




Next generation sequencing analysis to identify modifier gene candidates conferring pollen-part self-compatibility in sweet cherry ‘Cristobalina’

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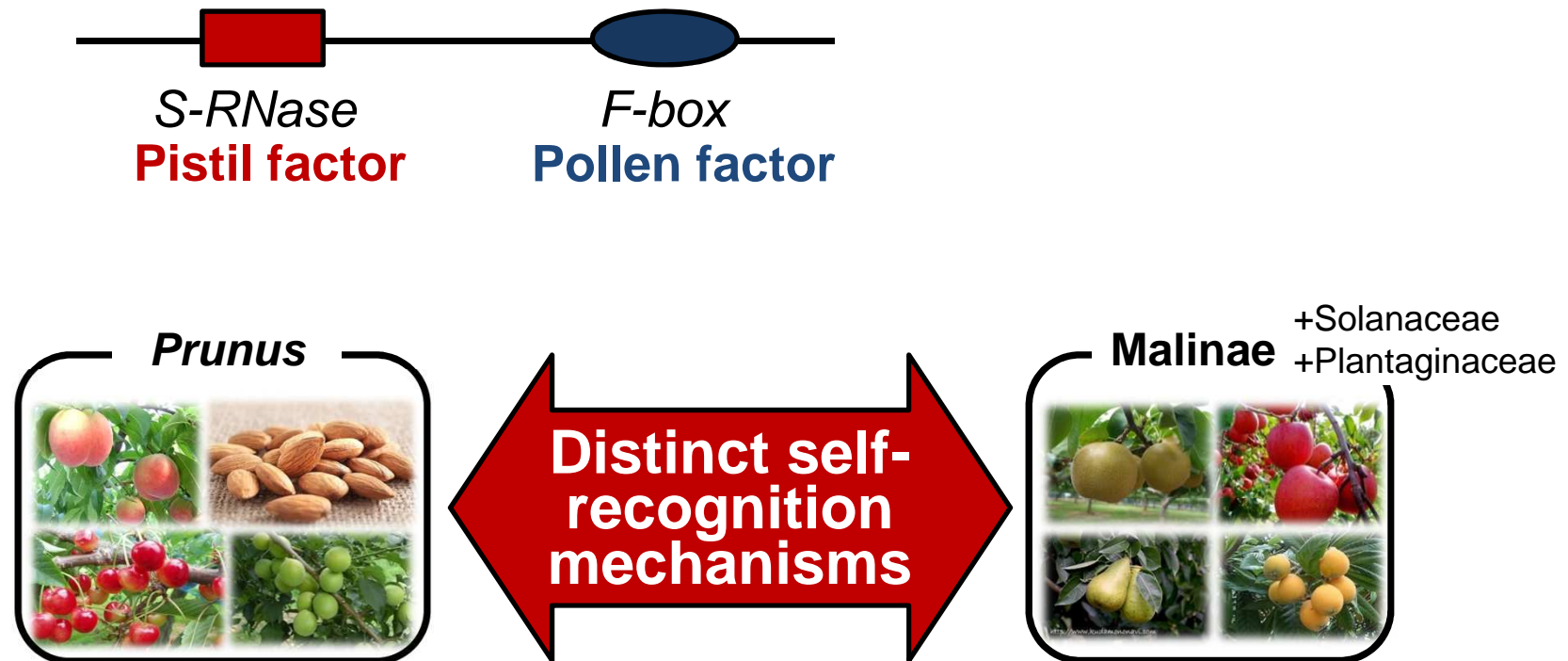
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Tecnología Agroalimentaria de Aragón (CITA)



S-RNase-based gametophytic self-incompatibility

- The Rosaceae, the Solanaceae, and the Plantaginaceae share the S-RNase-based gametophytic self-incompatibility.
- Self/ nonself recognition is determined by *S* locus



(Tao and Iezzoni, 2010, Sci. Hortic.)

Prunus-specific self-recognition mechanism

Toward elucidation of the *Prunus*-specific self-recognition mechanism...

- **Evolutionary analysis for *S* locus genes**

(Morimoto et al., 2015, Hort. J; Akagi et al., 2016, Plant Cell Physiol.)

- **Identification of a modifier factor (Today)**



(Tao and Iezzoni, 2010, Sci. Hortic.)

Self-incompatibility modifier factor

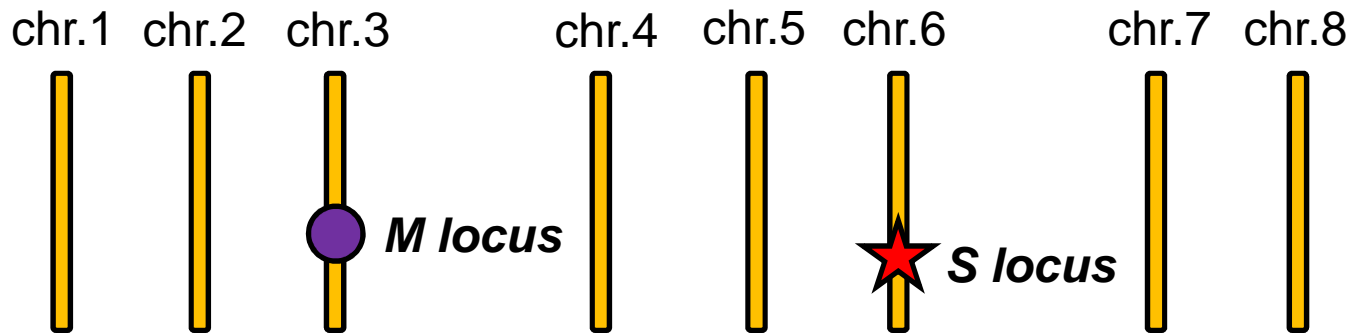
What is the modifier factor ?

- **non-specificity determinants**, but involves in self-incompatibility reaction

e.g.

- S-RNase import into pollen tube
- S-RNase degradation
- directing pollen tube growth *etc..*

- locates on **outside of S locus** (**M locus**)



Prunus-specific self-compatible mutant

Sweet cherry (*Prunus avium*)

‘Cristobalina’



- **Pollen-part self-compatible** cultivar
[mutation of the **modifier locus (*M locus*)**]

(Wünsch and Hormaza, 2004, Sex. Plant Reprod.)

