

Pathogen Genomics

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Wellcome Trust Sanger Institute

May 2012



Hughes Hall



UNIVERSITY OF
CAMBRIDGE



Bacterial Genomics 10 years ago

Nature 1998

article

Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence

S. T. Cole*, R. Brosch*, J. Parkhill, T. Garnier*, C. Churcher, D. Harris, S. V. Gordon*, K. Eiglmeier*, S. Gas*, C. E. Barry III†, F. Tekampas, K. Badcock, D. Basham, D. Brown, T. Chillingworth, R. Connor, R. Davies, K. Devlin, T. Feltwell, S. Gentles, N. Hamlin, S. Holroyd, T. Hornsby, K. Jagels, A. Kroghs, J. McLean, S. Moule, L. Murphy, K. Oliver, J. Osborne, M. A. Quail, M.-A. Rajandream, J. Rogers, S. Rutter, K. Seeger, J. Skelton, R. Squares, S. Squares, J. E. Sulston, K. Taylor, S. Whitehead & B. G. Barrell

Sanger Centre, Wellcome Trust Genome Campus, Hinxton CB10 1SA, UK

letters to nature 2001

Complete genome sequence of a multiple drug resistant *Salmonella enterica* serovar Typhi CT18

J. Parkhill*, G. Dougan†, K. D. James*, N. R. Thomson*, D. Pickard†, J. Wain†, C. Churcher*, K. L. Mungall*, S. D. Bentley*, M. T. G. Holden*, M. Sebahia*, S. Baker*, D. Basham*, K. Brooks*, T. Chillingworth*, P. Connor†, A. Cronin*, P. Davis*, R. M. Davies*, L. Dowd*, N. White‡, J. Farrar‡, T. Feltwell*, N. Hamlin*, A. Haque†, T. T. Hien§, S. Holroyd*, K. Jagels*, A. Krogh†, T. S. Larsen||, S. Leather*, S. Moule*, P. Ó'Gaora†, C. Parry§, M. Quail*, K. Rutherford*, M. Simmonds*, J. Skelton*, K. Stevens*, S. Whitehead* & B. G. Barrell*

* The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, UK

letters to nature 2000

The genome sequence of the food-borne pathogen *Campylobacter jejuni* reveals hypervariable sequences

J. Parkhill*, B. W. Wren†, K. Mungall*, J. M. Kettle‡, C. Churcher*, D. Basham*, T. Chillingworth*, R. M. Davies*, T. Feltwell*, S. Holroyd†, K. Jagels*, A. V. Kartyshev†, S. Moule*, M. J. Pallen§, C. W. Penn||, M. A. Quail*, M.-A. Rajandream*, K. M. Rutherford*, A. H. M. van Vliet¶, S. Whitehead* & B. G. Barrell*

* The Sanger Centre, The Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK

letters to nature 2001

Genome sequence of *Yersinia pestis*, the causative agent of plague

J. Parkhill*, B. W. Wren†, N. R. Thomson*, R. W. Titball‡, M. T. G. Holden*, M. B. Prentice§, M. Sebahia*, K. D. James*, C. Churcher*, K. L. Mungall*, S. Baker*, D. Basham*, S. D. Bentley*, K. Brooks*, A. M. Cerdeño-Tarraga*, T. Chillingworth*, A. Cronin*, R. M. Davies*, P. Davis*, G. Dougan||, T. Feltwell*, N. Hamlin*, S. Holroyd*, K. Jagels*, A. V. Kartyshev†, S. Leather*, S. Moule*, P. C. F. Oyston‡, M. Quail*, K. Rutherford*, M. Simmonds*, J. Skelton*, K. Stevens*, S. Whitehead* & B. G. Barrell*

