



# *High resolution typing of livestock-associated MRSA*

**Thijs Bosch**

**28-5-2013**

**Bilthoven**

28-05-2013

Innovatie in typering, whole genome mapping

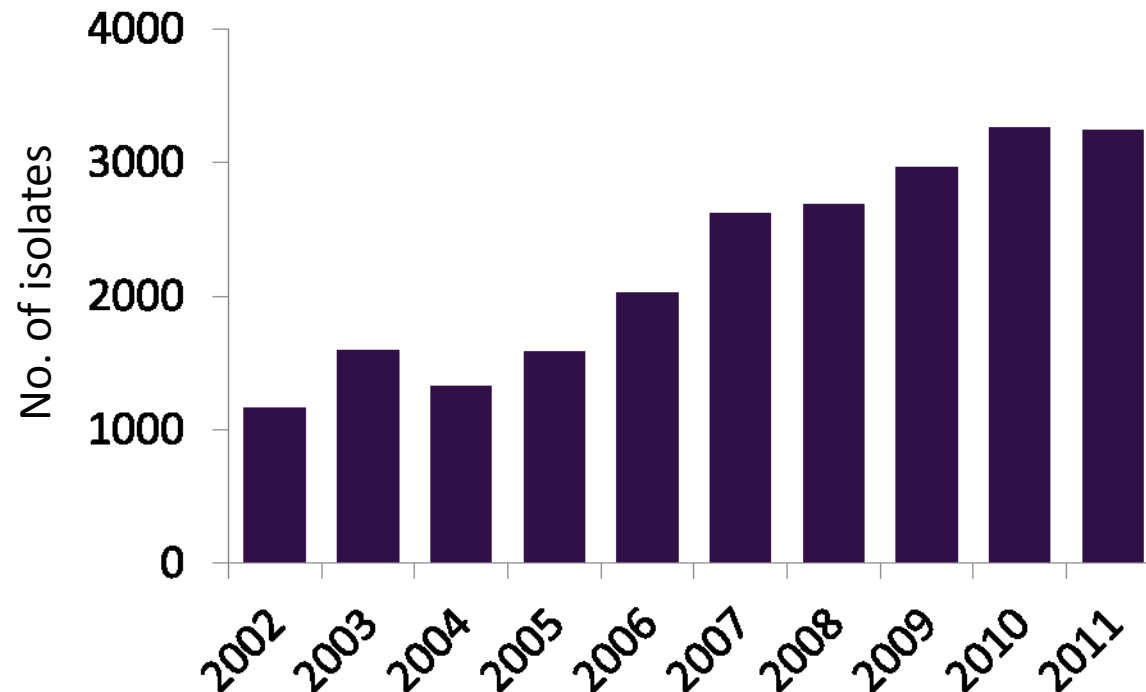




# ***RIVM is the national MRSA reference centre***

**1989 start surveillance**

**Gradual increase (50-100 strains/week)**





# *Emergence of a new MRSA clade in the Netherlands*

First isolated in 2003 (Voss et al. E.I.D. 2005)

Origin in livestock, mainly pigs (MC398)



No. of isolates



# *Emergence of a new MRSA clade in the Netherlands*

First isolated in 2003 (Voss et al. E.I.D. 2005)

Origin in livestock, mainly pigs (MC398)

Worldwide emergence

Guardibassi et al. Vet. Microbiol. 2007

Loeffler et al. J. Hosp. Infect. 2009

Ruzauskas et al. Pol J. Vet. Sci. 2013

Witte et al. E.I.D. 2007

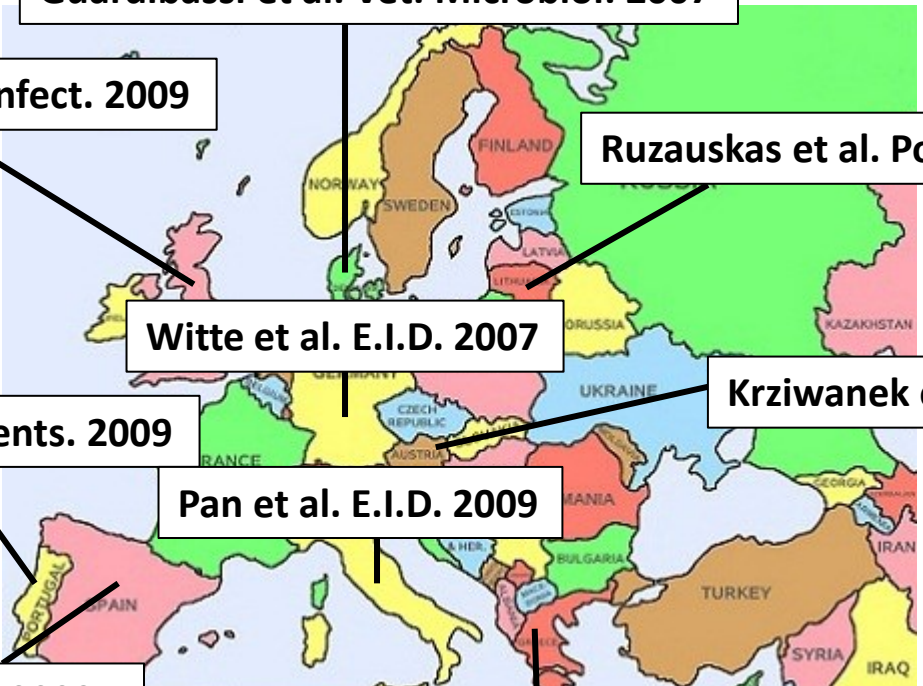
Pomba et al. Int. J. Anti. Agents. 2009

Krziwanek et al. E.I.D. 2009

Pan et al. E.I.D. 2009

Lozano et al. J. Anti. Chemo. 2009

Drougka et al. Indian. J. Med. Microb. 2012



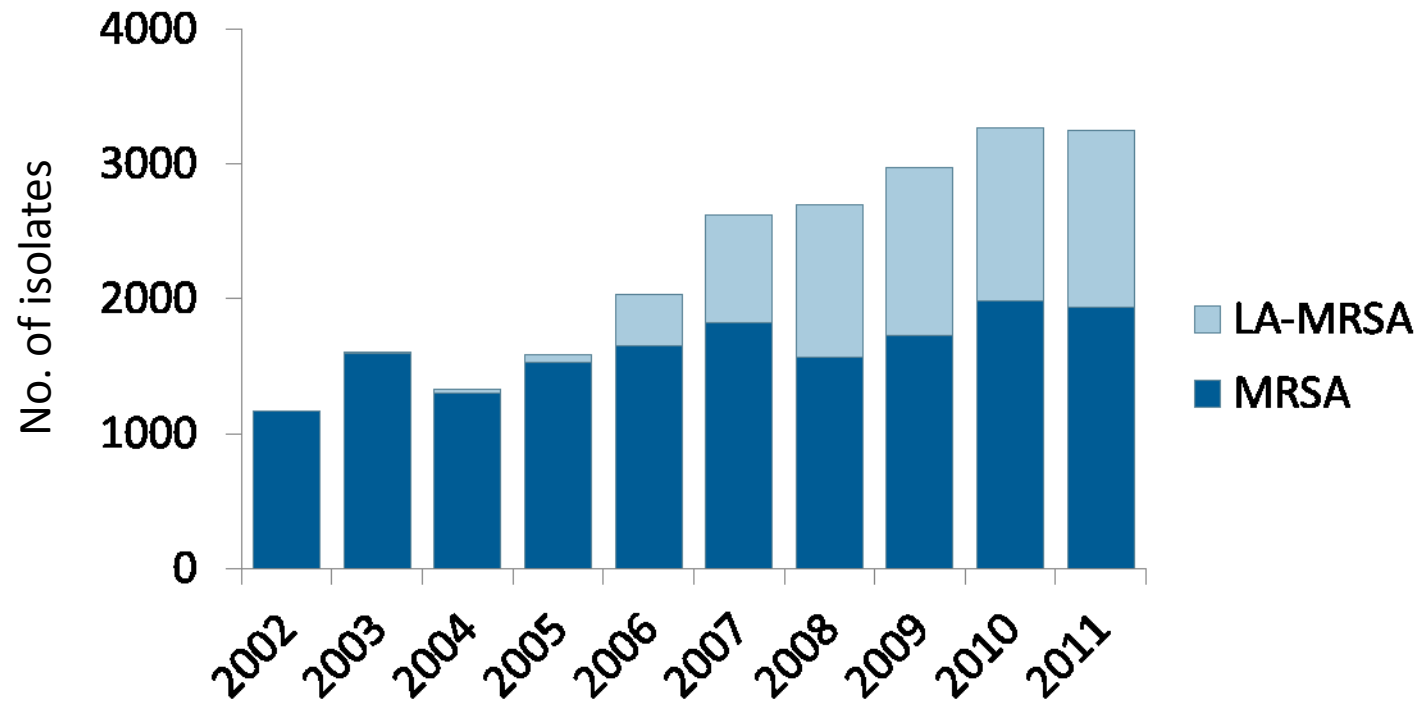


# *Emergence of a new MRSA clade in the Netherlands*

**First isolated in 2003** (Voss et al. E.I.D. 2005)

**Origin in livestock, mainly pigs (MC398)**

**Worldwide emergence**





# *Reasons for typing MRSA*

**Spot trends in the National surveillance**

**Identify emergence of new MRSA clades (LA-MRSA)**

**Outbreak management**

**Identifying possible transmission**

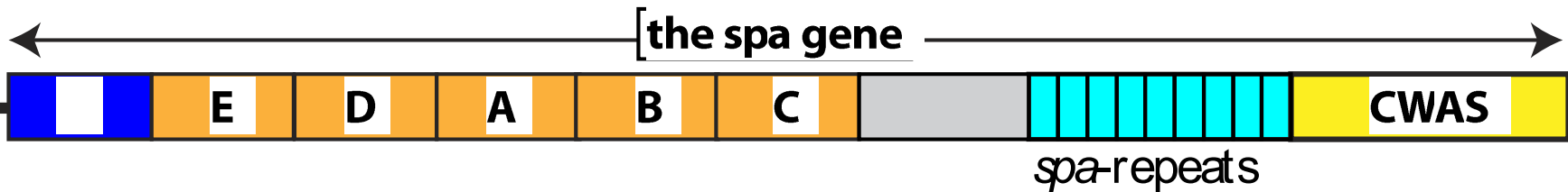


joe-ks.com



# Current typing of MRSA is sequence-based

2007 *spa*-typing



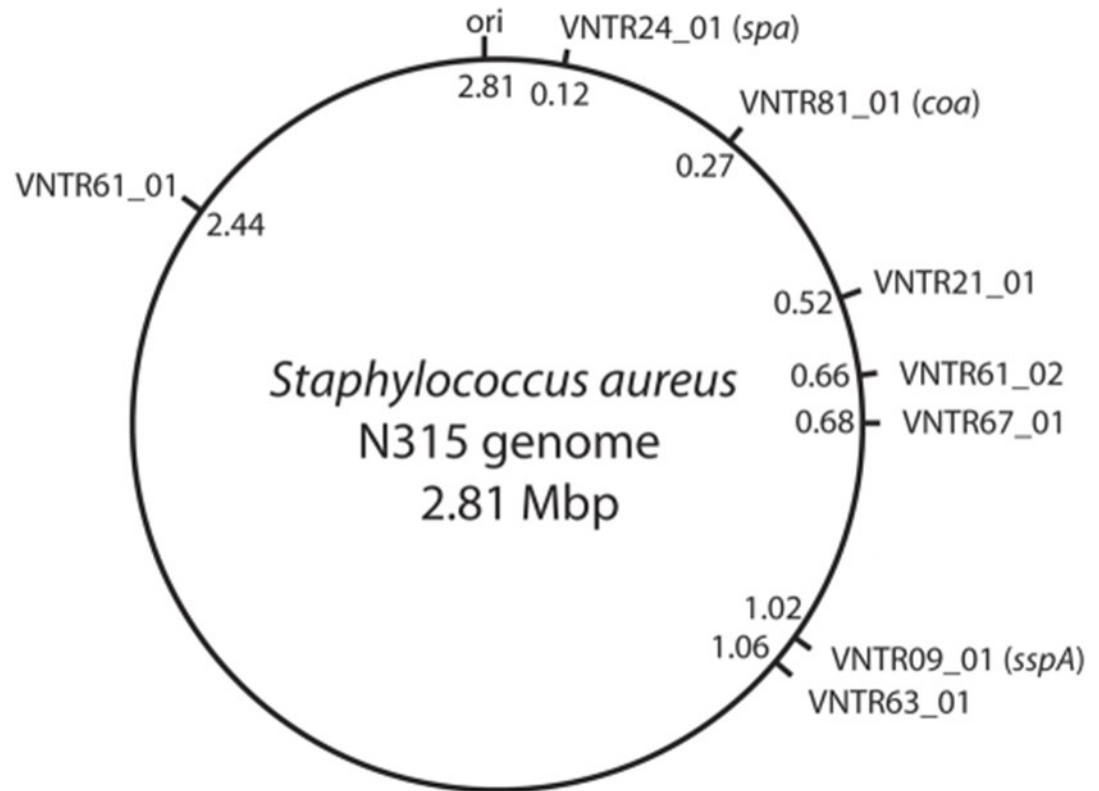
	Repeat succession	Number of repeats	Spa-type						
Strain I:	<table border="1"><tr><td>r08</td><td>r02</td><td>r16</td></tr></table>	r08	r02	r16	3	t818			
r08	r02	r16							
Strain II:	<table border="1"><tr><td>r08</td><td>r02</td><td>r16</td><td>r25</td><td>r34</td><td>r24</td></tr></table>	r08	r02	r16	r25	r34	r24	6	t3423
r08	r02	r16	r25	r34	r24				
Strain III:	<table border="1"><tr><td>r26</td><td>r23</td><td>r17</td><td>r16</td></tr></table>	r26	r23	r17	r16	4	t111		
r26	r23	r17	r16						