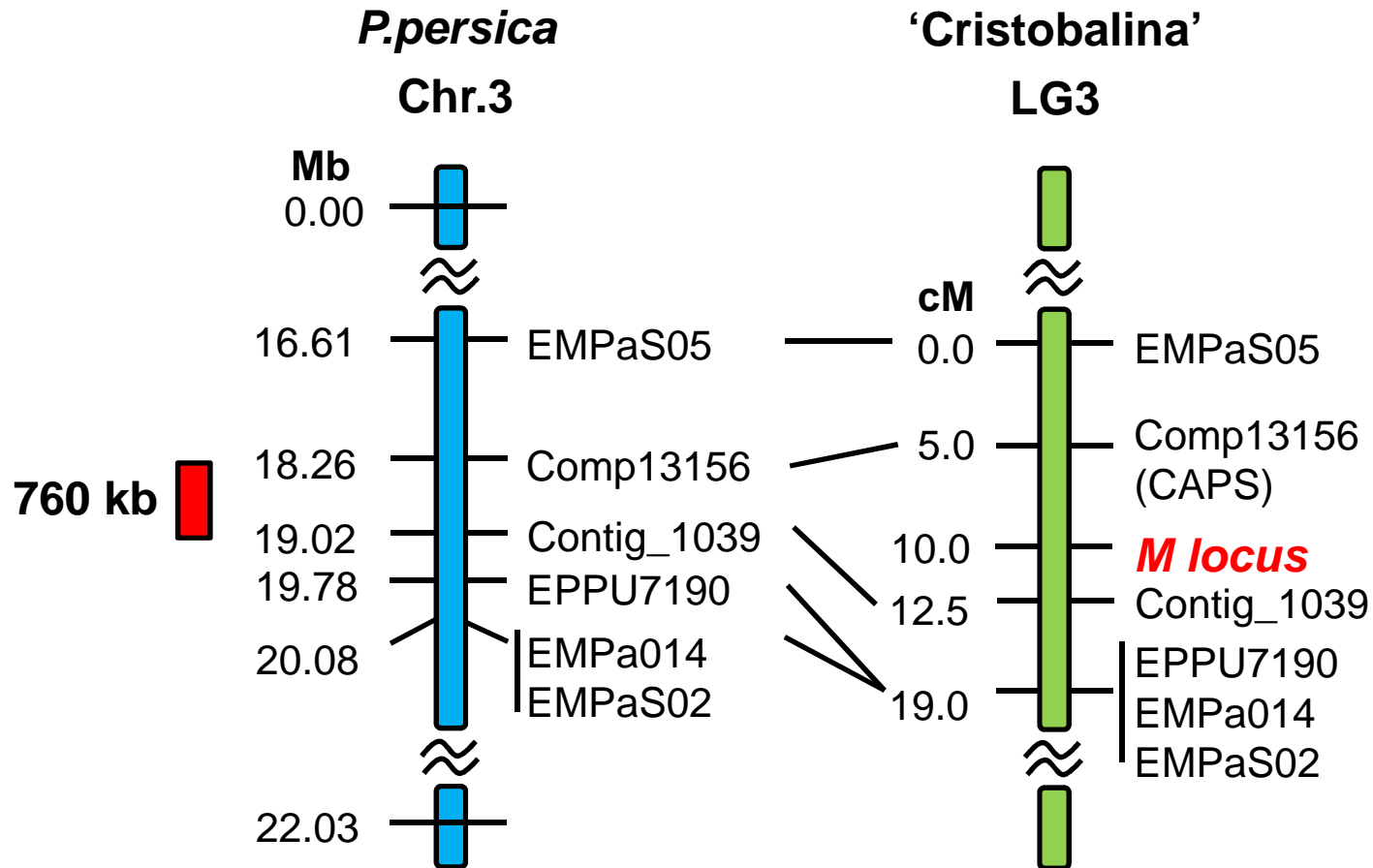
<Purpose>

Identification of pollen modifier factor in ‘Cristobalina’



- understanding the distinct self-recognition mechanism in *Prunus*
- breeding new self-compatible cultivars

Genetic mapping of *M locus* in 'Cristobalina'

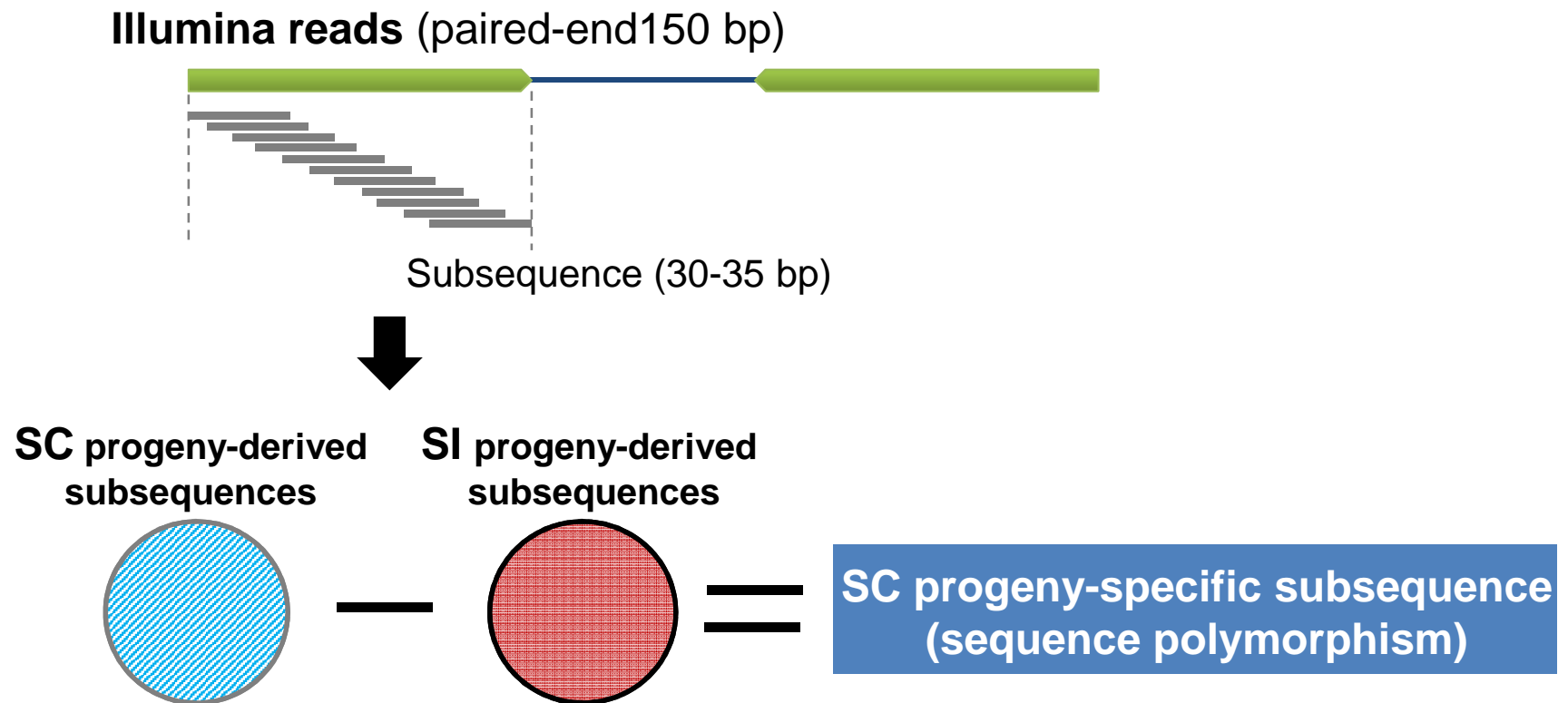


(Cachi and Wünsch, 2011, J. Exp. Bot.; Morimoto et al., unpublished data)

Strategy to identify pollen modifier factor (1)

★ Apply genome-wide subsequence analysis

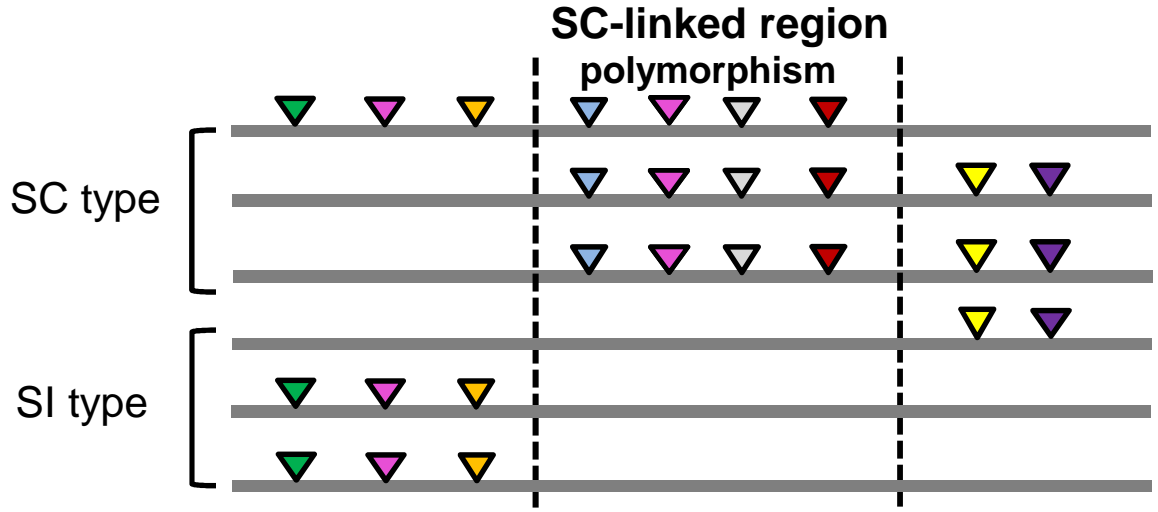
(Akagi et al., 2014, Science)



Strategy to identify pollen modifier factor (2)

Seedling analysis

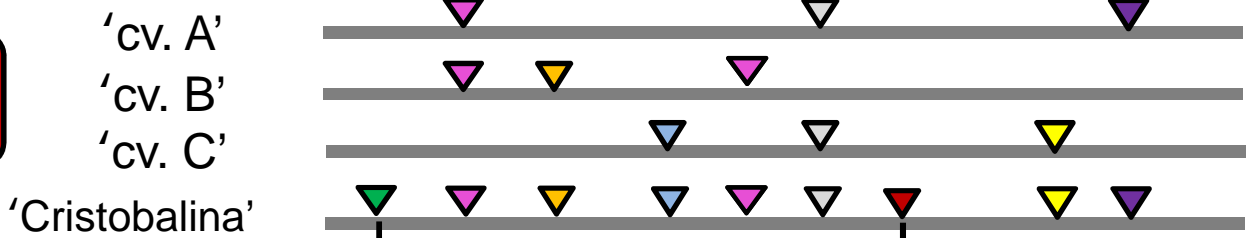
'Cristobalina'
F₁ population



Causal mutation for *M* locus

Integration of seedling and cultivar

Cultivar analysis



Cristobalina-specific polymorphism

Plant materials (for genome DNA-seq)

- 'Brooks' × 'Cristobalina'
(SC: 11 indiv., SI: 19 indiv.)

