Christian-Albrechts-Universität zu Kiel

Re-sequencing and hybrid assembly strategy of two nematode resistant *Beta vulgaris* translocation lines

Plant Breeding Institute

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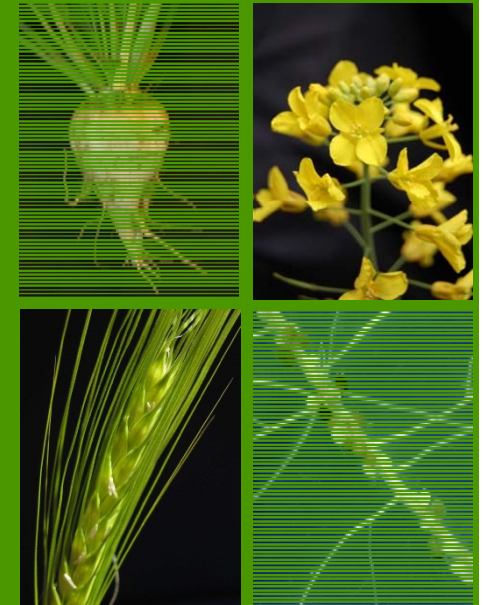
The *Heterodera schachtii - Beta vulgaris* pathosystem
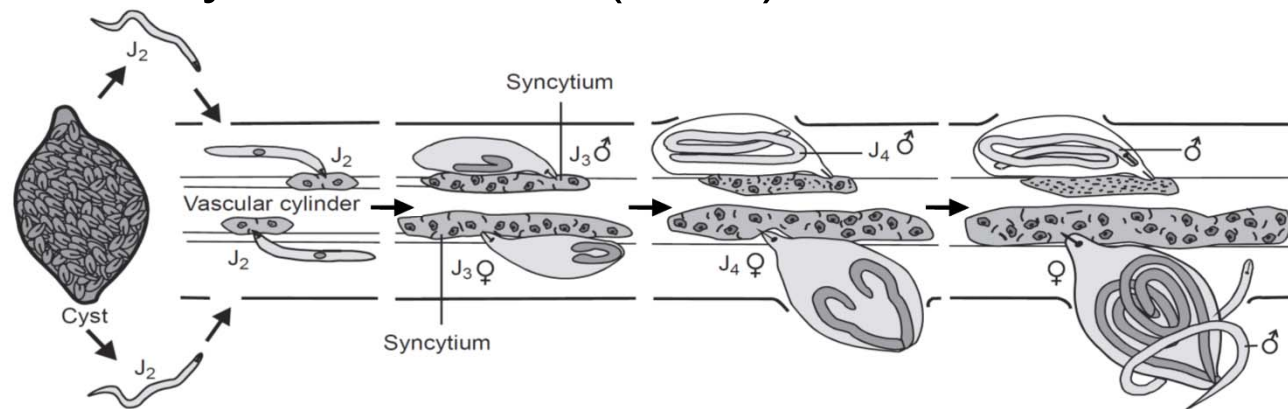
- Beet cyst nematode (BCN)

Figure 1: Life cycle of *Heterodera* (Jung and Wyss 1999)

- Host plants: *Chenopodiaceae* and *Brassicaceae*
- Distribution: Europe, USA, Canada, Australia
- Yield losses: 25-50%

➡ Solution: breeding of resistant/tolerant sugar beets

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Transferring resistance to the primary gene pool

- No resistance against the BCN in the primary gene pool of *Beta*

➡ Chromosomes of wild beet *Patellifolia* bear resistance genes

Fig. 1: Sugar beet compatible reaction

J4 females, 21 dpi

Table 1: Resistance to BCN on different chromosomes of the tertiary gene pool

Tertiary Genepool	Chromosome		
	1	7	8
Patellifolia procumbens	$Hs1^{pro-1}$ **Hs2**	$Hs2^{pro-7}$	$Hs3^{web-8}$
Patellifolia patellaris	$Hs1^{pat-1}$	---	---
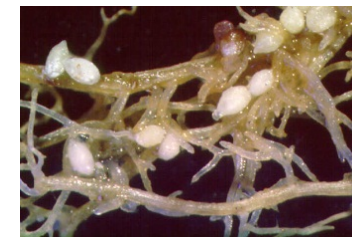
Fig. 2: Resistant beet incompatible reaction

stagnating female, 20 dpi

➡Transfer of resistance to *Beta vulgaris*: translocation lines

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Physical and genetic maps of 2 resistant translocation lines