## Current typing of MRSA is sequence-based

2007 *spa*-typing

2008 MLVA







## ***Current typing of MRSA is sequence-based***

2007 *spa*-typing

2008 MLVA



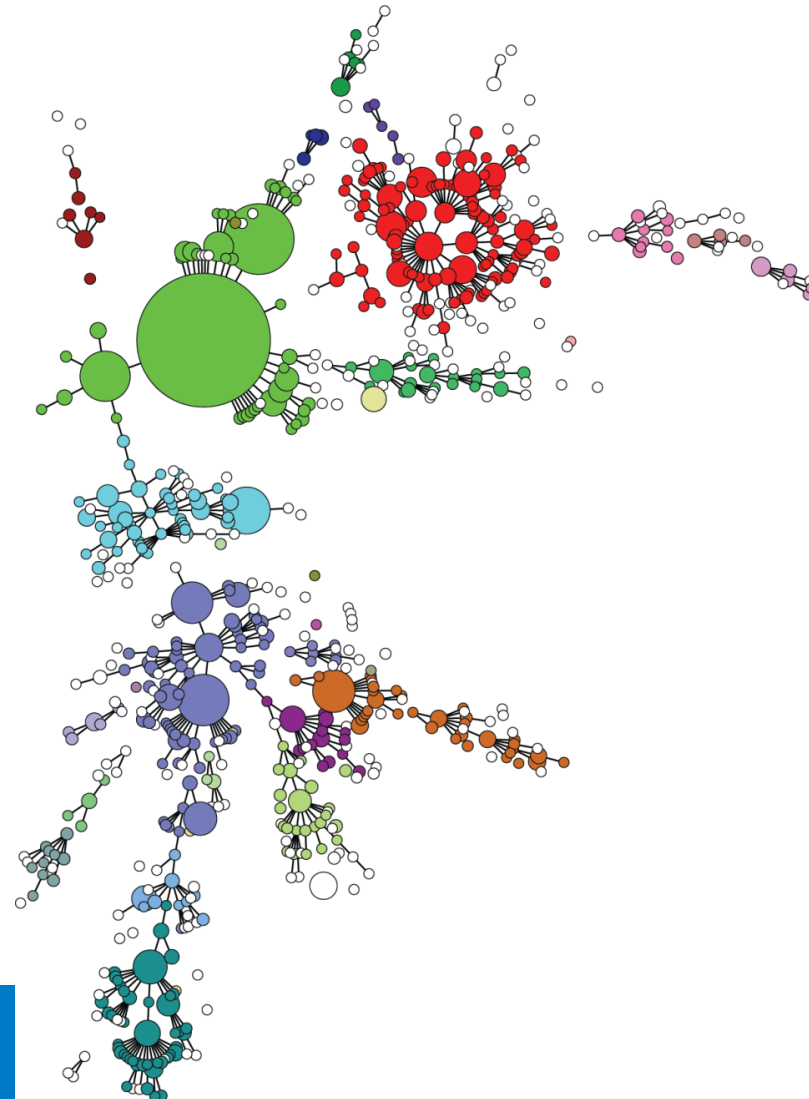
**MLVA profile of strain 1: 6-3-6-4-3-5-4-7**

**MLVA profile of strain 2: 6-3-4-4-2-5-7-7**



# *LA-MRSA has low genotypic diversity (MLVA)*

MST all MRSA isolates 2012 (n=3797)



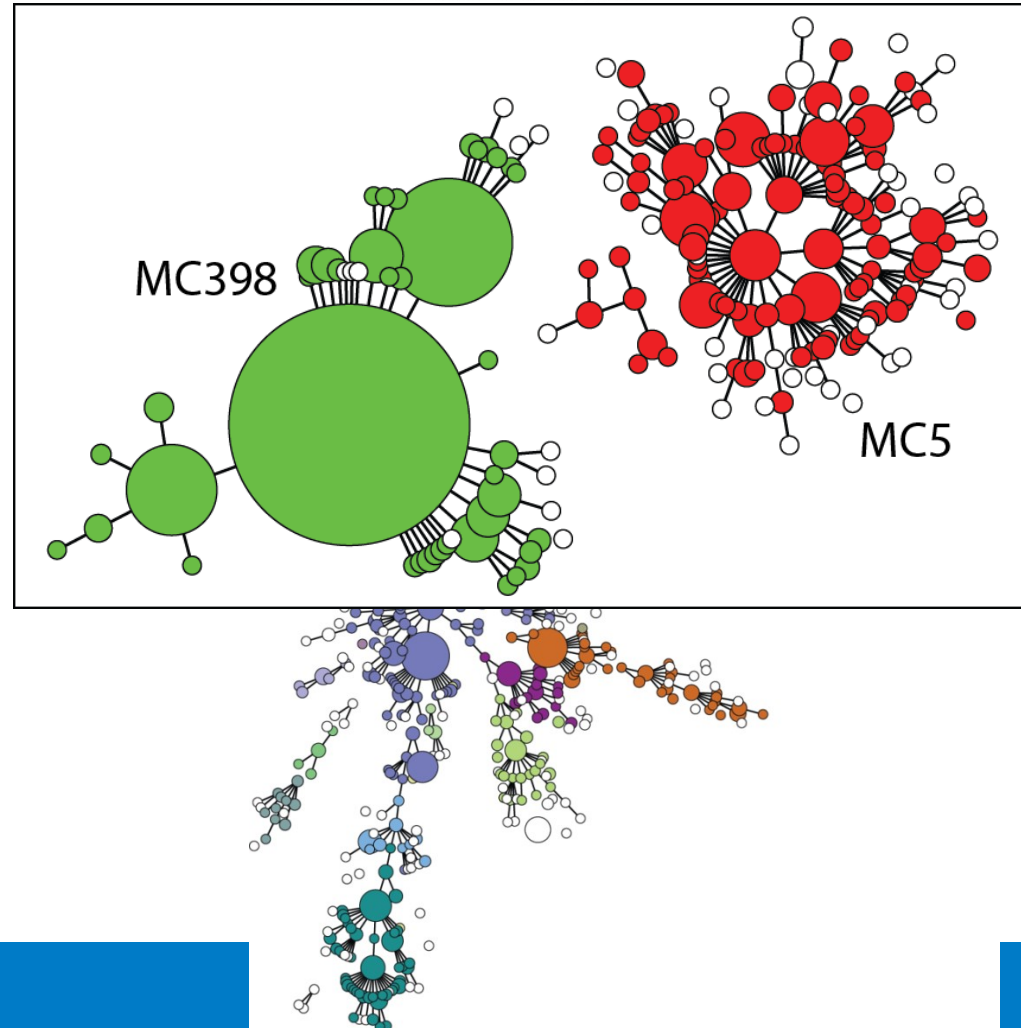


# *LA-MRSA has low genotypic diversity (MLVA)*

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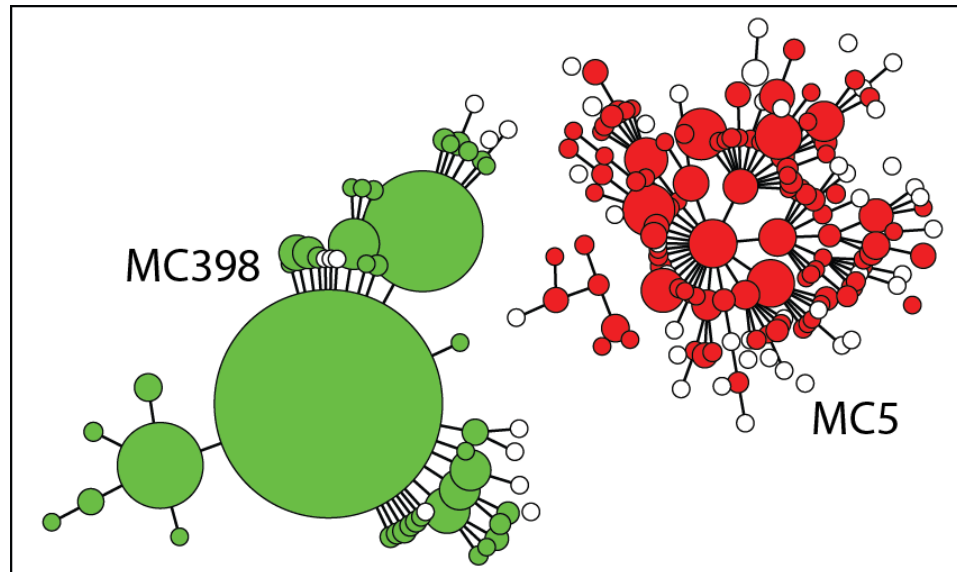
Some MLVA complexes (MC) and MLVA-types (MT) are found more frequently than others

**MC398 = LA-MRSA**





## ***LA-MRSA has low genotypic diversity (MLVA)***



MST of LA-MRSA (MC398) isolates 2008-2012  
 $n= 7066$ , 78 MLVA-types

MST of MRSA (MC5) isolates 2008-2012  
 $n= 2520$ , 205 MLVA-types

**Top 3 MLVA-types represent 83%**

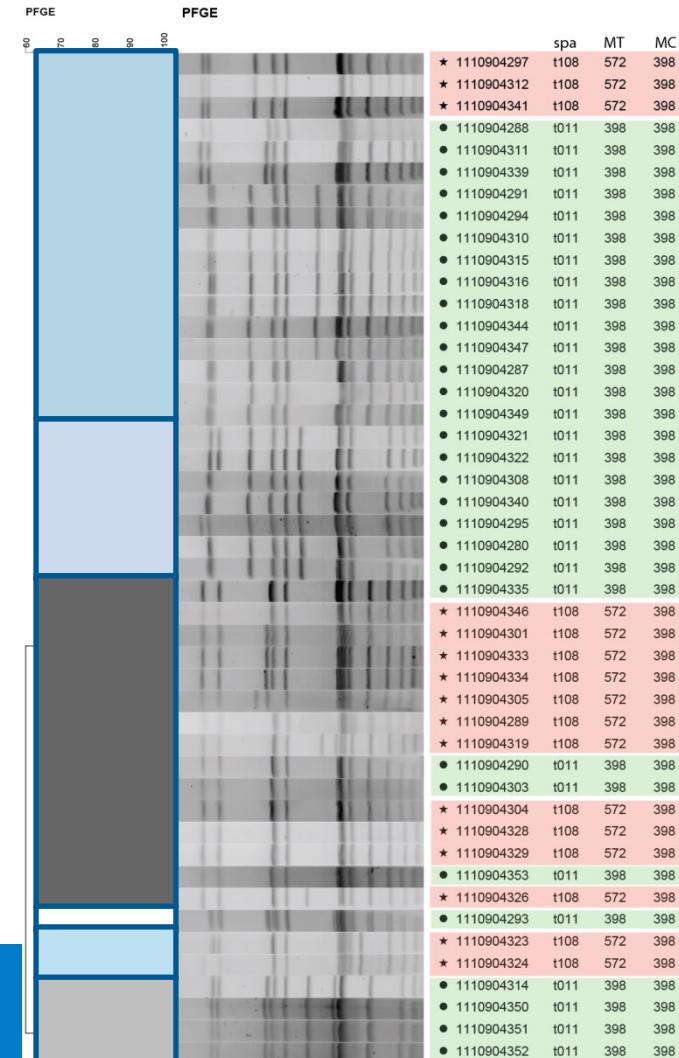
**Top 3 MLVA-types represent 21%**



# PFGE best method to differentiate LA-MRSA

Better differentiation between isolates  
with the same *spa*-/MLVA-type

(Green = *spa*-type t011, MT398, Red = *spa*-type t108, MT572)



Research article

Highly accessed

Open Access

## PFGE diversity within the methicillin-resistant *Staphylococcus aureus* clonal lineage ST398

Thijs Bosch<sup>1\*</sup>, Albert J de Neeling<sup>1†</sup>, Leo M Schouls<sup>1†</sup>, Kim W Zwaluw<sup>1†</sup>, Jan AJW Kluytmans<sup>2,3†</sup>, Hajo Grundmann<sup>1†</sup> and Xander W Huijsdens<sup>1†</sup>

\* Corresponding author: Thijs Bosch [thijs.bosch@rivm.nl](mailto:thijs.bosch@rivm.nl)

† Equal contributors

▼ Author Affiliations

<sup>1</sup> Centre for infectious disease control, National institute for public health and the environment (RIVM), Bilthoven, the Netherlands

<sup>2</sup> Department of medical microbiology and infection control, VU medical centre, Amsterdam, the Netherlands

<sup>3</sup> Laboratory for microbiology and infection control, Amphia hospital, Breda, the Netherlands  
For all author emails, please [log on](#).

