

Diversity analysis in *Erwinia amylovora* isolates from Central Asia

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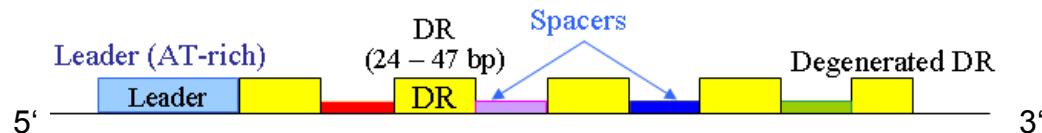
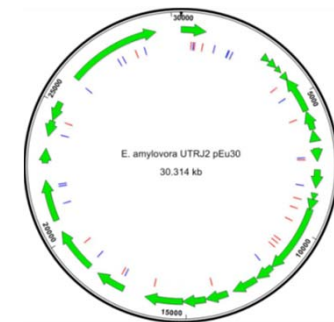
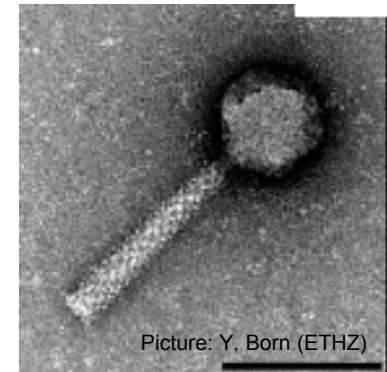
Objectives

- Use of CRISPR analysis as tool for strain typing in *E. amylovora*
 - > Sequence identity between strains on Maloideae (apple/pear) >99.99%
(i.e., 1 SNP in 30'000-100'000 bp, based on 5 genomes from Europe and 1 from U.S.)
- Clustering analysis of *E. amylovora* based on CRISPR data
 - > Using an international collection of strains obtained from PhytFire partners
- Hypothesis about epidemiology of fire blight in Central Asia
 - > Fire blight has reached the cradle of domesticated apple, but where does it come from?

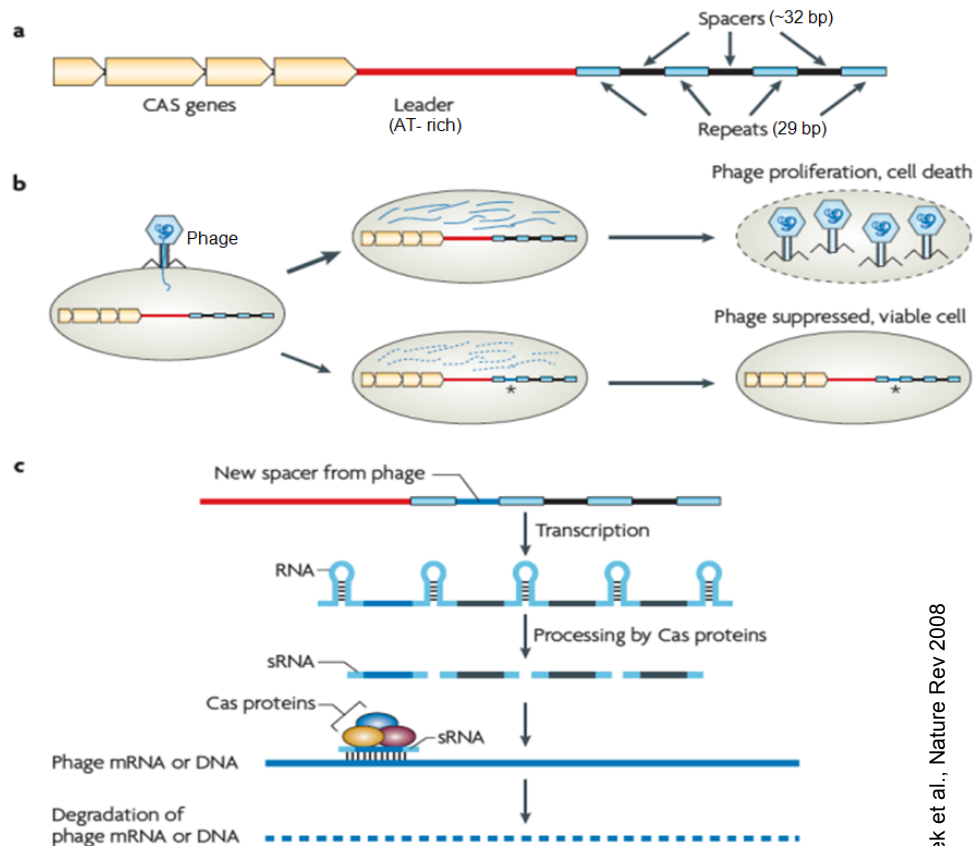
CRISPRs

Clustered Regularly Interspaced Short Palindromic Repeats

- Semi-repetitive genetic elements that confer acquired heritable immunity against mobile nucleic acid elements in prokaryotes
 - > Present in 90% of the *Archaea* and 40% of the *Bacteria*
 - > Protect against phage infection
 - > Limits horizontal gene transfer of plasmids
- Act via an RNA-interference-like mechanism
 - > In concert with CRISPR associated (Cas) proteins
- Hotspots for diversity!!!
 - > Constantly adapt to the changing plasmid and phage background
- Conserved direct DNA repeats interspersed by unique spacers



Three stages model of CRISPR/Cas system functionality



Semi-repetitive CRISPR repeat region (CRR) flanked by cas genes

Phage infection

- **Adaptation** of the array via recognition of alien DNA and integration of new spacers
> CRISPR regions grow polarly
- **Expression** of CRR and cas genes
> Processing of pre-crRNA to crRNA
- **Interference** of target DNA
> crRNA directs Cas protein complex that degrades foreign DNA

