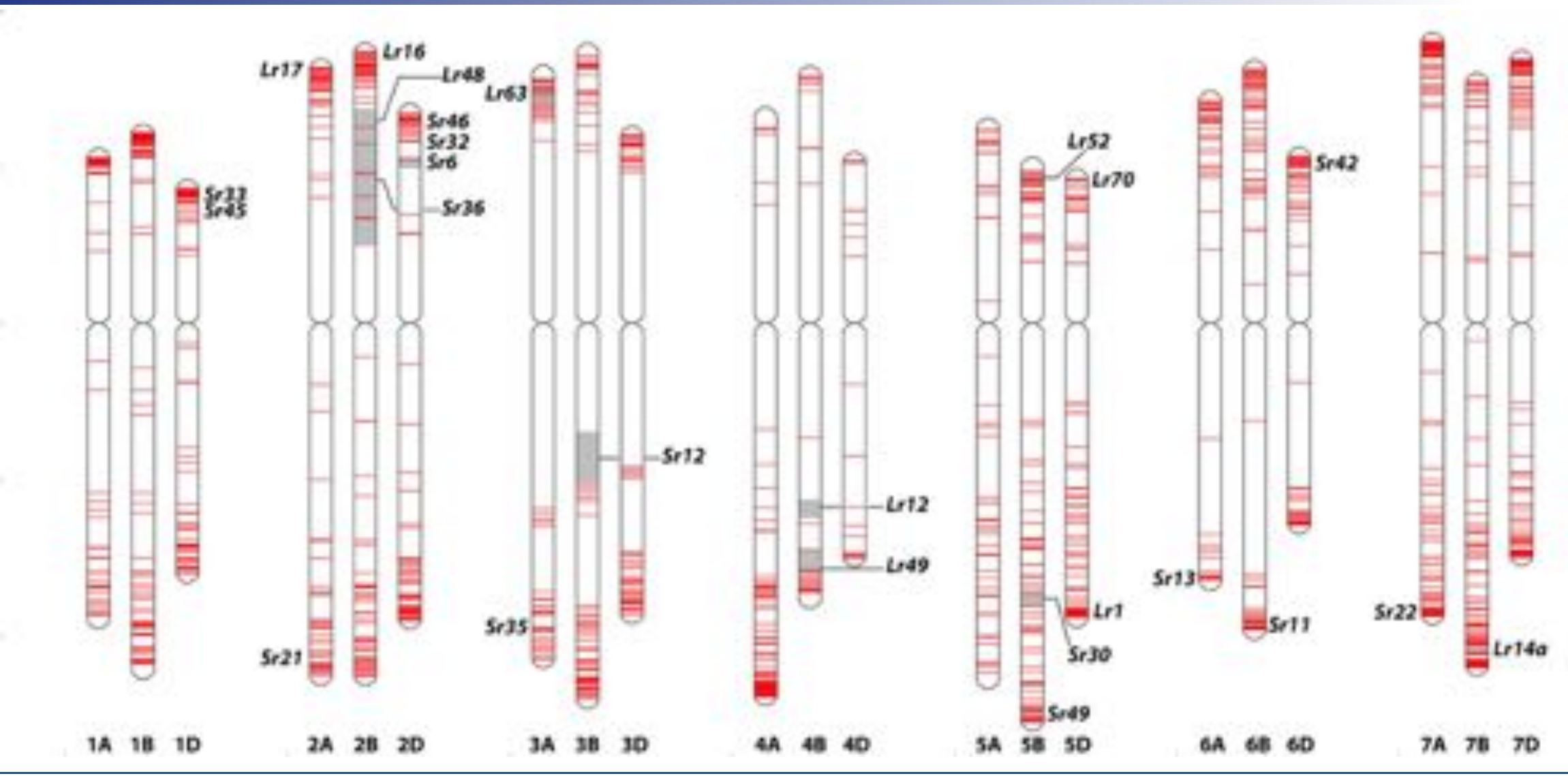
NLR-Annotator

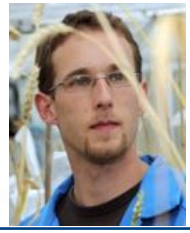


- NLR loci (vs. NLR genes)
  - No gene structure
  - No difference between pseudo-genes and genes

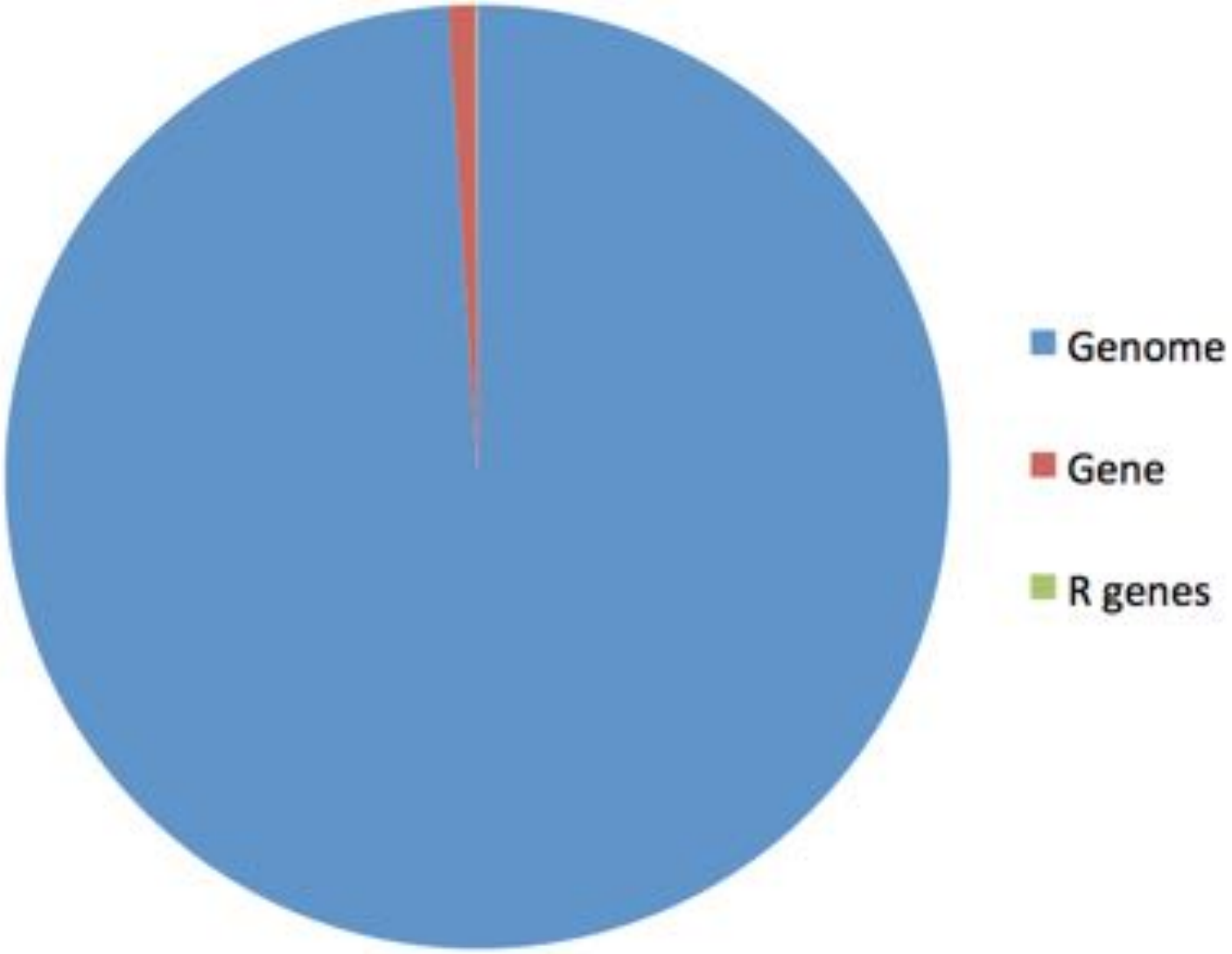
https://github.com/steuernb/NLR-Annotator

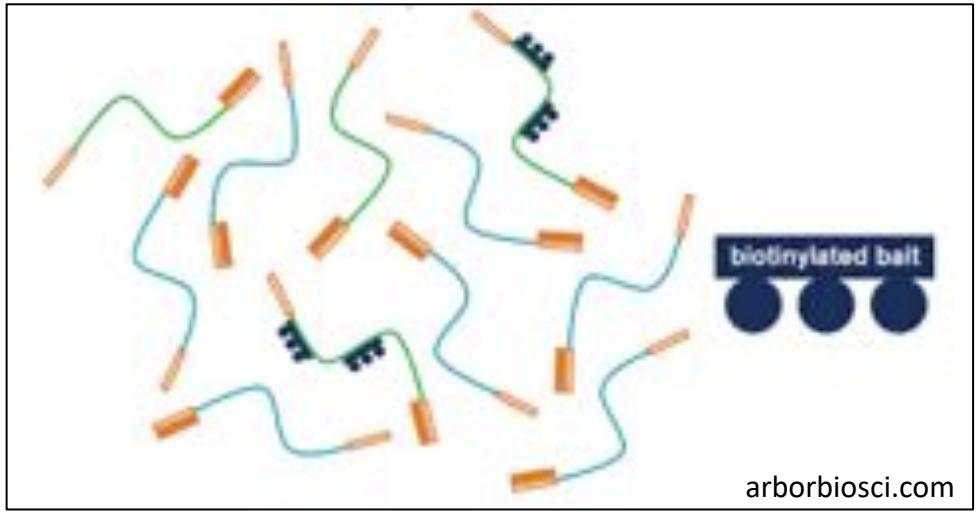
NLR-Annotation in Chinese Spring





Resistance Gene **En**richment **Seq**uencing (RenSeq)





