

*UTILIZING THE POTATO GENOME FOR APPLIED AND FUNDAMENTAL
RESEARCH INTO DIVERSE ASPECTS OF DISEASE RESISTANCE*

*TARGET ENRICHMENT SEQUENCING
ONE TOOL : MULTIPLE APPLICATIONS*



Ingo Hein

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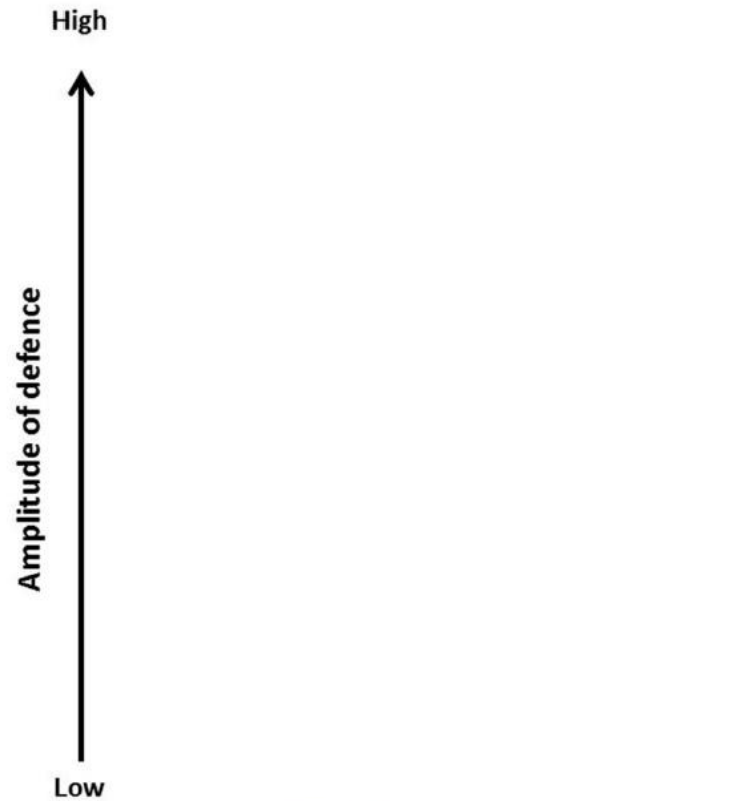
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INDUCIBLE RESISTANCES IN PLANTS

POTATO – LATE BLIGHT



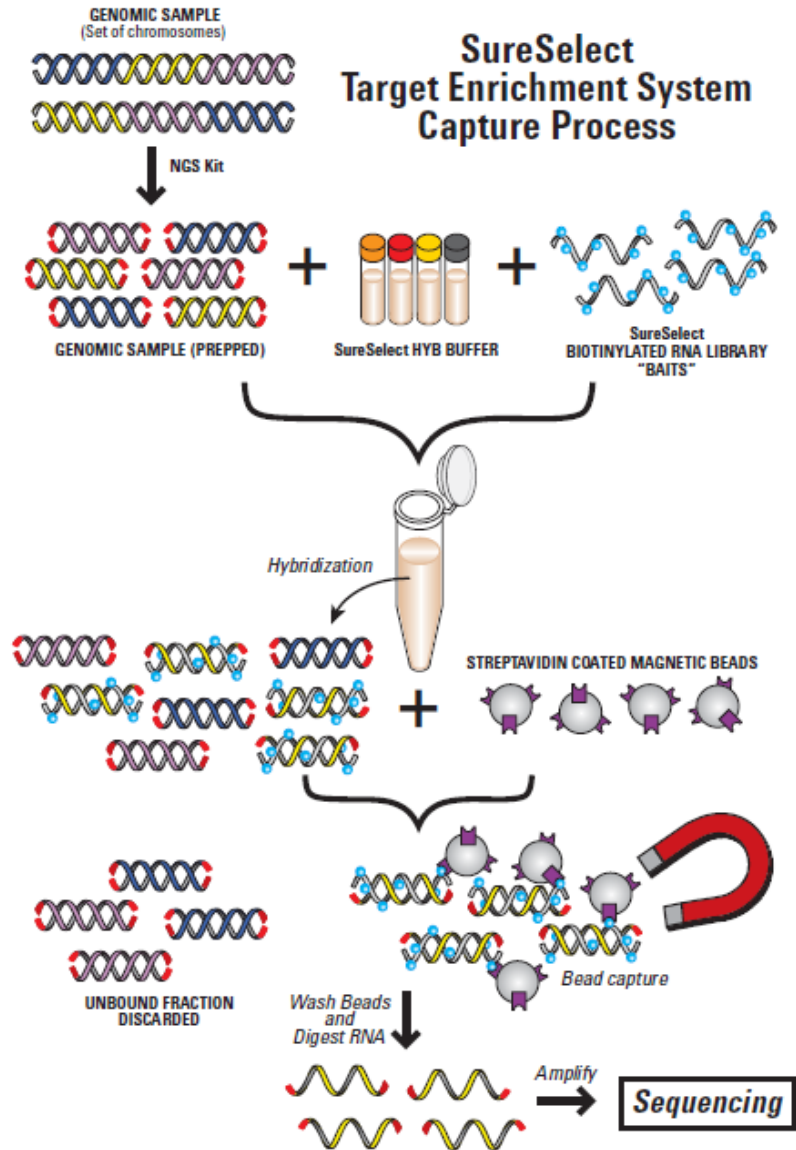
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ENRICHMENT AND SEQUENCING TO REDUCE GENOME COMPLEXITY