Sorek et al., Nature Rev 2008

CRISPRs in *E. amylovora*

- Three different CRISPR repeat regions were found in *E. amylovora*

> CRR1 and CRR2 are active and evolving

E. coli type (repeat, *cas* genes)

Similar to *E. pyrifoliae* CRR1 & CRR2

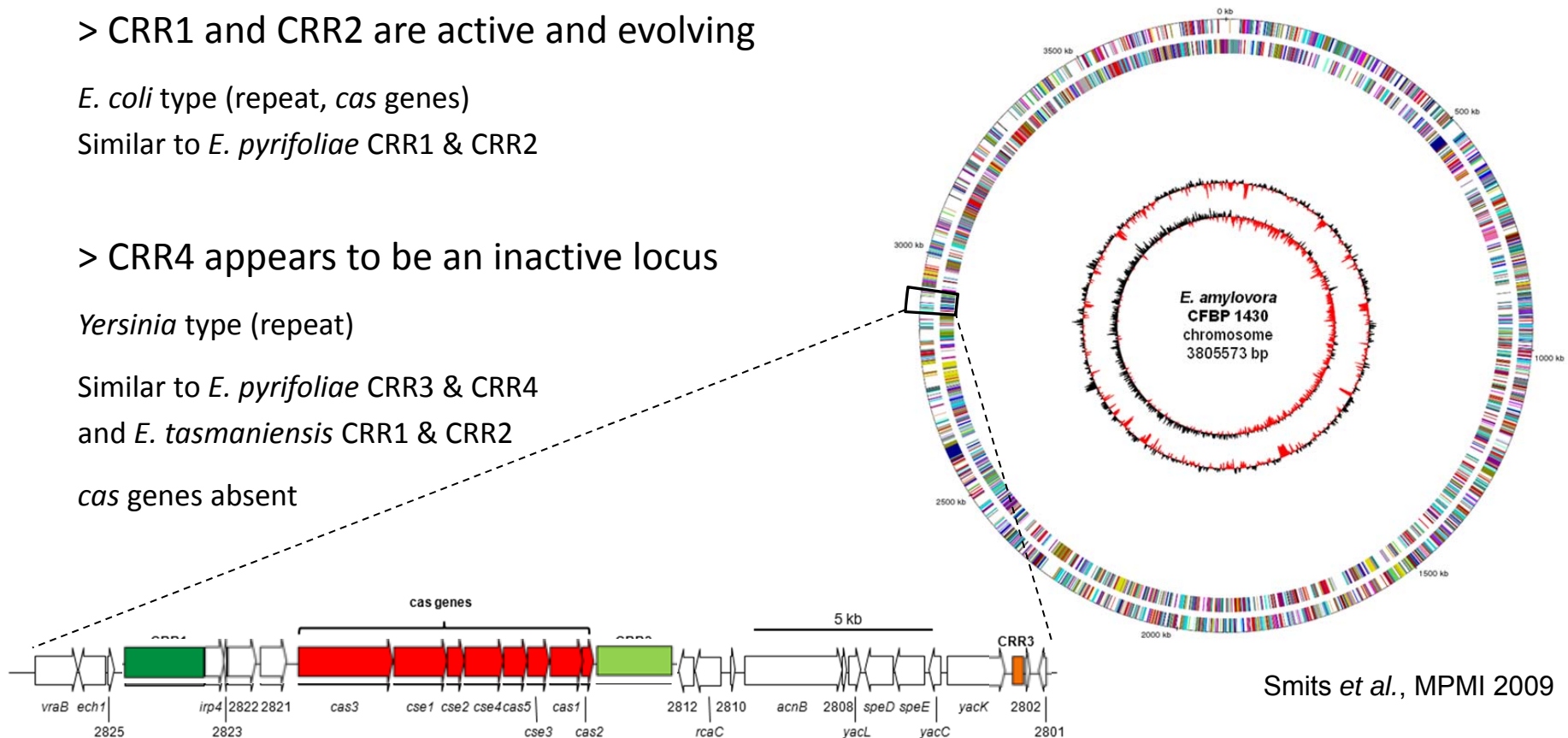
> CRR4 appears to be an inactive locus

Yersinia type (repeat)