RenSeq Bait Libraries

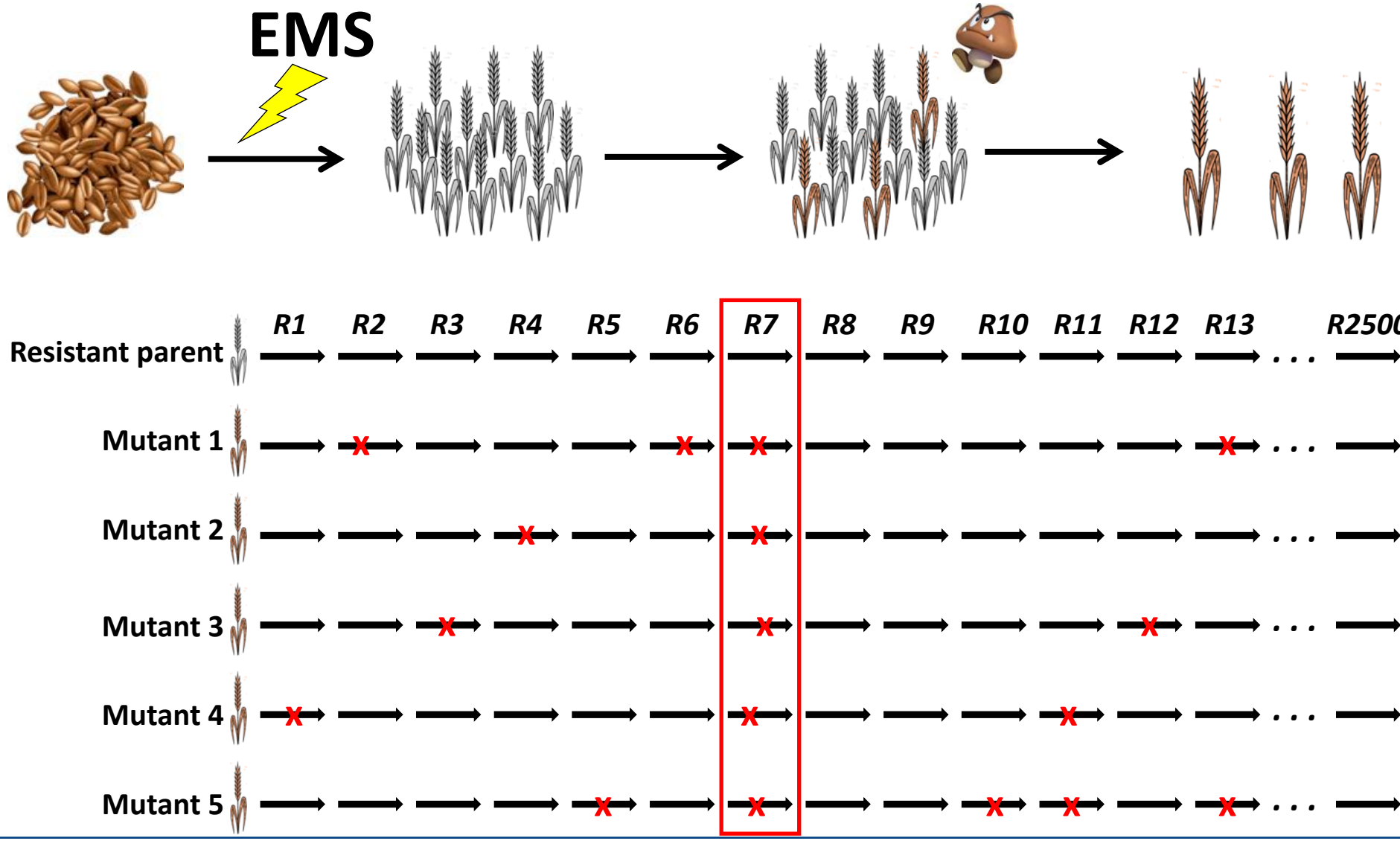
- Triticeae RenSeq v1 (60k baits)
- Triticeae RenSeq v2 (60k baits; improved sensistivity)
- Triticeae RenSeq v3 (220k baits; improved sensitivity; introns)
- *Ae. tauschii* RenSeq (60k baits; *Ae. tauschii* specific)

https://github.com/steuernb/MutantHunter

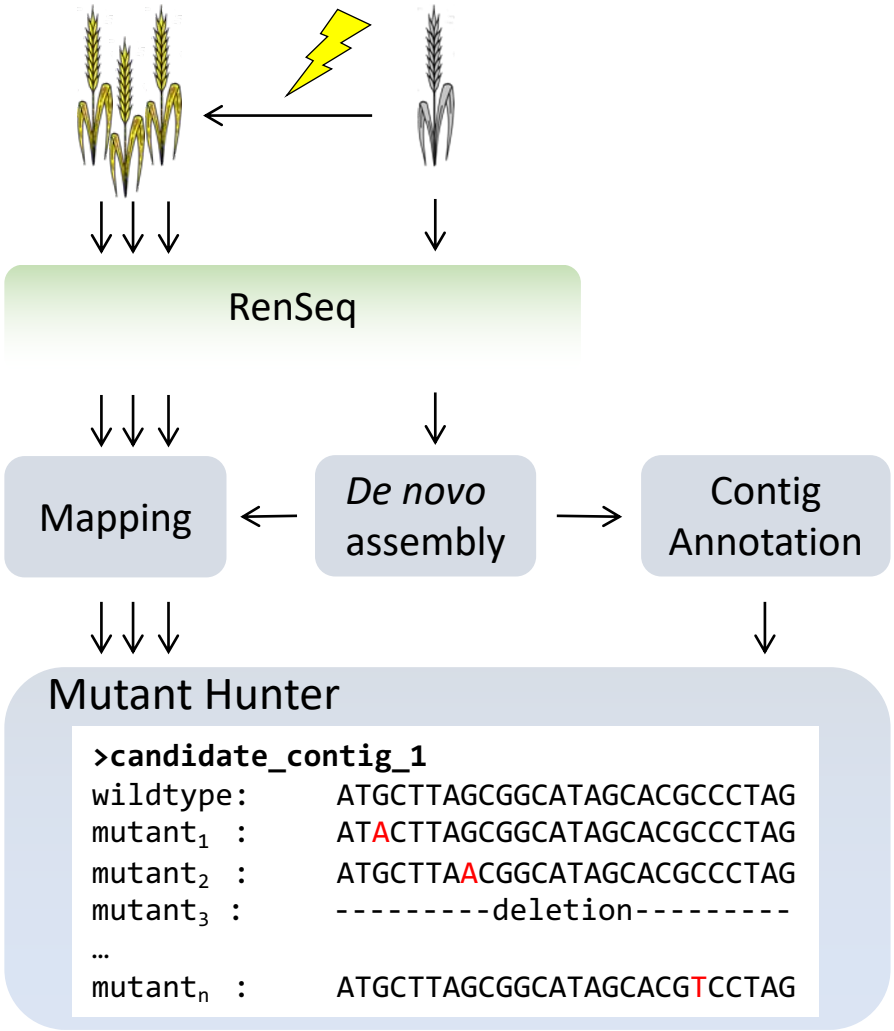
https://github.com/steuernb/AgRenSeq/releases/download/v1.0/

https://arborbiosci.com/applications/agrigenomics/resistance-gene-enrichment-sequencing-renseq/

