RESISTENZTAGUNG FULDA 2021 XII

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Fast evolution of rust pathogens leads to resistance gene breakage: could we track this speciation using GBS fingerprinting?

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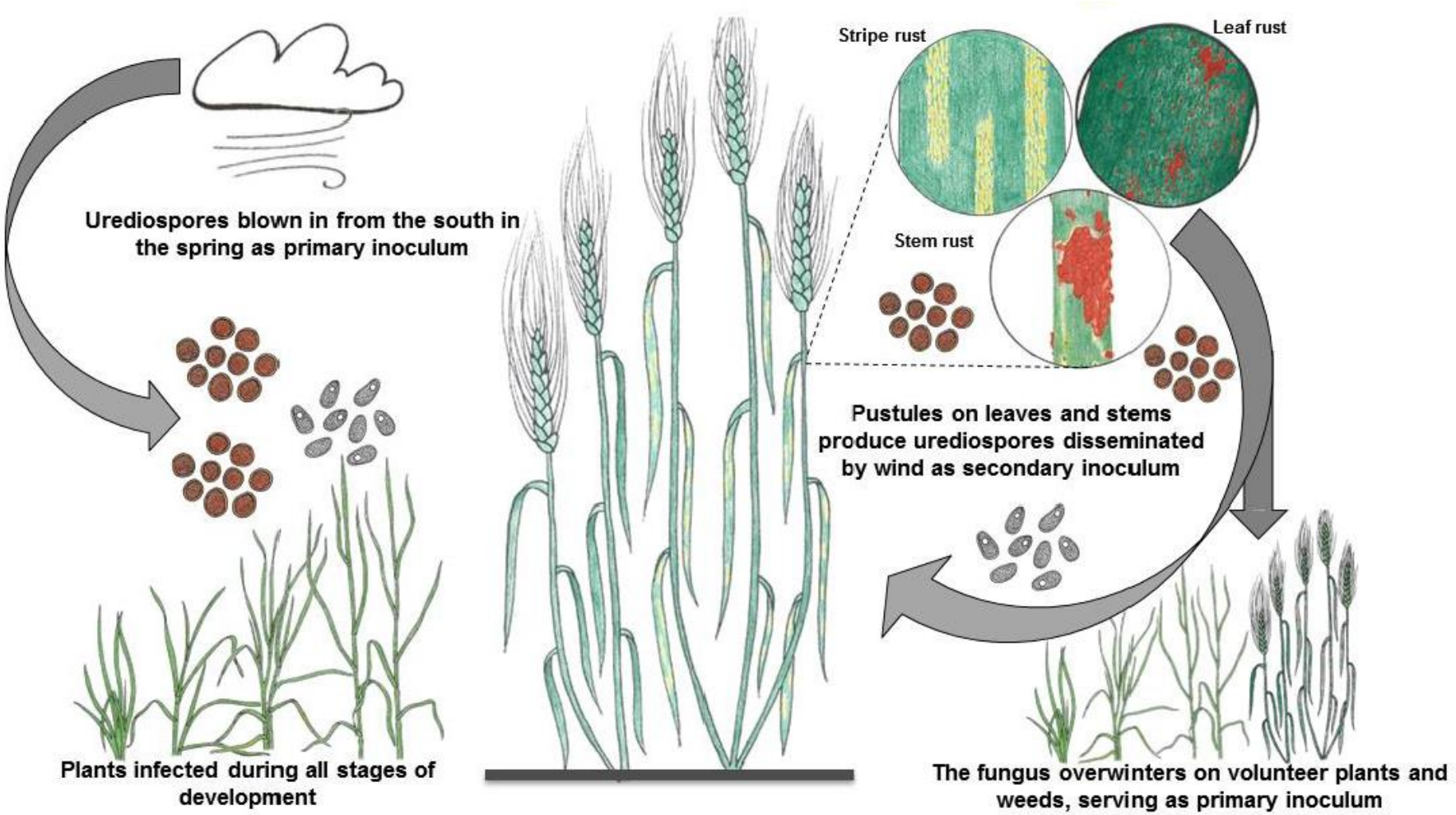
Introduction



