2019 Progress for applying genomic tools to accelerate breeding for disease resistance in confection sunflower

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NSA Research Forum
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Outline

- Background knowledge (DM, *R*-genes, *etc*.)
- Research objectives
- Research progress
  - **Updates of Pl_{17} and Pl_{19} fine mapping**
  - **Saturation mapping of DM *R*-gene Pl_{18}**
  - **Pl_{18} recombinant screening**
  - **Fine mapping of Pl_{18}**
- Future work
- Acknowledgements

Downy mildew

(Photo by Markell and Gong)

- *Plasmopara halstedii*
- Incidence: 16% of 2015 and 9% of 2017 (NSA surveys)
- Development of resistant hybrids is most effective management tool (economic & environmental)

DM threats sunflower production

- New DM races are emerging, making current DM *R*-genes ineffective
- Potential threat to sunflower production
  - Gilley (2014-2015) identified only five *R*-genes (Pl_{Arg}, Pl_{15}, Pl_{17}, Pl_{18}, and Pl_{33}) were resistant to 185 DM isolates collected in ND, SD, MN, NE
- New DM *R*-gene is continuously needed (Pl_{19}, Pl_{20}, Pl_{33}, Pl_{34}, Pl_{35}, and more)

Research objectives 2017-2019

- Analyze allelic relationships of the two DM *R*-genes, Pl_{17} and Pl_{19}
- Conduct high-resolution genetic and physical mapping of Pl_{17}, Pl_{19}, and Pl_{18}
- Identify candidate genes of DM resistance
- Develop user-friendly markers of these DM *R*-genes

Research objectives 2019

- Analyze allelic relationships of the two new DM *R*-genes, Pl_{17} and Pl_{19}
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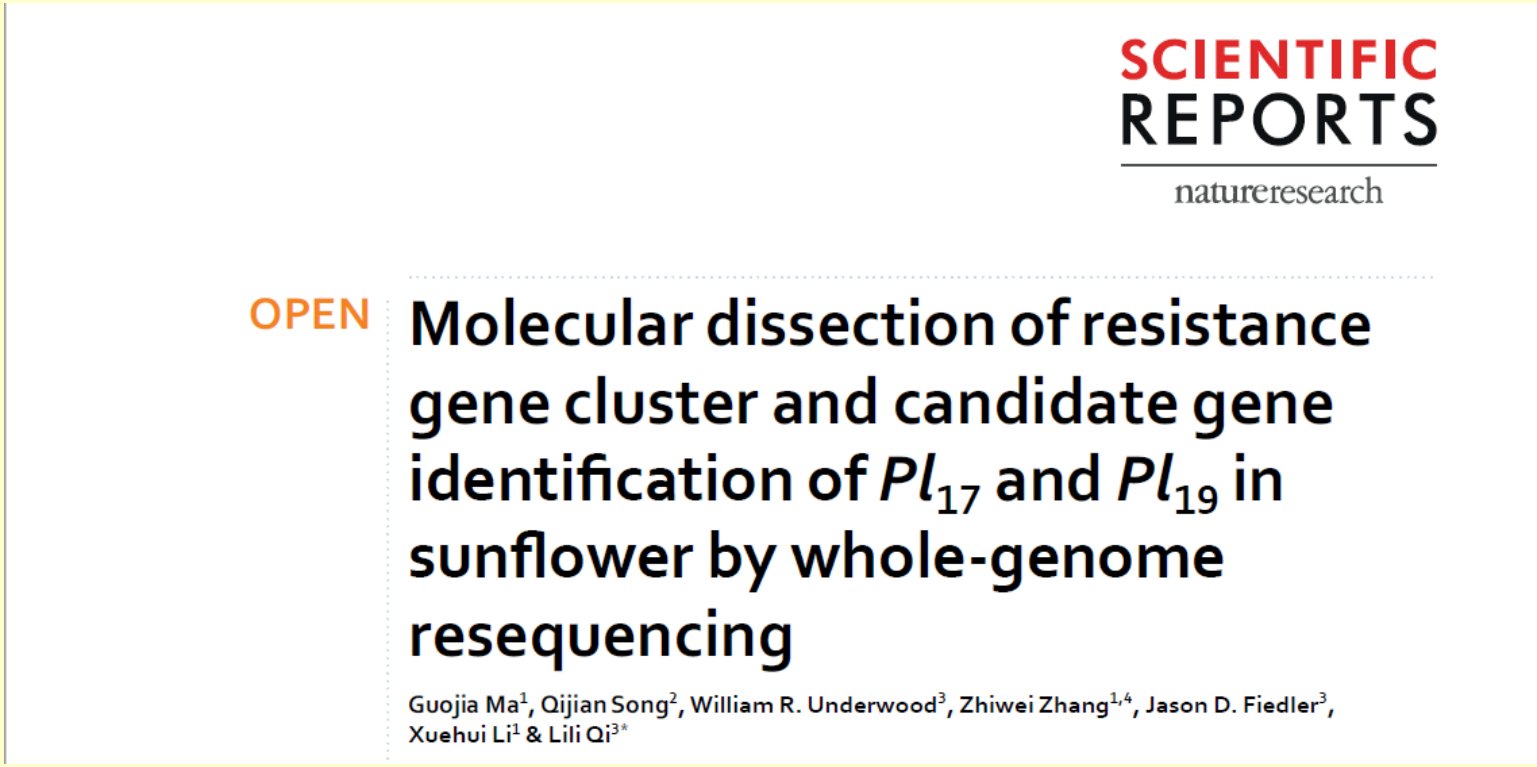
Updates of Pl_{17} & Pl_{19}