Similar to *E. pyrifoliae* CRR3 & CRR4

and *E. tasmaniensis* CRR1 & CRR2

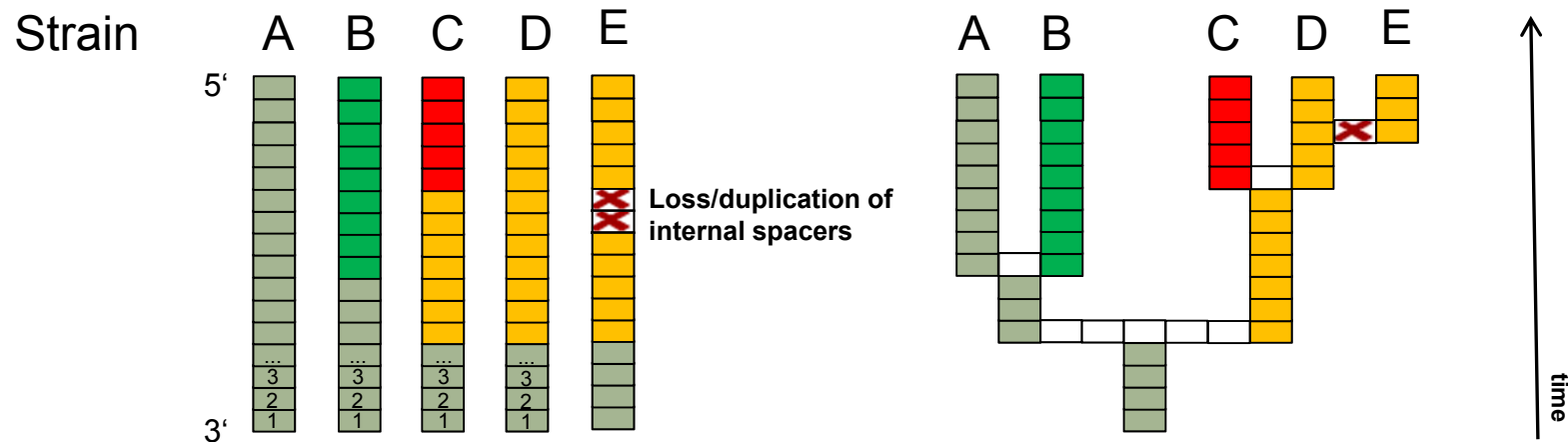
cas genes absent



Smits *et al.*, MPMI 2009

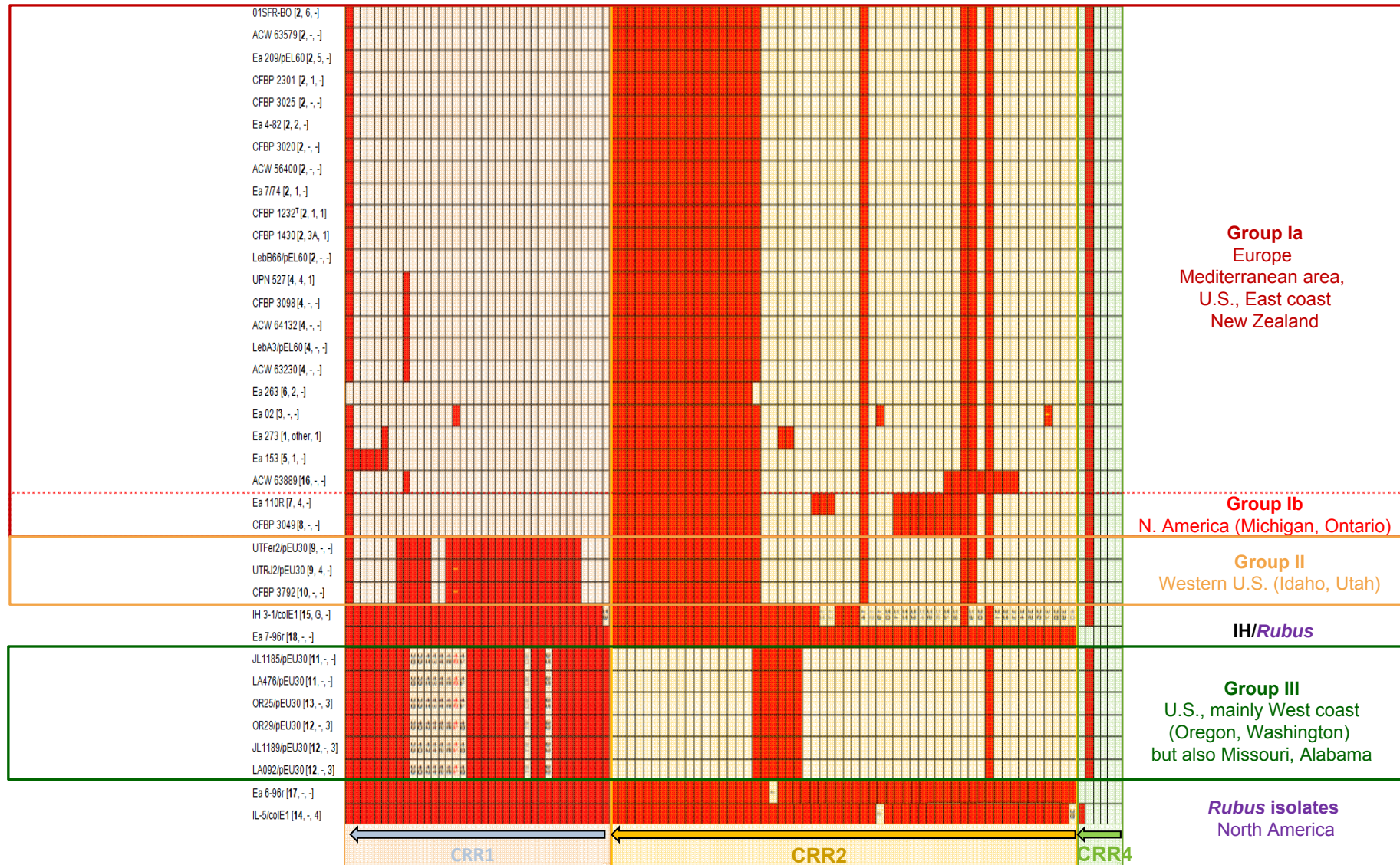
CRISPRs structure and assembly

- CRISPRs assembly
 - > New spacers inserted polarly at the 5' end of the cluster next to the leader
 - > Older spacers are frequently common whereas newer spacers are unique
 - > Chronological record of past encounters with foreign DNA