<u>Stripe Rust (Yellow Rust):</u>

- *Puccinia striiformis* infecting wheat

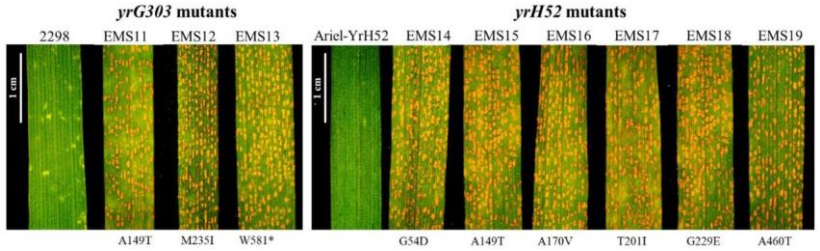
<u>Leaf Rust (Dwarf Rust):</u>

- *Puccinia hordei* infecting barley

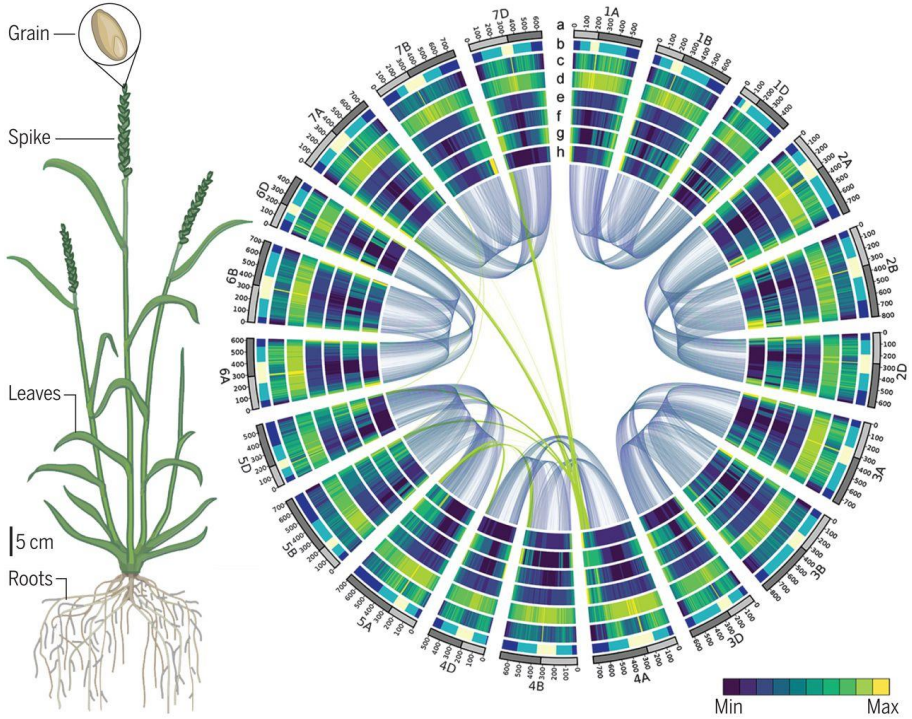
<u>Stem Rust (Black Rust):</u>

- *Puccinia graminis* infecting wheat

Introduction



www.biorxiv.org/content/10.1101/2020.01.02.892968v1.full



ohioline.osu.edu

Current approach:

- check for resistance genes
- improve breeding

Puccinia striiformis (wheat)
-> more than 83 resistance genes

Puccinia hordei (barley)
-> 26 resistance genes

Puccinia graminis (wheat)
-> less studied, but getting more important under climate change conditions

Major problem: rapid evolution of fungal genes break resistances!

Introduction

pl.pinterest.com

Complementary approach:

From the fungal perspective

Research topics:

1) Genome wide association study (GWAS) on most aggressive rusts in Germany

2) Gene evolution for specific rusts
   - Development of genes to break resistances of host plants

3) Identification of AVR-genes:
   -> elicitor-receptor model

Introduction

Focus of this presentation:

cropscience.bayer.co.uk

Organism: Puccinia striiformis infecting wheat

- InSilico digest vs. experimental digest
- genetic differentiation of the races Benchmark, Kalmar and Amboise belonging to aggressive Warrior(-) race (Pst10) using CaseControl studies

Methods

<u>From the spore to the sequence:</u>

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Spores of fungi
(Isolates)

DNA-Extraction
(modified CTAB-method)

„InSilico Enzyme Restriction Analysis“

->Package SimRad (Lepais & Weir 2014)