SCIENTIFIC REPORTS

nature research

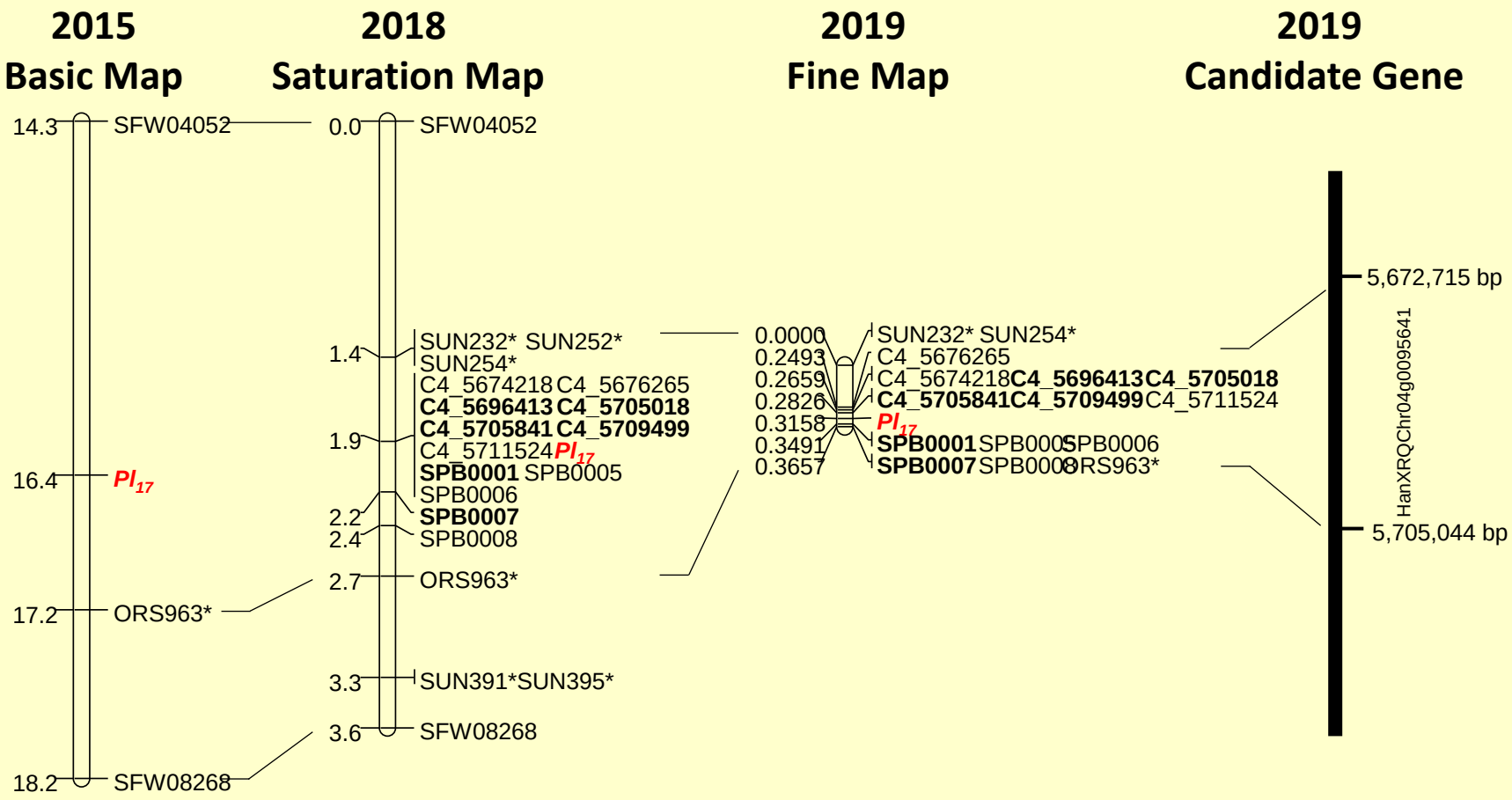
OPEN

Molecular dissection of resistance gene cluster and candidate gene identification of Pl_{17} and Pl_{19} in sunflower by whole-genome resequencing