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RenSeq: 755+ NB-LRR genes from Potato, Tomato and Pepper
48,500 probes (120 nt)

Rlp/kSeq: 1000+ RLP/RLK from potato including 40 cultivars
55,970 probes (120 nt)

GenSeq: 1980 low copy number genes including 1163 COS markers
19,137 probes (120 nt)

PathSeq: *P. infestans*, *P. capsici*, *Albugo*, *Hpa*, ...

RESISTANCE GENE ENRICHMENT AND SEQUENCING

[RENSEQ]



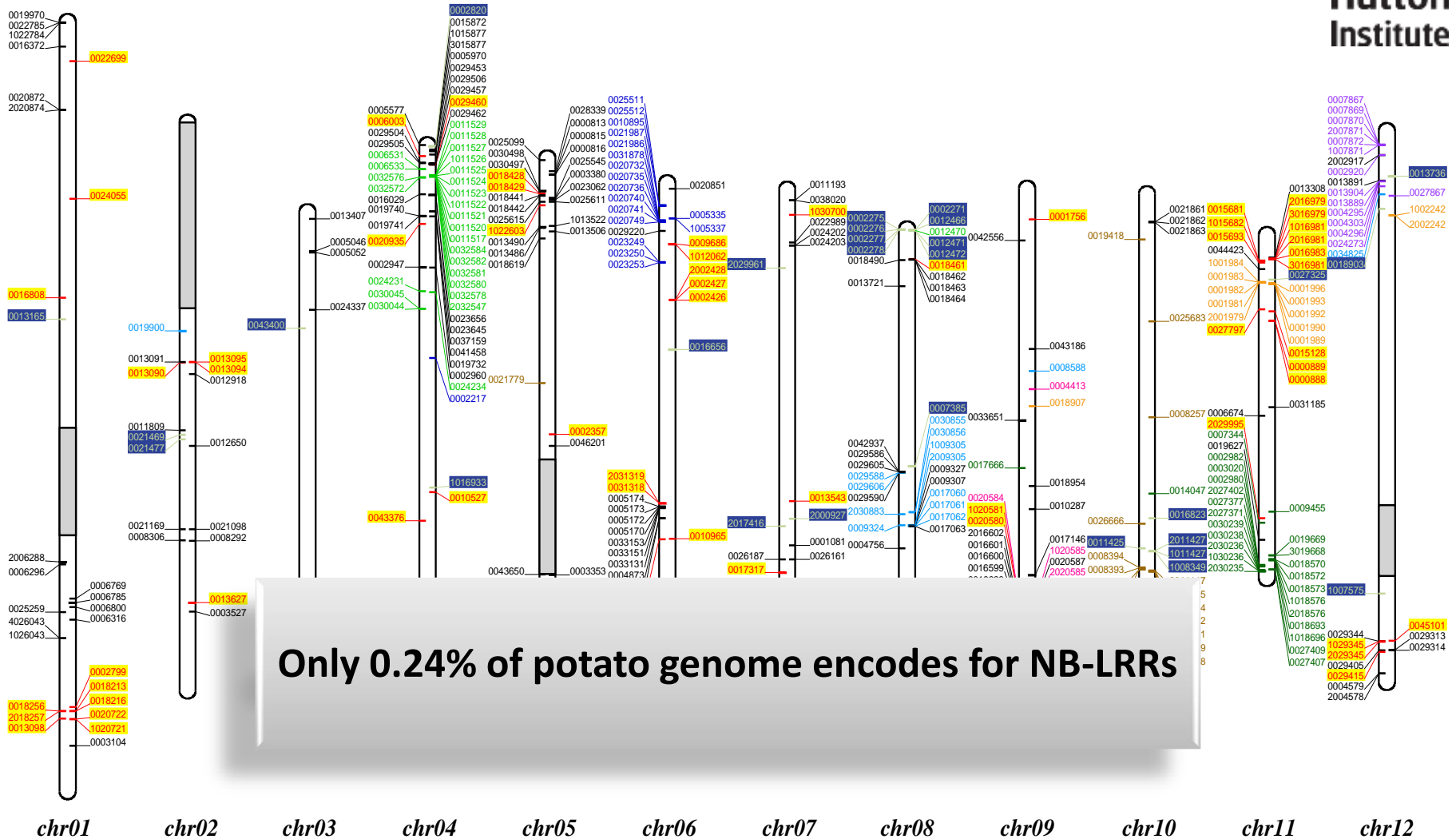
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- NB-LRR gene annotation
- Diagnostic tool
- Mapping of resistances
- Identifying candidate genes