BMC Microbiology 2010, 10:40

doi:10.1186/1471-2180-10-40



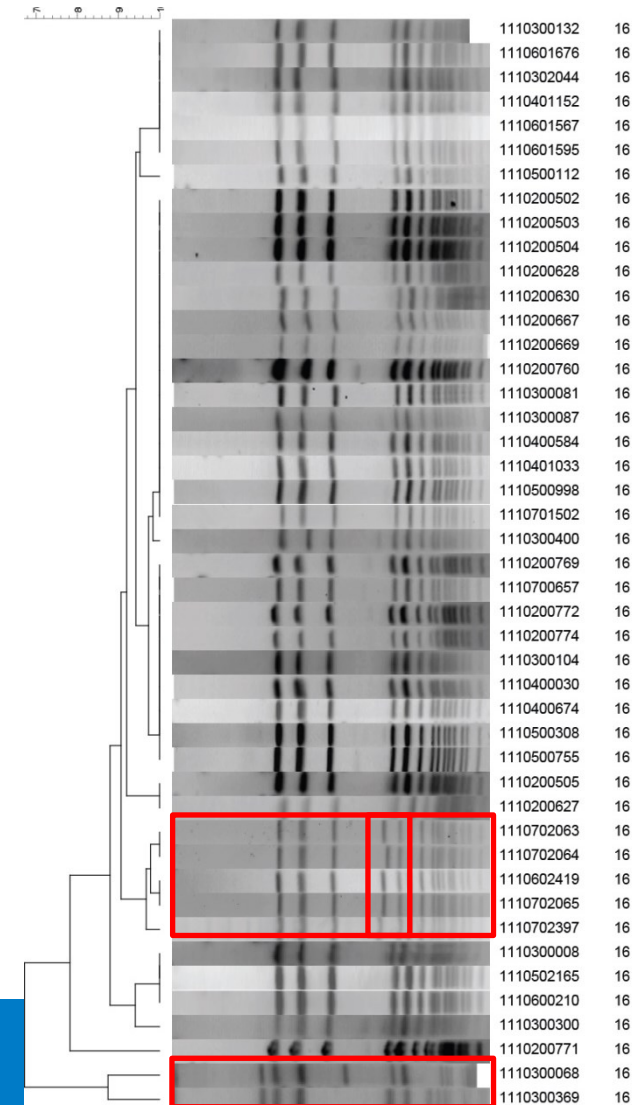
# Con's for typing LA-MRSA with PFGE

Time-consuming

Non-portable

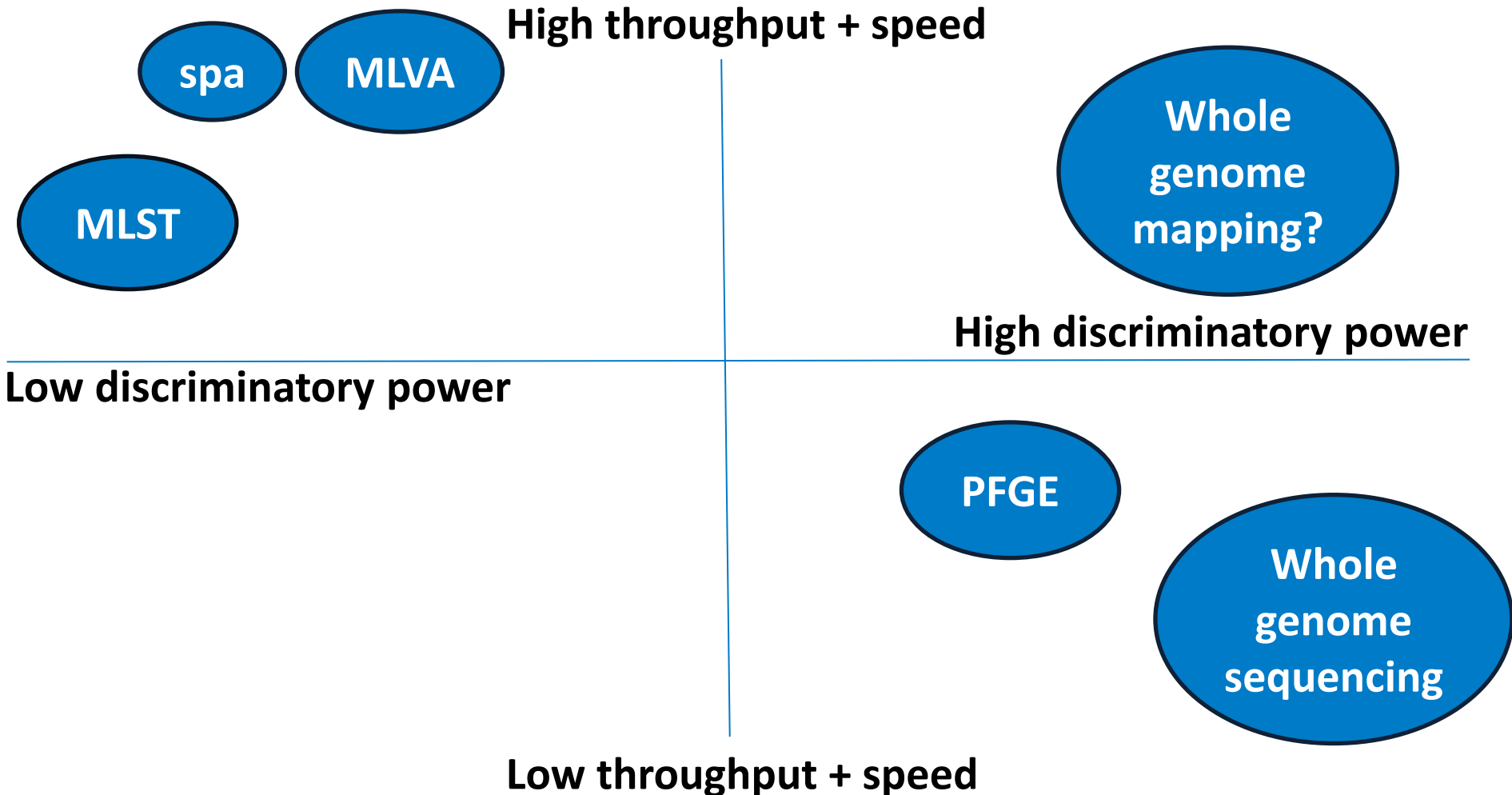
Difficult to interpret

Error prone



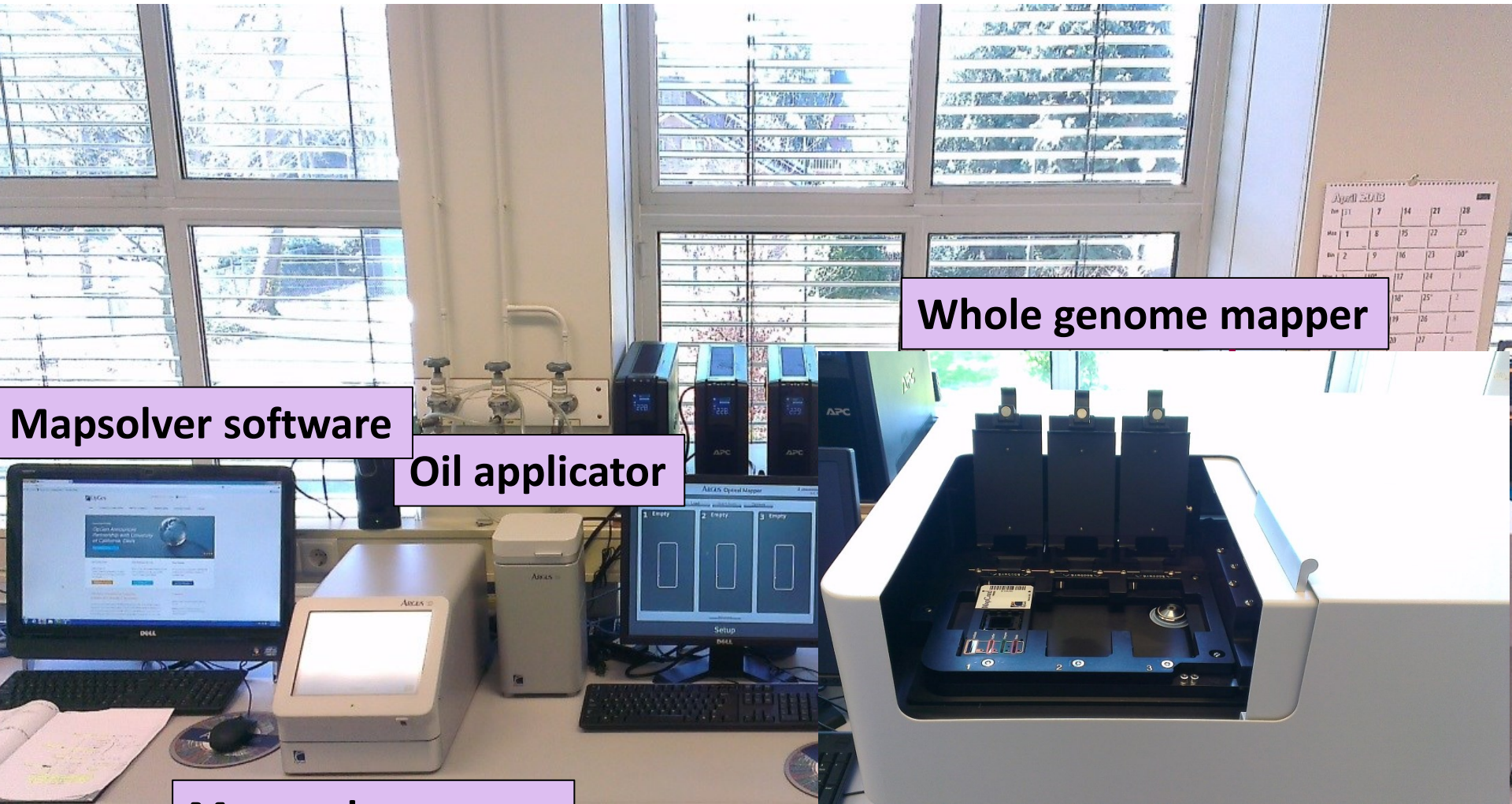


# *WGM, a fast and high resolution typing tool for LA-MRSA*





# Overview whole genome mapping system



Mapsolver software

Oil applicator

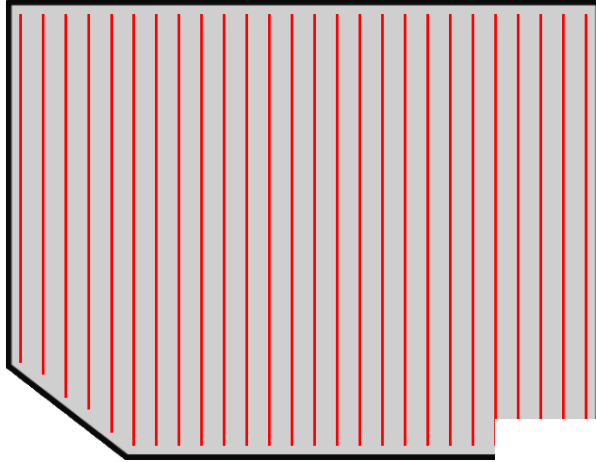
Whole genome mapper

Mapcard processor





# ***Principle whole genome mapping: mapcard assembly***

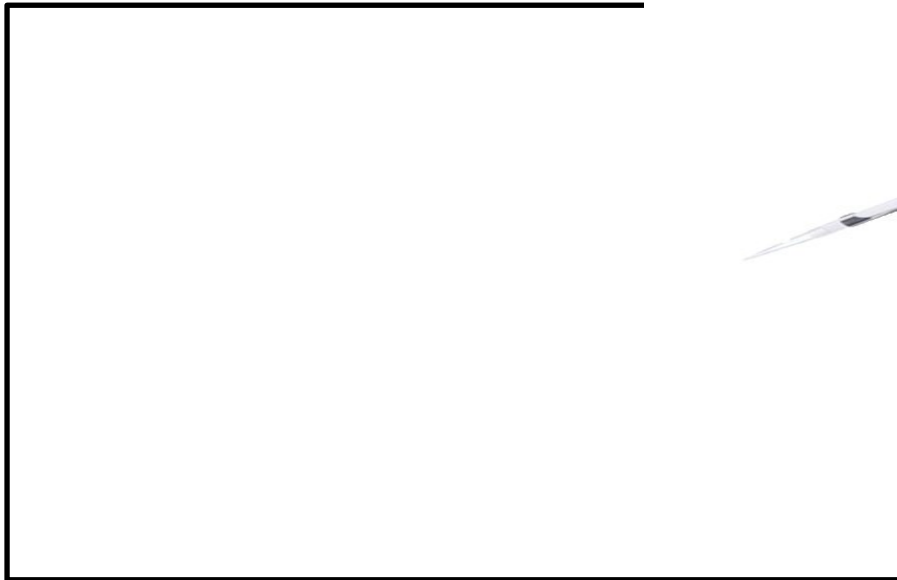


**Channel forming device with microchannels**

**High molecular weight DNA (>250kb)  
is added**



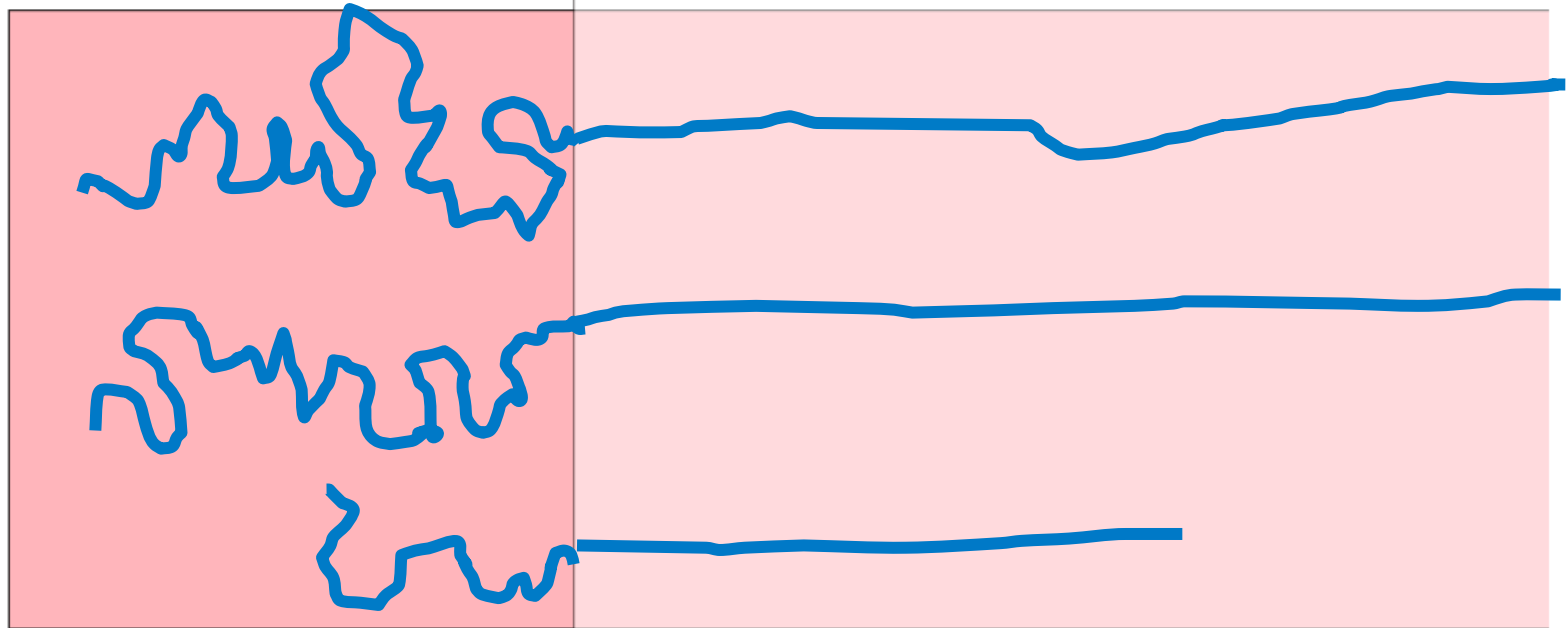