Patellifolia procumbens translocation chromosome 1

Beta vulgaris chromosome 9

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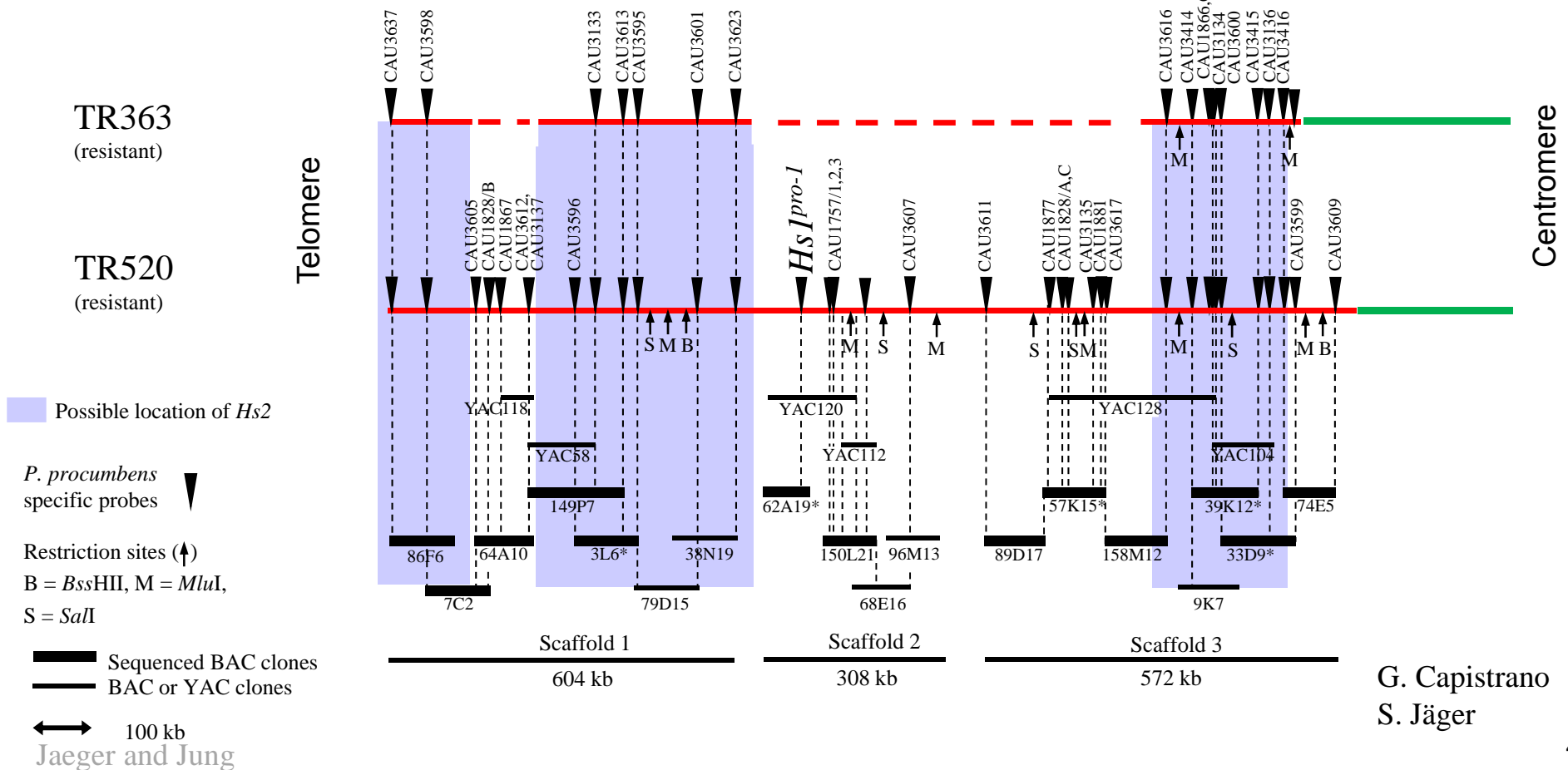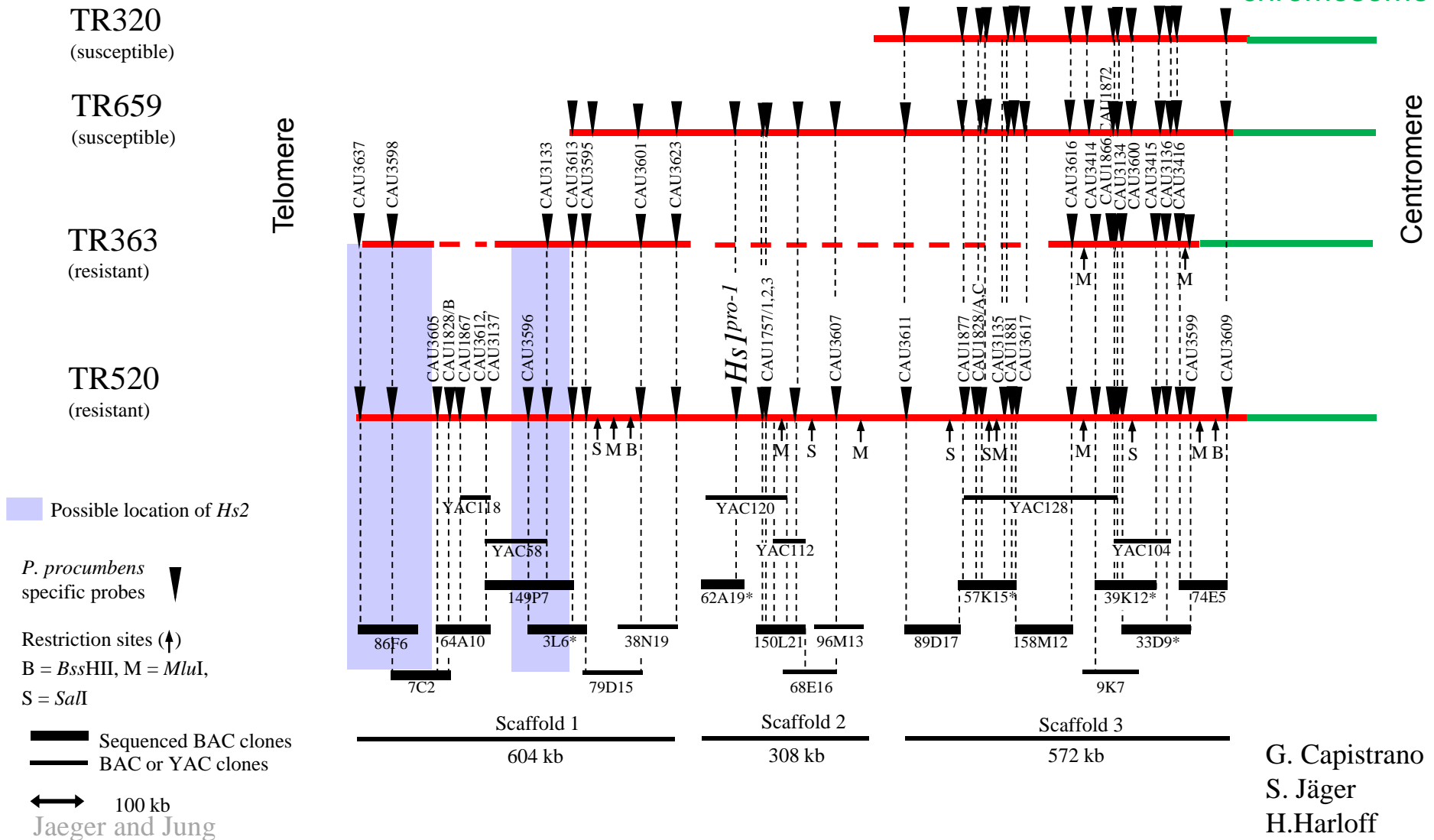
Genetic maps of 4 resistant and susceptible translocation lines