IDENTIFYING NB-LRR GENES IN DM



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Jupe *et al.*, (2012) BMC genomics 13:75

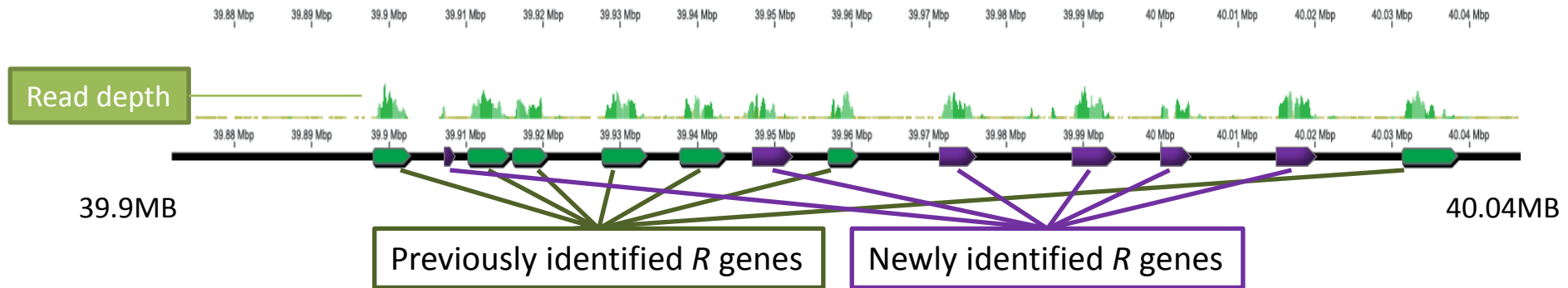
Jupe *et al.*, (2013) The Plant Journal

RENSEQ IN DM – IMPROVING THE NB-LRR GENE ANNOTATION

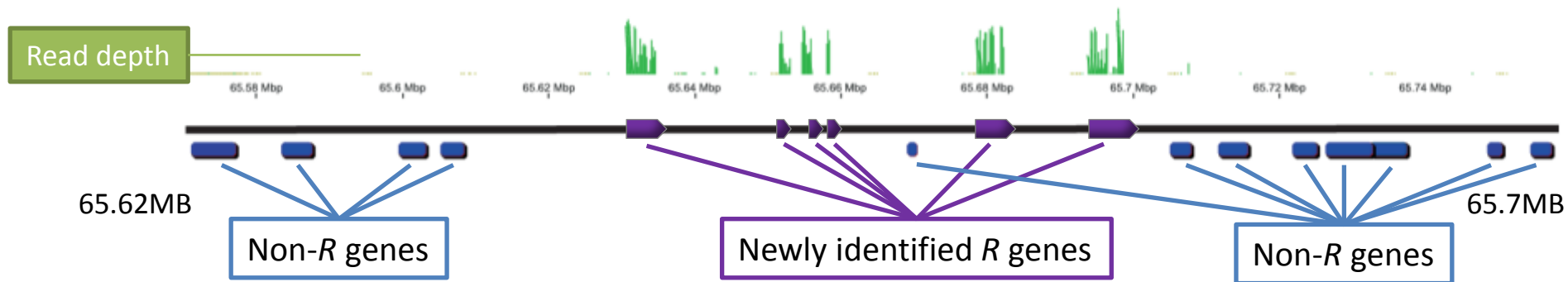


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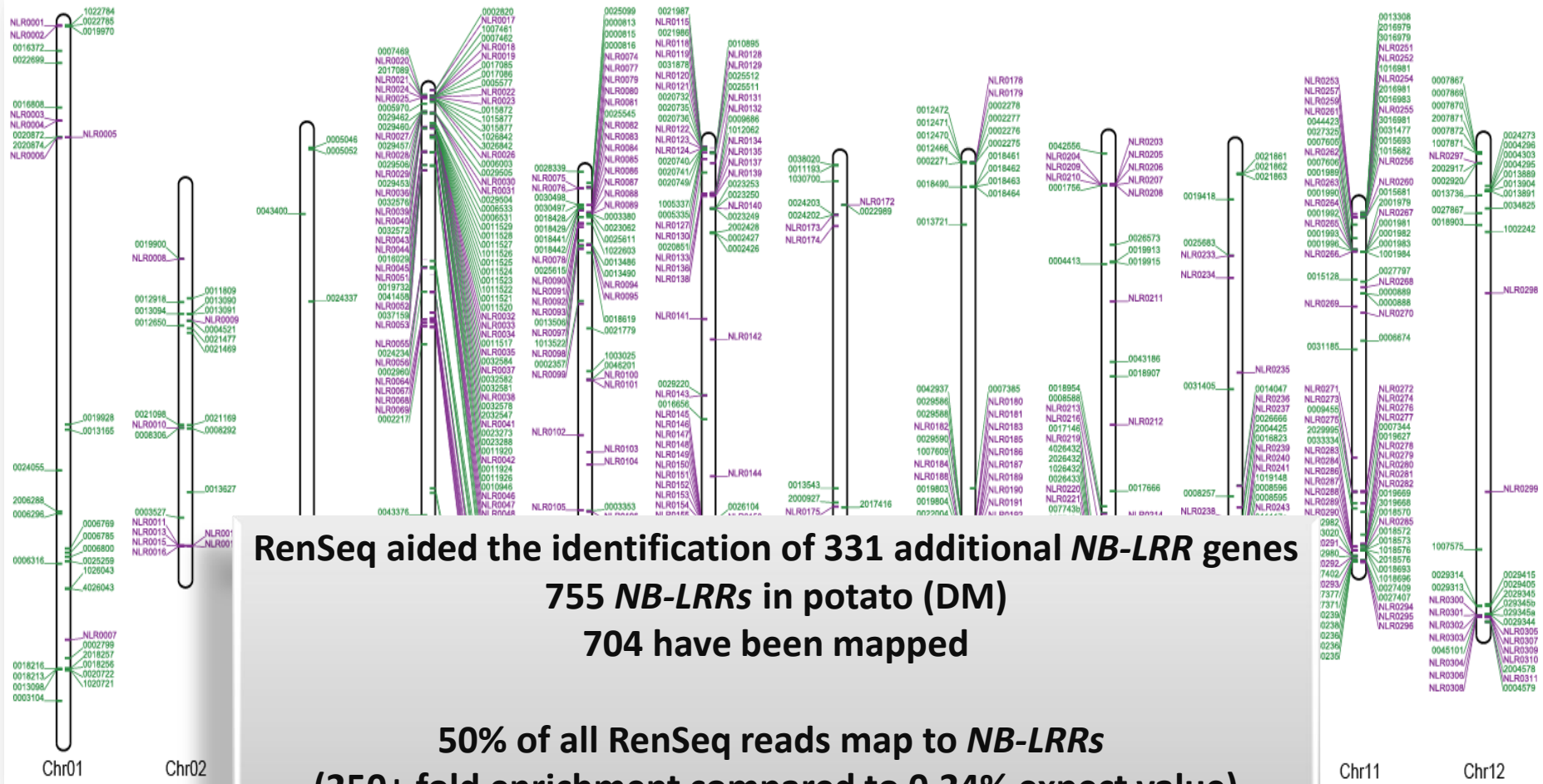
R3 locus on chromosome 11: high read depth identifies six new NB-LRRs



Gene 'sparse' region chromosome 2: discovery of six NRG1 homologues



RENSEQ IN DM – IMPROVING THE NB-LRR GENE ANNOTATION



RenSeq aided the identification of 331 additional NB-LRR genes
755 NB-LRRs in potato (DM)
704 have been mapped

50% of all RenSeq reads map to NB-LRRs
(250+ fold enrichment compared to 0.24% expect value)