Guojia Ma[1], Qijian Song[2], William R. Underwood[3], Zhiwei Zhang[1,4], Jason D. Fiedler[3], Xuehui Li[1] & Lili Qi[3*]

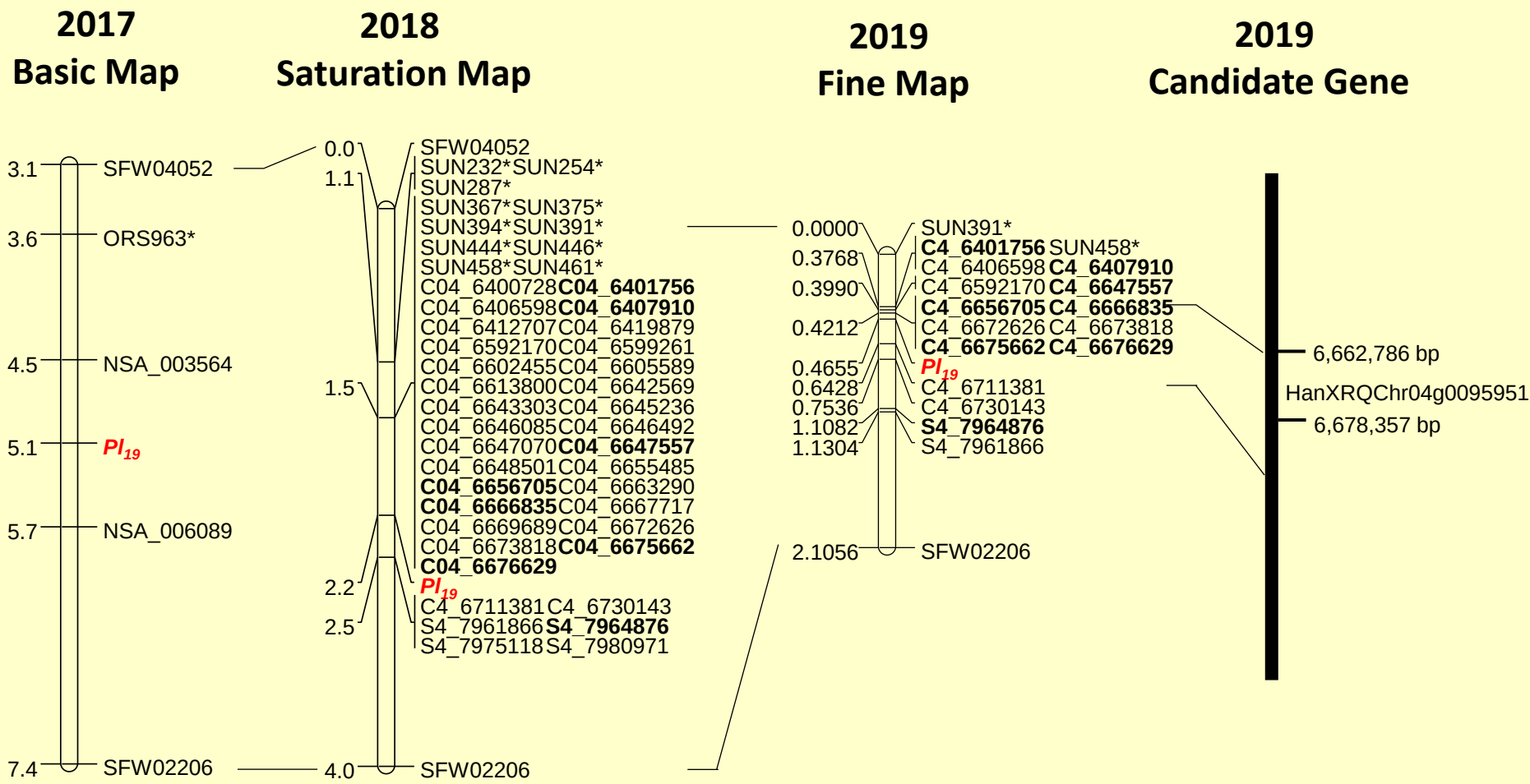
Scientific Reports *(2019) 9:14974*
(https://doi.org/10.1038/s41598-019-50394-8)