Identification of ancestral genotype

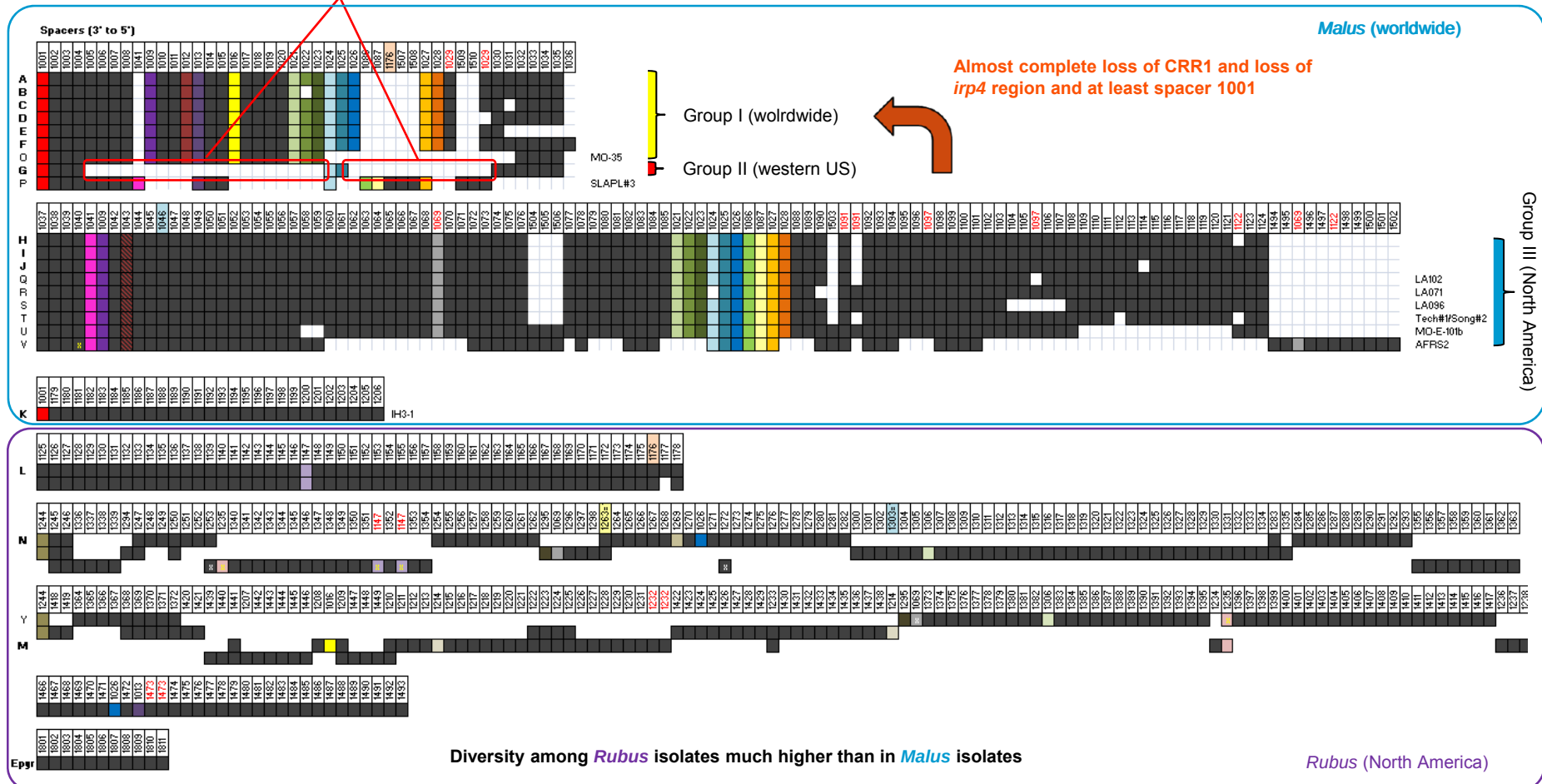
Strains that display recent incorporation of new spacers at 5' end
and/or
deletions in the central regions of the array
can not be ancestral