~80% identity sufficient between bait (120 nt) and target

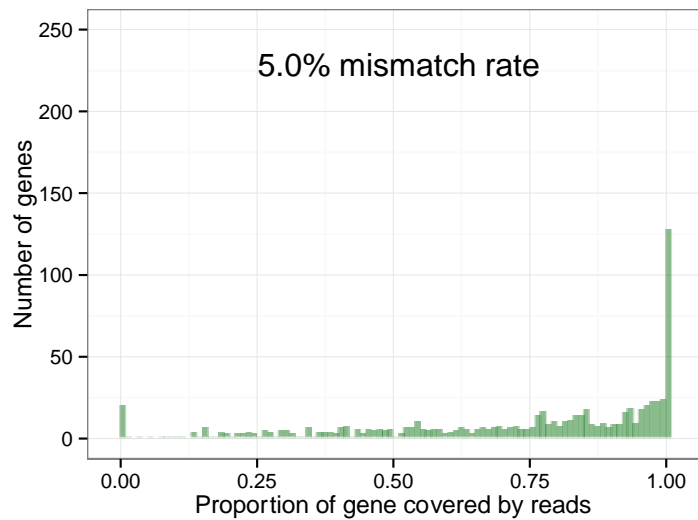
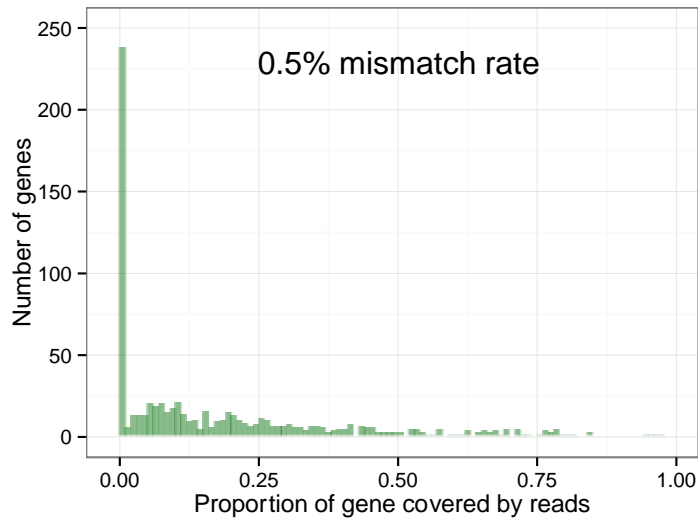
RENSEQ – A DIAGNOSTIC TOOL TO PRIORITISE NOVEL RESISTANCES WITIN GERMPLASM COLLECTIONS (CPC)

Species	Accessions	Blight	Known Rpi gene
	3761	Susceptible	
	3762	Resistance	Rpi_vnt1.1
<i>S. okadae</i>	7129	Resistance	?
	7625	Resistance	?
	7629	Resistance	?
	7775	Susceptible	

3762 - MAPPING AGAINST 755 NB-LRRs FROM DM 5% MISMATCH RATE OVERCOMES SPECIES DIFFERENCE



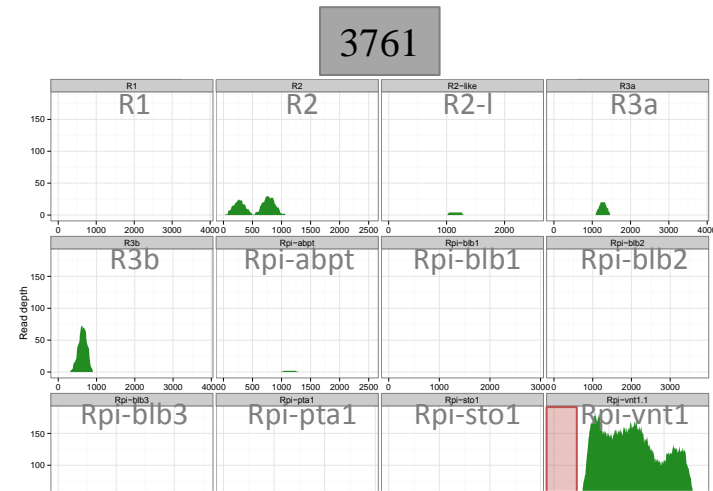
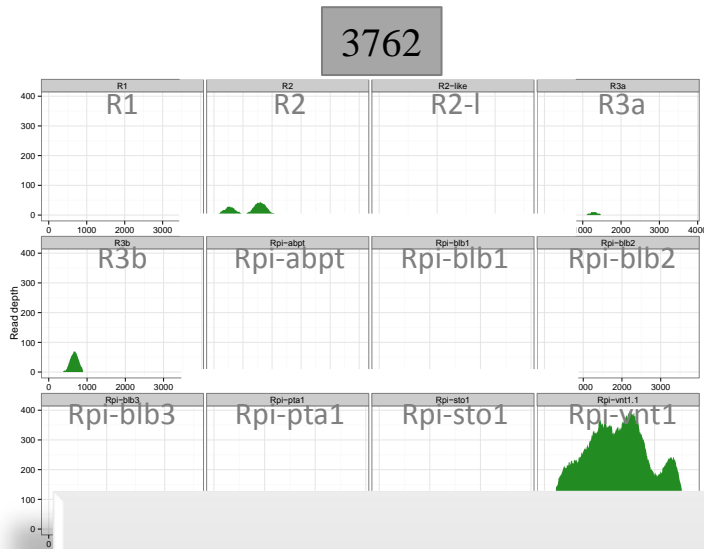
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HIGH STRINGENCY MAPPING (0.5 OR 1% MM) AGAINST REFERENCE SET OF 12 KNOWN FUNCTIONAL NB-LRR



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- *S. okadae* 7129 and 7625 also contain *Rpi_vnt1.1* (allele mining and dRenSeq)
 - dRenSeq to prioritise novel resistance in the CPC
 - RenSeq reads are subsequently used for mapping novel resistance
 - Tool for *R* gene stacking