**Glass surface that binds DNA**





## ***Filling of the mapcard in detail***

**Zoomed in on a microchannel**



**Added HMW DNA**

**DNA is pulled in by capillary force and stretched**

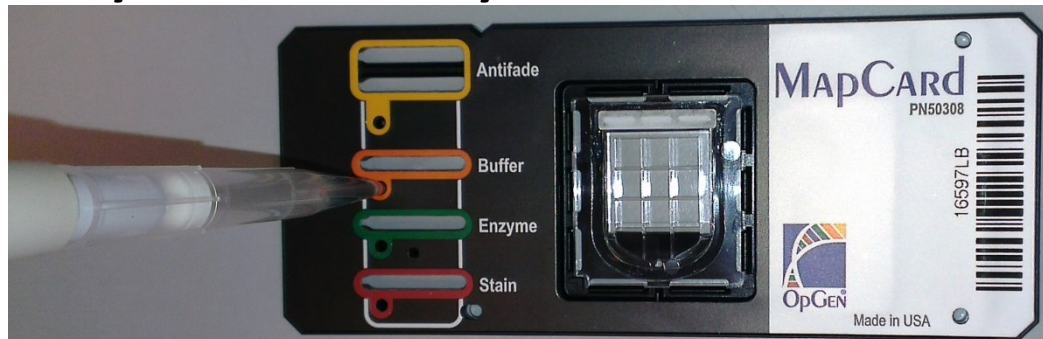


# *Cutting the DNA with a restriction enzyme*

Image of stretched DNA molecules



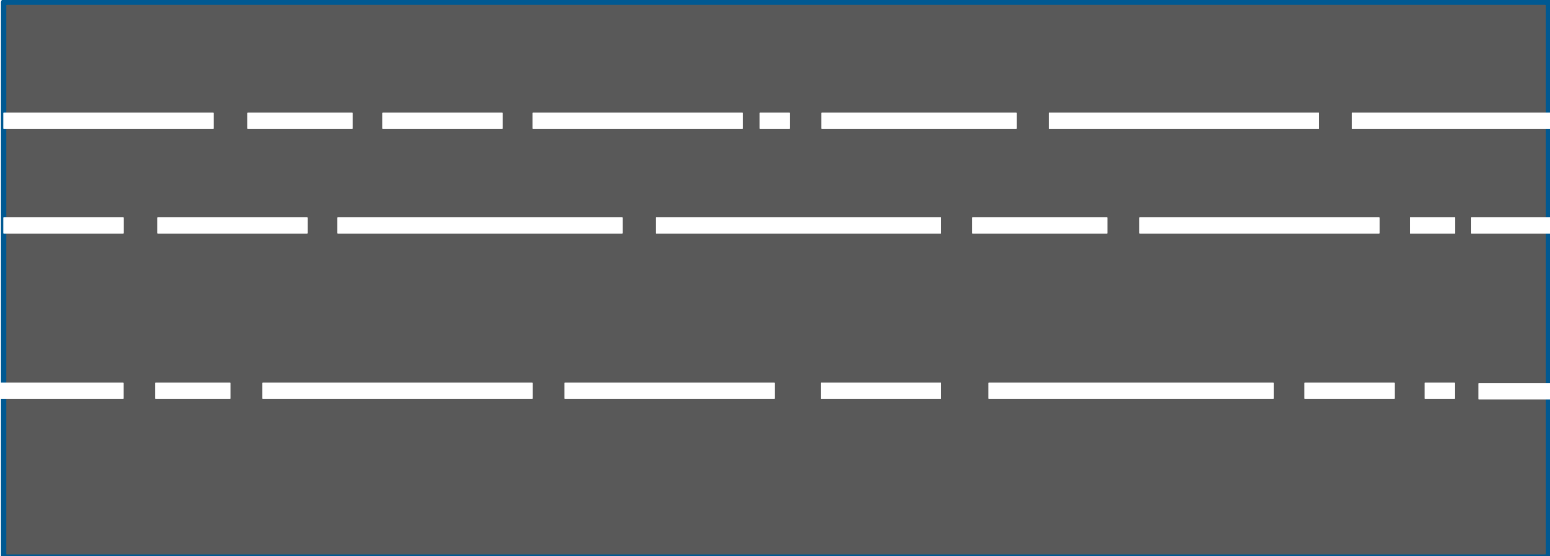
**Restriction enzyme is added by microfluids**

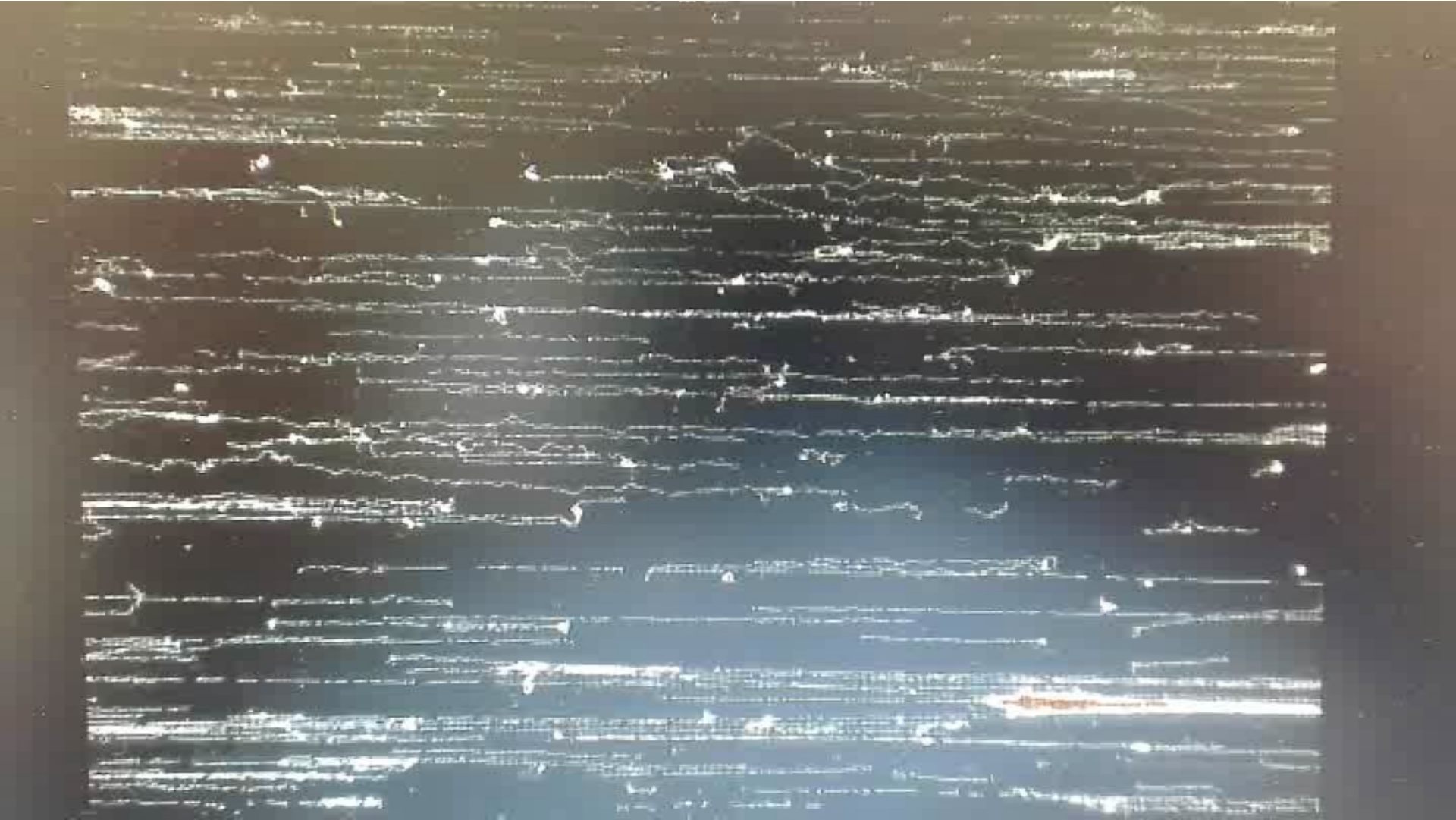




# *Cutting the DNA with a restriction enzyme*

**DNA is cut**







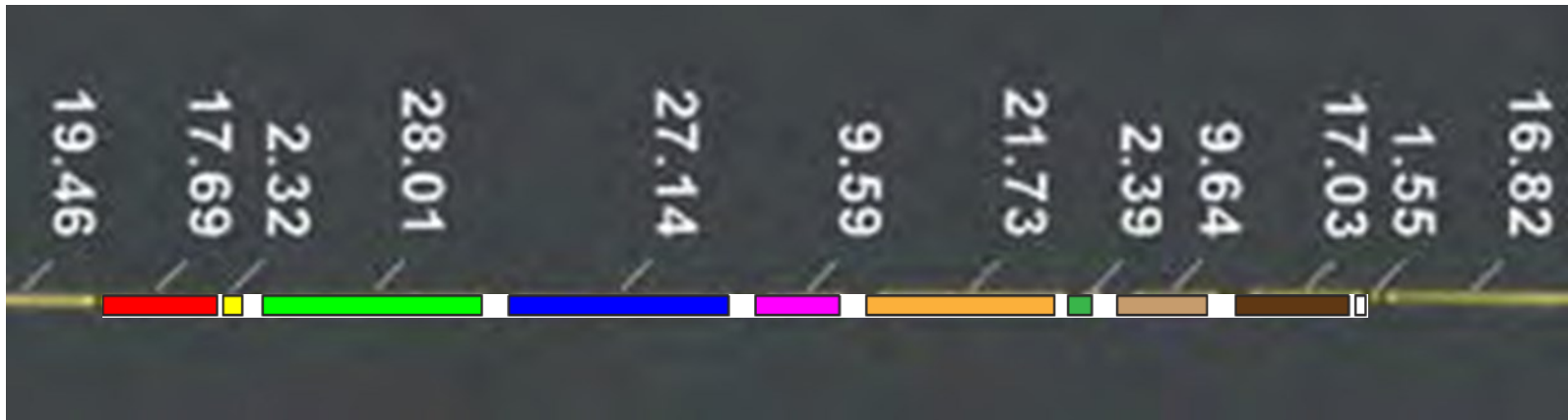
## *Creating maps from images*



**DNA molecules are sized**



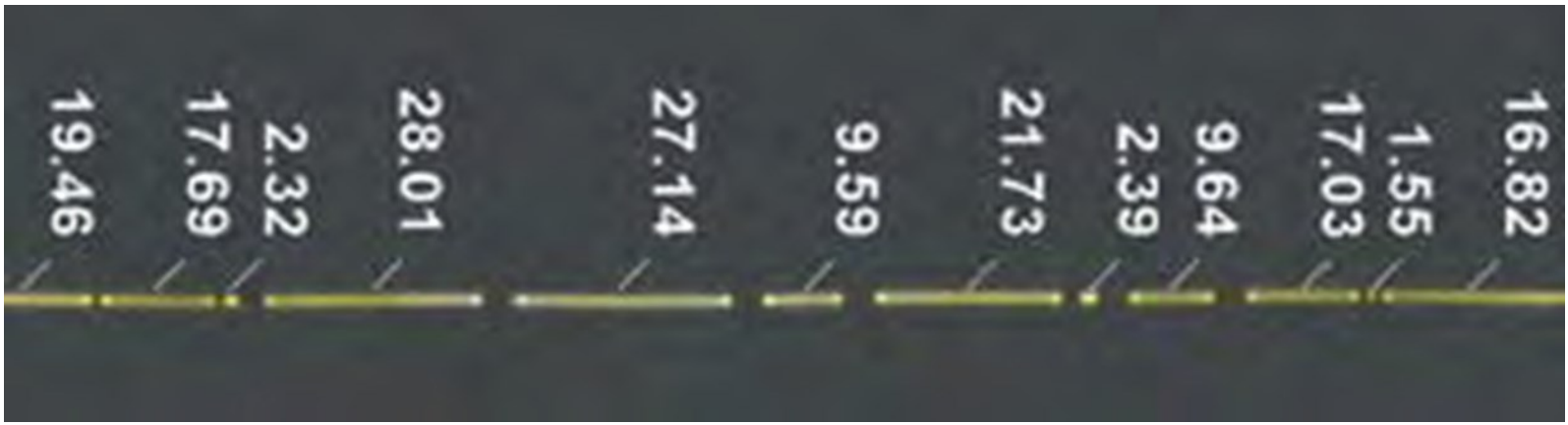
## *Creating maps from images*



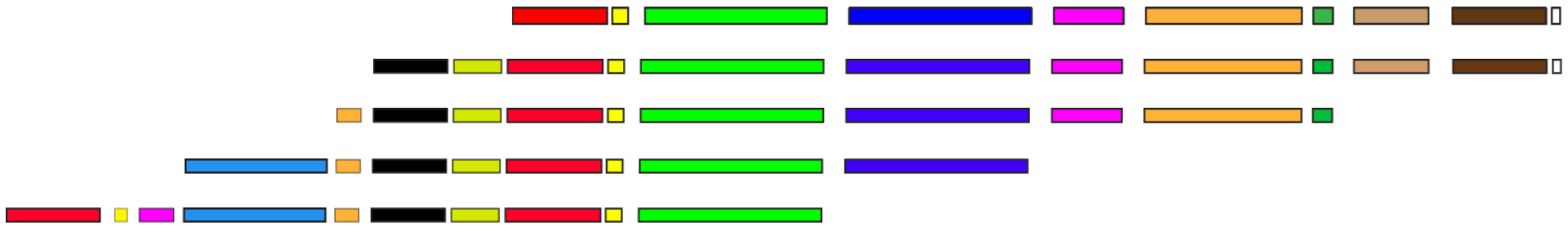
**The order and size of fragments is represented as colored blocks**