CRISPR Region 1

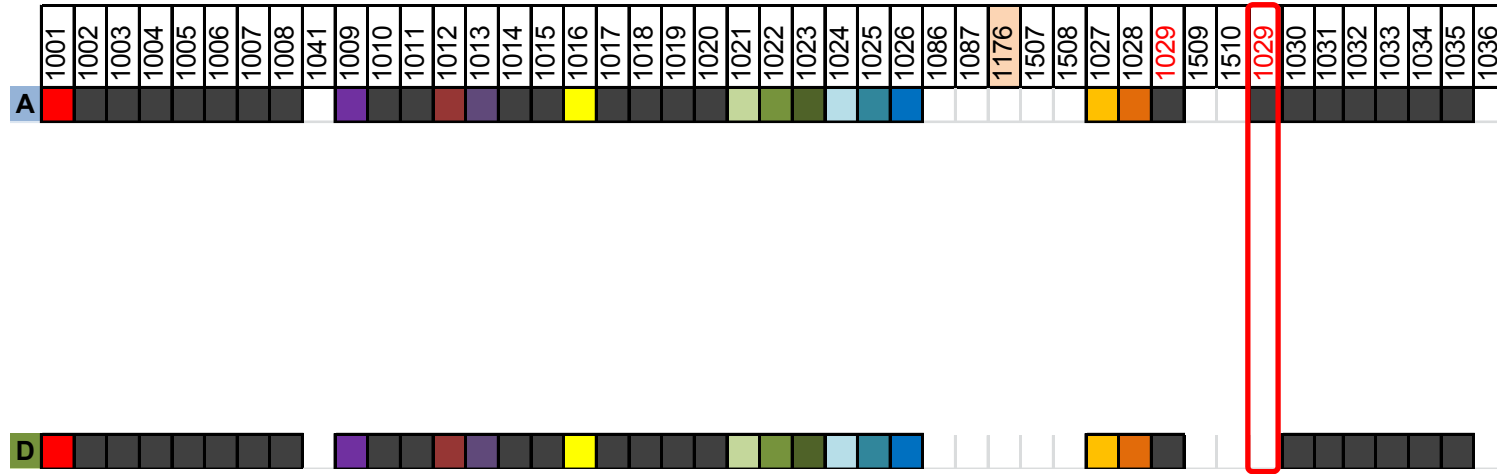
In worldwide strains

Internal deletions leading to Gr. II



Group Ia genotypes

CRISPR Region 1 in ancestral European strains

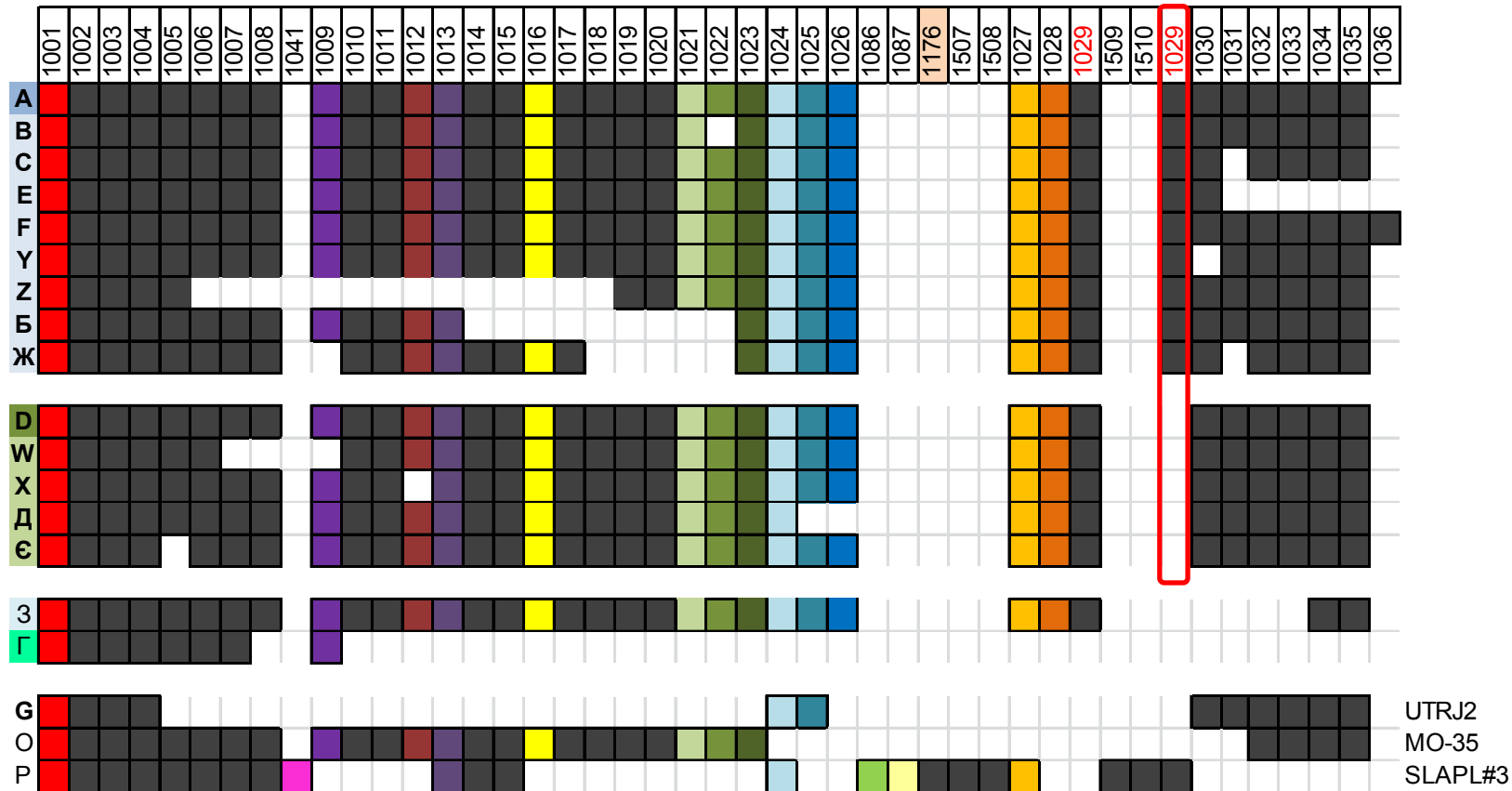


In North American strains



Group Ia genotypes