Patellifolia procumbens translocation chromosome 1

Beta vulgaris chromosome 9

TR320
(susceptible)

TR659
(susceptible)

TR363
(resistant)

TR520
(resistant)

Telomere

Centromere

CAU3637
CAU3598
CAU3133
CAU3613
CAU3595
CAU3601
CAU3623
CAU3616
CAU3414
CAU1866
CAU1872
CAU3134
CAU3600
CAU3415
CAU3136
CAU3416

CAU3605
CAU1828/B
CAU1867
CAU3612
CAU3137
CAU3596
Hs1pro-1
CAU1757/1,2,3
CAU3607
CAU3611
CAU1877
CAU1828/A,C
CAU3135
CAU1881
CAU3617
CAU3599
CAU3609

M   M

S M B   M S   M   S   SM   M   S   M B

Possible location of *Hs2*

P. procumbens specific probes

Restriction sites (↑)
B = *Bss*HII, M = *Mlu*I,
S = *Sal*I

Sequenced BAC clones
BAC or YAC clones

100 kb

YAC118

YAC58

149P7

86F6   64A10

7C2

YAC120

YAC112

62A19*

150L21 96M13

79D15

68E16

3L6*

38N19

YAC128

YAC104

57K15*

39K12*   74E5

89D17   158M12

33D9*

9K7

Scaffold 1
604 kb

Scaffold 2
308 kb

Scaffold 3
572 kb

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Physical mapping and sequence analysis of the nematode resistance translocation line TR520

- Physical map: 3 scaffolds, 18 BAC clones, total 1.482 Mbp

- Sequencing: 13 BAC clones = 1.015 Mbp
  - Repetitive elements: 11.14%
  - ORFs: 104 ORFs identified
  - Sequence similarity translocation – sugar beet: 66.39%

- Size of the translocation TR520: ~1.5 Mbp
  - 67% sequenced

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ORF702 as a candidate for the *Hs2* gene

- Sequence analysis:
    - Putative Avr9 elicitor response protein
    - 1 transmembrane domain
    - Conserved domain: Galactosyltransferase
    - Schaff et al., 2007: Root knot nematode - tomato

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- Functional analysis:
    - 15 transgenic *Beta vulgaris* hairy root clones
    - 24 transgenic *Arabidopsis thaliana* T2 families

    ➡ nematode resistance tests resulted in <u>no significant differences</u> between control and transgenic plants

- Conclusion: ORF702 is not the resistance gene *Hs2*

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# Nematode resistance gene candidates from *P. procumbens*: results from 15 years of research

Sequence candidates	*Beta vulgaris* translocation lines				*B.vulg.* genome	*P. proc.* genome	Predicted protein function	Functional analysis in *B. vulgaris* hairy roots and *A. thaliana*	References
	TR520 (res.)	TR363 (res.)	TR659 (susc.)	TR320 (susc.)					
Hs2	X	X	-	-	-	X	unknown	Significantly reduced cysts	This work
Hs1^pro-1	X	X	X	-	-	X	LRR-TM	Significantly reduced cysts	Cai et al., 1997
ORF702	X	X	-	-	-	X	Galactosyl-transferase	No differences in cyst number	Capistrano, 2009; This work
BpPIP1	X	-	X	X	-	X	Aquaporin	Significantly reduced cysts	Menkhaus, 2011
cZR 3	X	X	NA	NA	X	X	CC-NBS-LRR	Significantly reduced cysts	Tian et al., 2004 Knecht , 2009
cZR7	X	X	NA	NA	X	X	CC-NBS-LRR	Significantly reduced cysts	Tian et al., 2004 Knecht , 2009