Update of PI_{17}



- 6 diagnostic SNP markers for MAS
- Candidate gene for PI_{17} was identified, and functional analyses are underway (Dr. Md Shamimuzzaman)

Update of PI_{19}



- 9 diagnostic SNP markers for MAS
- Candidate gene for PI_{19} was identified, and candidacy needs validation

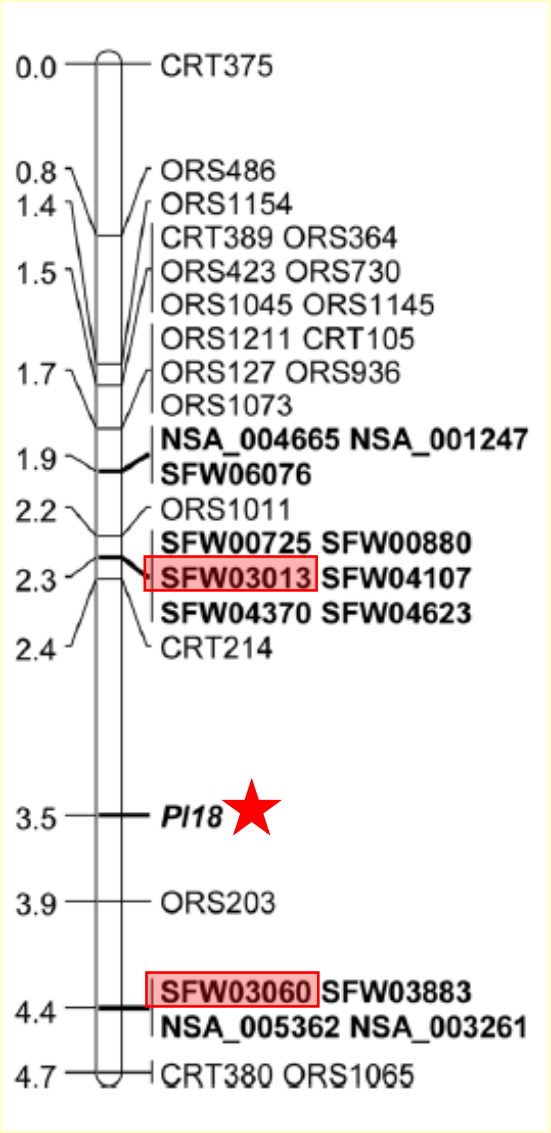
DM *R*-gene Pl_{18}

	Pl_{18}
Publication	Qi et al., 2015, TAG
Resistance origin	*H. argophyllus* (PI 494573), Texas
Chromosome location	Linkage Group 2
Resistance	All races identified in U.S.
Released germplasm	Oil: HA-DM1 (2015), Pl_{18} Confectionary: HA-DM4 (2017), Pl_{18}+R_{13a}

- Broad-spectrum and new resistance
- Long-term mission: making the *R*-genes easier to use (breeder-friendly markers)
  - Closer markers (best would be *R*-gene itself)
  - More unique markers