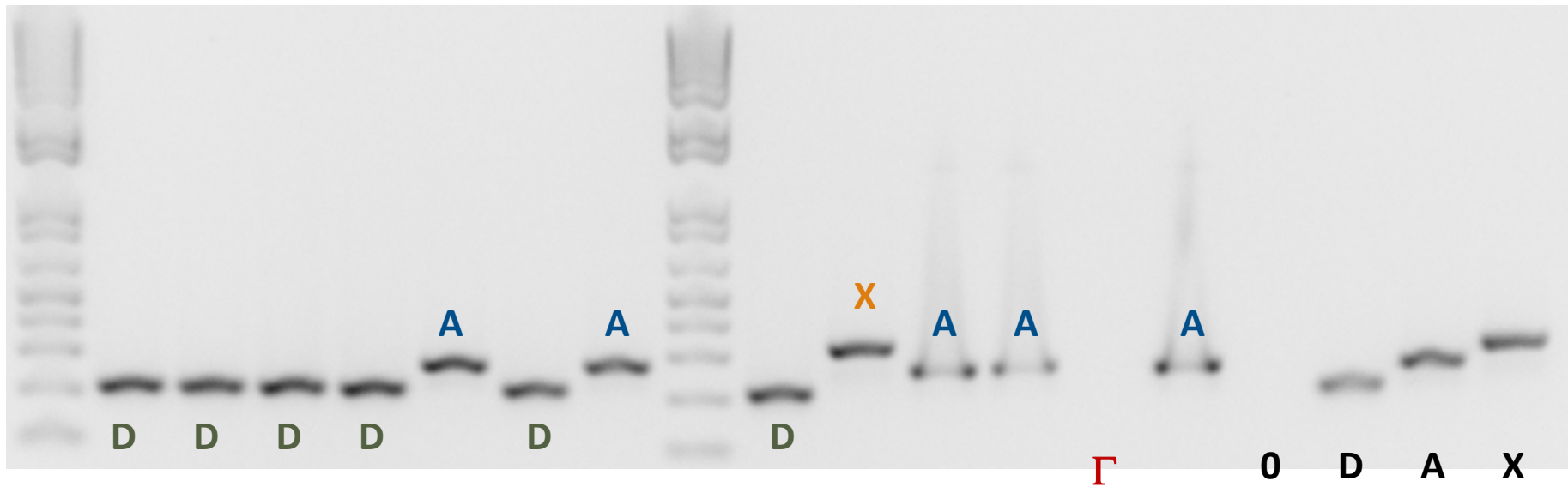
CRISPR Region 1 in European strains



CRR1 genotypes A and D are the ancestral genotypes in Europe

PCR test of CRISPR1 ancestral genotypes

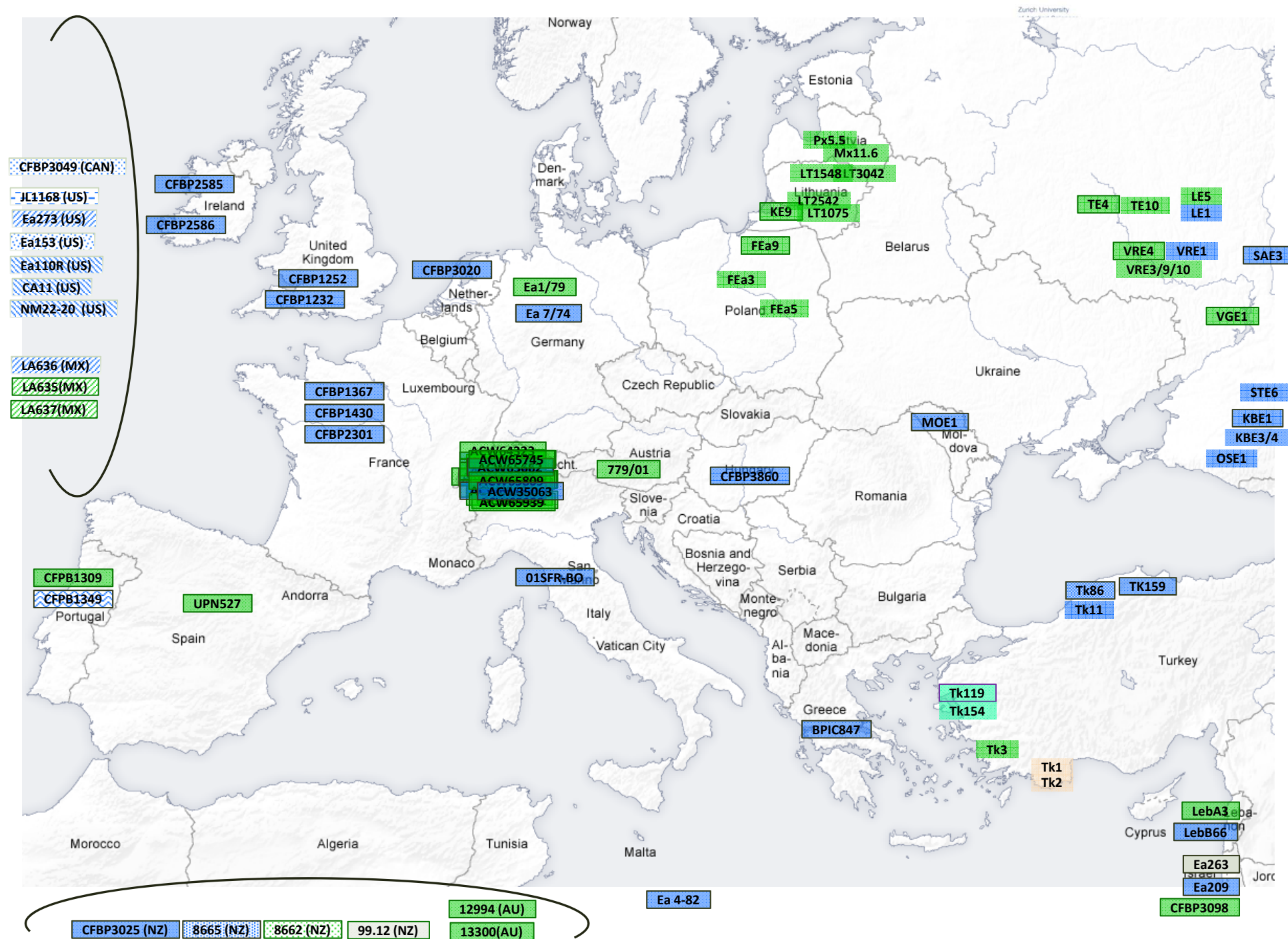
Allows quick discrimination of A- or D-derived strains