Mutational Genomics



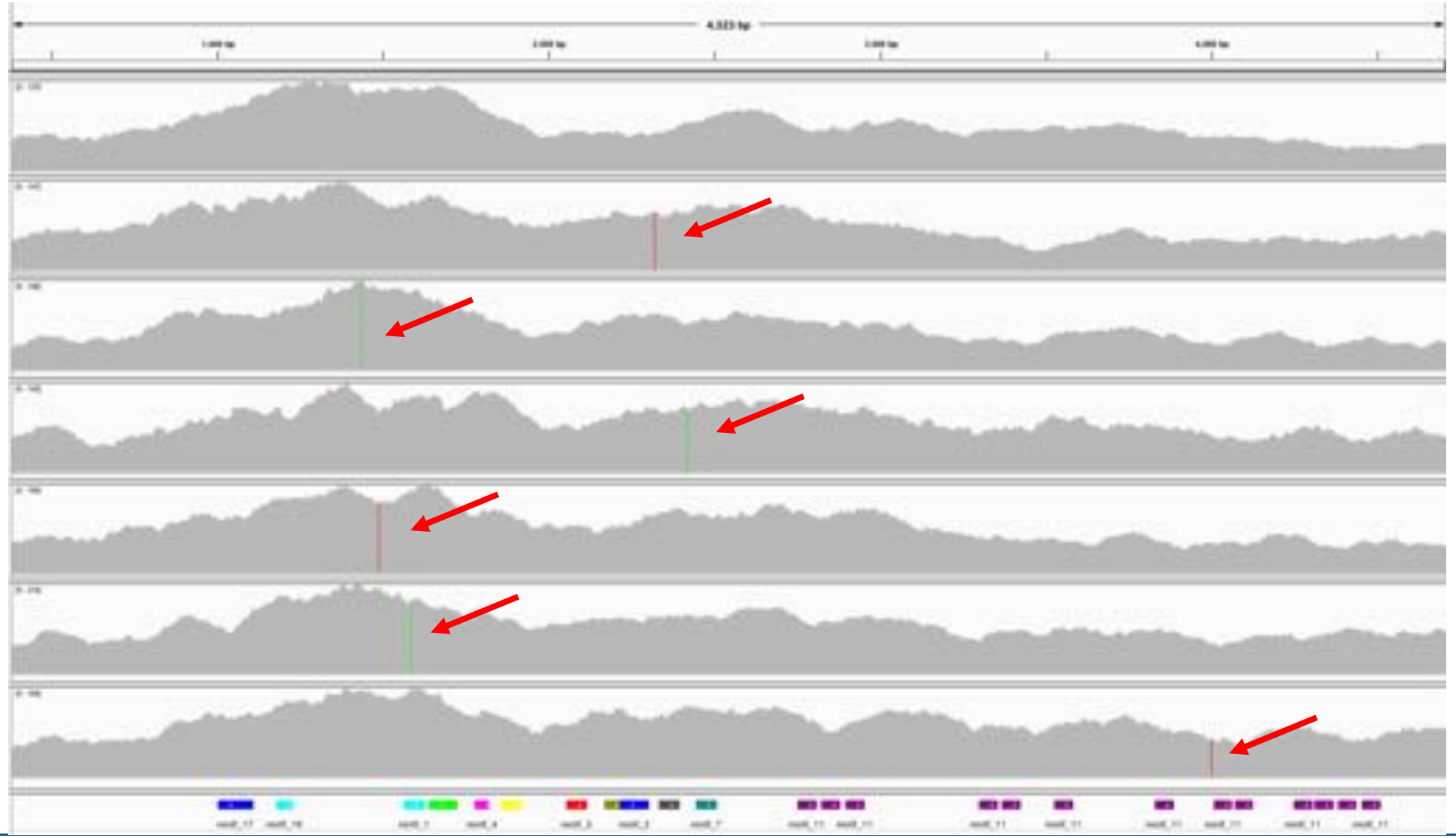
MutRenSeq



https://github.com/steuernb/MutantHunter

- EMS mutagenesis
- RenSeq on wild type and mutants
- Assembly, annotation and mapping
- Mutational genomics

MutRenSeq

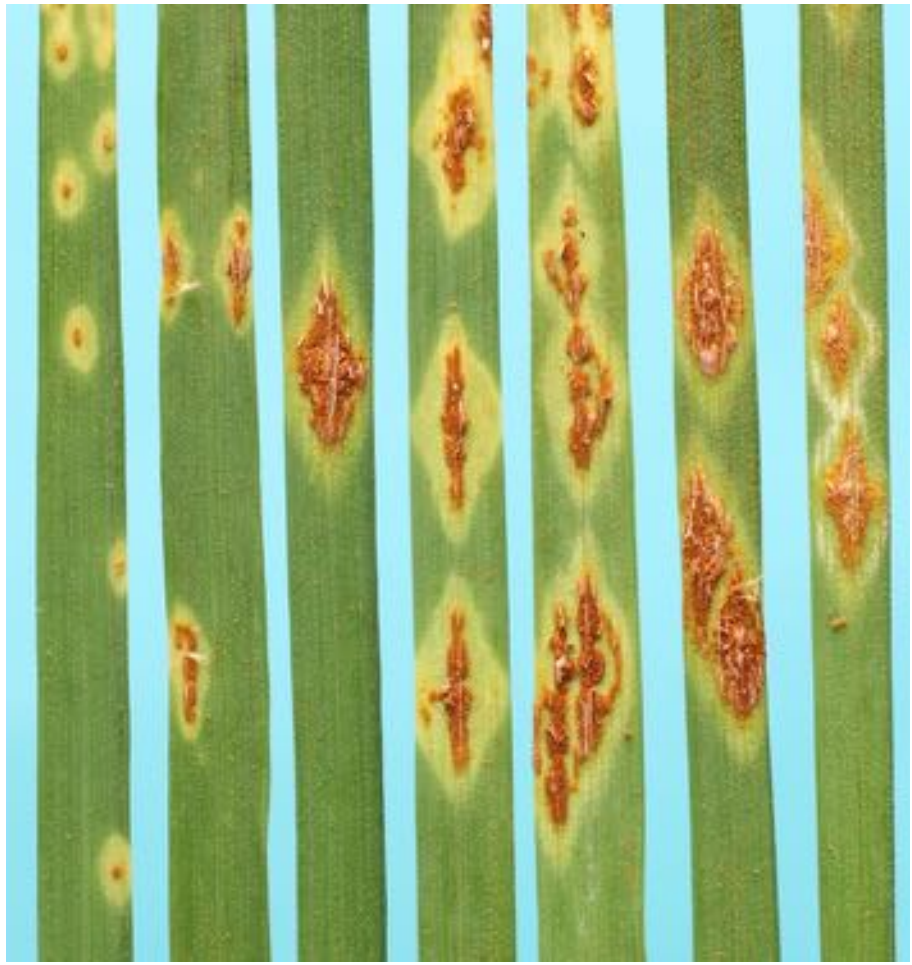


Sr22

- Introgressed into wheat from *T. boeoticum*
- Suppressed recombination prevents map-based cloning
- 6 EMS mutants