# Creating maps from images



## Map assembly from overlapping molecules



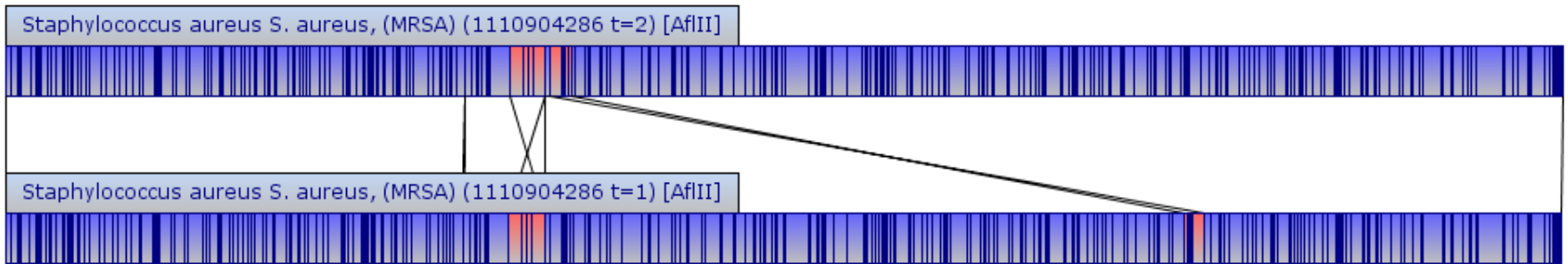
## A compiled whole genome map is created





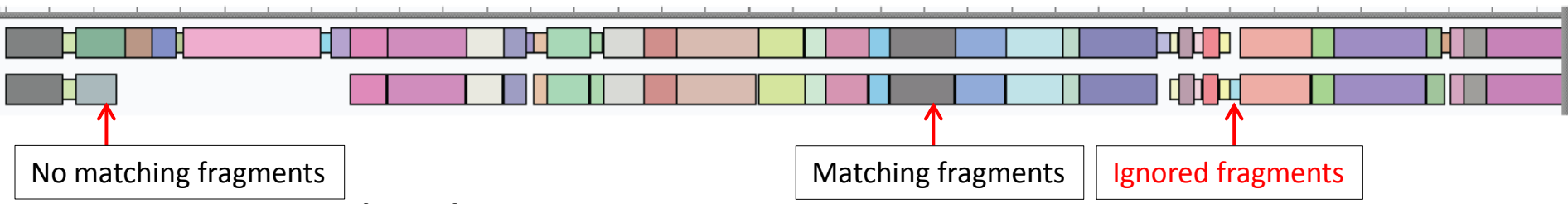


# *Bionumerics software is used for typing analysis*



**Alignment of 2 maps with Mapsolver software**

**Bionumerics software: Fragments represented in random chosen colors, matching fragments have identical colors**



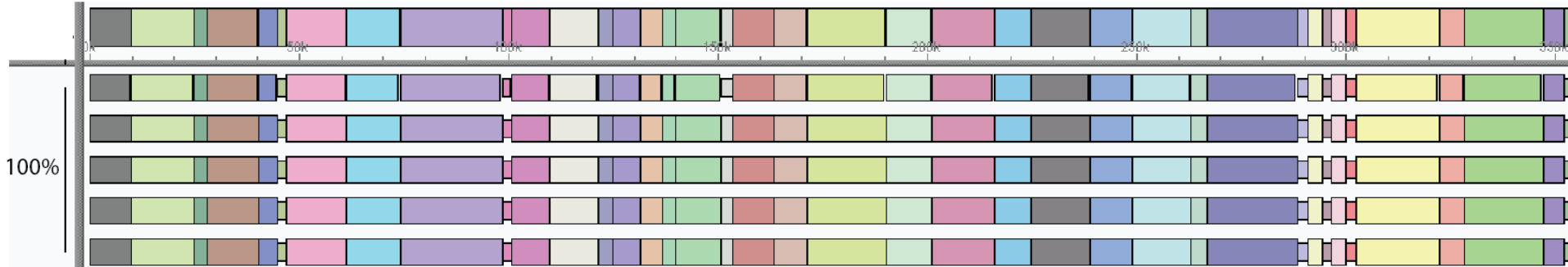
**Small fragments (<3kb) that are ignored by fragment filtering are represented as thinner blocks**

**Tolerance settings are used to correct for sizing errors**

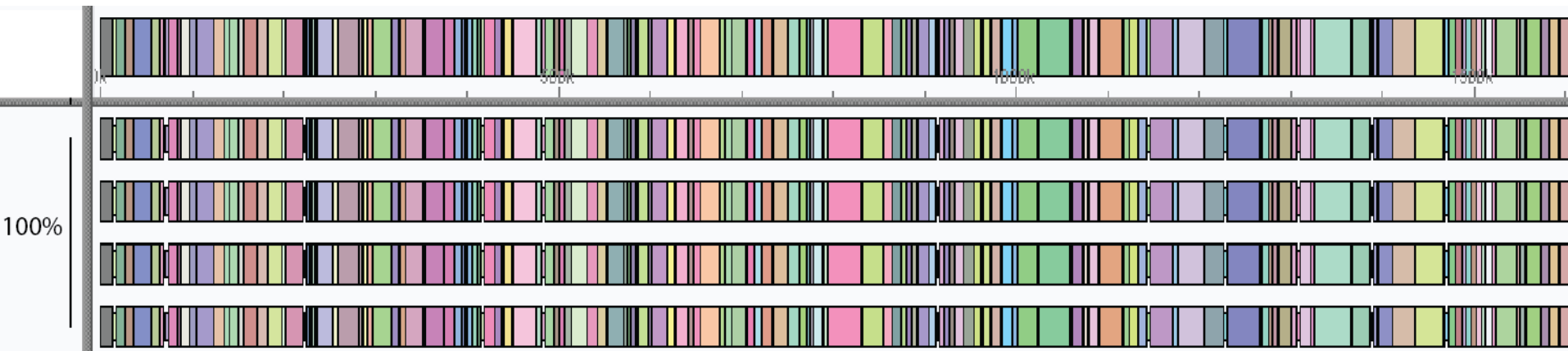


# *Reproducible assembly of whole genome maps*

**LA-MRSA, assembly repeated 5 times**



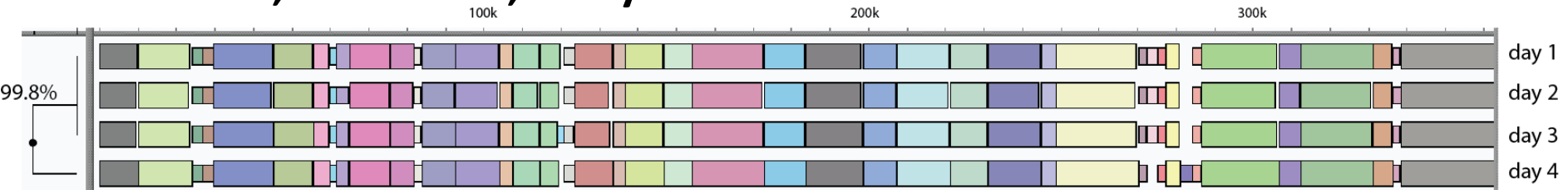
**MRSA, assembly repeated 4 times (zoomed out)**



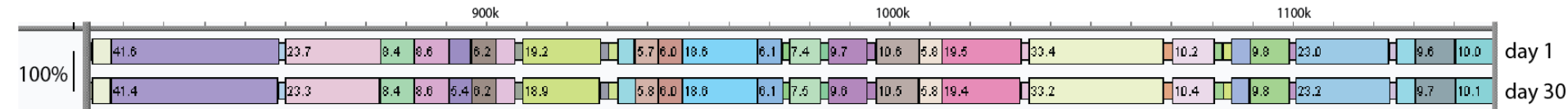


# Replicates yield highly similar WGMs

## LA-MRSA, same DNA, 4 days



## Subculturing MRSA for 30 consecutive days



## Similar results for other strains

Strain	% similarity
MRSA (2)	99.6
LA-MRSA (1)	99.4
LA-MRSA (2)	99.6

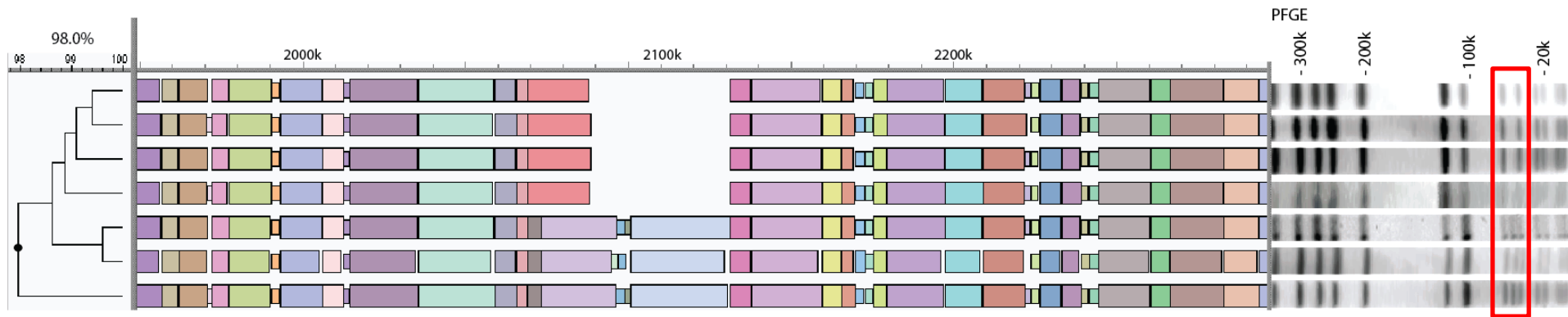


# *WGM confirms a published outbreak of CA-MRSA*

Outbreak in a Dutch beauty salon, highly similar maps

Additional DNA fragment in 3 WGMs

Extra fragment also present in PFGE



**~40kb, probably bacteriophage**



# ***Establishing a cut-off value for WGM of LA-MRSA***

**Replicates of the same DNA yielded >99% similarity**

**Well documented outbreak showing the effect of a mobile element ~98%**

***The cut-off value for indistinguishable profiles was set at  $\geq 98\%$***



# Not all isolates belong to a presumed outbreak of HA-MRSA

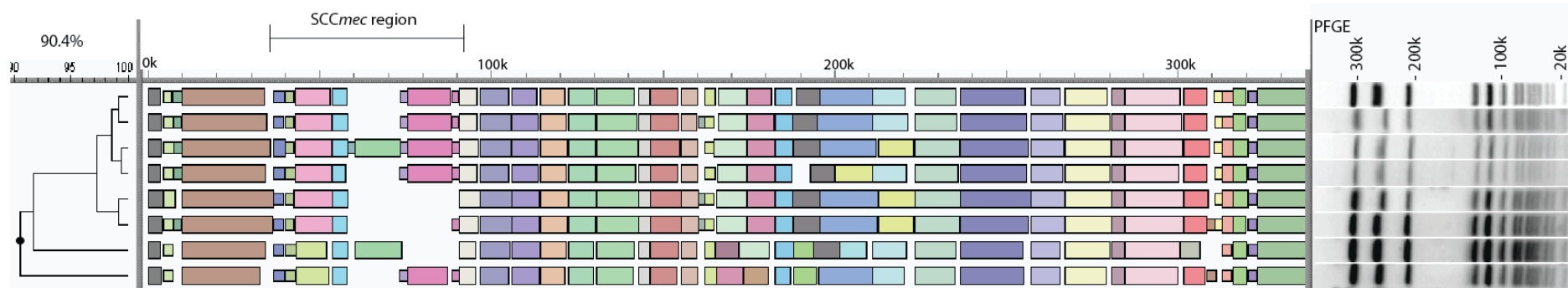
Multicentre outbreak (Mellis et al. 2004, ntvg)

8 isolates originating from one centre, identical *spa*, MT and PFGE

Considerable variation throughout the genome



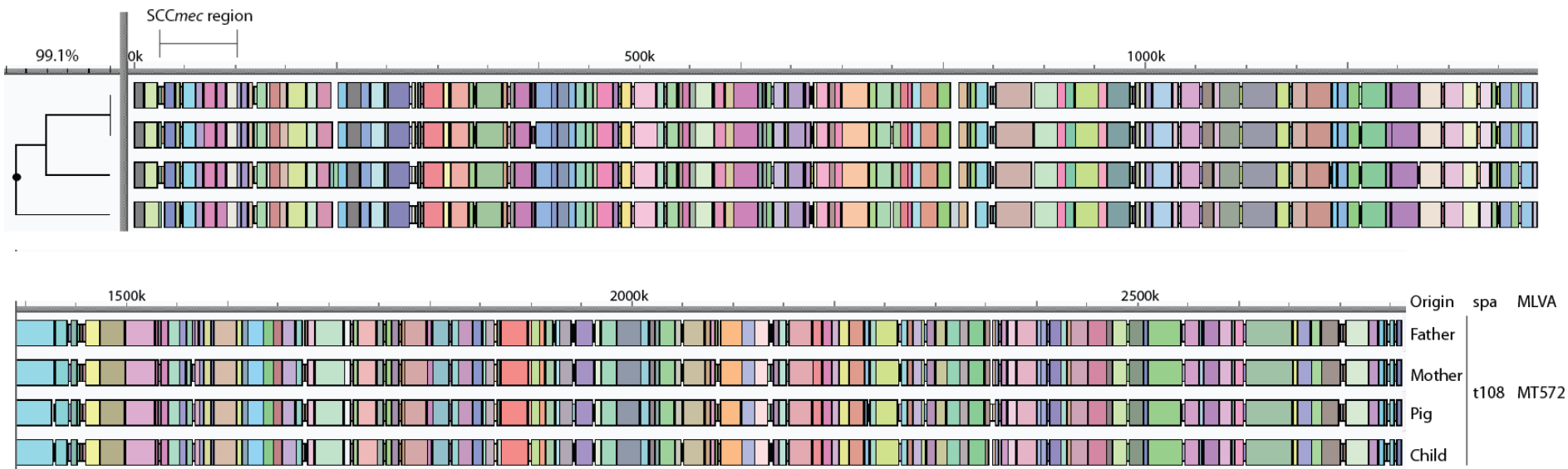
2/8 isolates differed considerably from the other 6 (~90%)