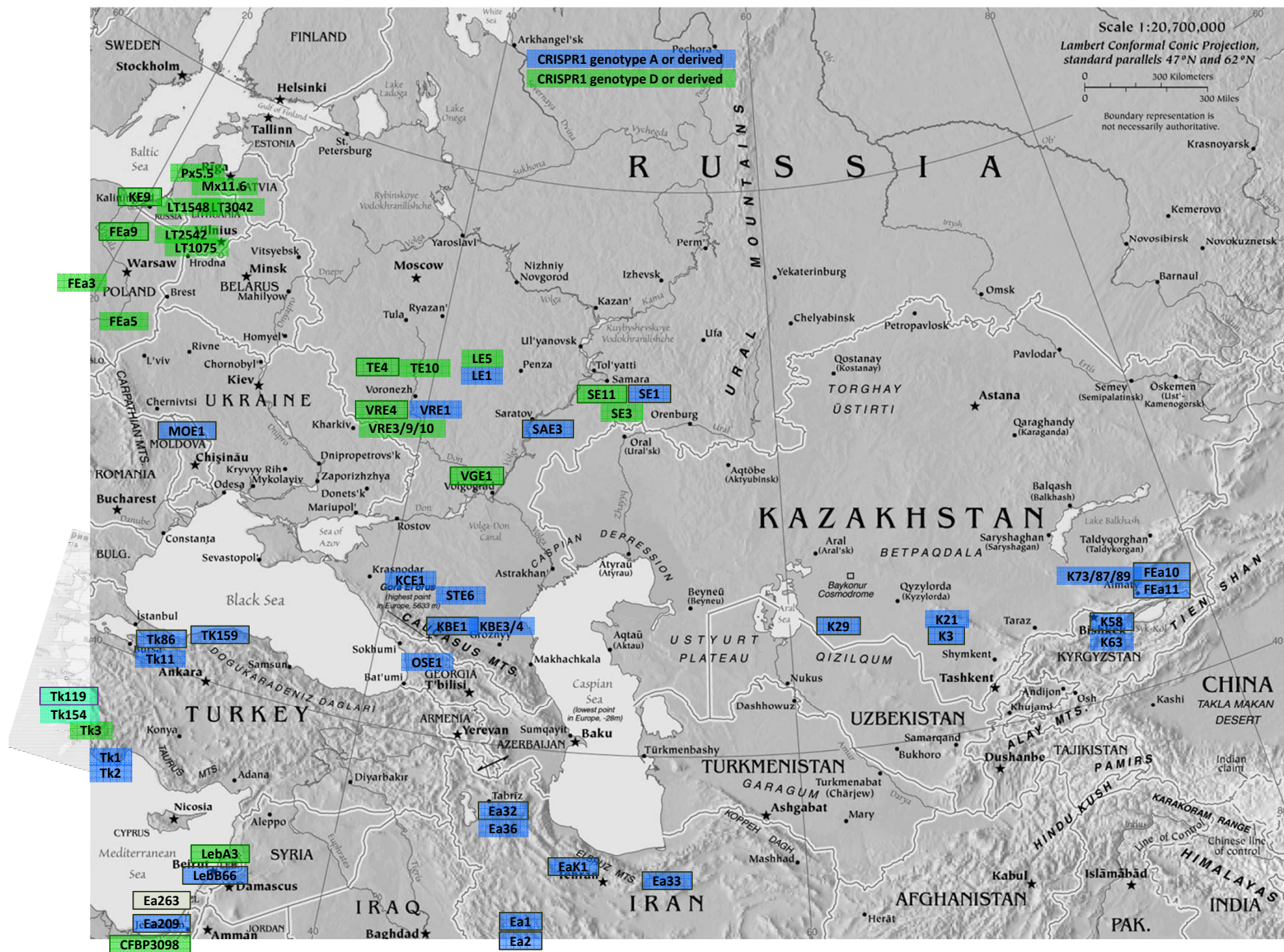


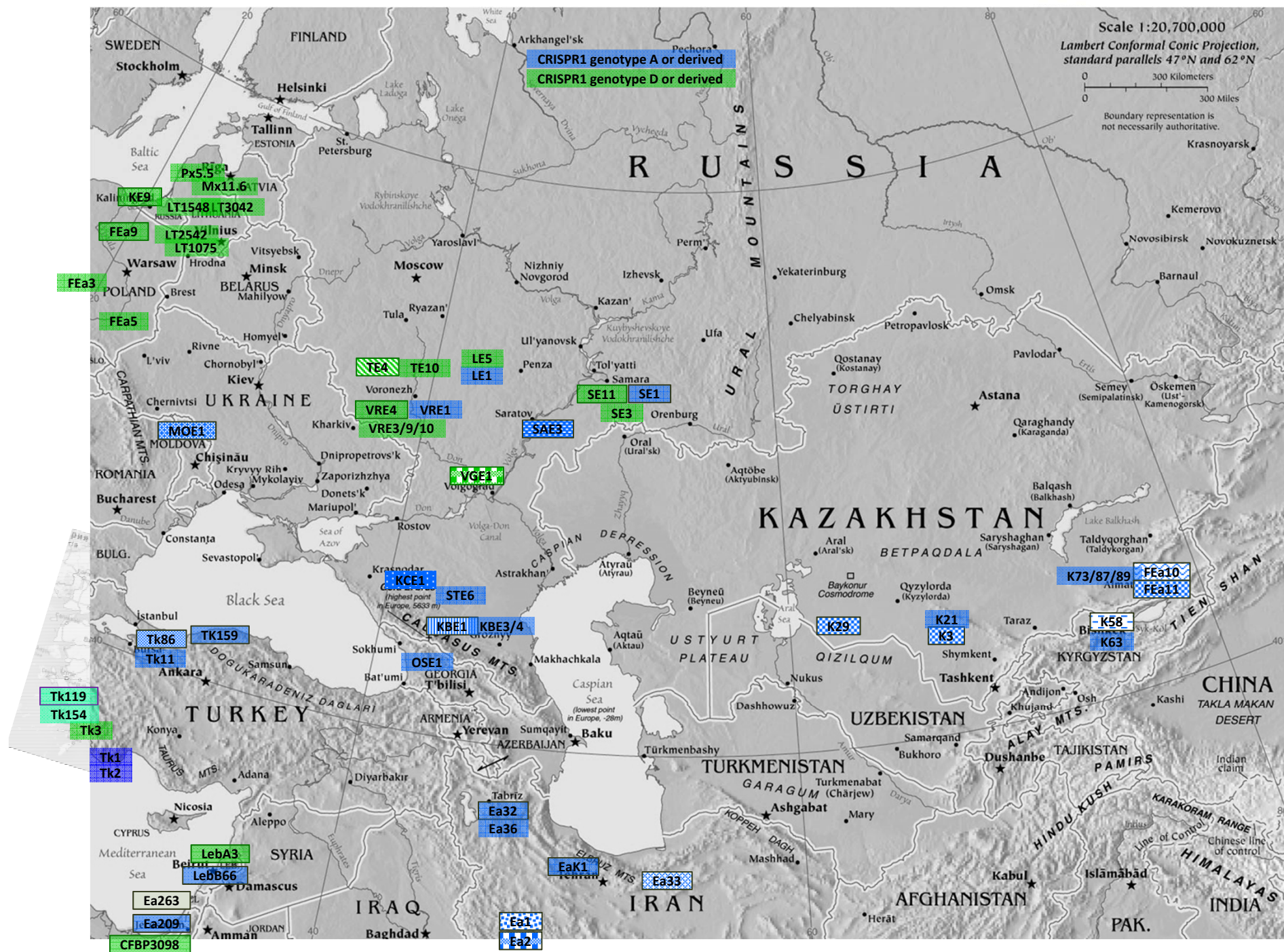
1951							CFBP1252	CFBP1232	1960
1961						First appearance in Europe (A genotype)			1970
1971	CFBP1430 CFBP1367		Ea 7/74					Ea1/79	1980
1981	Ea 4-82 CFBP2301 CFBP3020		BPIC847		CFBP2585 CFBP2586	CFBP3098			1990 326
1991		472	118	92	CFBP3860	Ea209 UPN527 467	Ea263 11 LebA3 LebB66	553	2000 281
2001	Ea02 ACW35063	ACW35250 KE9	ACW42138 ACW42134 EaK1	LT1548		ACW56400 MOE1 LT3042 TEa Tk86	ACW63230 ACW63889 ACW64132 ACW63579 ACW63106 ACW63561 ACW64051 LT2542	ACW64323 ACW64369 ACW65131 LT1075 VRE1 KBE1	2010 CFPB1309 ACW65422 ACW65662 Px5.5 Mx11.6 VRE3/9/10 KBE3/4
2011	FEa5 ACW65745 ACW65809 ACW65939 FEa9 K89 CFPB1349	LE5 TK159 STE6 SAE3 OSE1 FEa10 FEa11	K29 K21 K3 K73/87						