- 'Lambert' × 'Cristobalina'
(SC: 7 indiv., SI: 7 indiv.)

18 SC individuals

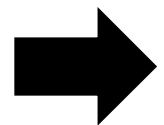
26 SI individuals

from CITA, Spain

(Cachi and Wünsch, 2011, J. Exp. Bot.)

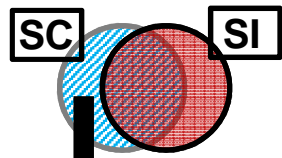
- 17 sweet cherry cultivars

'Ambrunes', 'Benishuho', 'Brooks', 'Cristobalina', 'Ferrovia', 'Hedelfingen',
'Lambert', 'Napoleon', 'Rainier', 'Sam', 'Satonishiki', 'Stella', 'Sue', 'Summit',
'Takasago', 'Van', 'Vic'



Run on Illumina HiSeq 2500/ 4000 (paired-end 150bp)

Exp.1 gDNA-seq (seedling analysis)



Subsequence (35mer) cataloging for seedlings

Extract **SC progeny-specific** subsequences

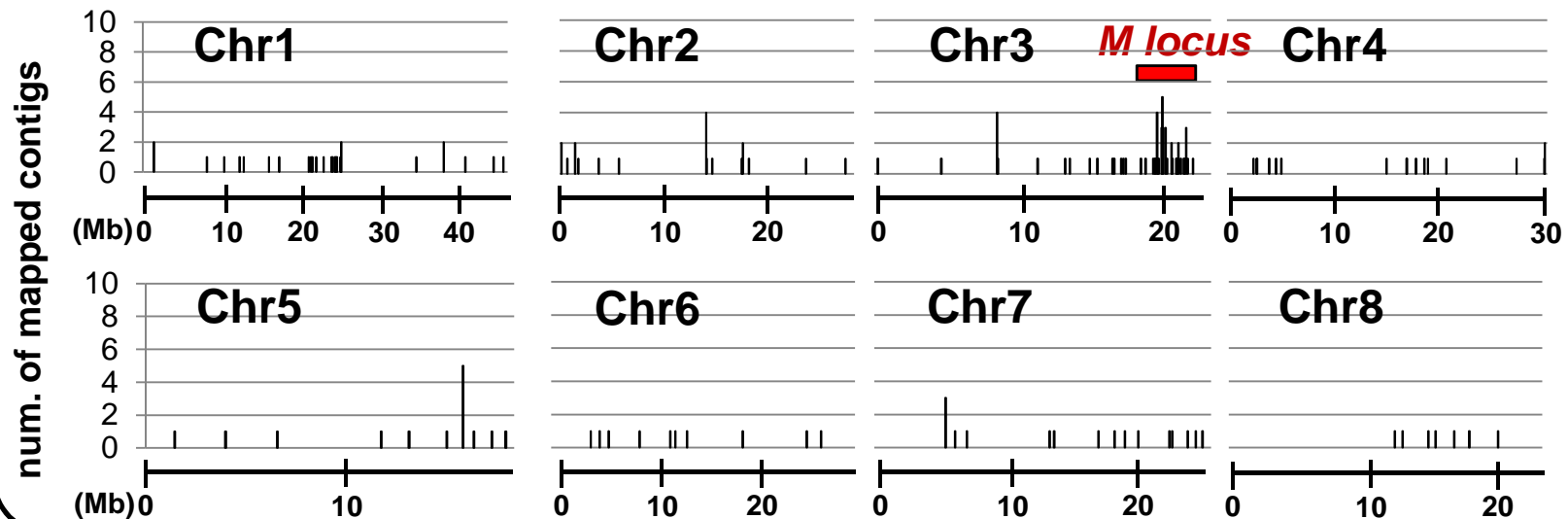
12,287 subsequences

De novo assembly (CLC assembler)

1,290 contigs

Confirmation of reliability of our method

(homology search against peach genome)



Exp.1 gDNA-seq (integration of cultivar analysis)

1,290 contig



Seedling analysis

1. Exclude **repetitive contigs** and **unlinked contigs**
2. Exclude **recombinant contigs**
 - ➔ completely linked **SC progeny-specific** contigs