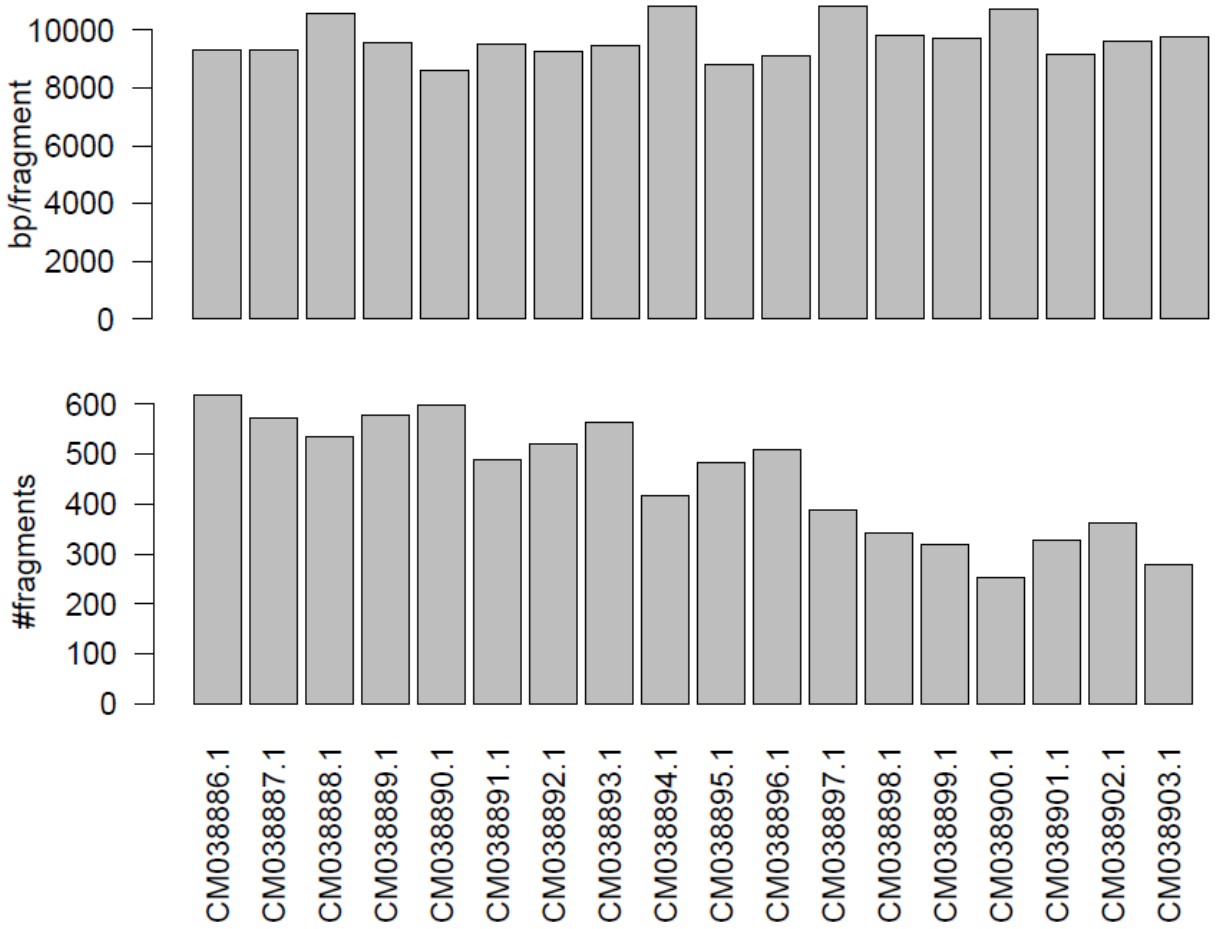
Methods

Representative genome: Puccinia striiformis f. sp. tritici (assembly Pst134E16_v1_alt)

Loc	Type	Name	RefSeq	INSDC	Size (Mb)	GC%	Protein	tRNA	Gene
	Chr	1	-	CM038886.1	5.74	44.5	1,267	47	1,228
	Chr	2	-	CM038887.1	5.34	44.3	1,115	38	1,075
	Chr	3	-	CM038888.1	5.65	44.3	1,111	36	1,084
	Chr	4	-	CM038889.1	5.53	44.8	1,125	29	1,071
	Chr	5	-	CM038890.1	5.14	44.4	1,134	51	1,127
	Chr	6	-	CM038891.1	4.65	44.4	996	33	975
	Chr	7	-	CM038892.1	4.84	44.3	1,006	28	980
	Chr	8	-	CM038893.1	5.34	44.2	1,083	41	1,064
	Chr	9	-	CM038894.1	4.51	44.4	972	16	915
	Chr	10	-	CM038895.1	4.25	44.8	927	25	904
	Chr	11	-	CM038896.1	4.65	44.5	900	27	878
	Chr	12	-	CM038897.1	4.2	44.5	848	16	832
	Chr	13	-	CM038898.1	3.37	44.2	681	26	668
	Chr	14	-	CM038899.1	3.09	44.2	604	19	593
	Chr	15	-	CM038900.1	2.71	44.4	539	20	533
	Chr	16	-	CM038901.1	2.99	44.3	576	17	561
	Chr	17	-	CM038902.1	3.48	44.1	646	48	663
	Chr	18	-	CM038903.1	2.72	44.4	532	15	516
	Un	-	.	-	78.18	44.4	16,062	532	15,667

Methods

InSilico Digest with MspI and PstI, Fragment sizes: 200-500 bp



Methods

<u>From the spore to the sequence:</u>

Spores of fungi
(Isolates)

DNA-Extraction
(modified CTAB-method)

Restriction
(restriction enzymes PstI and MspI)

„InSilico Enzyme Restriction Analysis“

->Package SimRad (Lepais & Weir 2014)