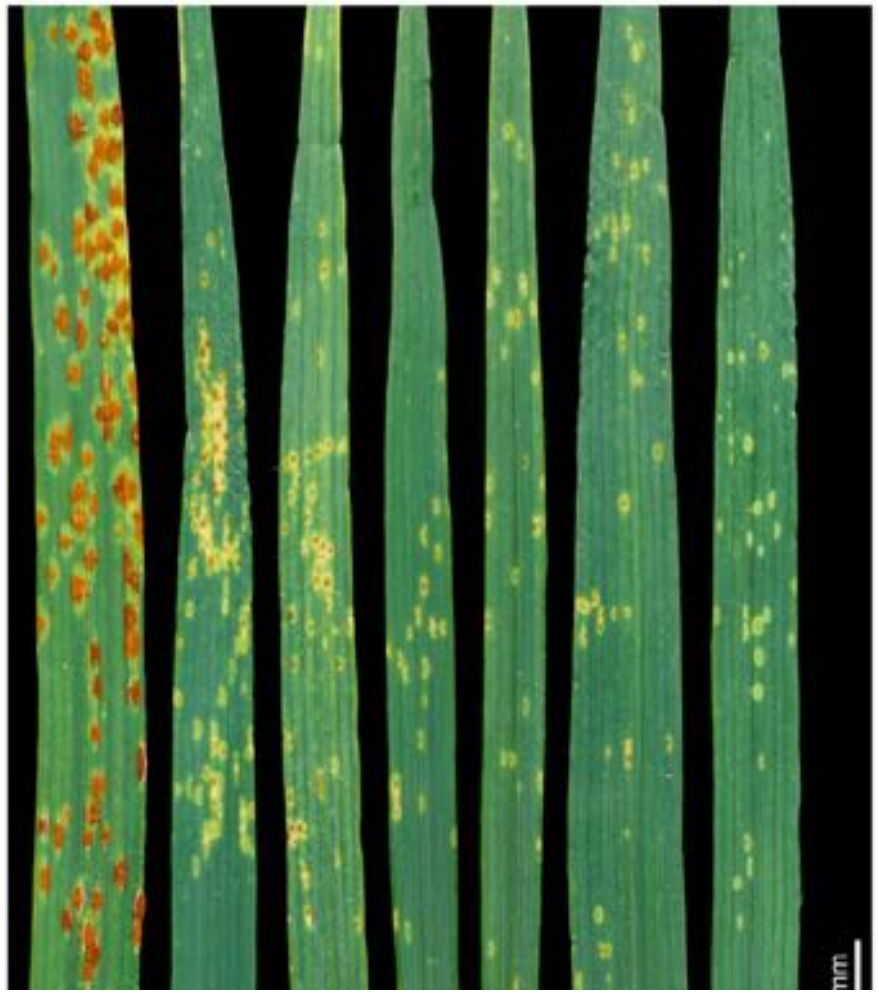
Sr22 sr22

Sr22



Sr22 Transgenics

Fielder Transgenics

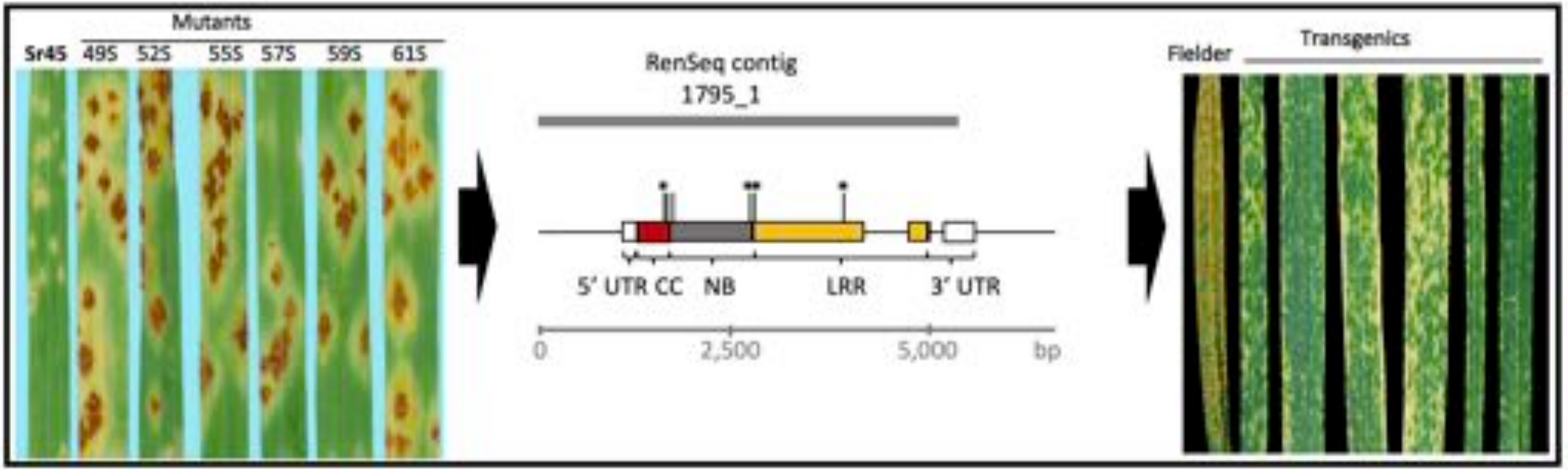


nature biotechnology

Rapid cloning of disease-resistance genes in plants using mutagenesis and sequence capture

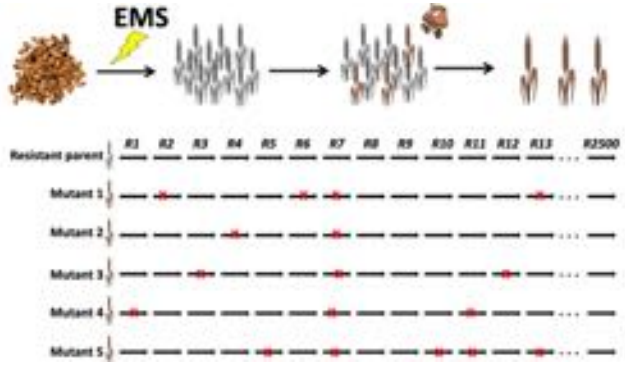
Burkhard Steuernagel[1,2,7], Sambasivam K Periyannan[3,7], Inmaculada Hernández-Pinzón[1], Kamil Witek[1], Matthew N Rouse[4], Guotai Yu[2], Asyraf Hatta[2,5], Mick Ayliffe[3], Harbans Bariana[6], Jonathan D G Jones[1], Evans S Lagudah[3] & Brande B H Wulff[1,2]

Sr45 (MutRenSeq)