# *WGM confirms published LA-MRSA-transmission*

Isolates from a pig farmer's family, complete whole genome maps

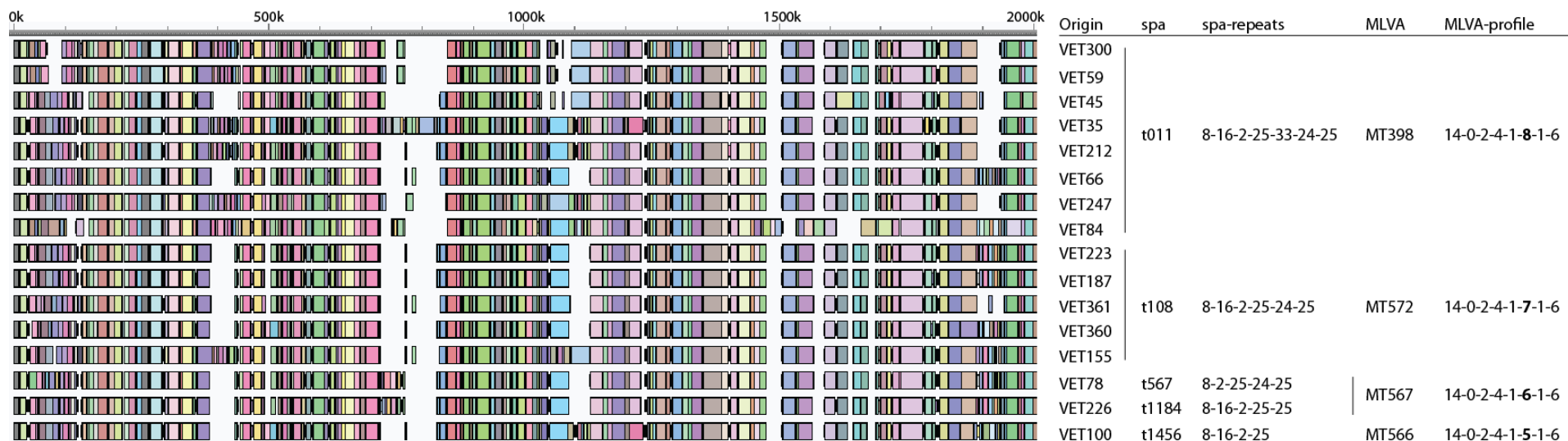


Huijsdens et al. 2006 ACMA



# Discriminatory power of WGM for LA-MRSA

## 16 LA-MRSA from epidemiologically unrelated veterinarians



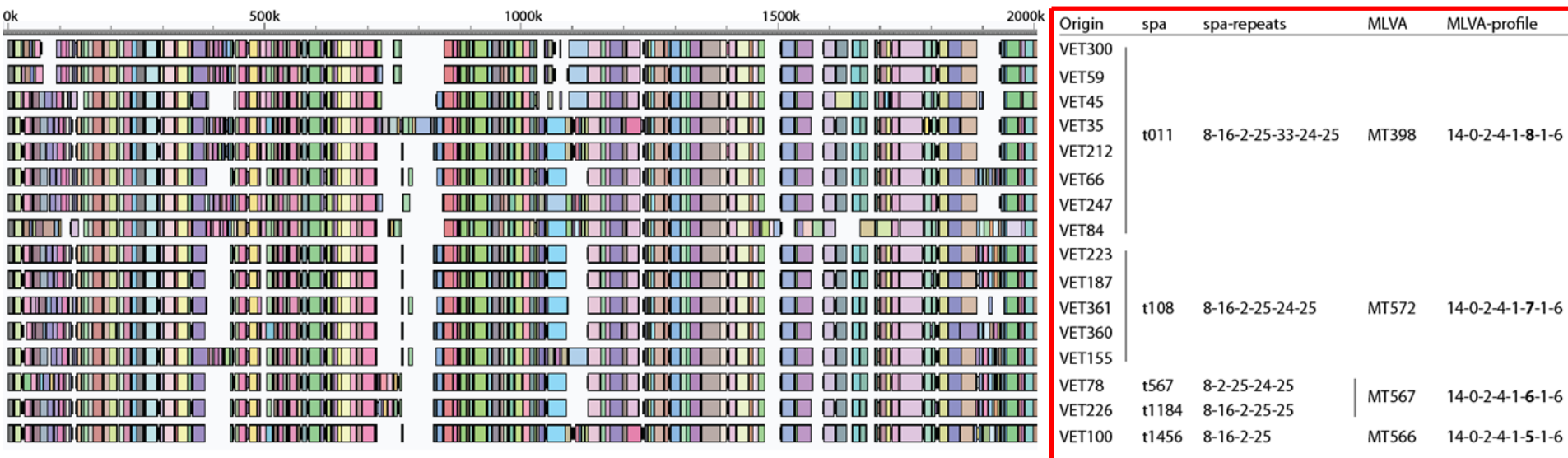




# Discriminatory power of WGM for LA-MRSA

16 LA-MRSA from epidemiologically unrelated veterinarians

Limited variation in MLVA and *spa*-types





# Discriminatory power of WGM for LA-MRSA

16 LA-MRSA from epidemiologically unrelated veterinarians

Considerable variation in WGMs of isolates with identical *spa*-type and MT

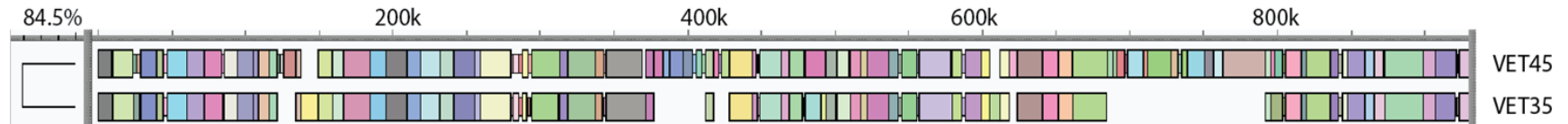


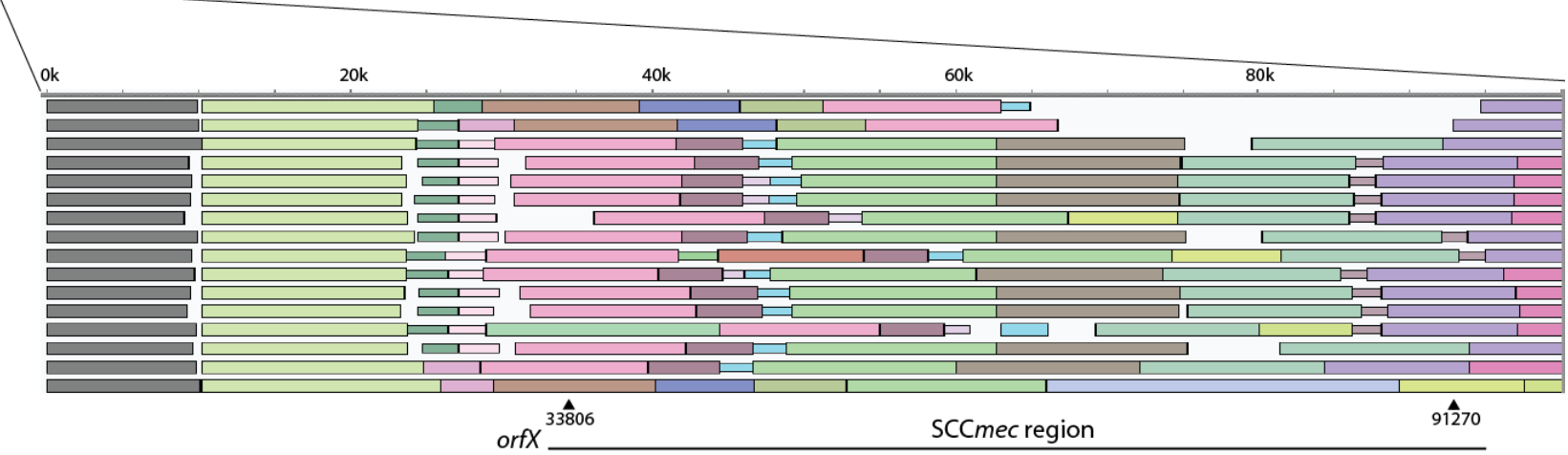
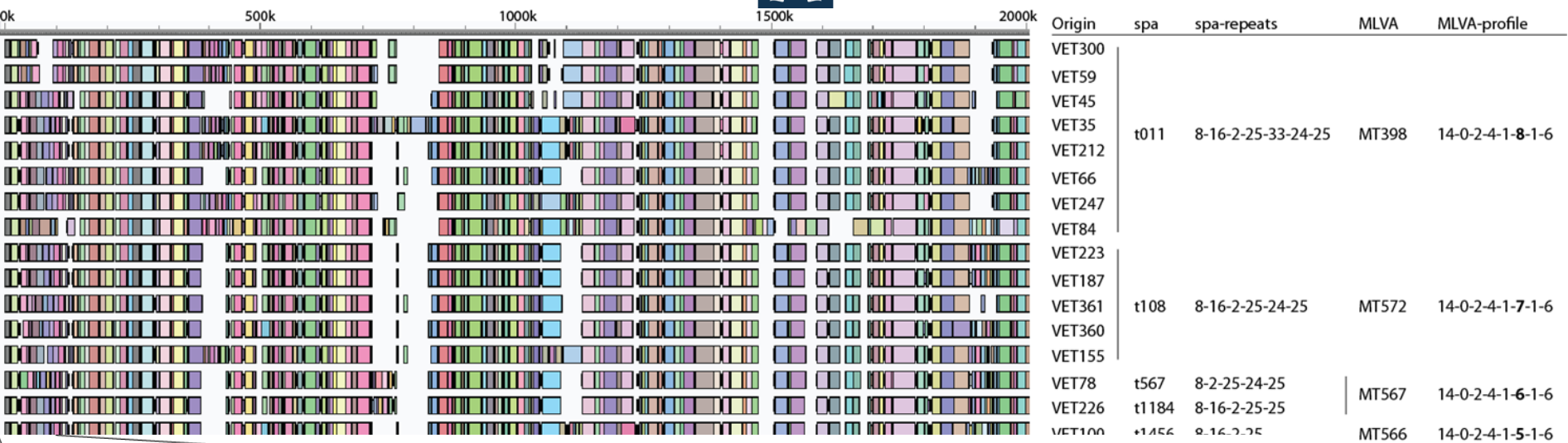


# ***Discriminatory power of WGM for LA-MRSA***

**16 LA-MRSA from epidemiologically unrelated veterinarians**

**Considerable variation in WGMs of isolates with identical *spa*-type and MT**





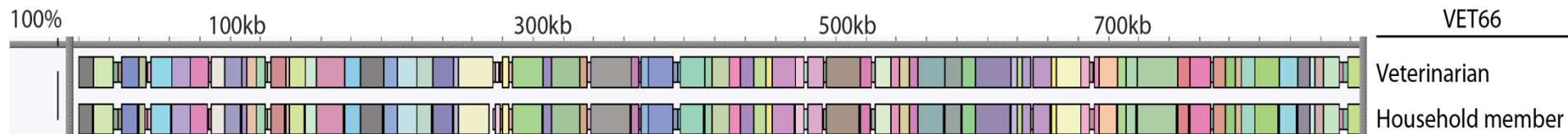
## High degree of variation in SCCmec region corroborated



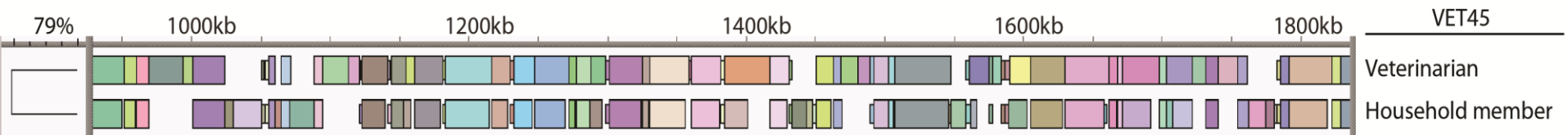
# ***WGM is capable of identifying LA-MRSA transmission***