Index-PCR

illumina

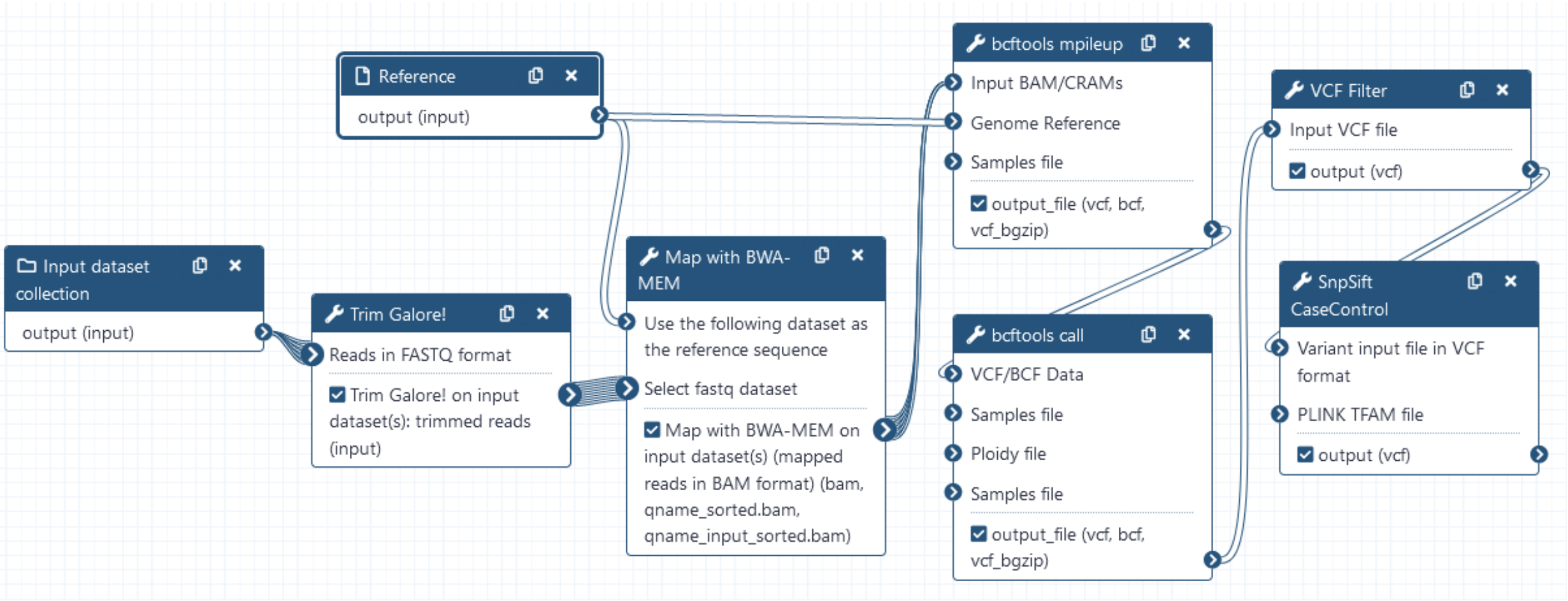
Sequencing

Analysis

Galaxy

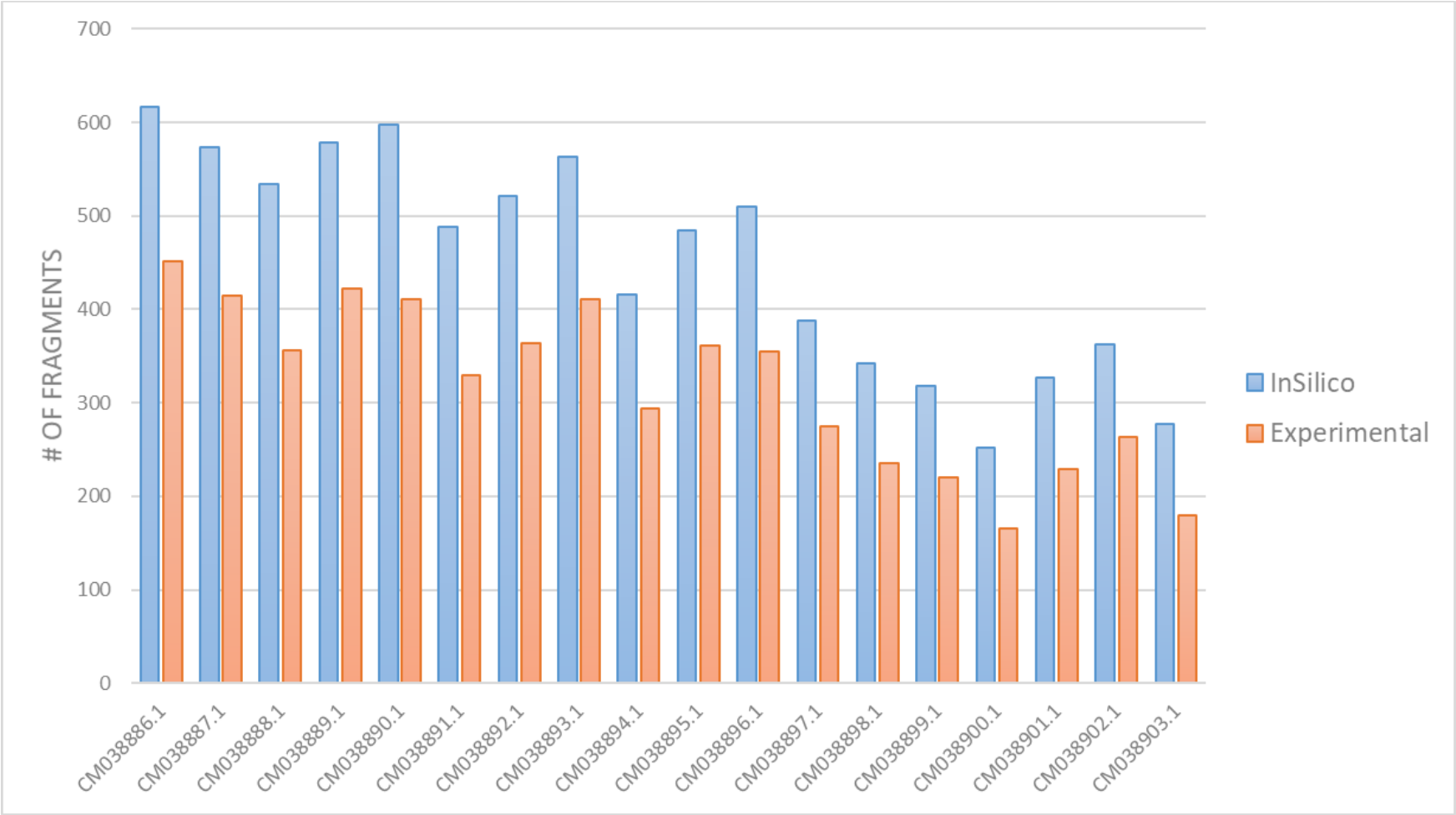
Methods

<u>Workflow in Galaxy:</u>



Results

Comparison: in-silico vs. results wet-lab



Fragments:

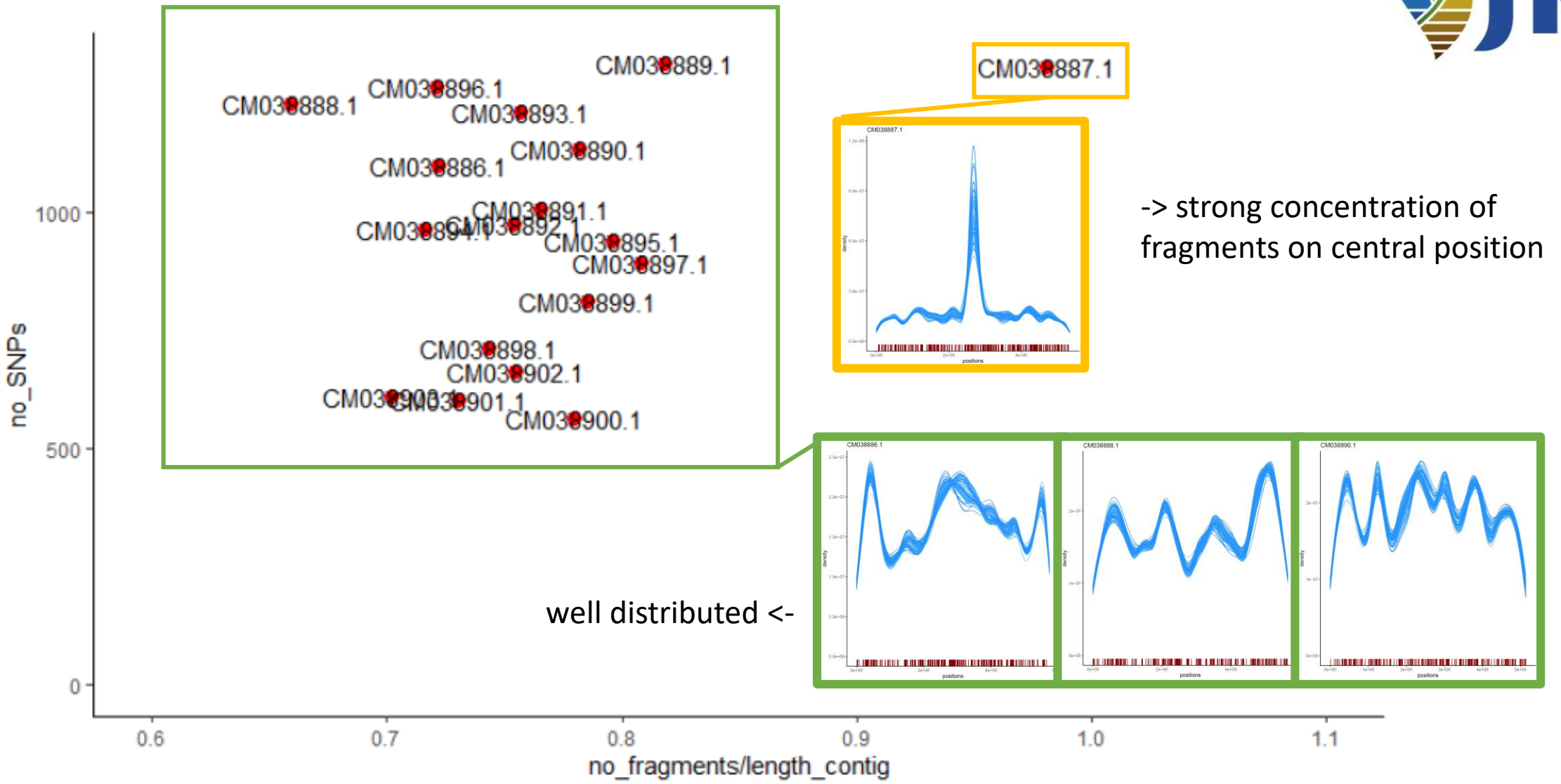
in-silico = 8148

wet-lab = 5738

makes ~ 70 % concordance

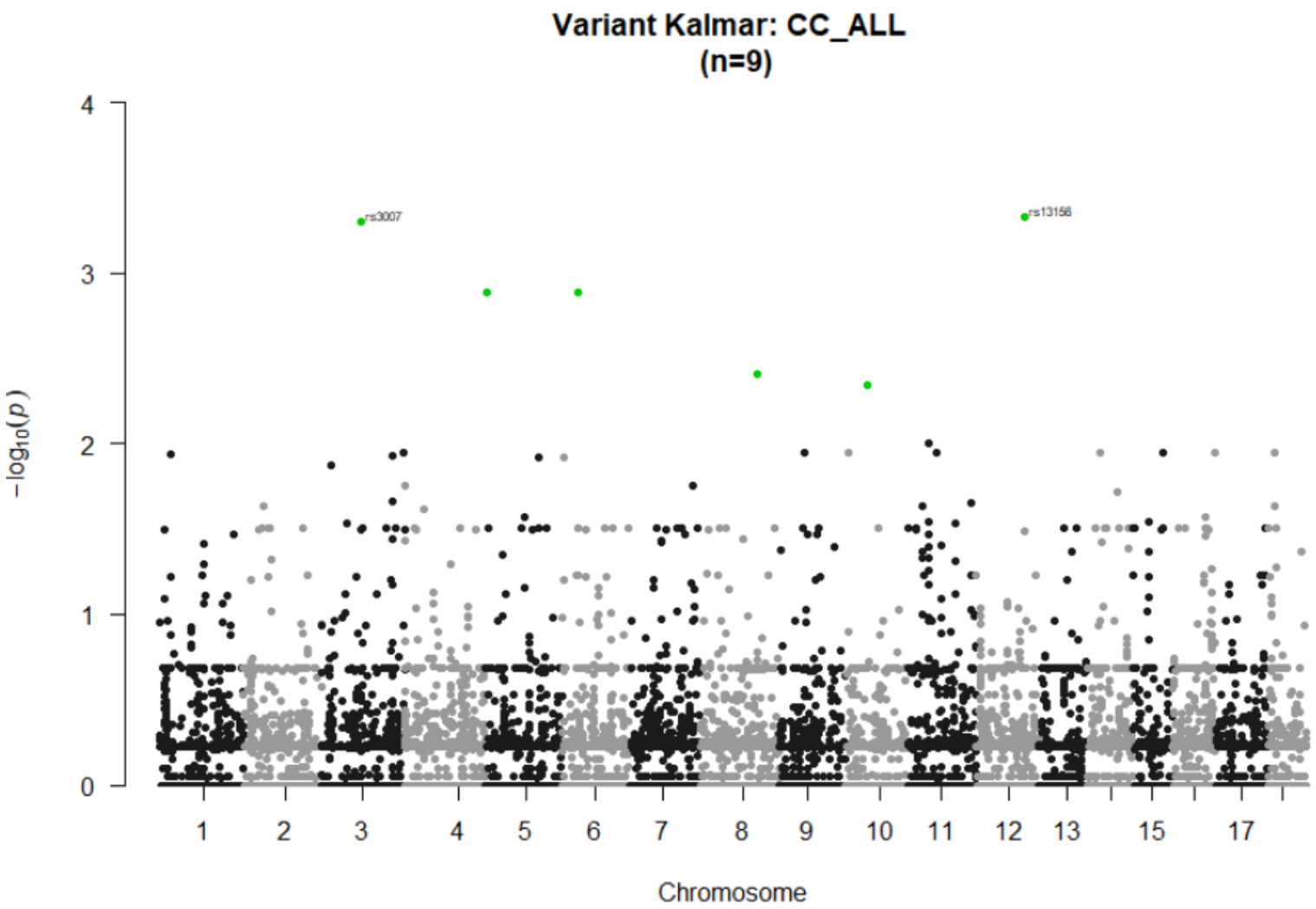
reason: methylation not implemented in in-silico analysis

Results


CM038889.1
CM038896.1
CM038888.1
CM038893.1
CM038890.1
CM038886.1
CM038891.1
CM038892.1
CM038895.1
CM038897.1
CM038899.1
CM038898.1
CM038902.1
CM038900.1
CM038887.1
1000
500
0
no_SNPs
0.6
0.7
0.8
0.9
1.0
1.1
no_fragments/length_contig
-> strong concentration of fragments on central position
well distributed <-