MutChromSeq

Mutational Genomics + Chromosome Flow Sorting

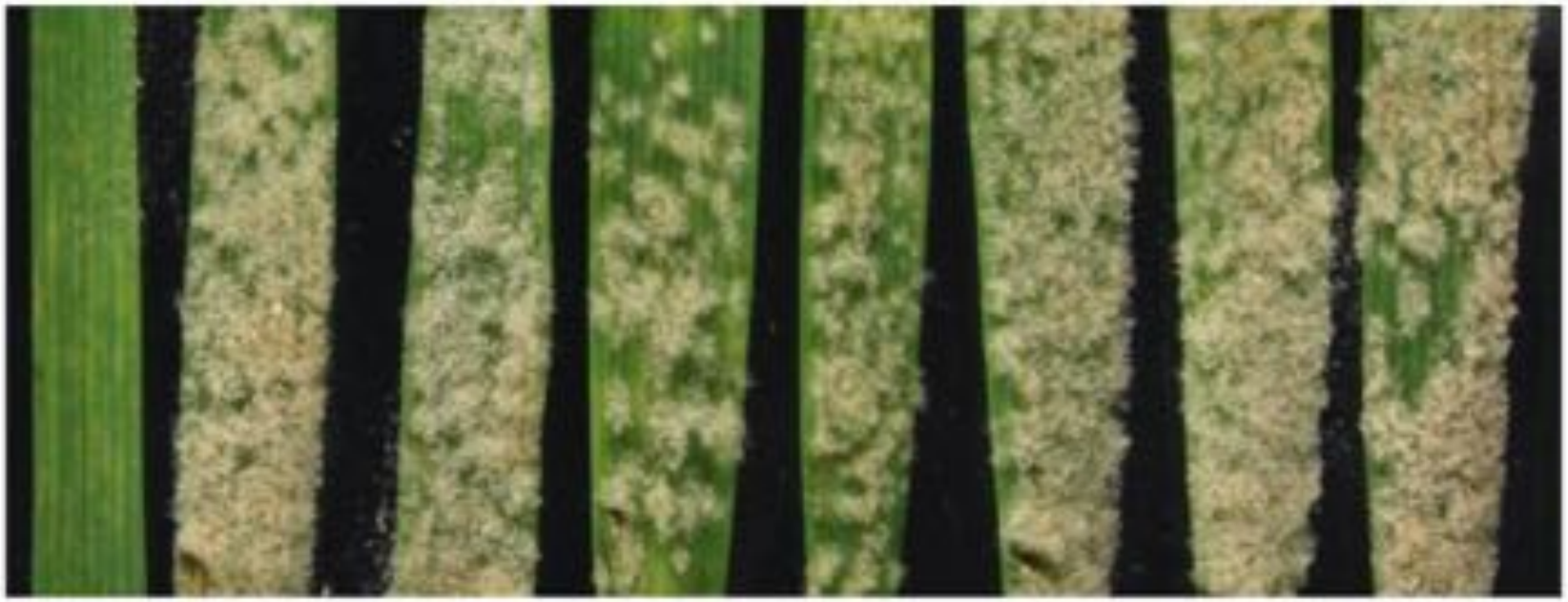


wild type

historical mutants

MutChromSeq *Pm2*

wild type EMS mutants Chancellor



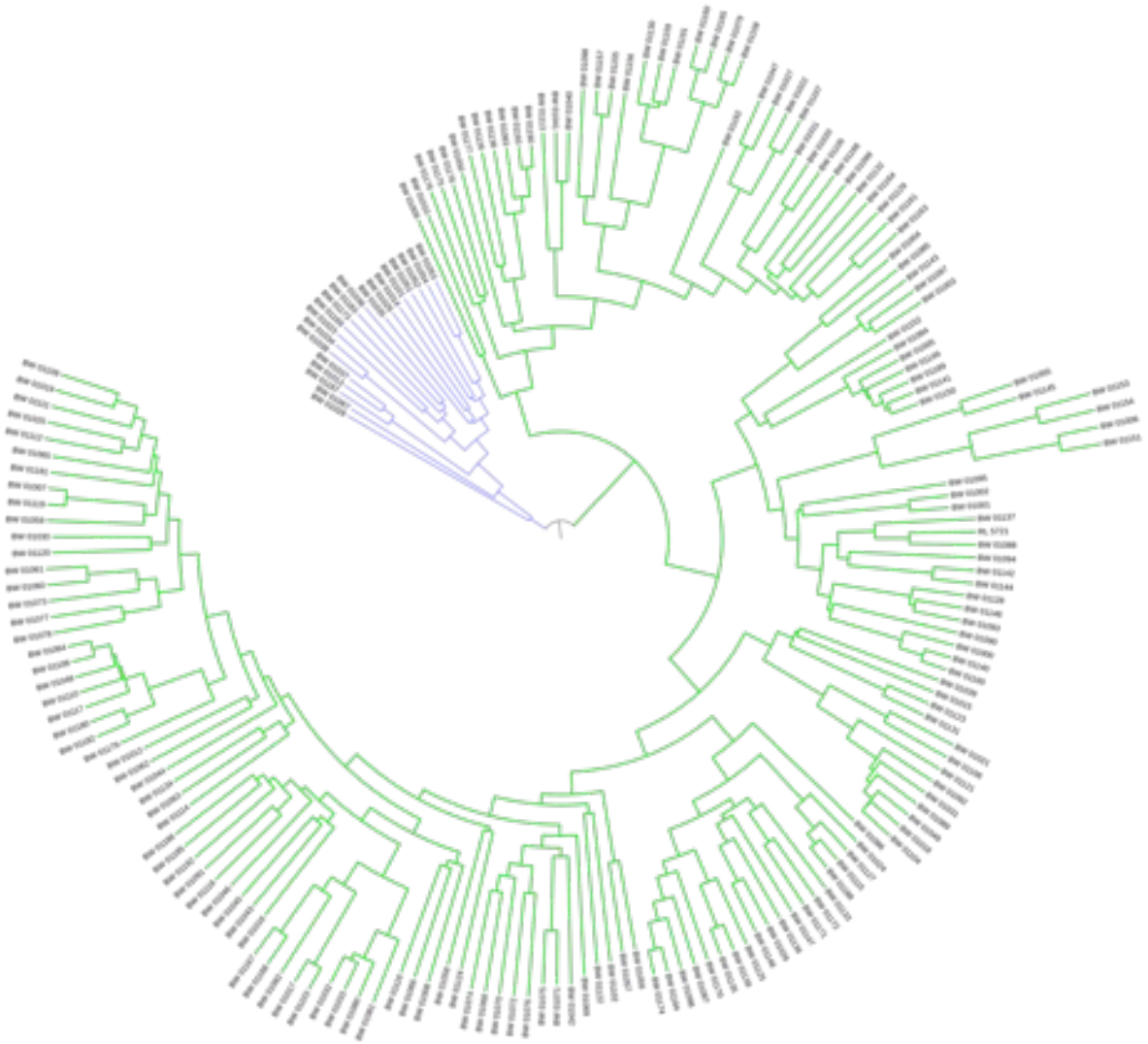
Mutational Genomics

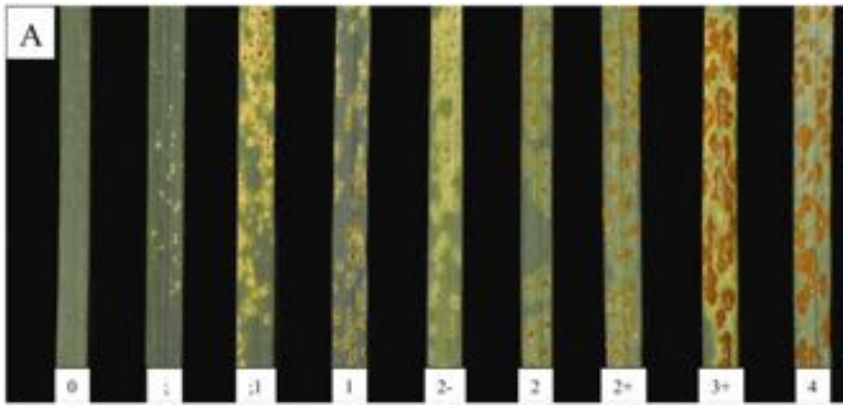