88 contig



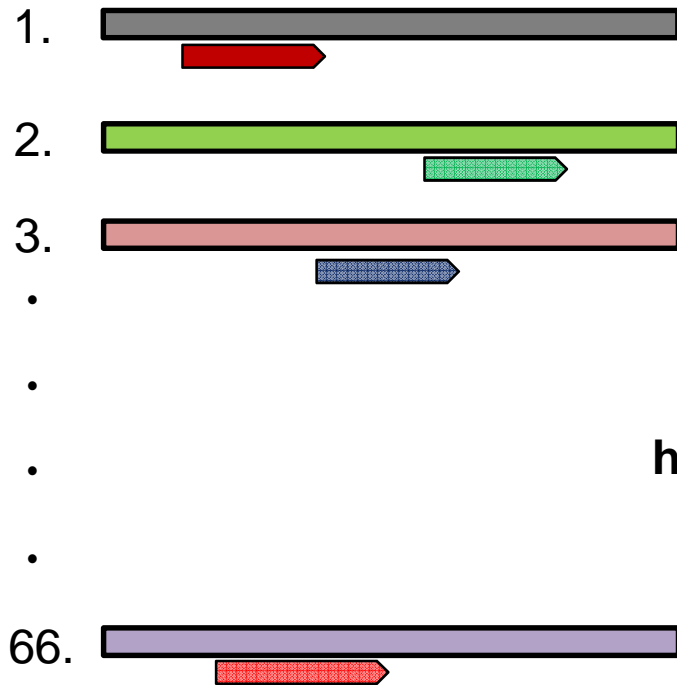
Cultivar analysis

- Identify 'Cristobalina'-specific contigs
 - ➔ **SC-specific** genome contigs

66 contig

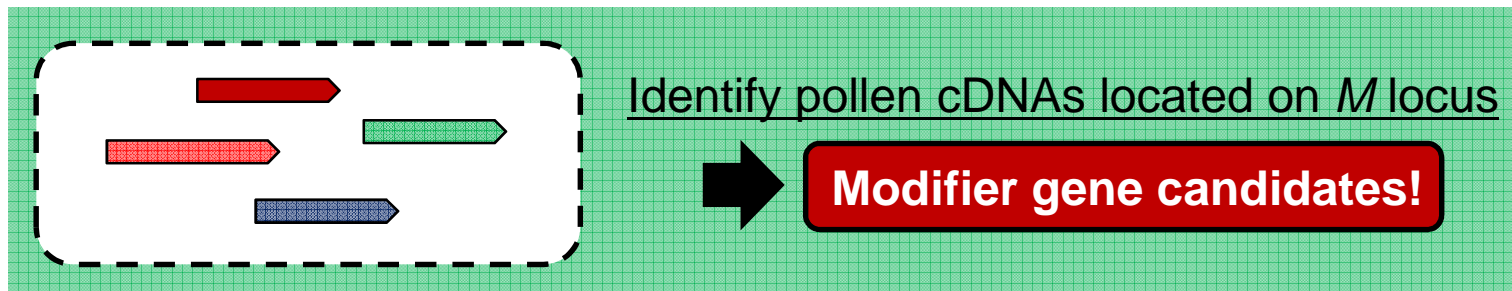
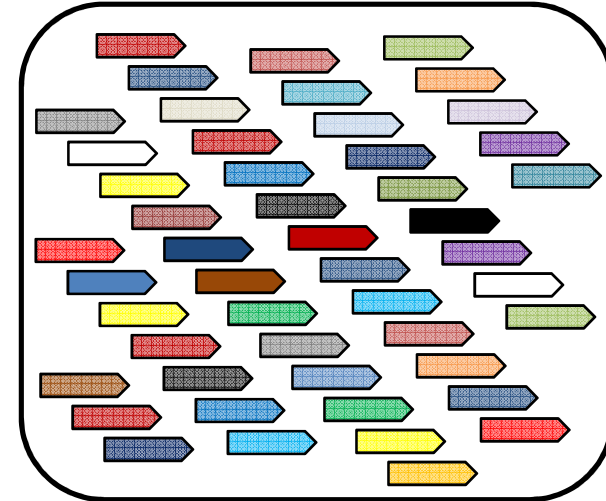
Exp.2 mRNA-seq strategy to identify candidate genes

66 SC-specific genome contig



homology search

Pollen cDNAs



Exp.2 mRNA-seq (1)

1. Construct *de novo* cDNA contig from 'Cristobalina' pollen grain mRNA-seq

2. Identify cDNA contigs showing homology to SC-specific genome contig

⇒ 135 cDNA contigs

3. Identify cDNAs with significant expression in pollen (RPKM>2)

(reads per kilobase per million reads)

⇒ **17 cDNA contigs**

Exp.2 mRNA-seq (2)

Pollen cDNAs expressed on SC-specific genome contig (RPKM>2)

	cDNA contig	peach genome	RPKM	TAIR		
		v2.1 (Mb)	Cristobalina	accession		annotation
①	comp10608_c0_seq2	chr3: 24.18	29.13	AT1G69230.2	SP1L2 SPIRAL1-like2	Microtubule-associated
	comp10608_c0_seq3		20.64	AT1G69230.2	SP1L2 SPIRAL1-like2	
	comp10608_c0_seq6		21.57	AT1G69230.2	SP1L2 SPIRAL1-like2	
②	comp12220_c0_seq1	unknown	8.92	AT4G23160.1	CRK8 cysteine-rich RLK	transposon
	comp12220_c0_seq2		2.99	AT4G23160.1	CRK8 cysteine-rich RLK	
	comp12220_c0_seq3		3.36	AT4G23160.1	CRK8 cysteine-rich RLK	
	comp12220_c0_seq4		3.65	AT4G23160.1	CRK8 cysteine-rich RLK	
	comp12220_c0_seq5		3.86	AT4G23160.1	CRK8 cysteine-rich RLK	
③	comp12607_c0_seq1	chr2: 25.74	104.16	AT5G24318.1	O-Glycosyl hydrolases	Glycosylation
	comp12607_c0_seq2		114.14	AT5G24318.1	O-Glycosyl hydrolases	
④	comp12910_c2_seq1	chr4: 16.31	5.97	AT3G57650.1	LPAT2 lysophosphatidyl acyltransferase	Lipid metabolism
⑤	comp12969_c1_seq1	chr2: 9.11	2.77	AT5G16010.1	3-oxo-5-alpha-steroid 4-dehydrogenase	Lipid metabolism
⑥	comp13316_c0_seq1	chr1: 7.82	3.85	AT5G57800.1	FLP1, YRE, CER3, WAX2	Lipid metabolism
⑦	comp6077_c0_seq2	chr7: 13.85	58.19	no hit		Drought resistance
	comp6077_c0_seq5		84.77	AT2G35460.1	Late embryogenesis abundant (LEA)	
⑧	comp7820_c0_seq1	chr3: 24.15	3.06	AT5G39000.1	Malectin/receptor-like protein kinase	Receptor kinase
	comp7820_c0_seq3		3.27	AT5G39000.1	Malectin/receptor-like protein kinase	



Conclusions

- Genomic contigs covering *M* locus were constructed by applying genome-wide **subsequence cataloging**.
- **Integration of association analysis** using various cultivars identified the 66 SC-specific genomic contigs.
- Based on homology between genomic contigs and pollen cDNA, several candidate genes were detected.

VIII International Cherry Symposium



2017

Thank you for your attention

Jun 5th – 9th, 2017
<http://cherry2017.jshs.jp>



8th International Cherry Symposium 2017, in YAMAGATA, Japan



第8回国際アウトウシンポジウム

Welcome to "YAMAGATA" !!

The ISHS International Symposium for cherry researchers and growers is held every 4 years. 8th symposium will be held for the first time in Japan (June, 2017).

Program

- 5th, June
 - Registration
 - Technical Seminar for Cherry Growers
 - Welcome Reception
- 6th, June
 - Opening Ceremony
 - Plenary Lecture
- 6th to 8th, June
 - Oral Sessions
 - Poster Sessions
- 8th, June
 - ISHS Business Meeting
 - Gala Dinner
 - Closing Ceremony
- 9th, June
 - Technical Tour in Yamagata

Venue

YAMAGATA TERRA
1-2-3, Futaba, Yamagata
5min. walk from JR Yamagata Station

Oral and Poster Sessions

Breeding, Genetics and Biotechnology
Crop Production and Orchard Management
Rootstocks and Varieties Evaluation/Propagation
Nutrient and Irrigation
Tree Fruit Physiology, Plant Growth, and Floral Biology
Physiological Disorder and Pests and Diseases Management
Postharvest Technology, Fruit Quality, Health Related Issues



Technical Seminar for Cherry Growers

特別講演会

国際アウトウシンポジウムは4年に一度、世界中のアウトウの研究や生産・流通関係者が集まって開催されます。日本での開催は今回の山形開催が初めてです。日本の一般生産者向けに日本語通訳付きの特別講演会の開催を企画しています。海外から講師を招聘し、海外のアウトウ栽培の最新技術についてお話しいただきます。

日時：2017年6月5日(月)午後
場所：山形テルザ
山形市双葉町1-2-3 JR山形駅から徒歩5分

当日の参加も可能です。
詳しくはホームページをご覧ください。
<http://cherry2017.jshs.jp>

Important Dates

2016 Oct Registration Start
2017 Feb 15 Deadline for Abstract Submission and Early-Bird Registration
2017 May 15 Deadline for Online Registration

Registration Fees

Early-Bird Registration, Student, ISHS Member and One Day Registration

Symposium committee

Conveners

Prof. Dr. Satoshi Taira (Yamagata Univ.)
Prof. Dr. Ryutarō Tao (Kyoto Univ.)
Prof. Dr. Hideki Murayama (Yamagata Univ.)



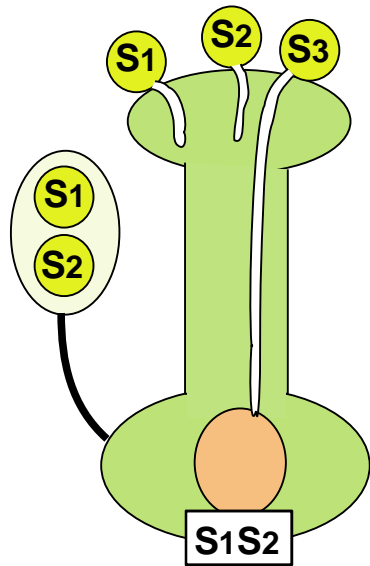
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<http://cherry2017.jshs.jp>