Results

SnpSift CaseControl



-> 6 SNPs of Interest

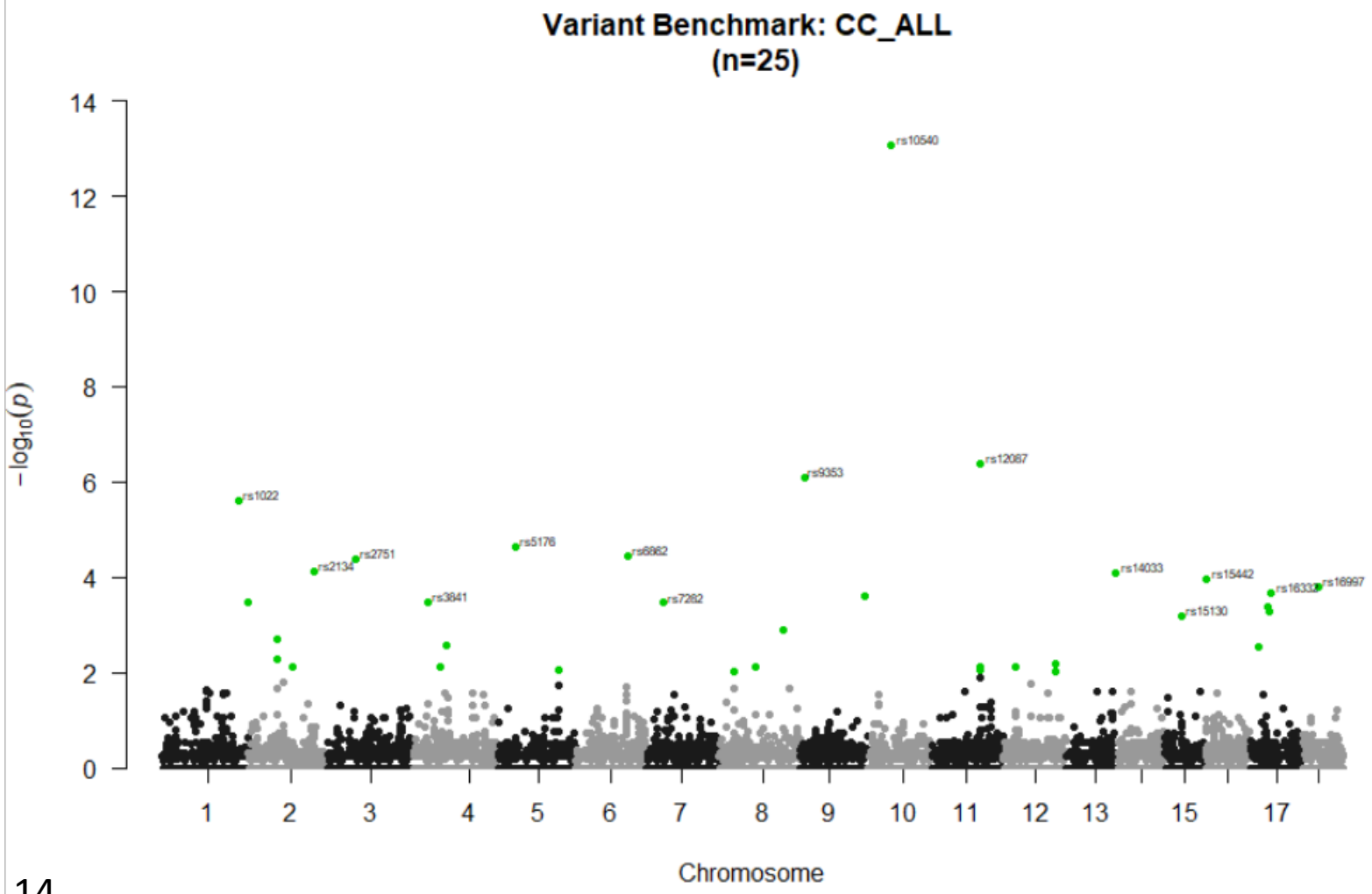
SNP	CHR	BP	P	contig
rs3007	3	2724354	0.0005022	CM038888.1
rs10540	10	1518668	0.004527	CM038895.1
rs13156	12	3218314	0.0004668	CM038897.1