Forward genetics to clone single dominant genes

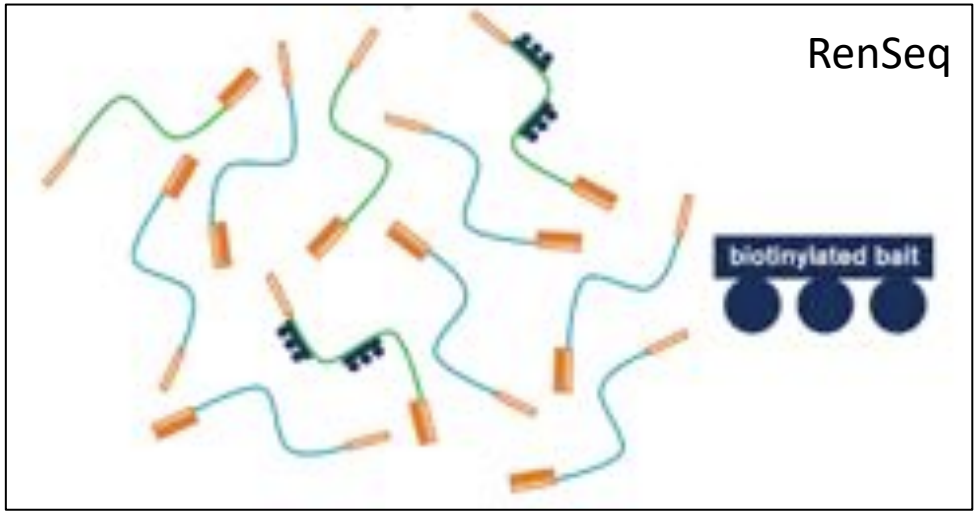
Pursue single genes

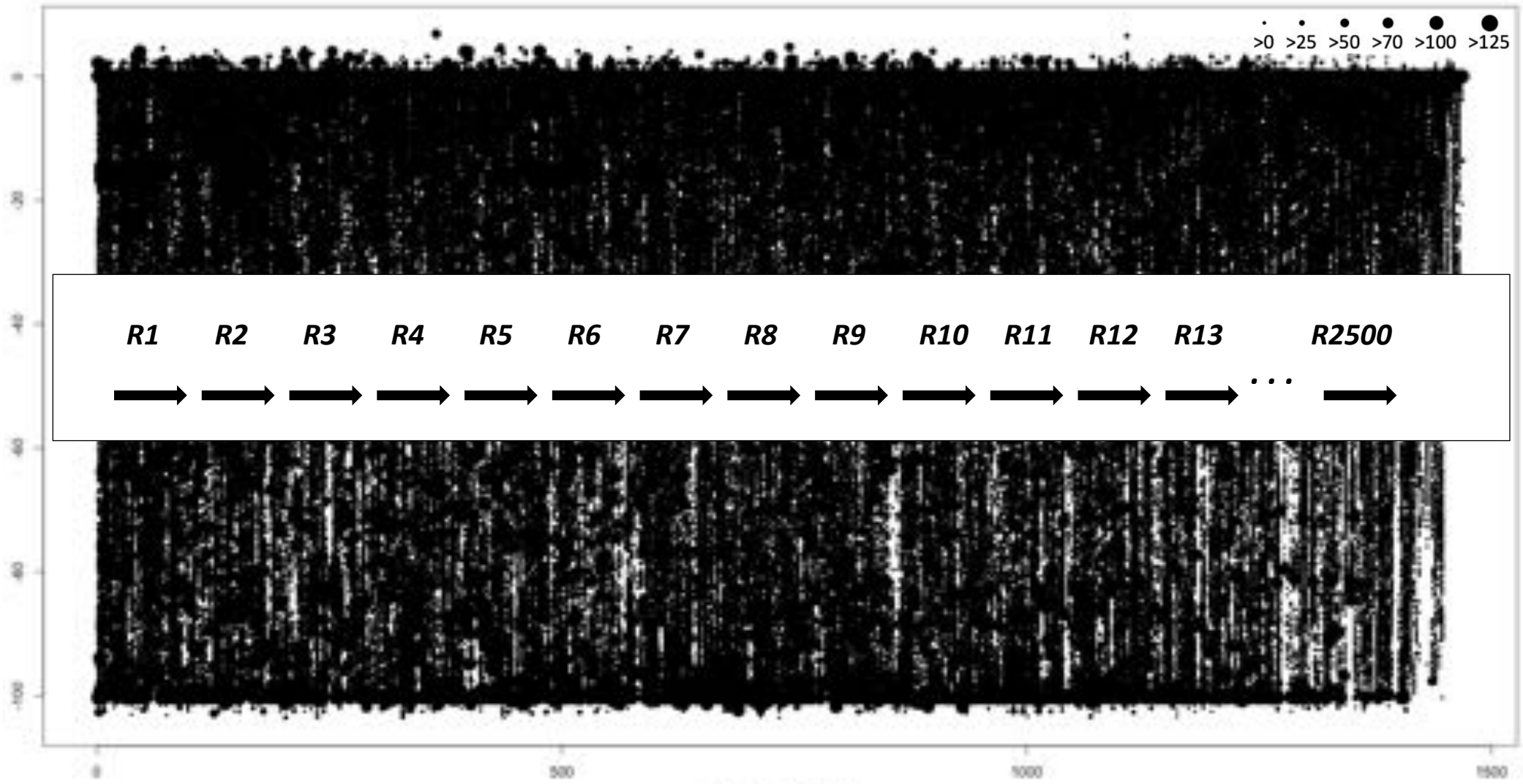
EMS mutagenesis

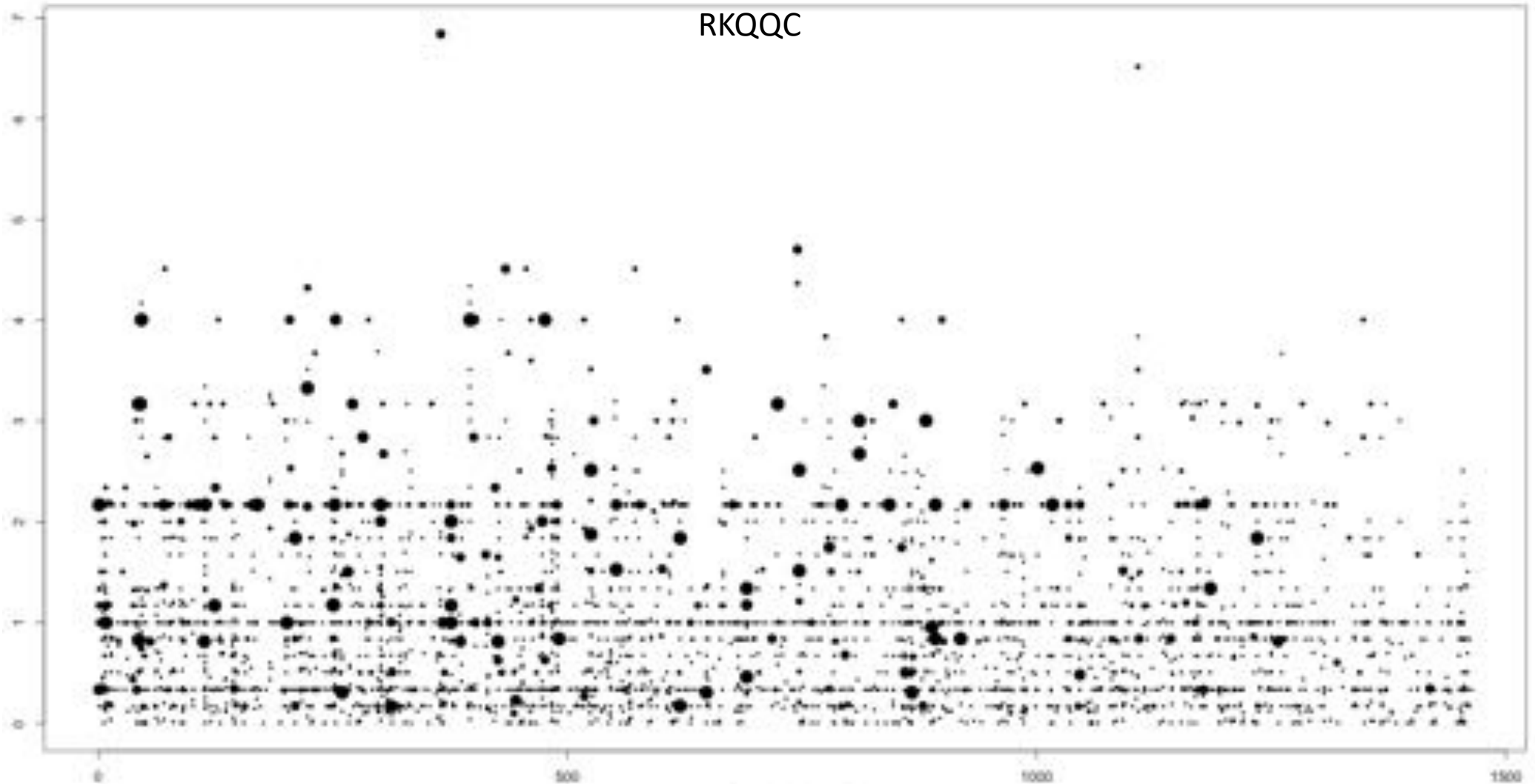
Association **G**enetics using **RenSeq** (AgRenSeq)