BULKED SEGREGANT RENSEQ ANALYSIS

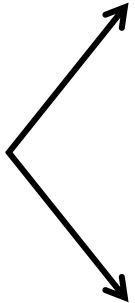


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Diploid populations (*S. berthaultii*, *S. okadae*; *S. verrucosum*)

F1

	SS	S	S
RS			
R	RS	RS	
S	SS	SS	



F1 Parent resistant
(heterozygous: R/S)

Bulk resistant
(heterozygous: R/S)

F1 Parent susceptible
(homozygous: S/S)

Bulked susceptible
(homozygous: S/S)



RenSeq enrichment
MiSeq 300 bp PE sequencing

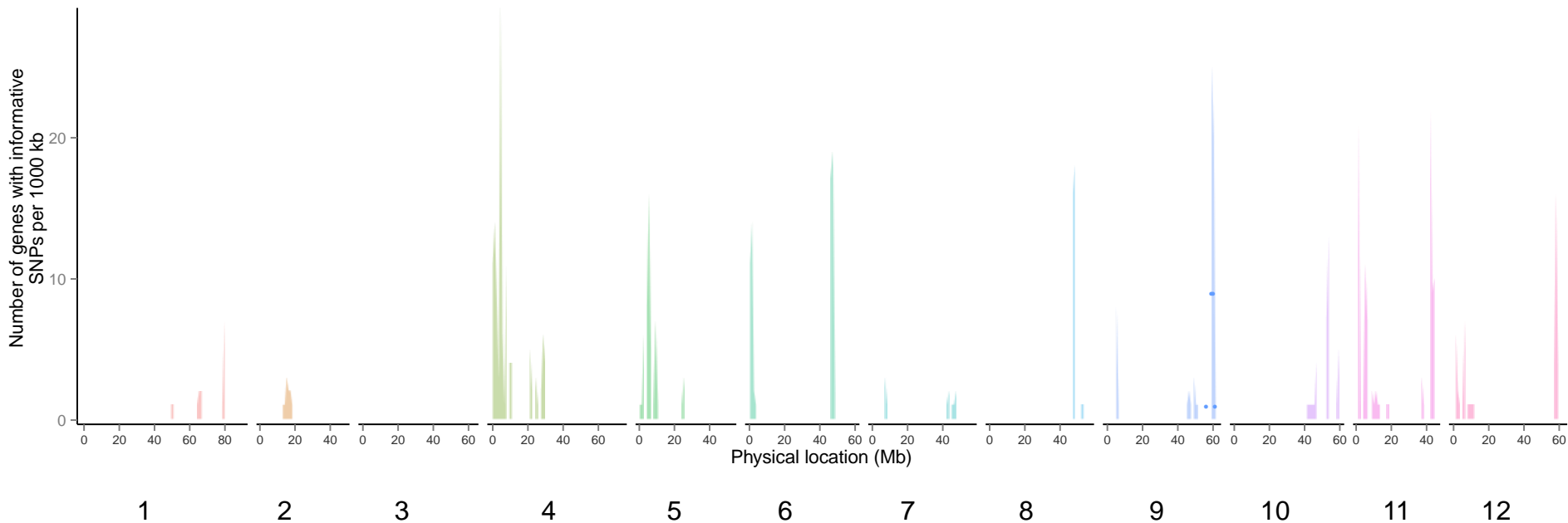
DM-BASED RENSEQ MAPPING AND SNP FILTERING

EXAMPLE LATE BLIGHT RESISTANCE GENE ON LG9



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Number of Genes with informative SNPs between parental *R* genes and bulks