First appearance of D-derived genotype

First appearance of A-derived genotype







CRISPR Region 2 provides an additional layer of information

[illegible]

a = ancestral European genotype

t= Kyrgyz genotype displaying a spacer deletion

u= Kazakh genotype displaying a spacer duplication

← Spacer duplication

← Spacer deletion

This does not imply that FEa10 derives directly from K58, but outrules the inverse path!

Conclusions

- CRISPR analysis is suitable for typing *E. amylovora* at regional or national level
- First introduction in Europe due to 1-2 genotypes from Eastern U.S.
 - > Bottleneck leading to high genetic homogeneity, it is not clear if genotype D correspond to a second introduction from the U.S. or originated in Europe from genotype A
- Recent European strains show increased genetic diversity
- Strains present in Central Asia are derived from genotype A and correspond to the southern European/Mediterranean genotype
- The Caucasus region lies at the crossroad between the two genotypes

Outlook

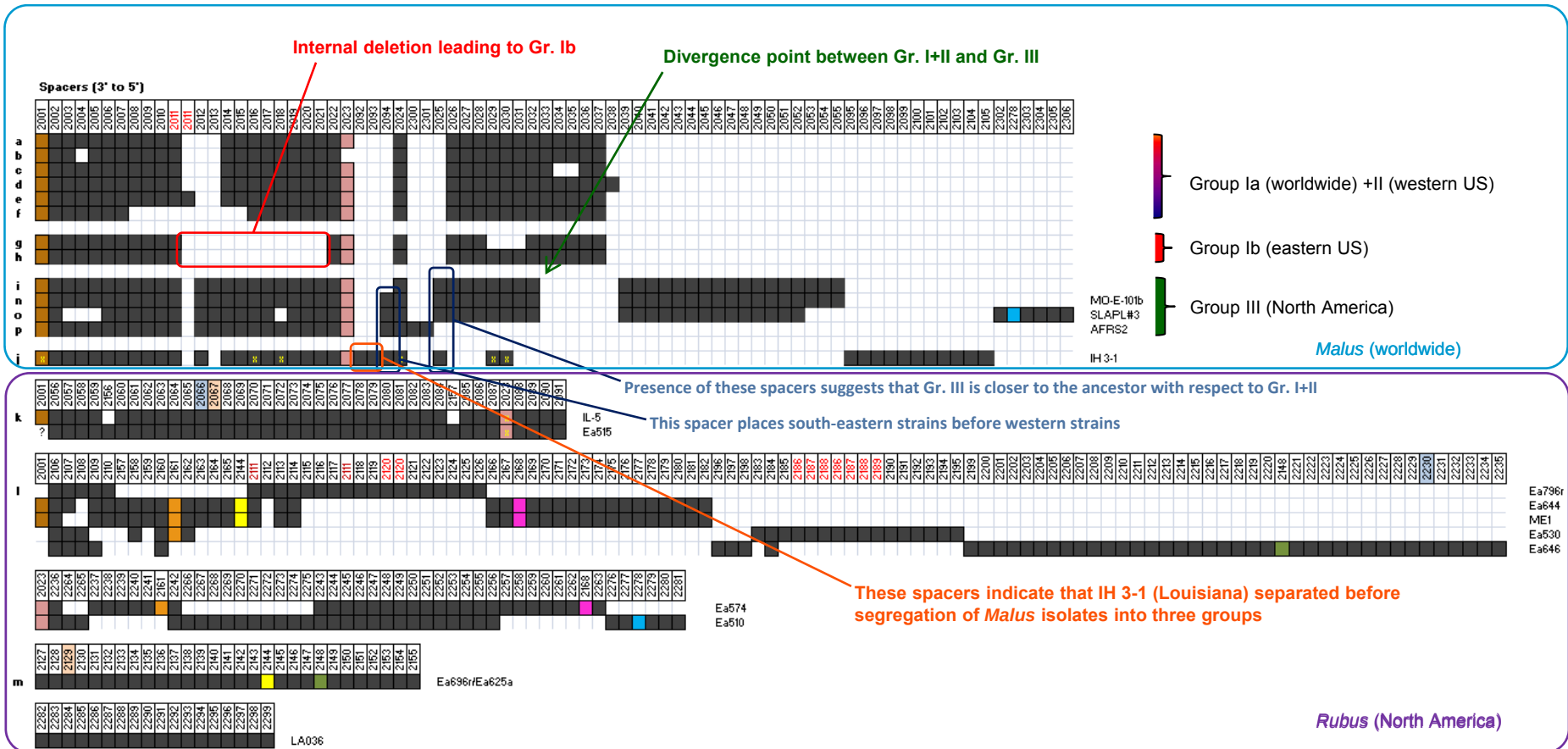
We have just scratched the surface, there are still a lot of data (mainly from WGS) that expect evaluation and can be used to refine our picture: VNTR, SNPs, plasmid information, etc.

Acknowledgments

- The PhytFire (extended) community for providing the strains
 - > Turkey, Russia, Kazakhstan, Kyrgyzstan, Lithuania, Lettonia, Portugal, (Iran)
- The other participants to WP6
- Hatice and her team of local organizers for setting up the meeting in such a wonderful location

CRISPR Region 2

What does it tell us?



Diversity among *Rubus* isolates much higher than in *Malus* isolates