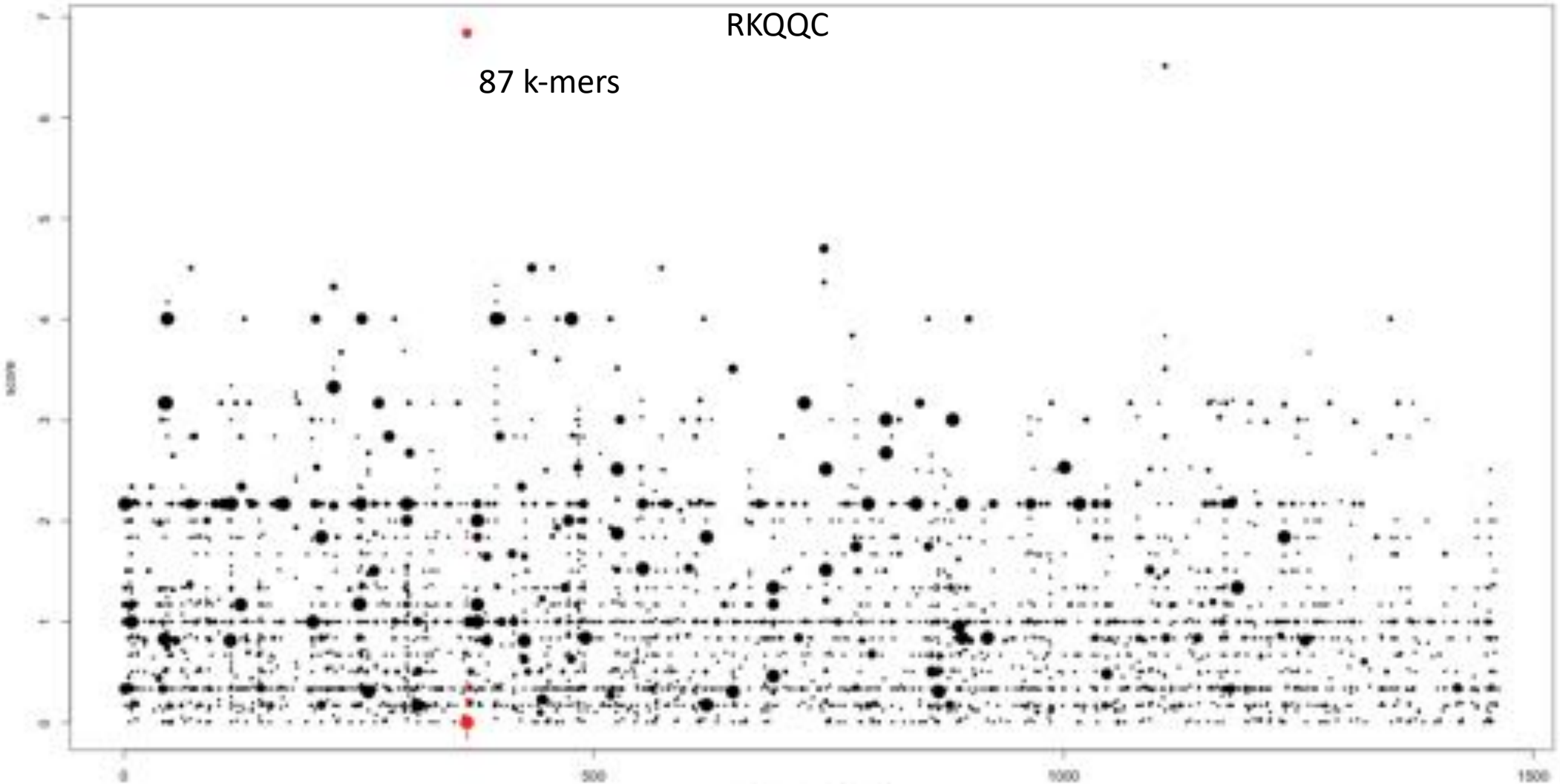



>0 >25 >50 >70 >100 >125
R1 R2 R3 R4 R5 R6 R7 R8 R9 R10 R11 R12 R13 . . . R2500

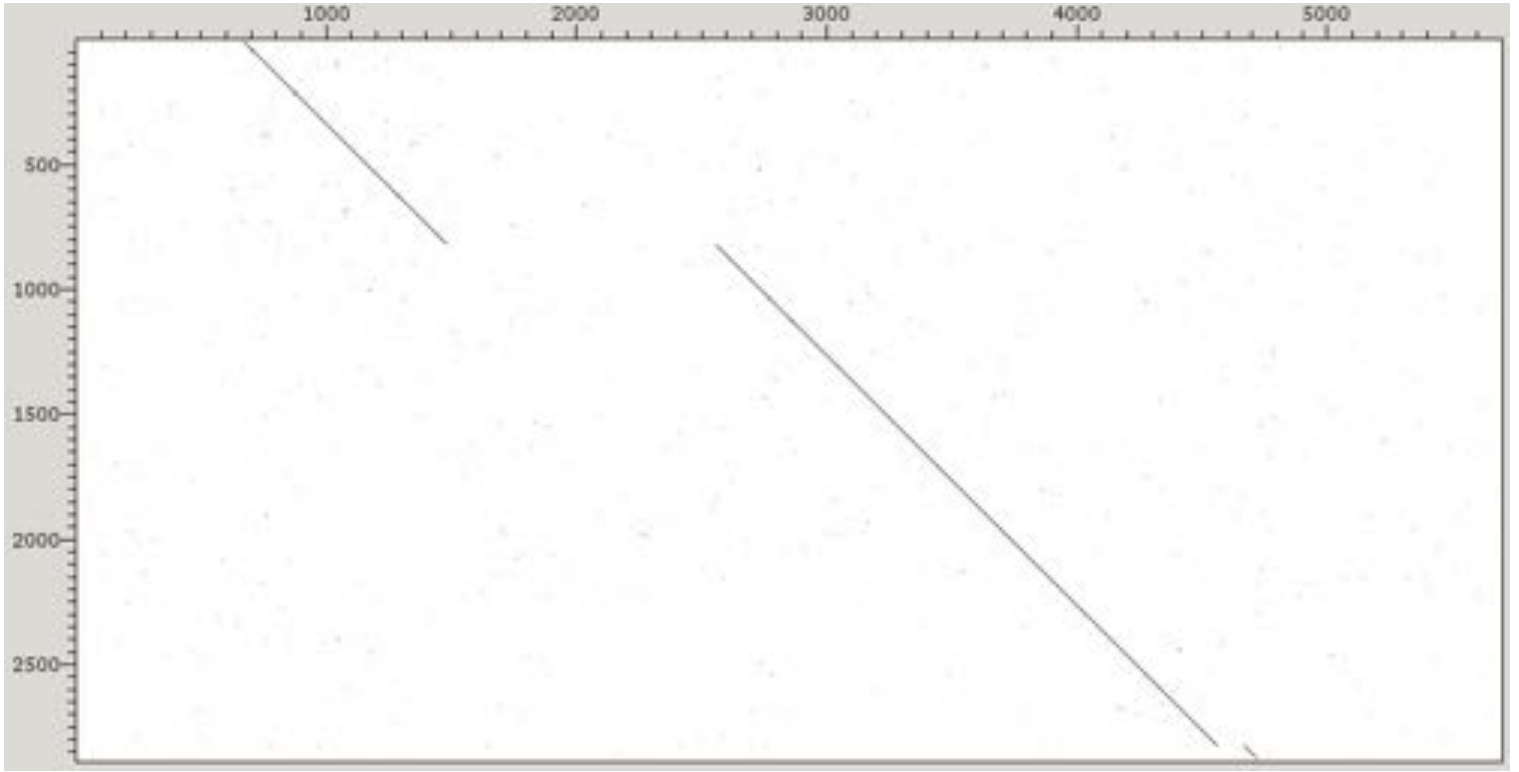

RKQQC
0
500
1000
1500

RKQQC

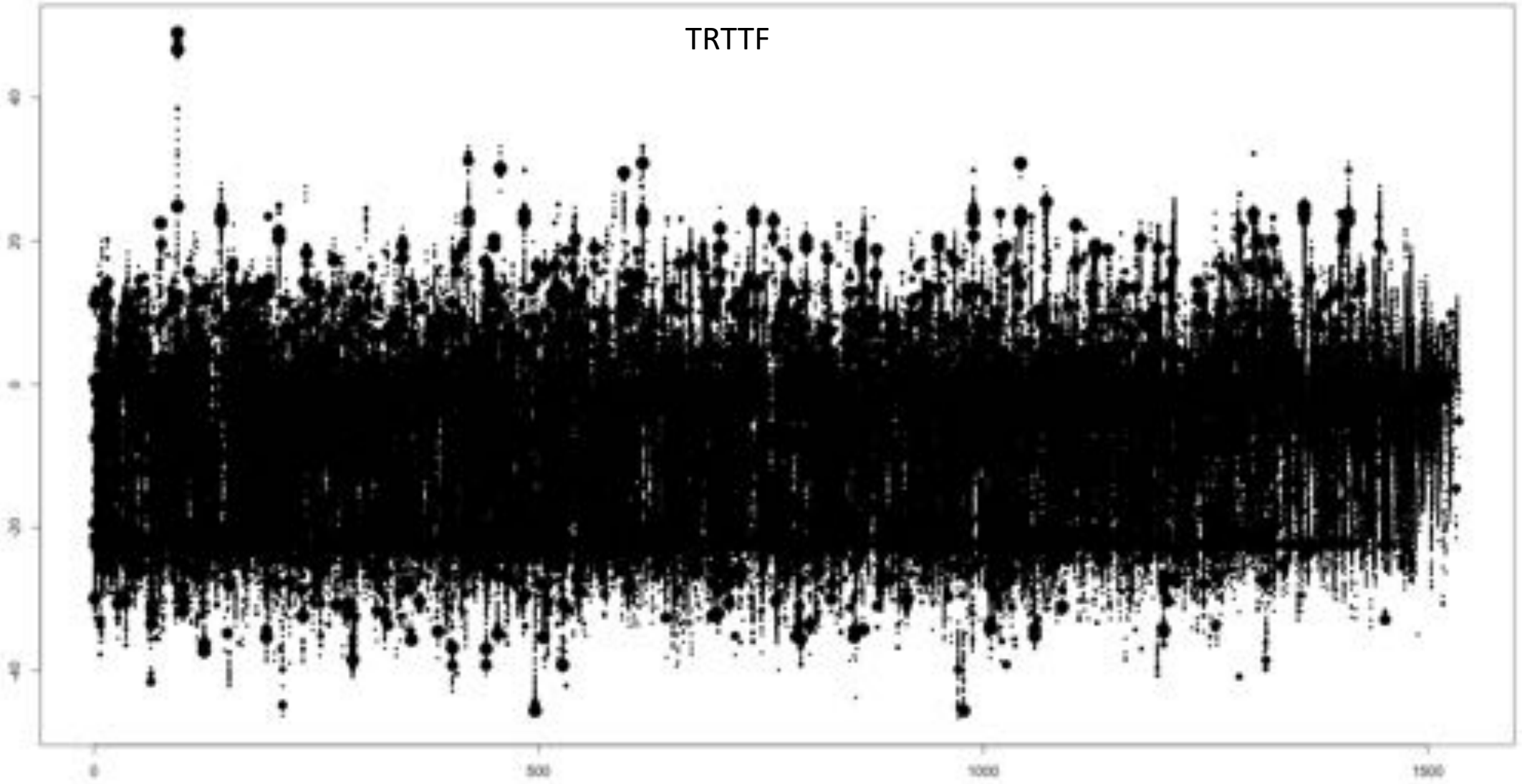
87 k-mers



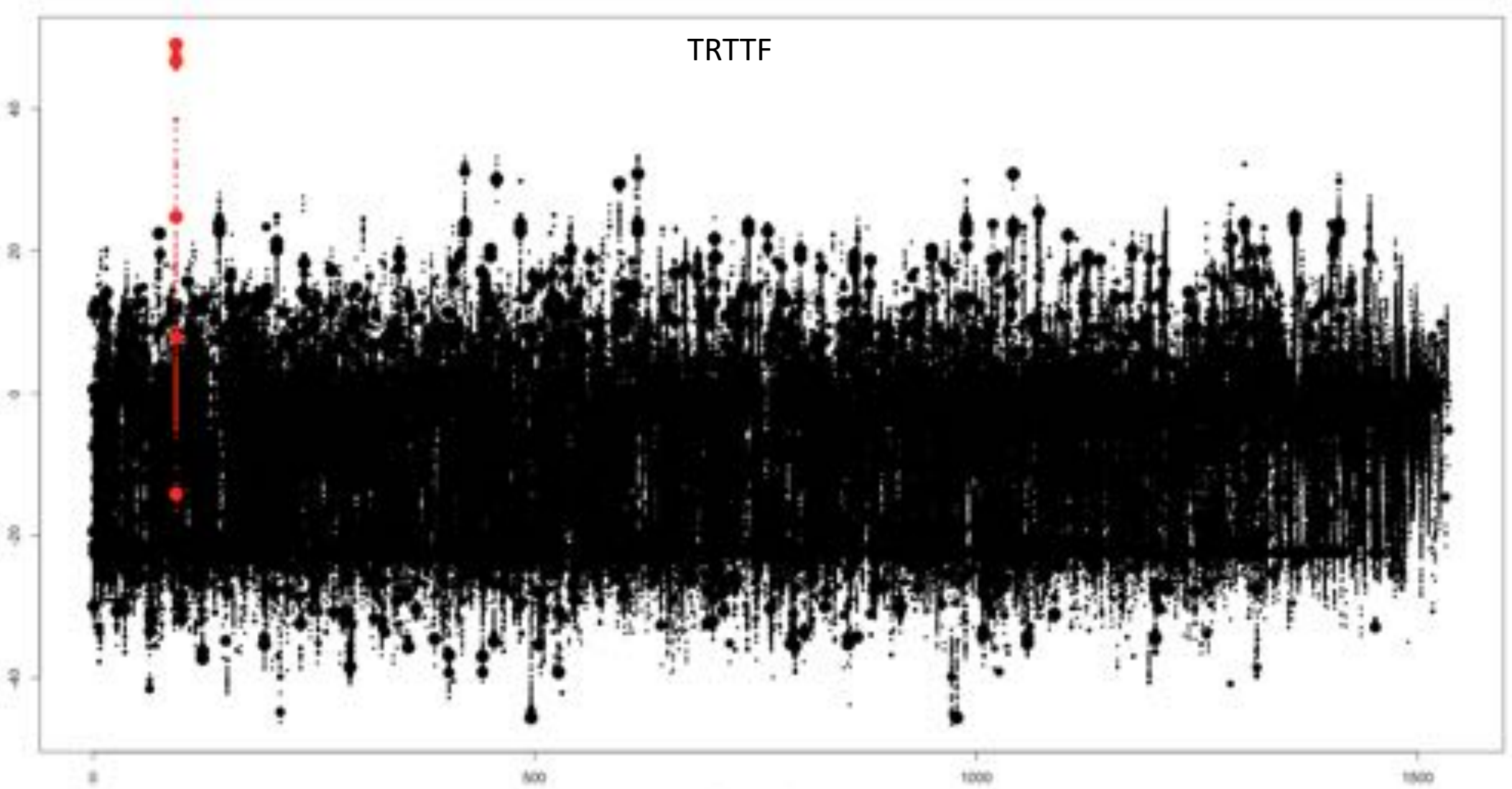
Candidate contig



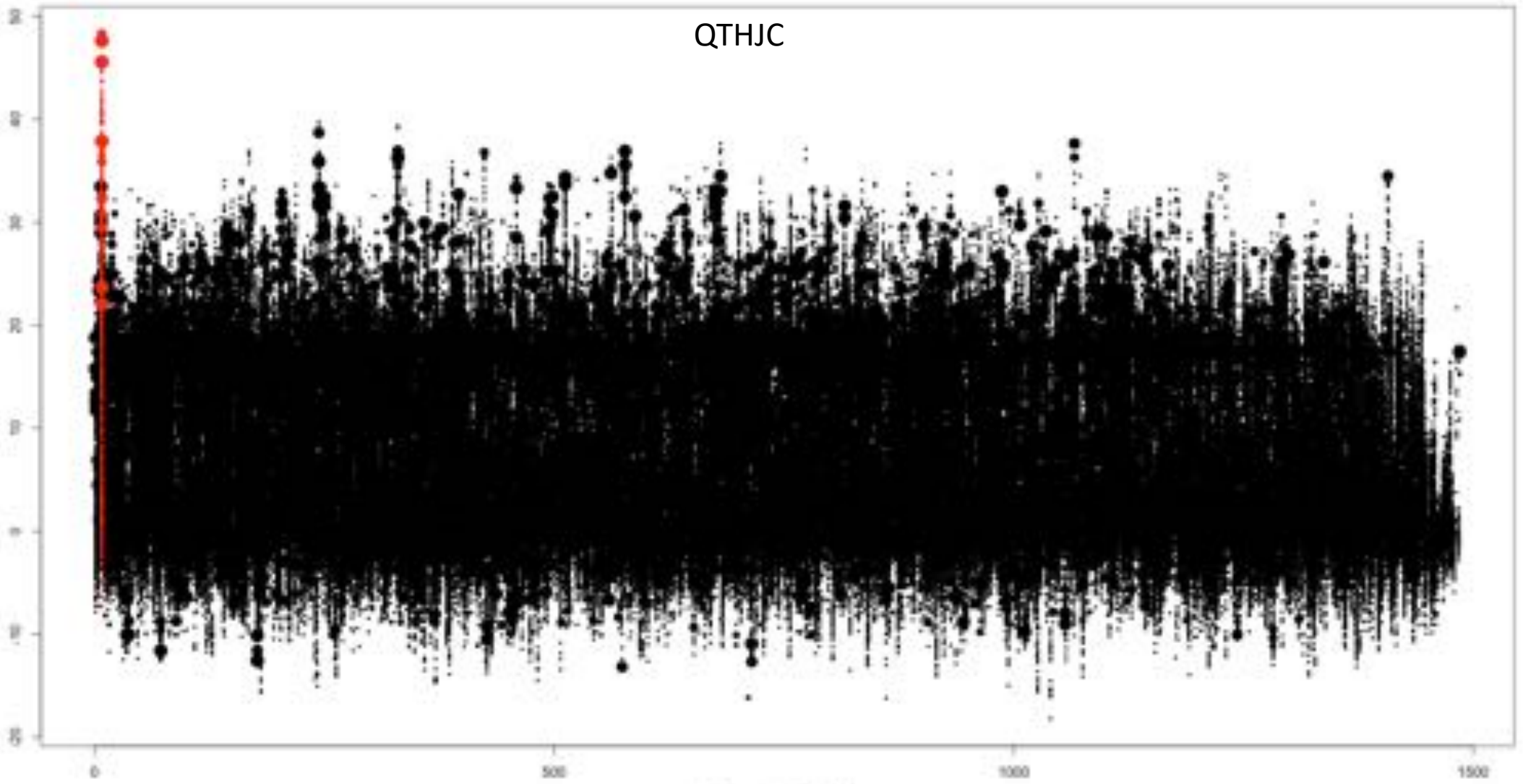
Novel Gene 1



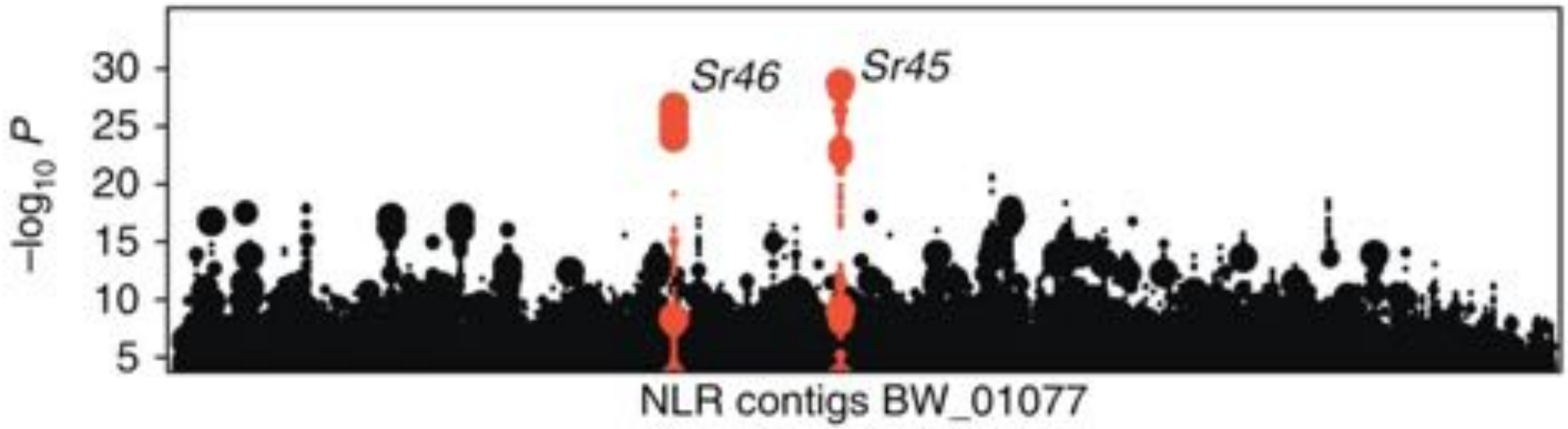
Sr46



SrTA1662



AgRenSeq

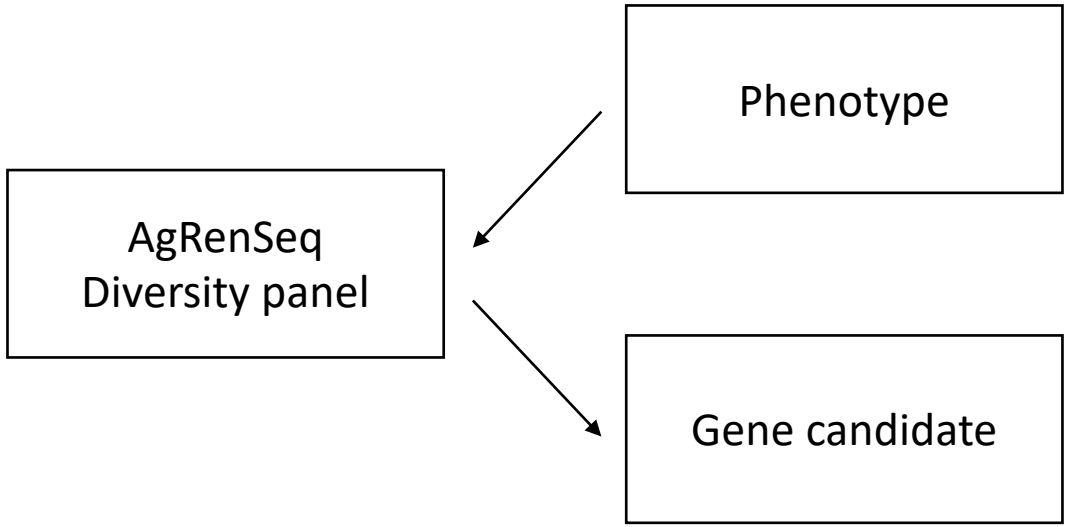


nature biotechnology
LETTERS

Resistance gene cloning from a wild crop relative by sequence capture and association genetics

Sanu Arora, Burkhard Steuernagel, Kumar Gaurav, Sutha Chandramohan, Yunming Long, Osadi Matny, Ryan Johnson, Jacob Enk, Sambasivam Periyannan, Narinder