Sequence is x = present or - = absent on translocation line; NA =not availible

➡ re-sequencing the translocation line

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- ## Aim:

  Identification of the nematode resistance gene *Hs2*

- ## Objective:

  Whole genome shotgun (WGS) sequencing of the two resistant translocation lines TR520 and TR363

Identification of new translocation regions and *Hs2*

B. vulgaris

BAC

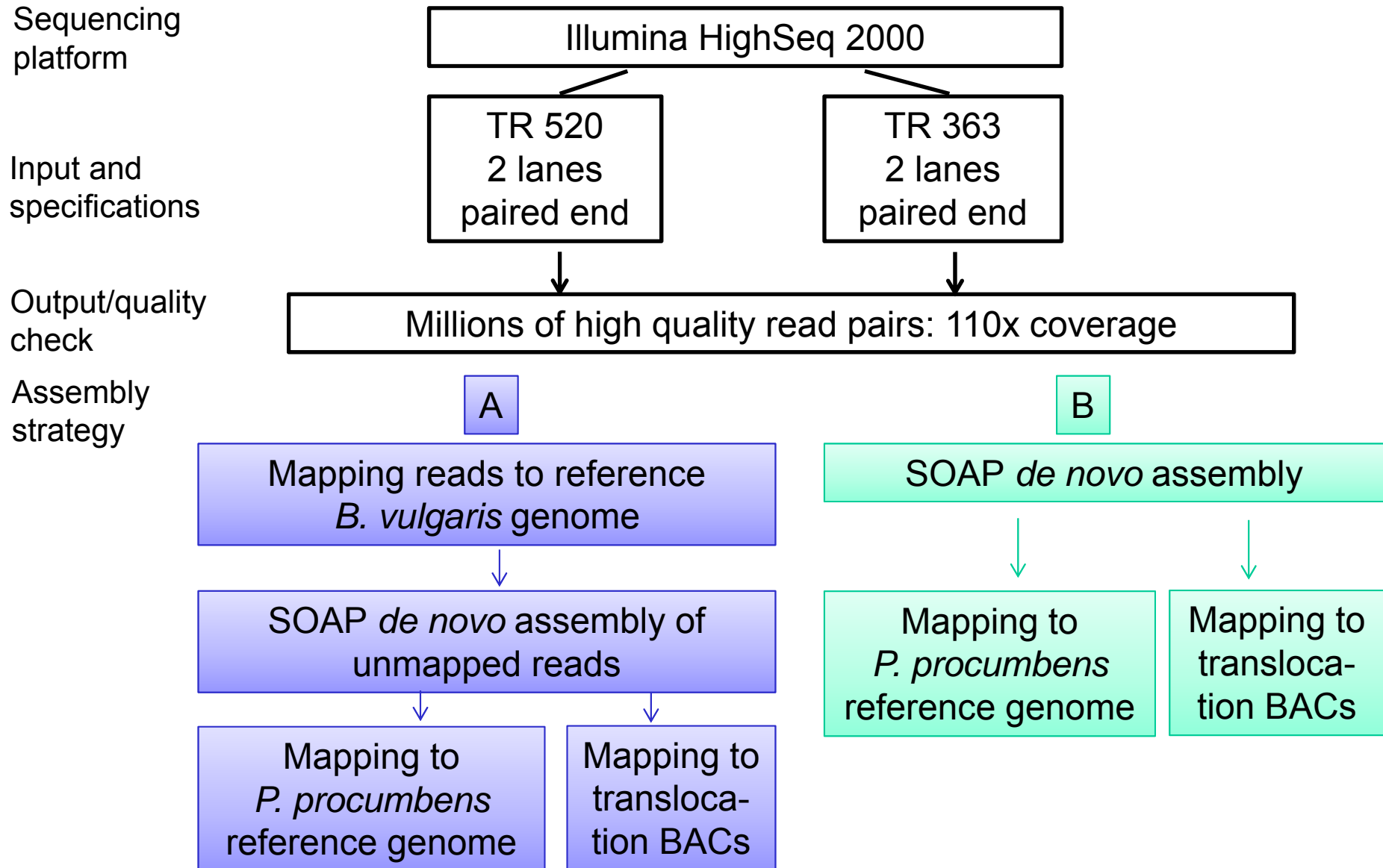P. procumbens

Translocation line

Hs2
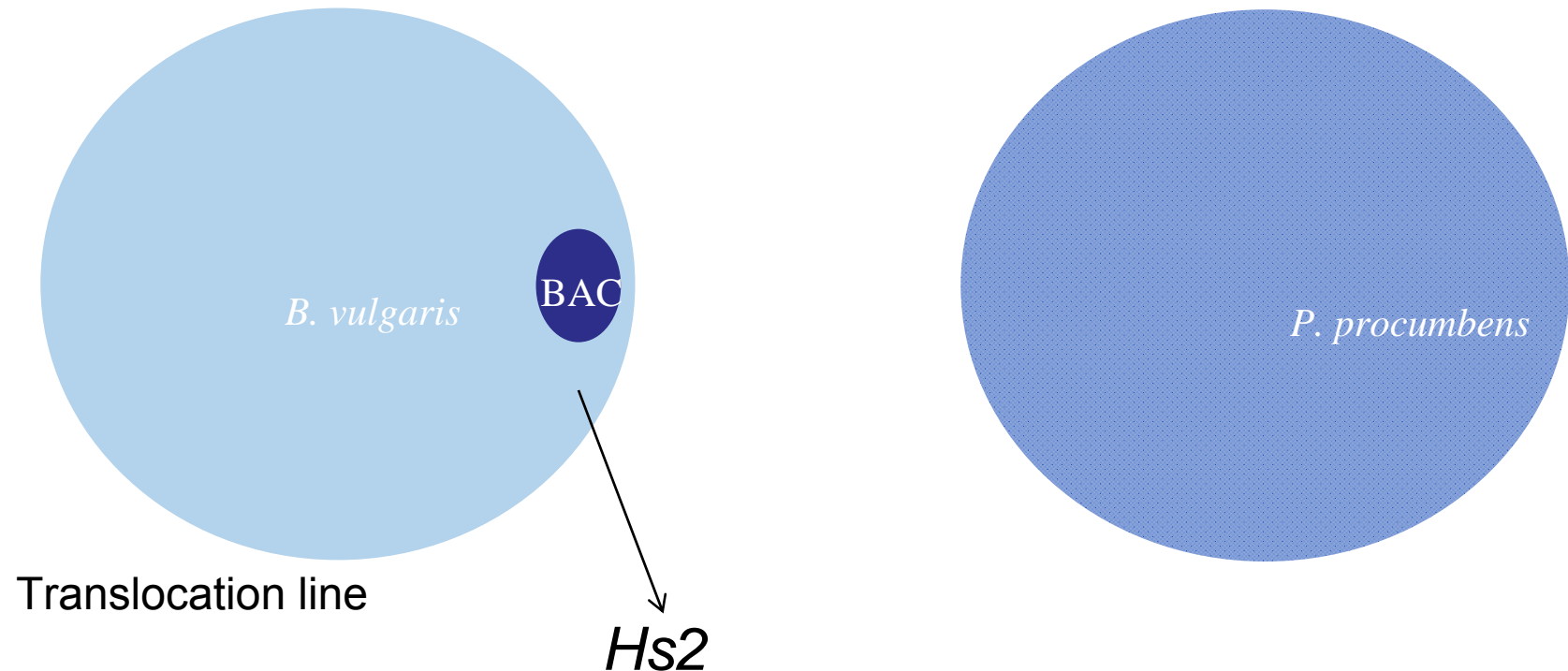
Reference sequences

B. vulgaris
996.7 Mb
N50: 358 kb

13 translocation
specific (*P. proc.*)
BAC sequences
1015 kb

P. procumbens
641 Mb
N50: 38 kb

(Genome data 28.06.2011: H.Himmelbauer, J.C. Dohm, A.E. Minoche, GABI Beetseq)   (BAC data: S. Jäger; G. Capistrano 2009)