Contact us! cherry2017@tds1.ty.yamagata-u.ac.jp

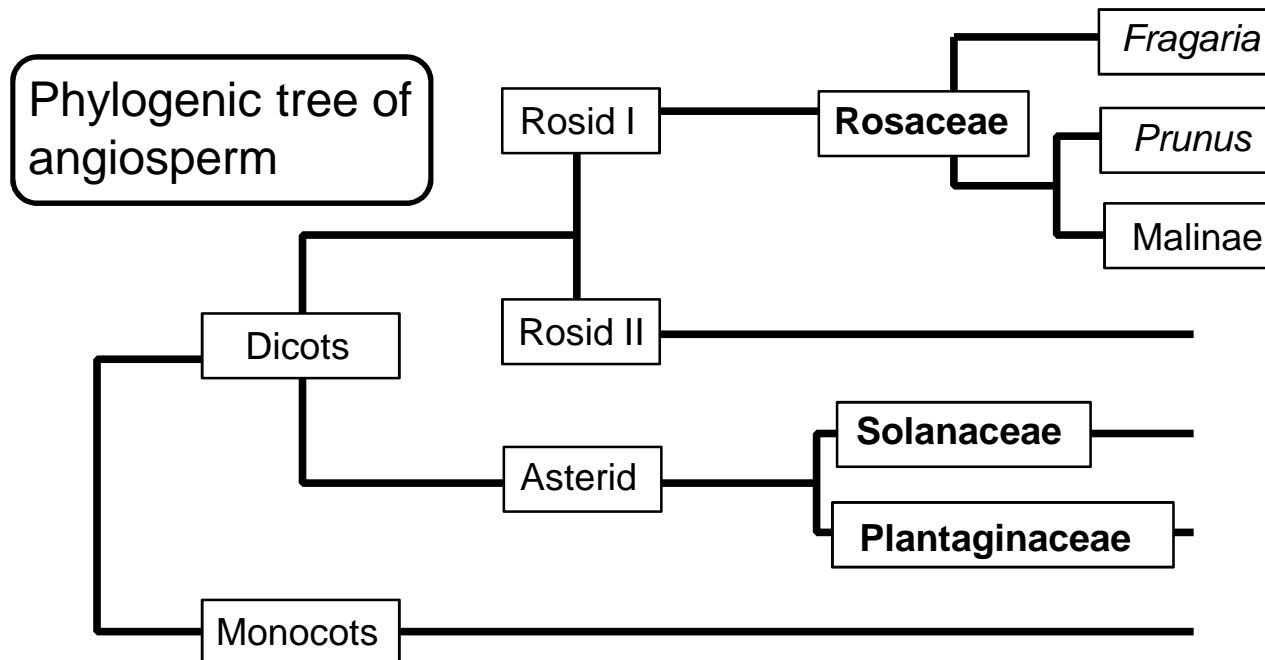
Organization
主催・後援





S-RNase-based gametophytic self-incompatibility

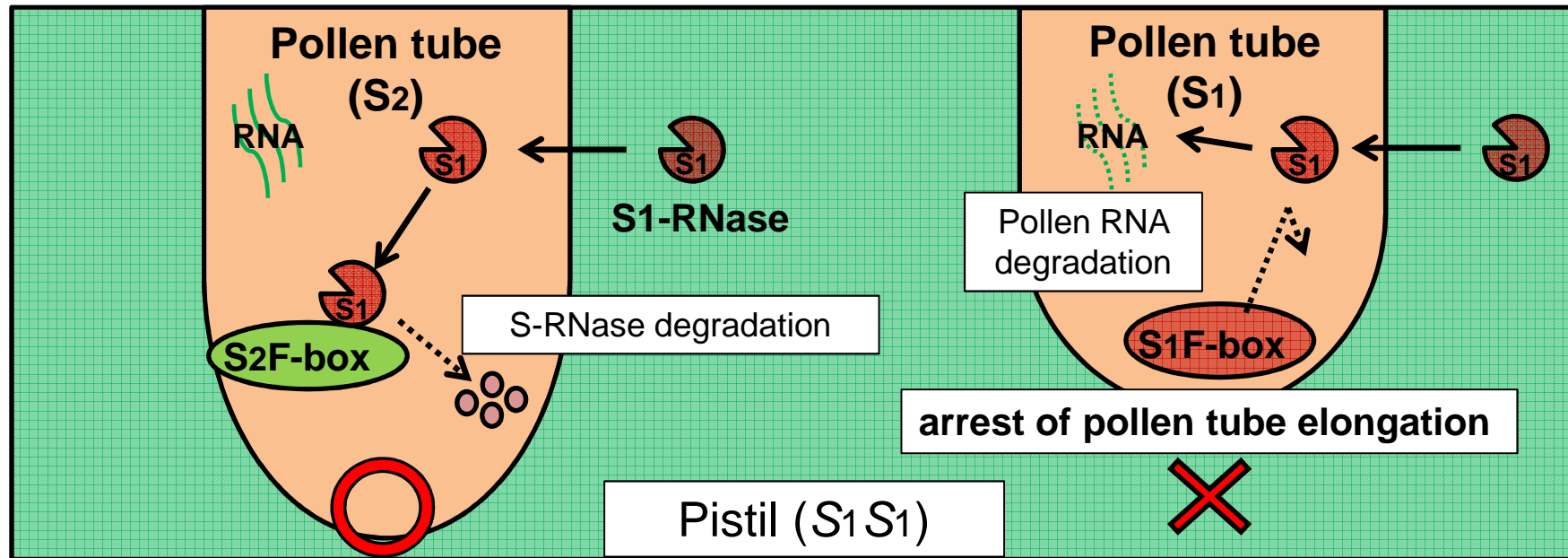
- self/ nonself recognition is determined by **S locus**



In Solanaceae, Plantaginaceae, and subtribe Malinae

Cross pollination (compatible)

Self pollination (incompatible)