**Veterinarian and household member same sampling moment**

**MLVA, *spa*, and WGMs indistinguishable -> Likely transmission event**



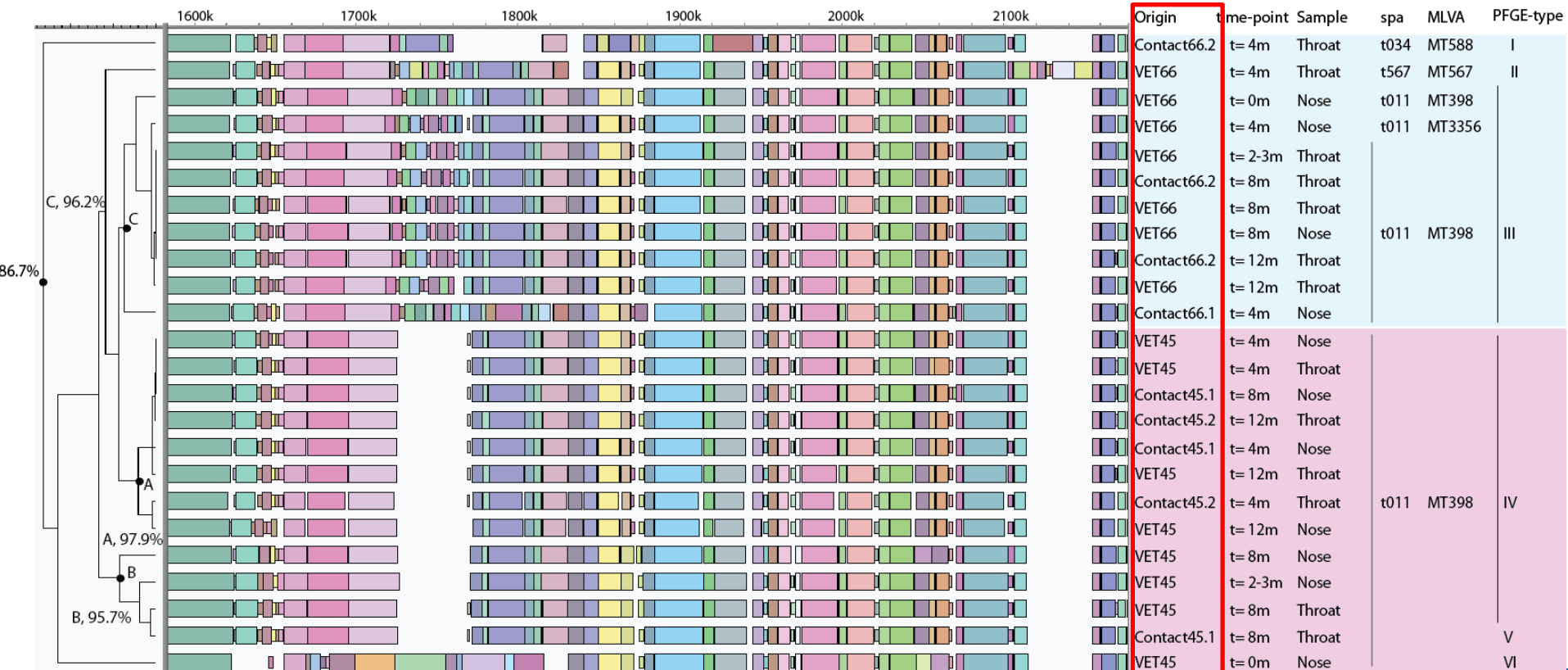
**MLVA, *spa* indistinguishable, clearly different WGMs -> No transmission**





# WGM to study suspected transmission of LA-MRSA

## 2 veterinarians and their family members (VET45, VET66)

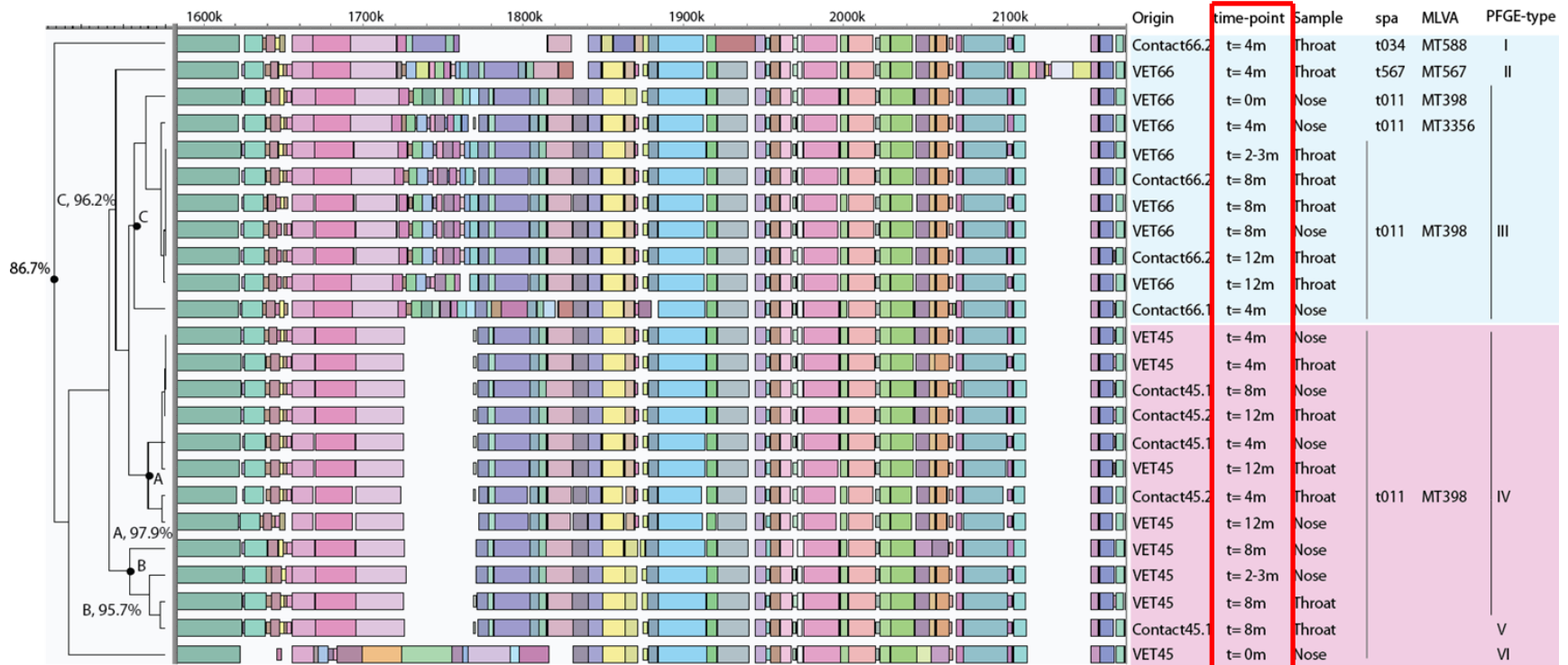




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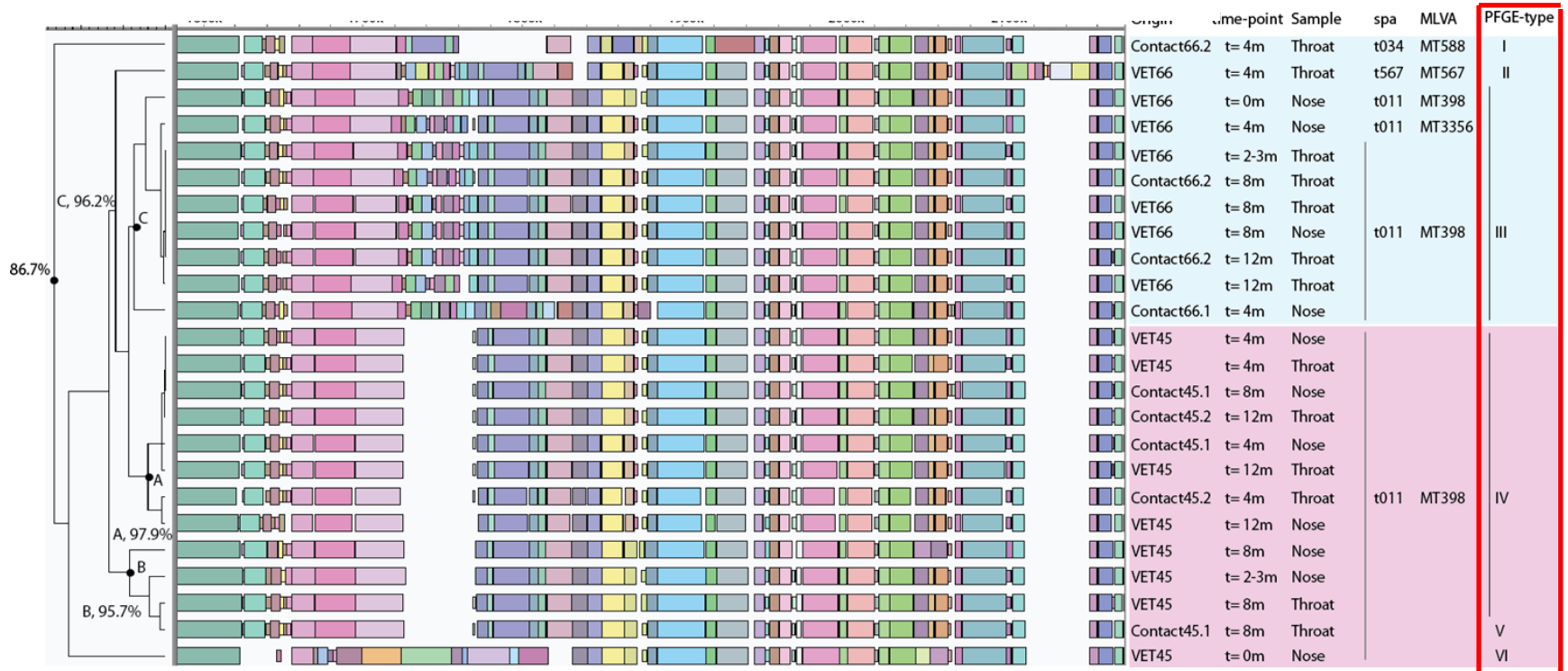
One year follow-up





# WGM to study suspected transmission of LA-MRSA

In contrast to MLVA/spa, PFGE divided both



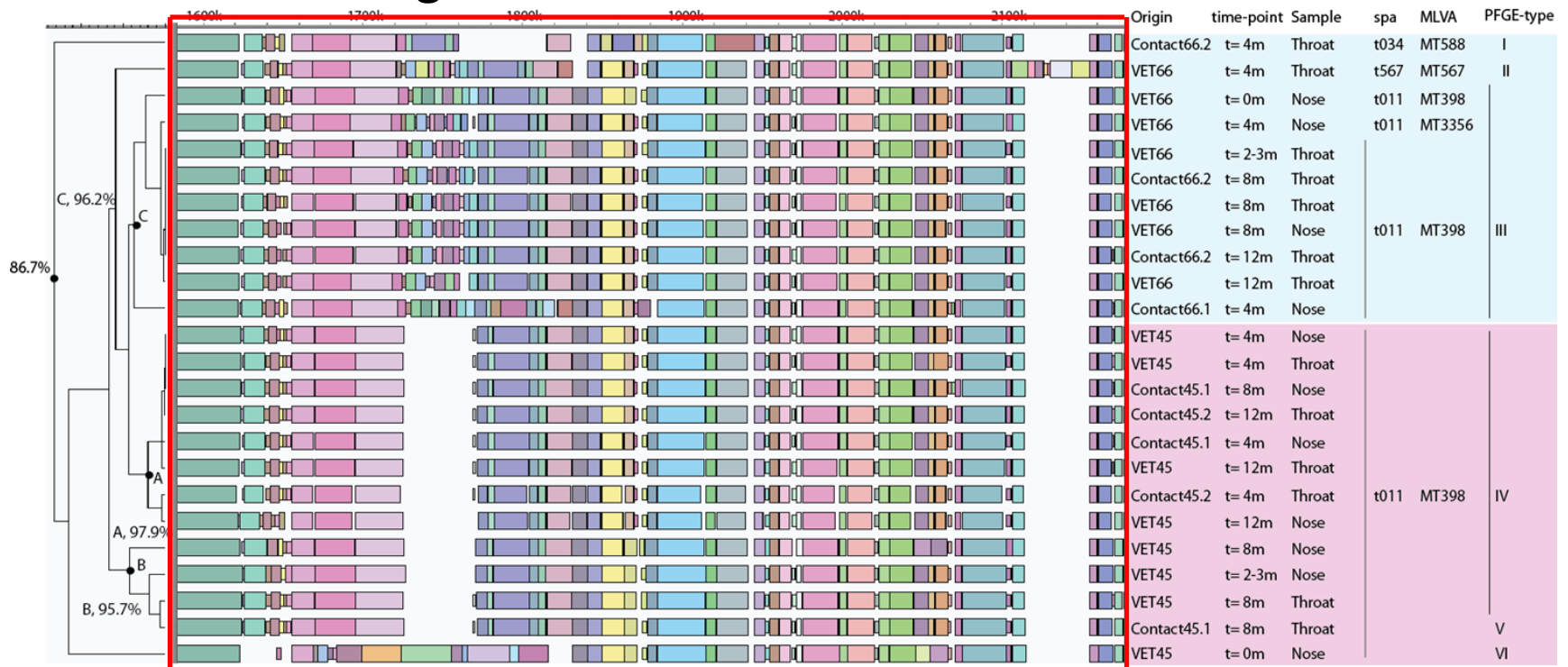




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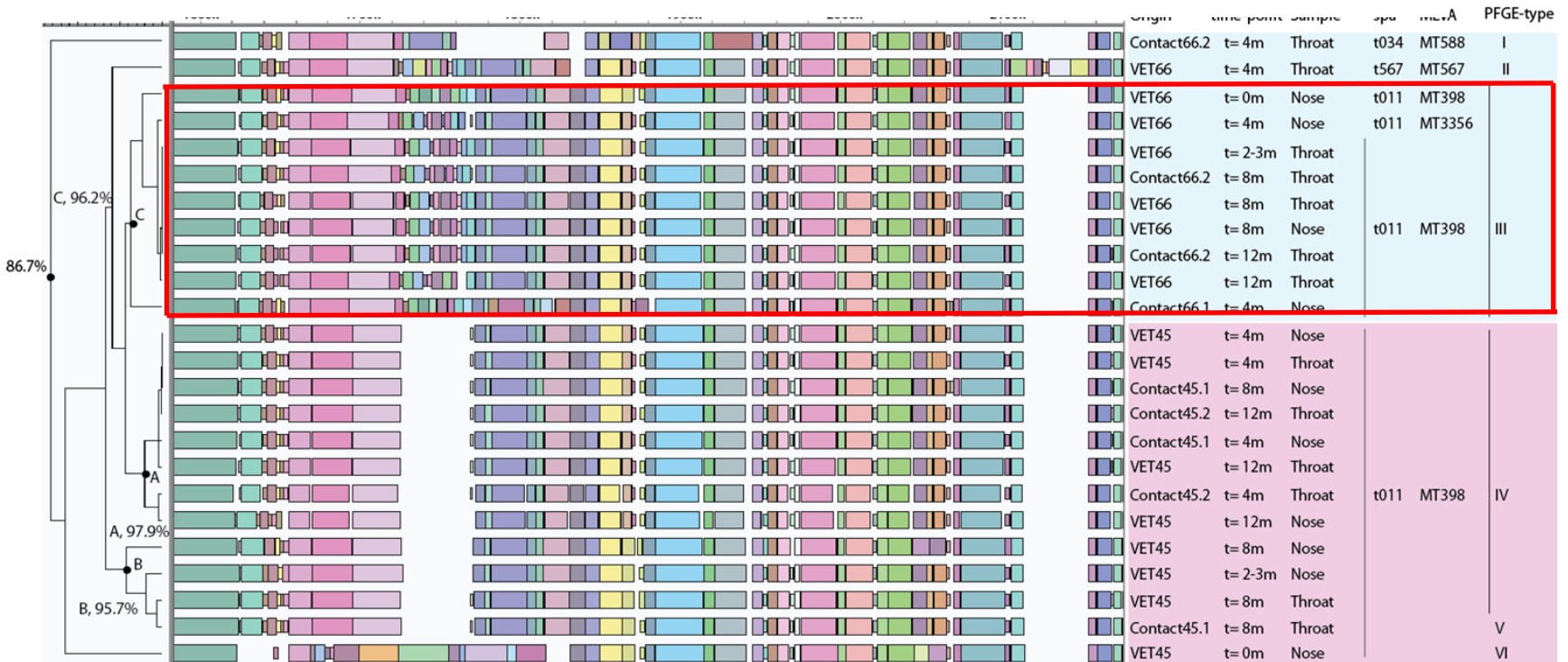
WGM could distinguish the families even further