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Hybrid assembly strategy

Sequencing platform

Illumina HighSeq 2000

Input and specifications

TR 520
2 lanes
paired end

TR 363
2 lanes
paired end

Output/quality check

Millions of high quality read pairs: 110x coverage

Assembly strategy

A

B

Mapping reads to reference
B. vulgaris genome

SOAP *de novo* assembly

SOAP *de novo* assembly of
unmapped reads

Mapping to
P. procumbens
reference genome

Mapping to
transloca-
tion BACs

Mapping to
P. procumbens
reference genome

Mapping to
transloca-
tion BACs

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Criteria for *Hs2* sequence candidates

- Plant resistance genes

- Conserved domains

- Genes up- or downregulated upon nematode infection
    - (Soybean: Klink et al., 2007, 2009; Ibrahim et al., 2011; Mazarei et al., 2011; Tomato: Bhattarai et al., 2008; Schaff et al., 2007; Cotton: de Deus Barbosa et al.,2009)

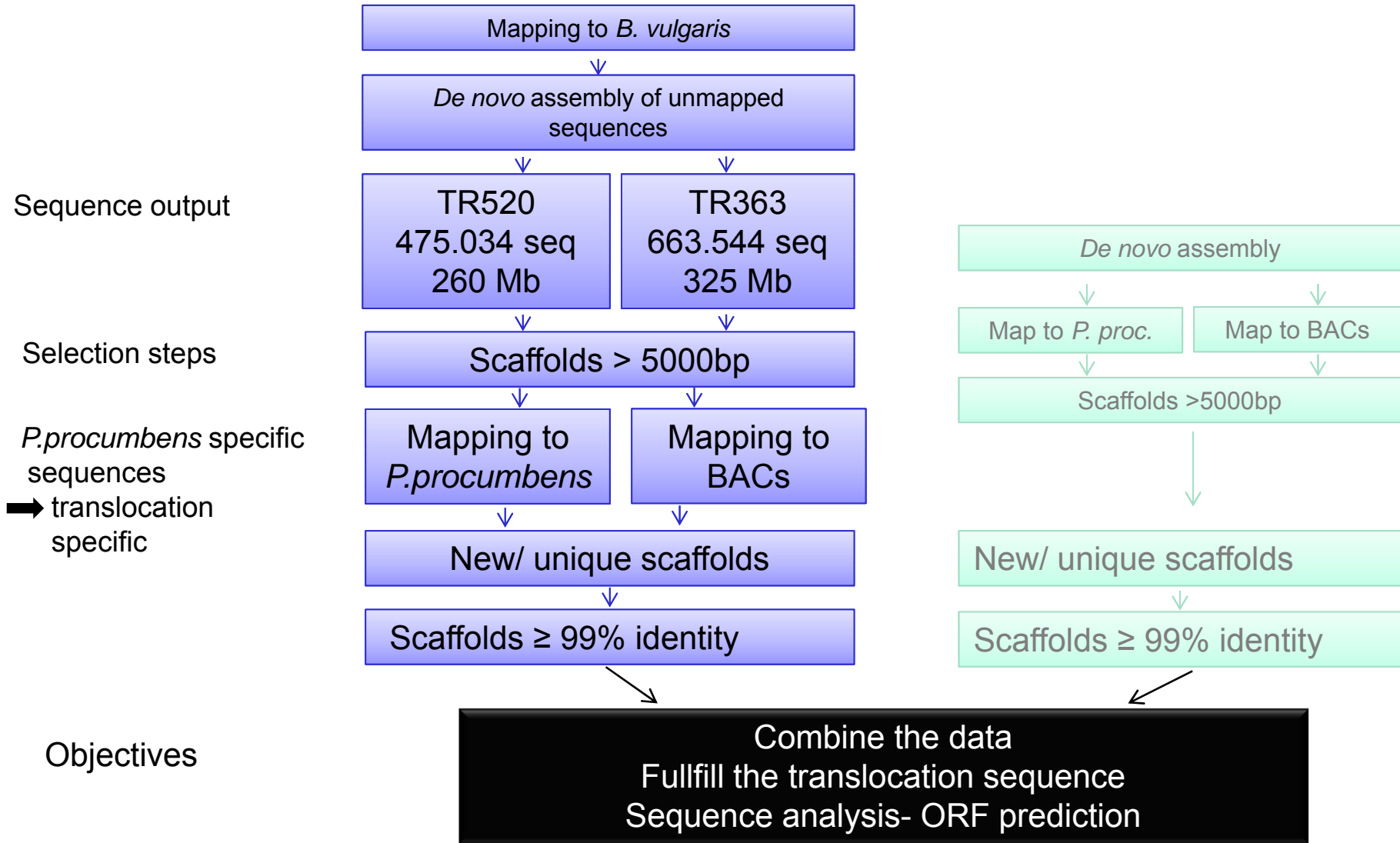## 11 cloned nematode resistance genes and their functional domains