Position of *PI*$_{18}$

LG 2



100 kb in HA412-HO;
780 kb in XRQ

Qi et al., 2015, TAG

Saturation mapping of *PI_{18}*

- ***STEP 1-sequence-based SSR marker development***
  - *PI_{18}* was delimited to 100-kb and 780-kb regions on LG2 of HA412-HO and XRQ, respectively
  - 100 and 200 kb genomic sequences of HA412-HO and XRQ were extracted from database
  - 28 pairs of SSR primers were designed
  - Polymorphism screening between HA 89/PI 494573 (*PI_{18}*)
  - Genotyped 142 F_2 individuals with 16 polymorphic SSR markers

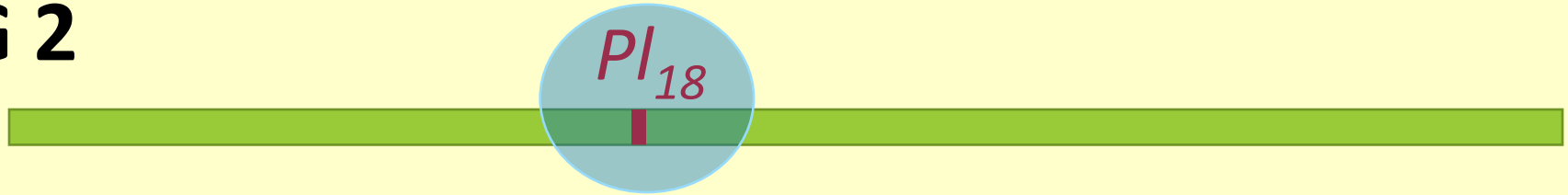
Saturation mapping of *PI_{18}*

- ***STEP 2-sequence-based SNP marker development***
  - HA-DM1 (*PI_{18}*) was whole genome re-sequenced at 40× coverage
  - Whole genome sequence was aligned with two reference genomes, and variants (SNPs & InDels) were called
  - 150 SNPs/InDels potentially around *PI_{18}* were chosen (101 from XRQ and 49 from HA412-HO)
  - Genotyped 142 F_2 individuals with 43 polymorphic SNP markers

❖ **Outcome**: 43 SNP markers were mapped around *PI_{18}*, reducing gene interval to 12 kb for both references

Saturation mapping of *Pl*$_{18}$ in 2019

LG 2



100 kb in HA412-HO &
780 kb in XRQ

<u>SSR</u> marker development from reference seq

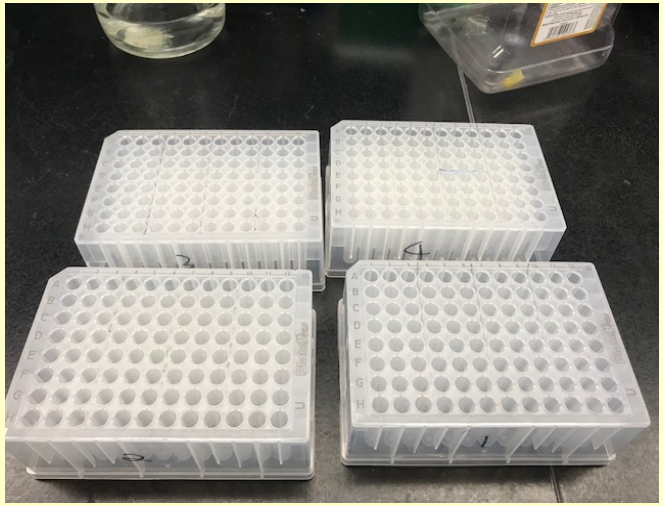
<u>SNP</u> marker development from whole genome re-sequencing