PACBIO RENSEQ-BASED R-PARENT REFERENCE FOR CANDIDATE DISCOVERY



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F1

	SS	S	S
RR		R	R
R	RS	RS	RS
R	RS	RS	RS

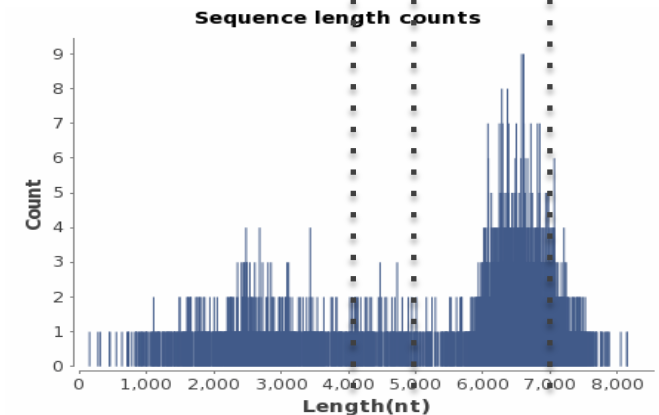
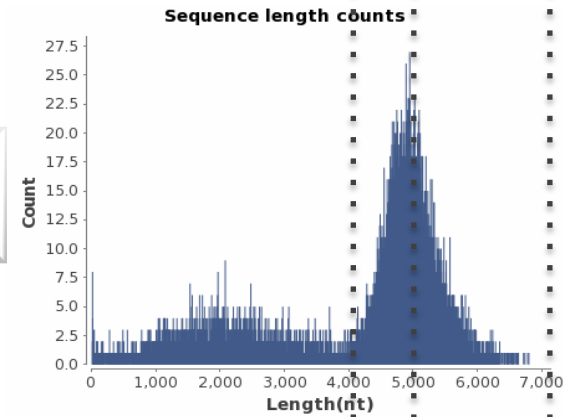
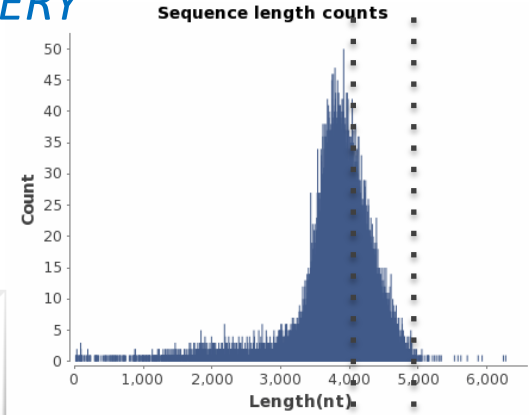
Parent resistant
(homozygous: R/R)

RenSeq enrichment
PacBio sequencing of 4, 5 and 7 kb fragments

Bulked resistant
(heterozygous: R/S)

Bulked susceptible
(homozygous: S/S)

SNP discovery plus
Presence/Absence variation





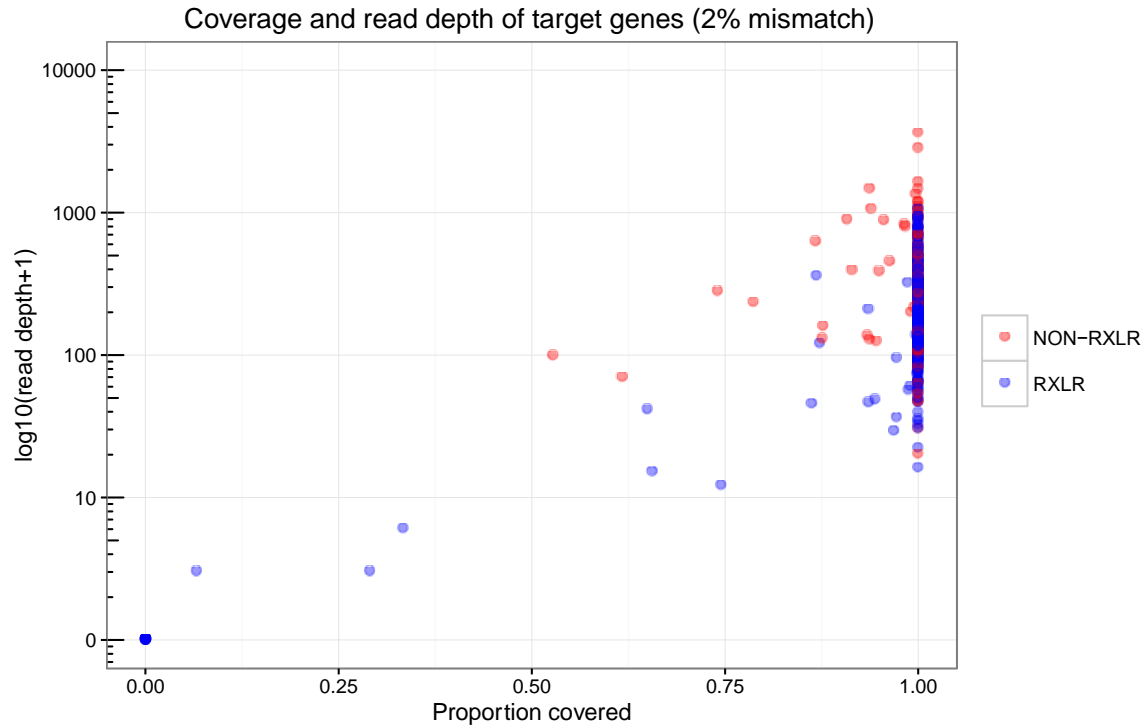
- 1162 *P. infestans* and *P. capsici* genes with a signal peptide [gene space ~0.83 MB]
- *P. capsici*:
 - 509 RXLRs
 - 65 CRNs
- *P. infestans*:
 - 457 RXLR from T30-4 reference isolate
 - 6 novel RXLR from 3928A
 - 125 additional genes [e.g. drug targets]