Prunus-specific self-recognition mechanism

- Disruption of pollen S F-box

Solanaceae, Plantaginaceae, Malinae

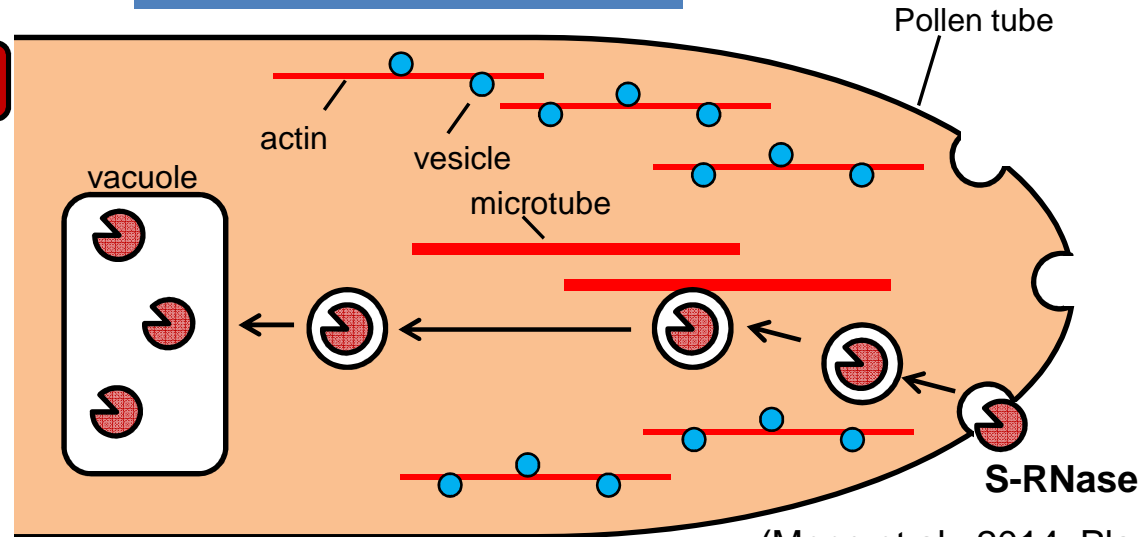
➡ self, cross **incompatible**

Prunus

➡ self, cross **compatible**

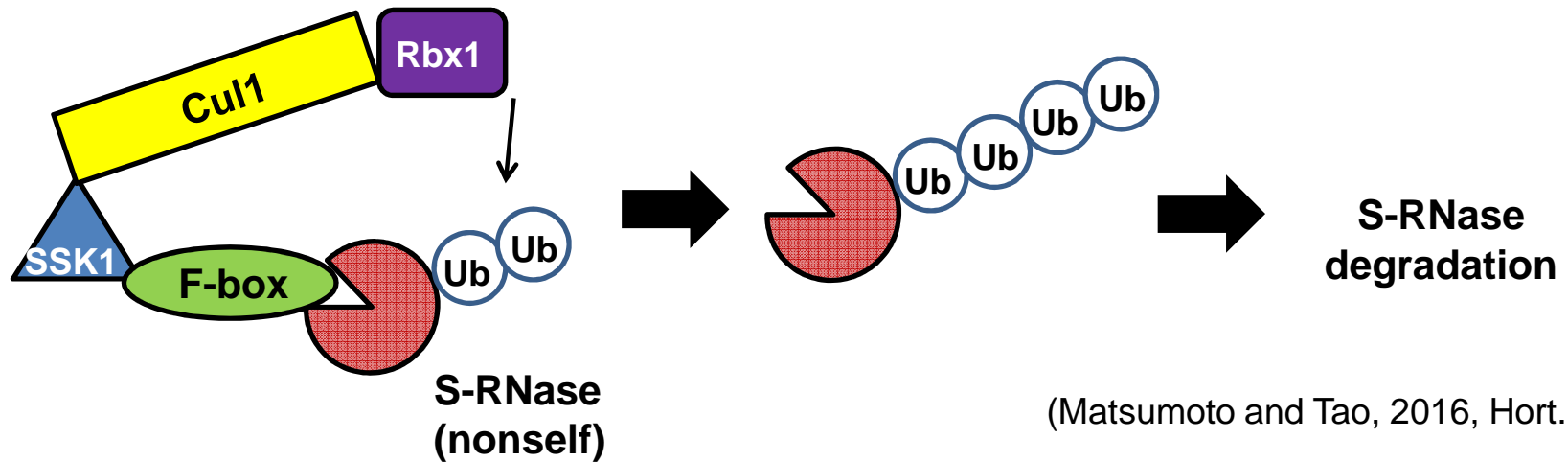
Modifier factor

S-RNase import



(Meng et al., 2014, Plant Cell Physiol.)

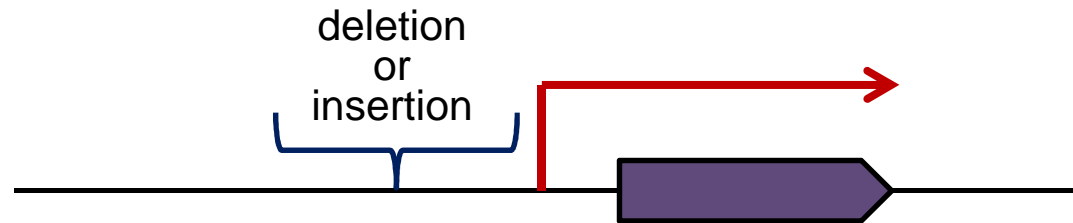
S-RNase degradation



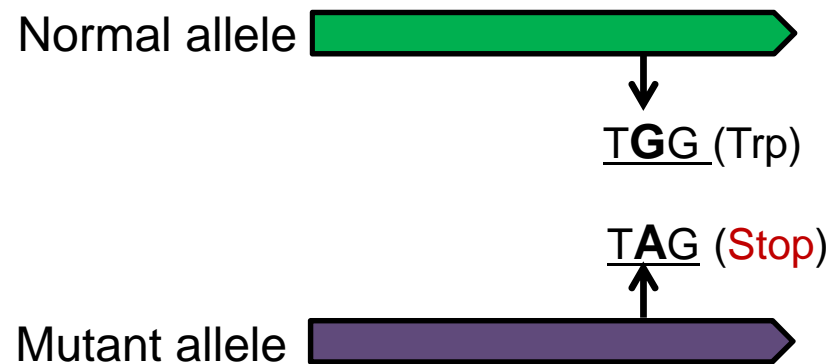
(Matsumoto and Tao, 2016, Hort. J)

● Causal mutations responsible for *M* locus

1. Differential expression (up- or down-regulation)



2. Functional mutation (nonsynonymous substitution)



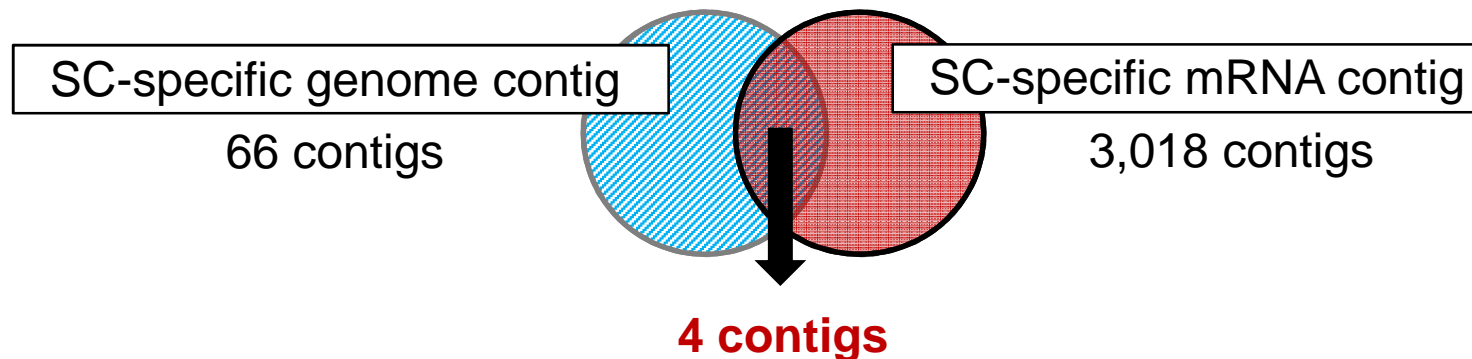
Exp.2 mRNA-Seq

Pollen cDNAs located on *M* locus (RPKM>1)

cDNA contig	RPKM		TAIR	
	Cristobalina	accession	description	e-value
comp10826_c0_seq2	1.20	AT4G26190.1	Haloacid dehalogenase-like hydrolase	8.00E-27
comp10826_c0_seq4	1.09	AT3G29760.1	Haloacid dehalogenase-like hydrolase	1.00E-47
comp13483_c2_seq3	1.74		no hit	
comp13676_c2_seq2	1.13	ATMG00300.1	ORF145A Gag-Pol-related retrotransposon	5.00E-09
comp13725_c0_seq19	1.05	AT4G32060.2	calcium-binding EF hand family	2.00E-84
comp13725_c0_seq32	1.20	AT4G32060.2	calcium-binding EF hand family	1.00E-83
comp13725_c0_seq37	1.29	AT4G32060.2	calcium-binding EF hand family	6.00E-84
comp13725_c0_seq44	1.31	AT4G32060.2	calcium-binding EF hand family	3.00E-84
comp13725_c0_seq50	1.11	AT4G32060.2	calcium-binding EF hand family	4.00E-84
comp39203_c0_seq1	1.37		no hit	
comp8358_c1_seq1	1.07	AT5G15640.1	Mitochondrial substrate carrier	3.00E-129