# WGM to study suspected transmission of LA-MRSA

Most isolates of VET66 belonged to one cluster (C), transmission

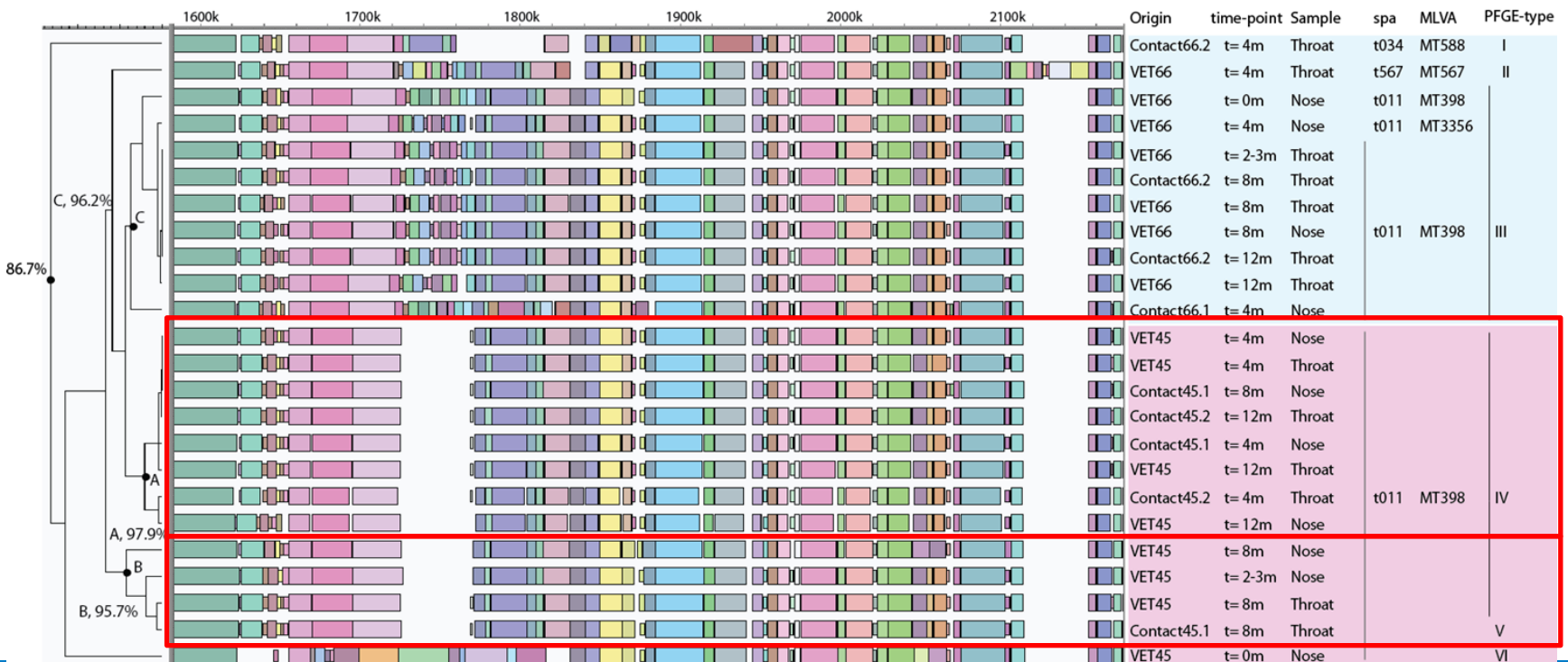




# WGM to study suspected transmission of LA-MRSA

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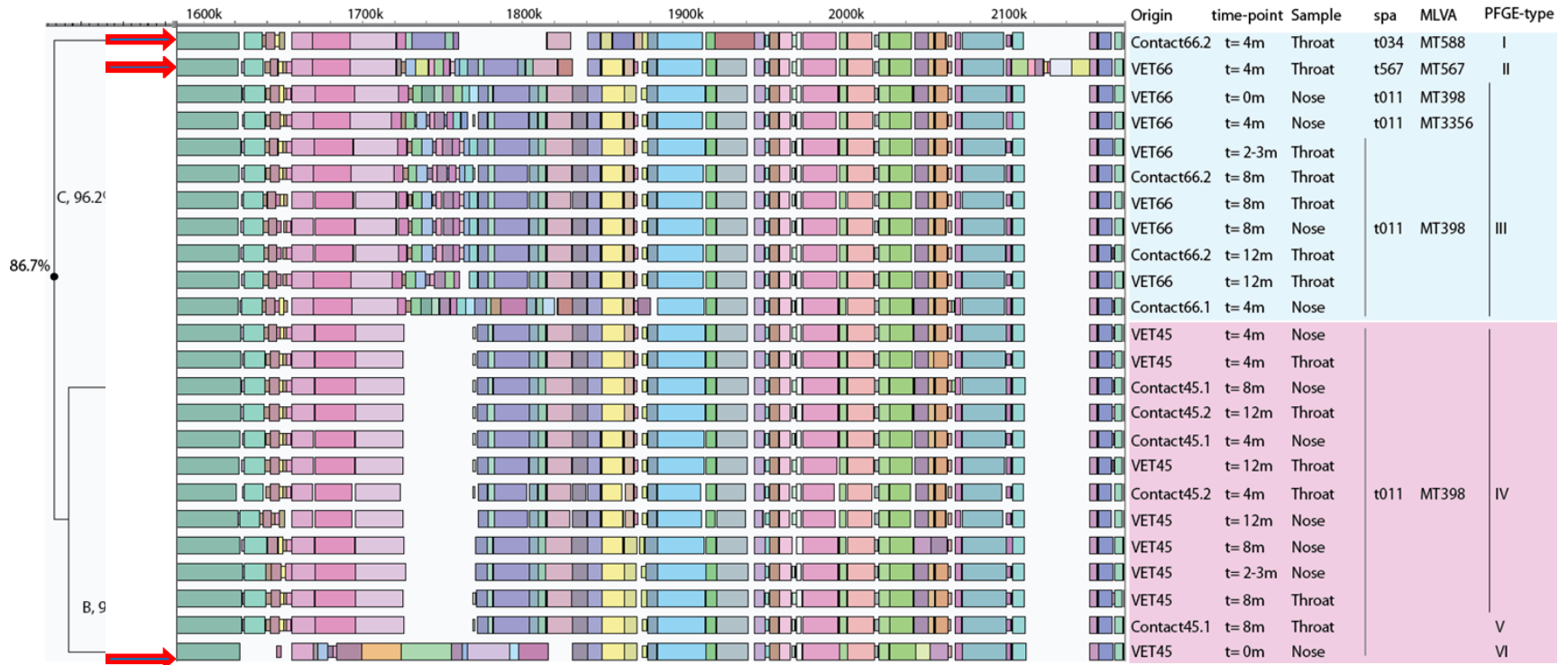
VET45 isolates were divided into 2 clusters (A, B), transmission





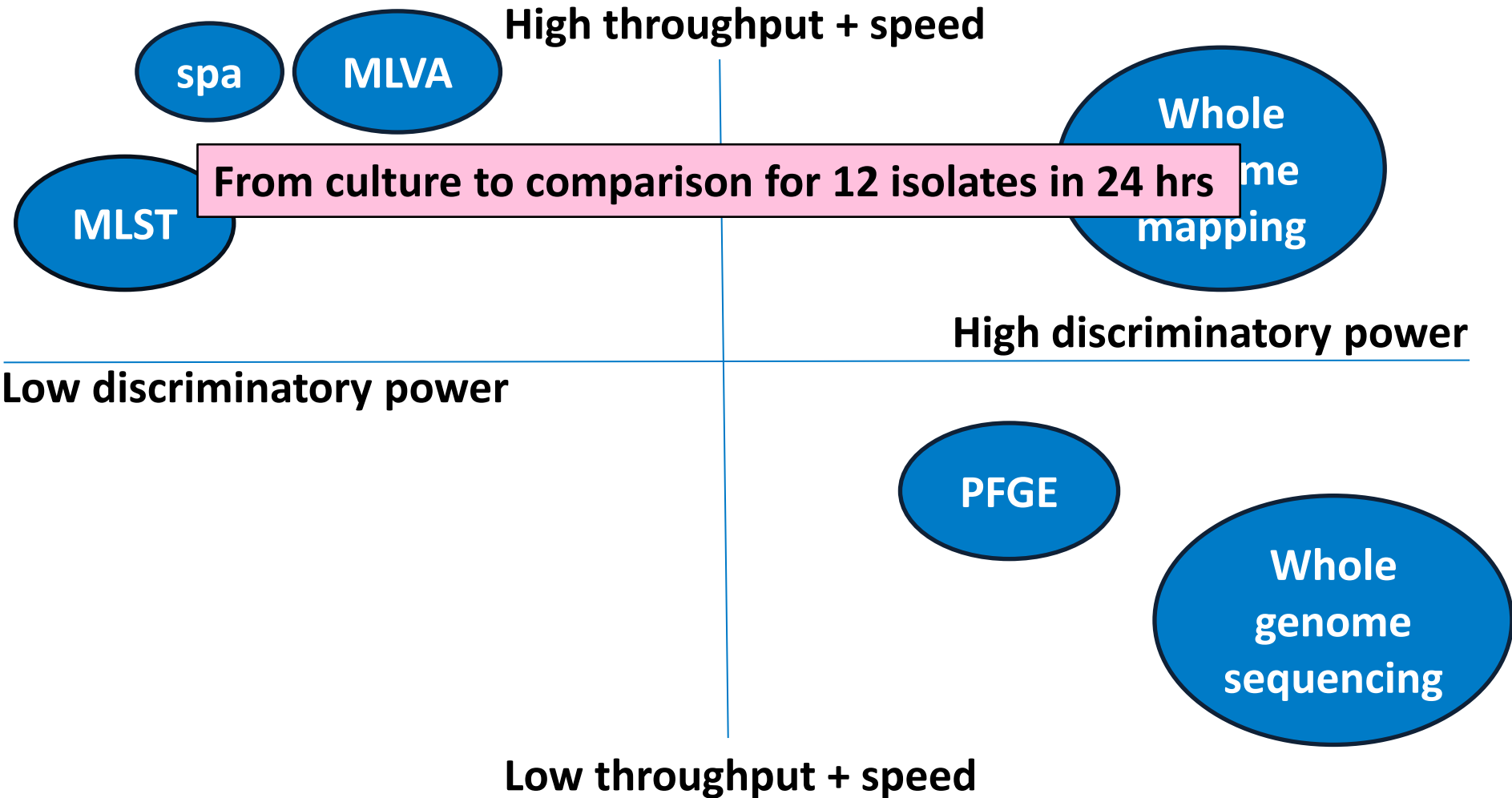
# WGM to study suspected transmission of LA-MRSA

## 3 isolates appeared to be clearly different strains, no transmission





# *WGM, a fast and high resolution typing tool for LA-MRSA*





# ***Comparing WGM with whole genome sequencing***

## Whole genome mapping

+

**Fast, comparison within 24h**

**Simple data analysis**

**Data easily exchanged**

-

**No phylogenetic relationships**

**No population structures**

**No info on gene composition**

## Whole genome sequencing

+

**Phylogenetic relationships**

**Population structures**

**Gene composition**

-

**Slow, only retrospective studies**

**Difficult data analysis**

**Data not easily exchanged**

**Although we believe that WGS is the ultimate typing method, there may be a number of drawbacks for outbreak investigations leaving a niche for methods like WGM!**



## ***Ongoing application of WGM for LA-MRSA***

- **Transmission from veterinarians to household members (180 WGMs done)**
- **LA-MSSA vs LA-MRSA (30 WGMs done)**
- **Association with virulence e.g. PVL, *spa*-type t571 (25 WGMs done)**
- **Persistence of LA-MRSA carriage (70 WGMs done)**
- **Presumed nosocomial LA-MRSA outbreaks (10 of 70 WGMs done)**
  
- **Implementation for other pathogens e.g. *Klebsiella pneumoniae*, *E. coli*, *Salmonella*, VRE, etc...**



# *Acknowledgements*

## RIVM

- Leo Schouls
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- Kenny Knecht

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- Erwin Verkade

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