Singh, M. Asyraf Md Hatta, Naveenkumar Athiyannan, Jitender Cheema, Guotai Yu, Ngonidzashe Kangara, Sreya Ghosh, Les J. Szabo, Jesse Poland, Harbans Bariana, Jonathan D. G. Jones, Alison R. Bentley, Mick Ayliffe, Eric Olson, Steven S. Xu, Brian J. Steffenson, Evans Lagudah and Brande B. H. Wulff

AgRenSeq

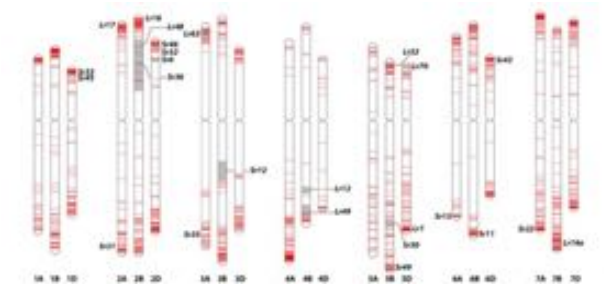


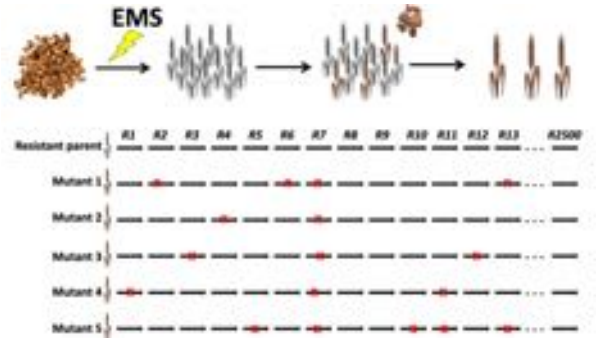
Summary

- NLR-Annotator
  to annotate NLR loci in wheat

- Mutational Genomics
  to pursue single dominant genes
  - MutRenSeq
  - MutChromSeq

- AgRenSeq
  screen diversity panels





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Javi Sanchez

Beat Keller

Marie Kubaláková

Jan Vrána

Jaroslav Doležel

Jacob Enk

Brian Steffenson

Jesse Poland