Gene	Origin	Nematode	Functional domains	References
Hs1[pro-1]	*P. procumbens*	*H. schachtii*	**TM-LRR**	Cai et al., 1997
Mi 1.2	*S. peruvianum*	*M. incognita*	**CC-NBS-LRR**	Milligan et al., 1998 Vos et al., 1998
Gpa2	*S. tuberosum* **ssp.** *andigena*	*G. pallida*	**CC-NBS-LRR**	Van der Vossen et al., 2000
Hero	*S. pimpinelli-folium*	*G. rostochiensis*	**CC-NBS-LRR**	Ernst et al., 2002
Rhg4	*G. max*	*H. glycines*	**TM-LRR**	Lightfoot and Meksem 2002
Gro1-4	*S. spegazzinii*	*G. rostochiensis*	**TIR-NBS-LRR**	Paal et al., 2004
rhg1	*G. max*	*H. glycines*	**TM-LRR**	Ruben et al., 2006
Mi-9	*S. peruvianum*	*M. incognita*	**CC-NBS-LRR**	Jablonska et al., 2007
CaMi	*C. annuum*	*M. incognita*	**CC-NBS-LRR**	Chen et al., 2007
XiR1	*V. arizonica*	*X. index*	**non-TIR-NBS-LRR**	Hwang et al., 2010
Ma	*P. cerasifera*	*M. incognita*	**TIR-NBS-LRR**	Claverie et al., 2011

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Sequence analysis

Strategy

A   B

Sequence output

Selection steps

P.procumbens specific
sequences
➡ translocation
specific

```
Mapping to B. vulgaris
        ⇓
De novo assembly of unmapped
        sequences
   ⇓              ⇓
TR520          TR363
475.034 seq    663.544 seq
260 Mb         325 Mb
   ⇓              ⇓
   Scaffolds > 5000bp
   ⇓              ⇓
Mapping to     Mapping to
P.procumbens   BACs
   ⇓              ⇓
   New/ unique scaffolds
        ⇓
   Scaffolds ≥ 99% identity
```

```
De novo assembly
   ⇓              ⇓
Map to P. proc.  Map to BACs
   ⇓              ⇓
   Scaffolds >5000bp
        ⇓
   New/ unique scaffolds
        ⇓
   Scaffolds ≥ 99% identity
```

Objectives

Combine the data
Fullfill the translocation sequence
Sequence analysis- ORF prediction

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Current status of the project:

- ✓ Illumina paired-end sequencing of TR520 and TR363
- ✓ Mapping to *Beta vulgaris* to <u>select *P. procumbens* specific sequences</u>
- o *De novo* assembly of <u>unmapped reads</u> of TR520 and TR363
- o ORF analysis

Future work:

- • *De novo* assembly of TR363 and TR520
- • Combination of the sequences
  - • Identification of *P. procumbens* specific sequences of TR520 and TR363
  - ⟶ overlapping sequences between TR520 and TR363
  - • ORF prediction and sequence analysis for *Hs2*

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