Pl$_{18}$

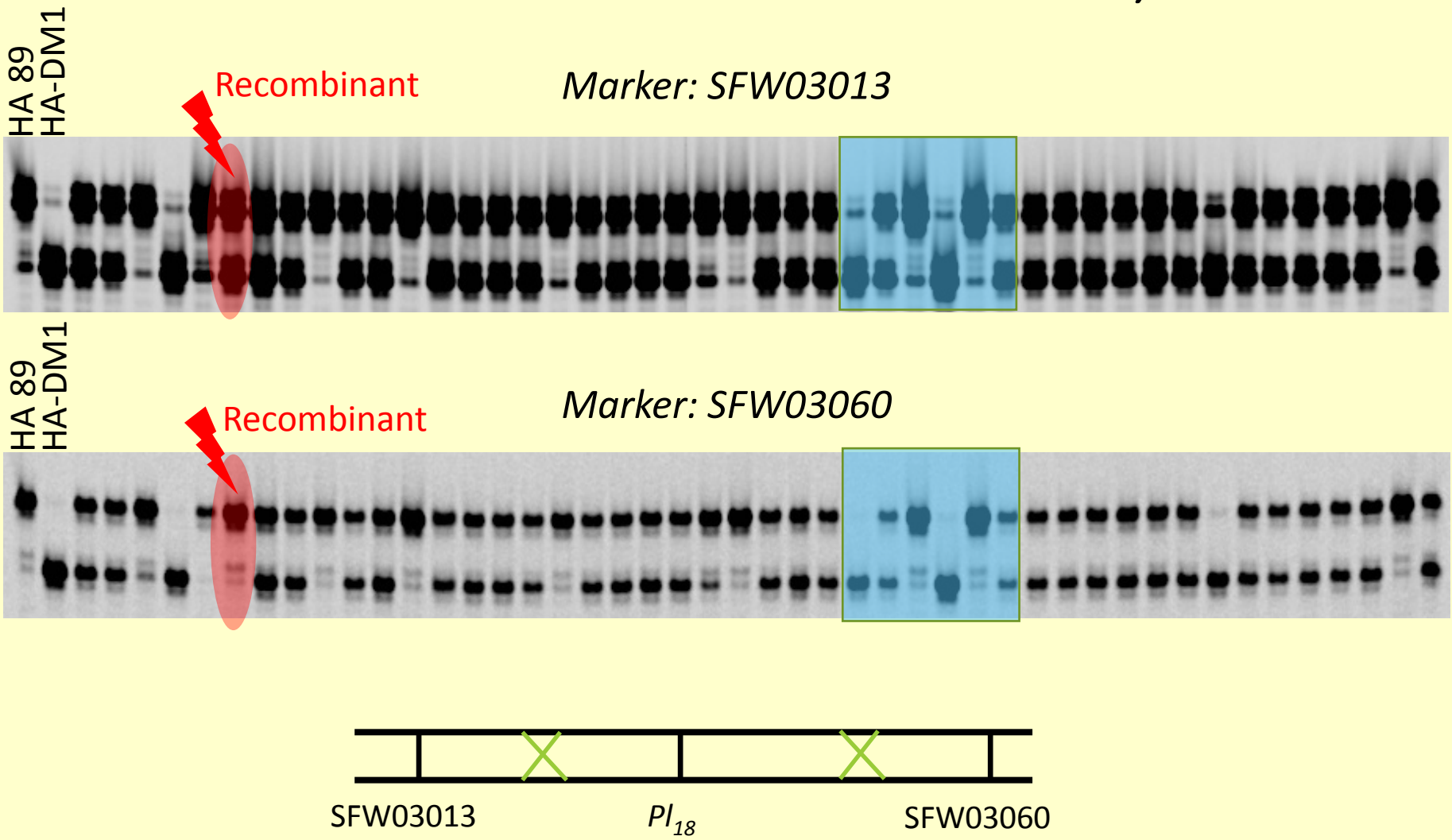
12 kb **(both in the HA412-HO & XRQ genome assemblies)**

Recombinant selection of *PI*$_{18}$

- A total of 2,904 F_2 individuals were tested with flanking markers



Recombinant selection of *PI_{18}, cont'd*



- Recombinants were advanced for further progeny tests

Fine mapping of *PI*$_{18}$

- 78 recombinants were detected from large F_2 pop (2,904 plants) with two flanking markers
- Phenotyping 78 recombinants
- Genotyping 78 recombinants with previous 43 mapped SNP markers
- Linkage analysis between genotyping and phenotyping data for fine mapping

Future work (2020)

- Finish fine mapping of Pl_{18}
- Test and validate diagnostic markers for Pl_{18}
- Identify candidate gene of Pl_{18}
- Prepare manuscript

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