Exp.2 mRNA-Seq

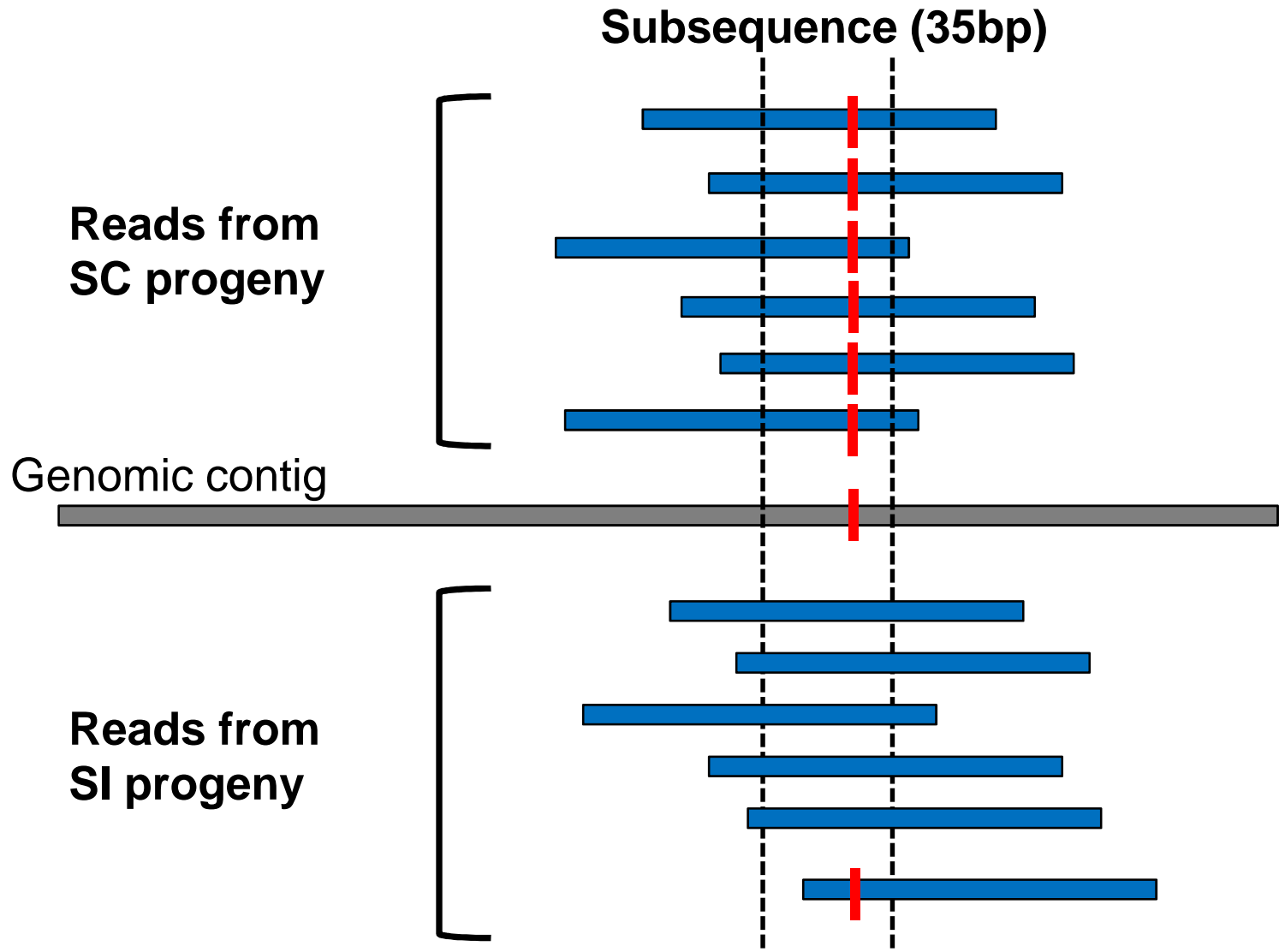
- Identify SC-specific subsequence
⇒ 146,344 subsequences
- de novo assembly for reads including SC-specific subsequence
⇒ 3,018 contigs
- Extract common contigs between gDNA and mRNA contigs



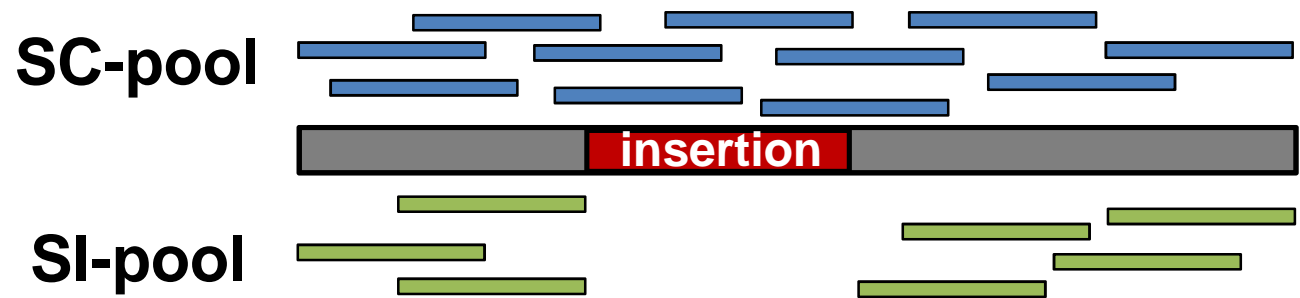
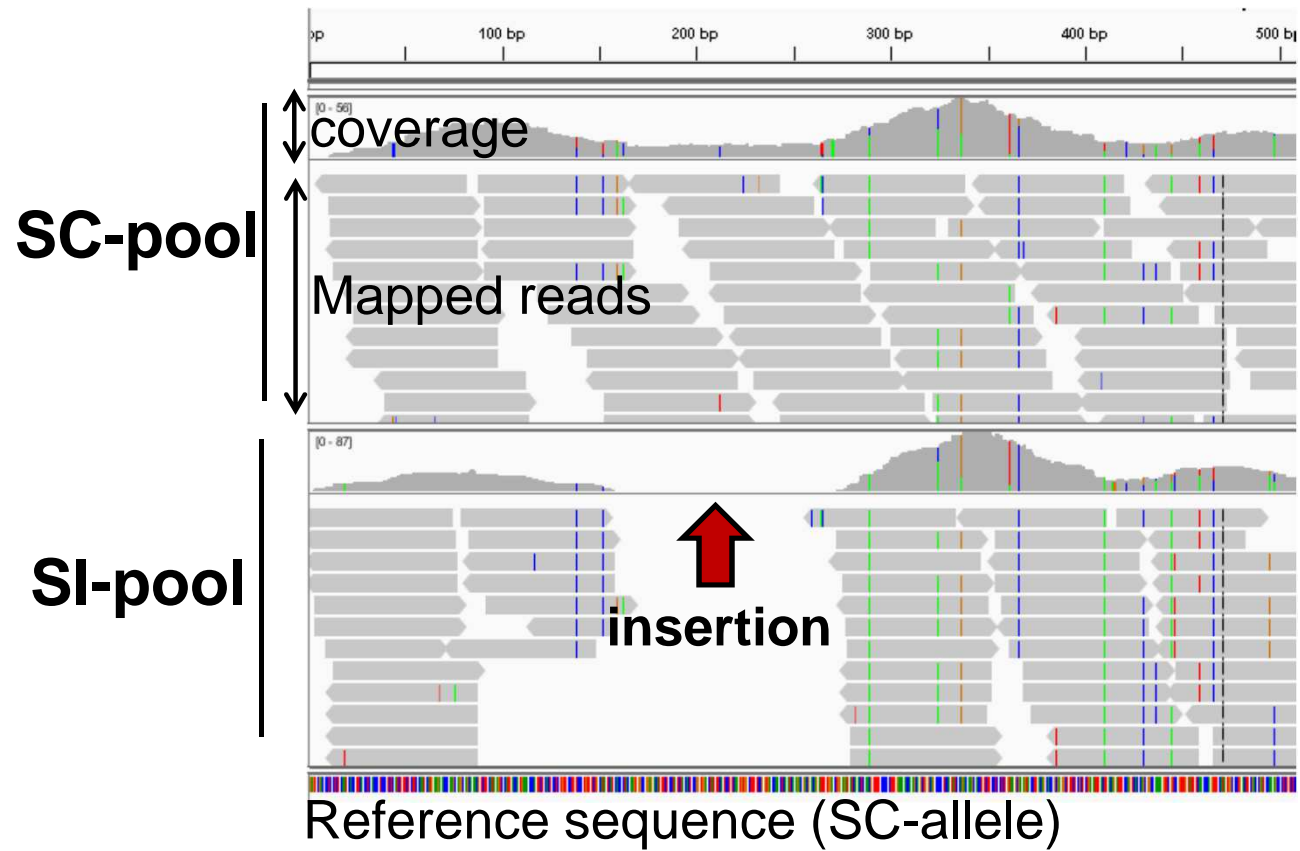
Exp.2 mRNA-Seq