SNP	REF	ALT
rs3007	TG	T
rs10540	G	A
rs13156	GC	GCAAAGGTGCC

-> all SNPs in non-coding regions

Results

SnpSift CaseControl



-> 37 SNPs of Interest

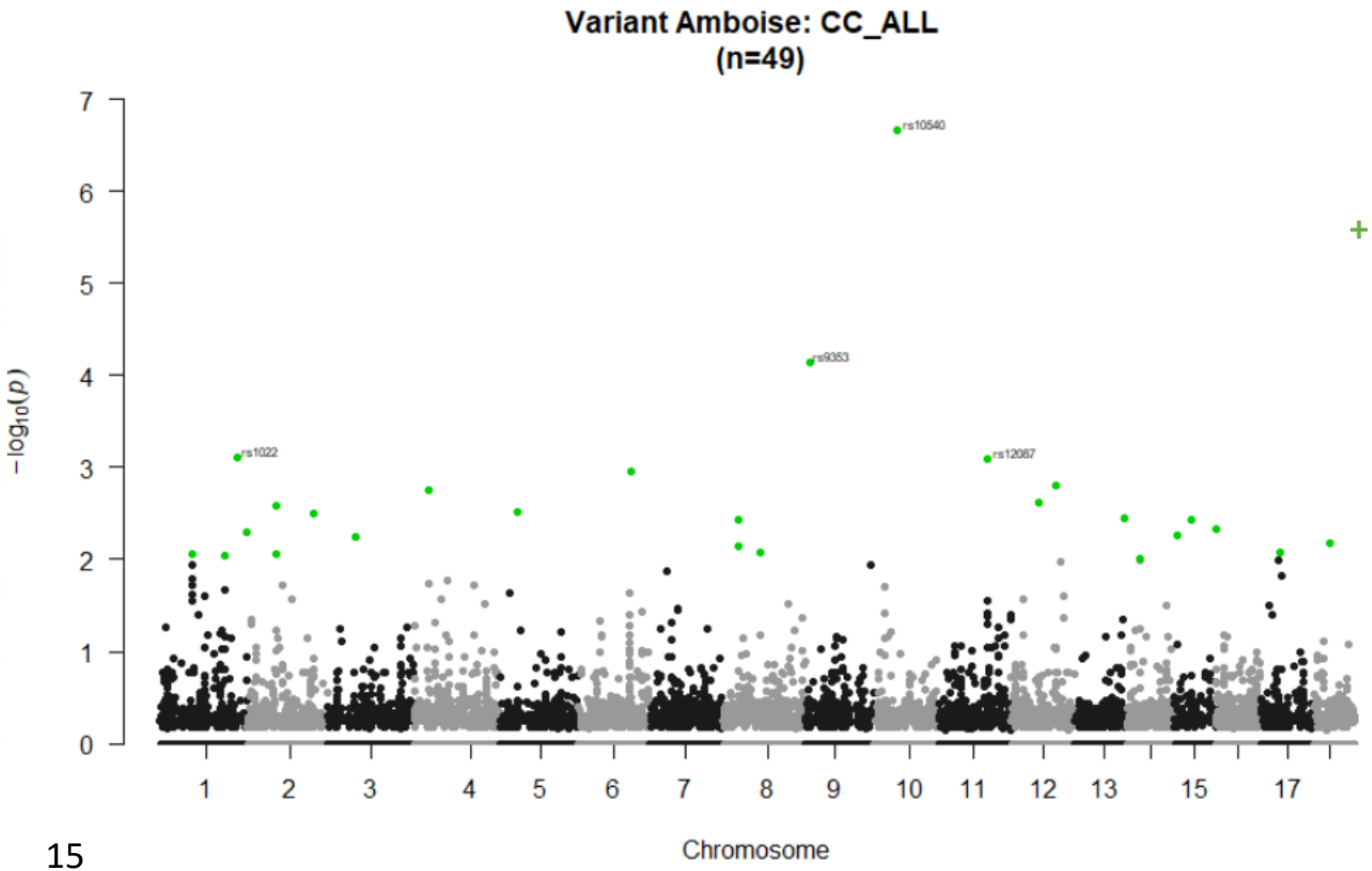
	SNP	CHR	BP	P	contig
+	rs1022	1	5027579	2,53E-03	CM038886.1
	rs9353	9	280733	7,82E-04	CM038894.1
	rs10540	10	1518668	8,33E-11	CM038895.1
	rs12087	11	3161840	4,17E-04	CM038896.1

SNP	REF	ALT
rs1022	A	G
rs9353	C	G
rs10540	G	A
rs12087	AGGGG	AGGGGG

-> besides +, all SNPs in non-coding regions

Results

SnpSift CaseControl



-> 27 SNPs of Interest

	SNP	CHR	BP	P	contig
+	rs1022	1	5027579	0.000772	CM038886.1
	rs9353	9	280733	7,21E-02	CM038894.1
	rs10540	10	1518668	2,15E-04	CM038895.1
	rs12087	11	3161840	0.0007987	CM038896.1

-> besides +, all SNPs in non-coding regions

-> yellow underlined SNP is represented in all CaseControl studies

Summary

- InSilico Digest provided good estimation of fragment numbers, at least for MspI and PstI system as applied in this study

- In total 83 isolates of Puccinia striiformis were genotyped and compared to phenotypic data:

  -> Significant SNPs were detected for differentiation of Kalmar, Benchmark and Amboise isolates, whereby one SNP was significant for all of the three

  -> One of the highly significant SNPs of Benchmark and Amboise lies in a coding-region

Next step

- verification of SNPs for differentiation of Kalmar, Benchmark and Amboise isolates
  -> KASP-marker development

Thanks to…

<u>JKI Quedlinburg:</u>

Prof. Dr. Frank Ordon
Dr. Andreas Stahl
Thomas Berner
Ina Lemke
Katy Niedung

<u>JKI Kleinmachnow:</u>

Daniel Kriegel
Anne-Kristin Schmitt

Julius Kühn-Institut
Federal Research Centre for Cultivated Plants

Thank you for your attention