Species	strain
<i>P. infestans</i>	Blue13
<i>P. infestans</i>	88069
<i>P. infestans</i>	T30-4
<i>P. infestans</i>	US23
<i>P. infestans</i>	US24
<i>P. infestans</i>	EC1
<i>P. capsici</i>	LT123
<i>P. capsici</i>	Pc204
<i>P. capsici</i>	LT1534
<i>P. capsici</i>	LT6535
<i>P. capsici</i>	Y006
<i>P. capsici</i>	Q108

PATHSEQ – COVERAGE OF T30-4 TARGET GENES



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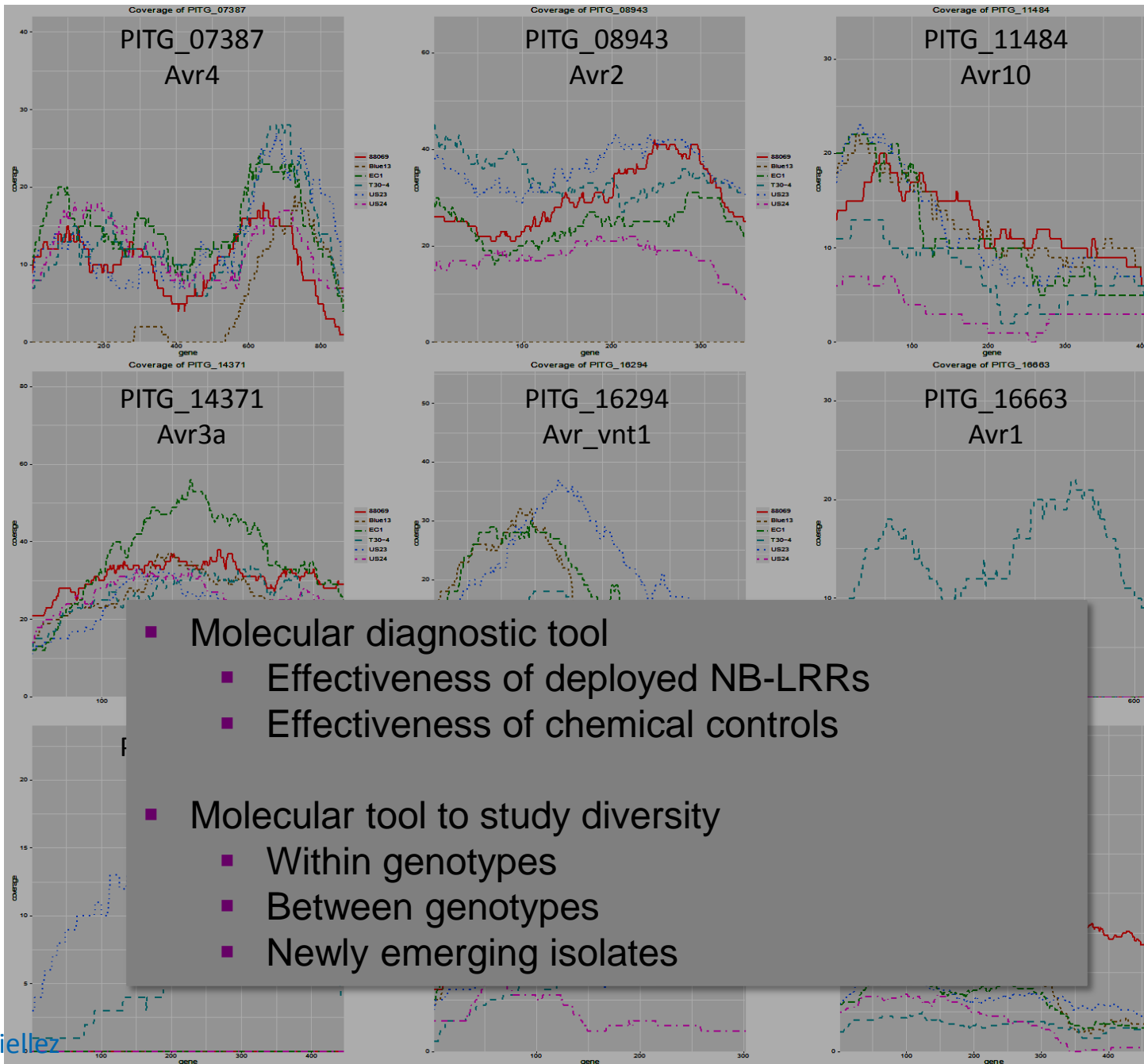
On-target rate only about 10%

1500+ putative SP-RXLR containing genes identified

PATHSEQ – AVR GENE DIVERSITY



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