Pollen cDNAs with SC-specific polymorphism in coding sequence

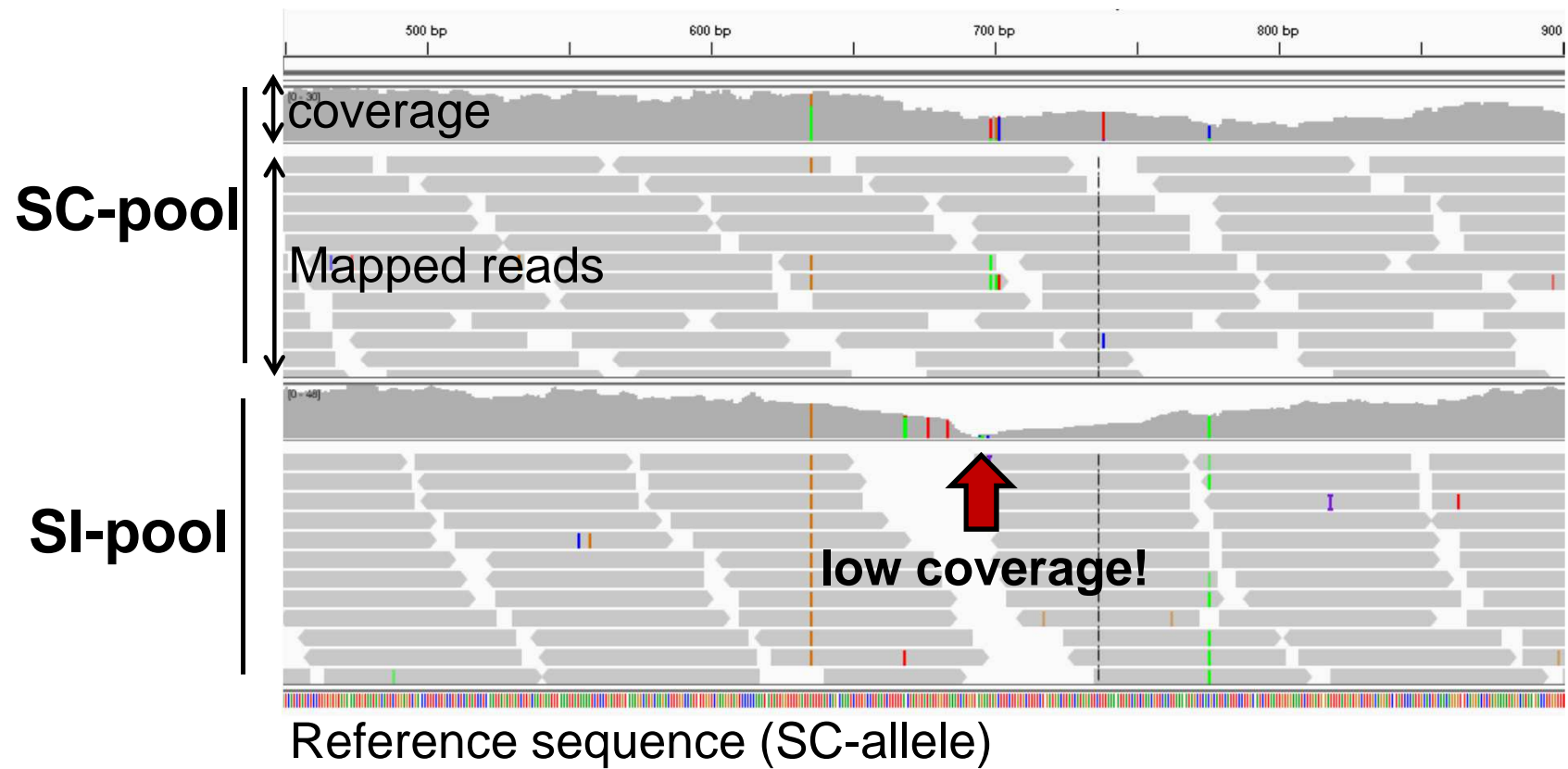
mRNA contig ID	AA substitution	TAIR annotation			RPKM (Cristobalina)
		Acc.No	Description	e-value	
SC-spe-contig_14	yes (SNP) T→S	AT4G26190.1	Haloacid dehalogenase-like hydrolase	3E-46	1.81
SC-spe-contig_2547	yes (SNP) I→F	AT1G21280.1	Retrotransposon gag protein	3.00E-06	14.35
SC-spe-contig_2643	yes (SNP) E→A		no hit		1.31
SC-spe-contig_2925	no	AT3G51550.1	Feronia receptor-like kinase	3.00E-06	0.53



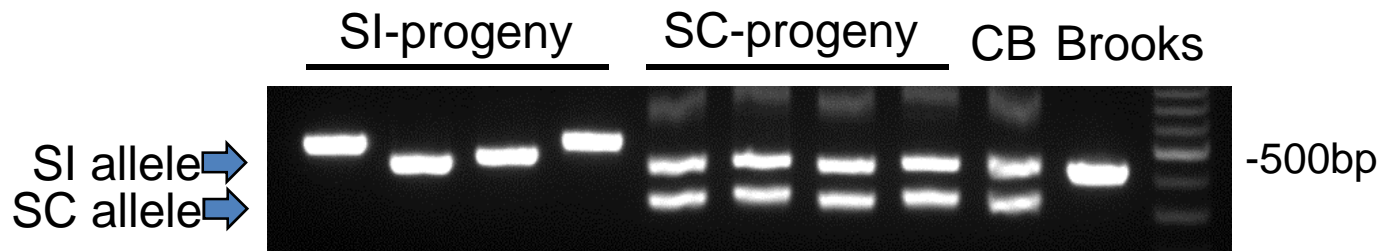
Identification of indels using NGS data -insertion-



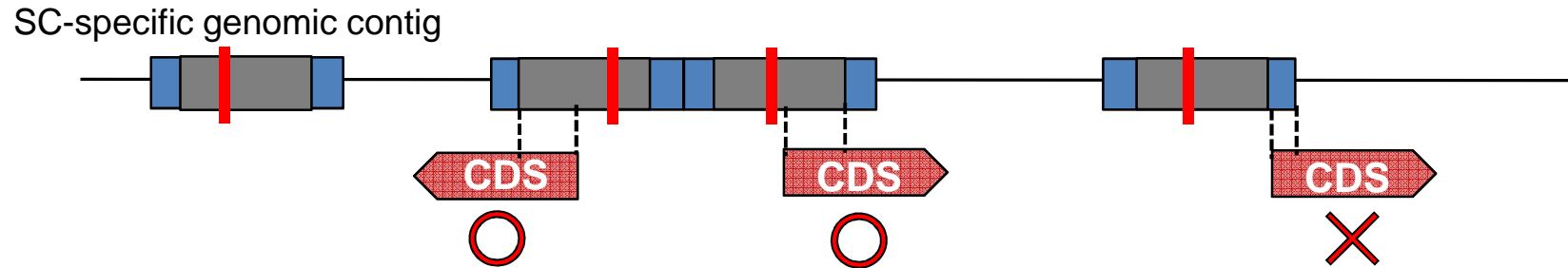
Identification of indels using NGS data -deletion-



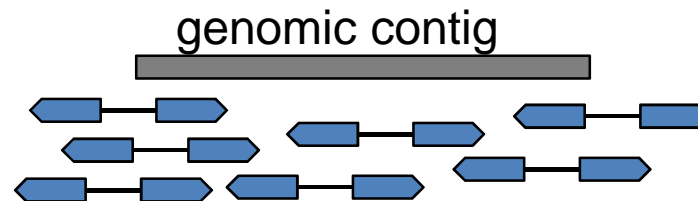
(screen shot from Integrated Genome Viewer; IGV)



Exp.2 mRNA-Seq



1. Map reads from SC progeny



2. Assemble
(expanding distal sequence)



3. Homology search between genomic contig (expanded) and pollen cDNA

Evolutionary analysis for S locus genes

1. Insights into the *Prunus*-specific S-RNase-based self-incompatibility system from a genome-wide analysis of the evolutionary radiation of S locus-related F-box genes

(T. Akagi, I.M. Henry, T. Morimoto and R. Tao, Plant and cell physiology. 2016)

- Evolutionary path that gave rise to *Prunus*-specific self-recognition system

2. Evolutionary analysis of genes for S-RNase-based self-incompatibility reveals S locus duplications in the ancestral Rosaceae

(T. Morimoto, T. Akagi and R. Tao, The Horticulture Journal. 2015)

- S locus evolution in ancestral Rosaceae