* The Sanger Centre, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, UK

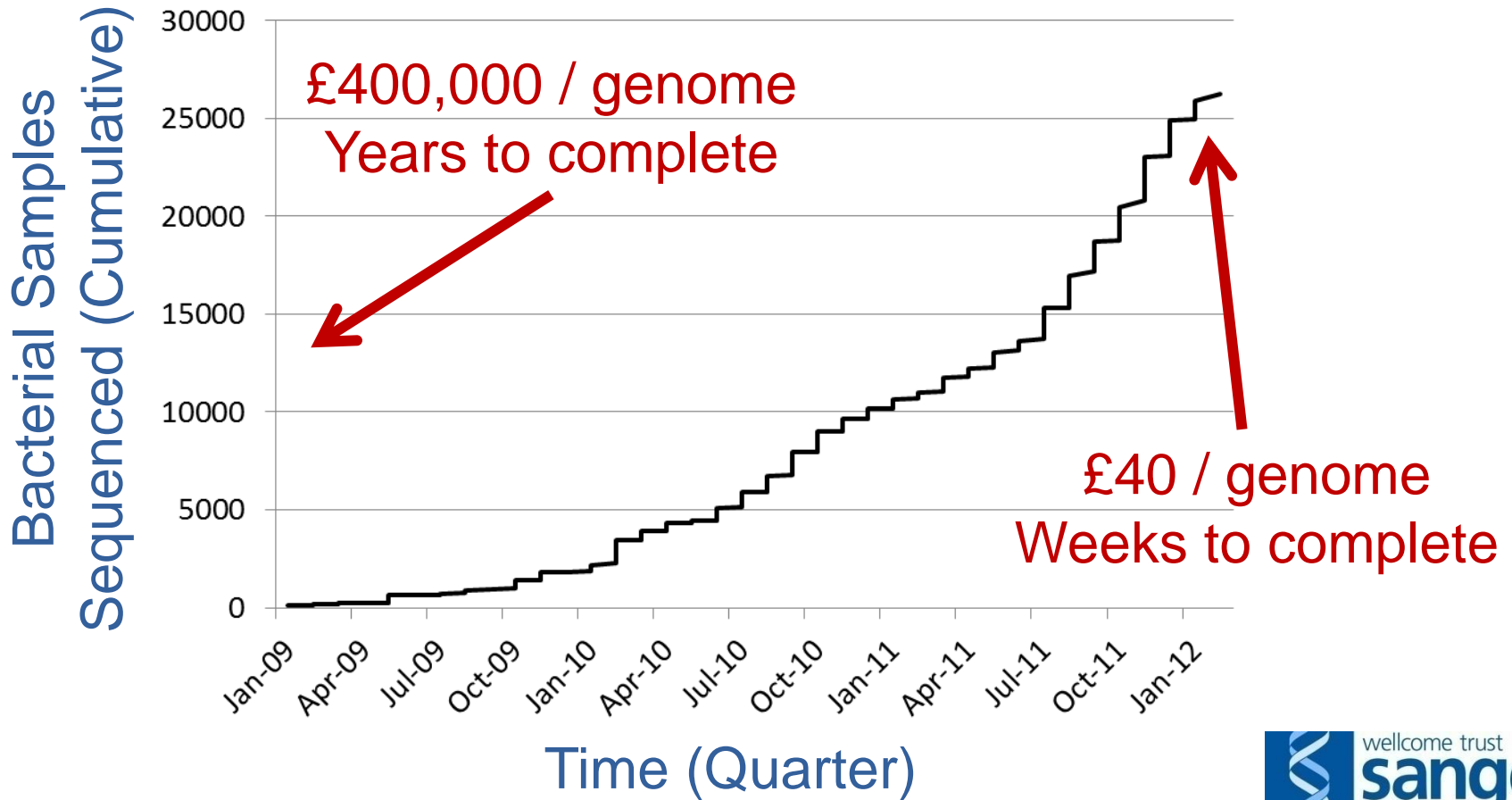
PNAS 2004

Complete genomes of two clinical *Staphylococcus aureus* strains: Evidence for the rapid evolution of virulence and drug resistance

Matthew T. G. Holden*, Edward J. Feil†, Jodi A. Lindsay‡, Sha'oun J. Peacock§, Nicholas P. J. Day¶, Mark C. Enright*, Tim J. Foster†, Catrin E. Moore§, Laurence Hurst†, Rebecca Atkin*, Andrew Barron*, Nathalie Bason*, Stephen D. Bentley*, Carol Chillingworth*, Tracey Chillingworth*, Carol Churcher*, Louise Clark*, Craig Corton*, Ann Cronin*, Jon Doggett*, Linda Dowd*, Theresa Feltwell*, Zahra Hance*, Barbara Harris*, Heidi Hauser*, Simon Holroyd*, Kay Jagels*, Keith D. James*, Nicola Lennard*, Alexandra Line*, Rebecca Mayes*, Sharon Moule*, Karen Mungall*, Douglas Ormond*, Michael A. Quail*, Ester Rabinovitsch*, Kim Rutherford*, Mandy Sanders*, Sarah Sharp*, Mark Simmonds*, Kim Stevens*, Sally Whitehead*, Bart G. Barrell*, Brian G. Spratt**, and Julian Parkhill**††

*The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge CB10 1SA, United Kingdom; †Department of Biology and

Impact of Next Generation Sequencing at Sanger



'Next Generation' Bacterial Genomics

Evolution of MRSA During 2010; Hospital Transmission and Intercontinental Spread

Simon R. Harris,^{1*} Edward J. Feil,^{2*} Matthew T. G. Holden,¹ Michael A. Quail,¹ Emma K. Nickerson,^{3,4} Narisara Chantratita,³ Susana Gardete,^{5,6} Ana Tavares,⁵ Nick Day,^{3,7} Jodi A. Lindsay,⁸ Jonathan D. Edgeworth,^{9,10} Hermínia de Lencastre,^{5,6} Julian Parkhill,¹ Sharon J. Peacock,^{3,4} Stephen D. Bentley^{1†}

SCIENCE VOL 327 22 JANUARY 2010

Rapid Pneumococcal Evolution in Response to Clinical Interventions

Nicholas J. Croucher,¹ Simon R. Harris,¹ Christophe Fraser,² Michael A. Quail,¹ John Burton,¹ Mark van der Linden,³ Lesley McGee,⁴ Anne von Gottberg,⁵ Jae Hoon Song,⁶ Kwan Soo Ko,⁷ Bruno Pichon,⁸ Stephen Baker,⁹ Christopher M. Parry,⁹ Lotte M. Lambertsen,¹⁰ Dea Shahinas,¹¹ Dylan R. Pillai,¹¹ Timothy J. Mitchell,¹² Gordon Dougan,¹ Alexander Tomasz,¹³ Keith P. Klugman,^{4,5,14} Julian Parkhill,¹ William P. Hanage,^{2,15} Stephen D. Bentley^{1*}

28 JANUARY 2011 VOL 331 SCIENCE 2011; 240 strains

Evolutionary dynamics of *Clostridium difficile* over short and long time scales

Miao He^a, Mohammed Sebahia^{a,1}, Trevor D. Lawley^a, Richard A. Stabler^a, Lisa F. Dawson^b, Melissa J. Martin^b, Kathryn E. Holt^{a,2}, Helena M.B. Seth-Smith^a, Michael A. Quail^a, Richard Rance^a, Karen Brooks^a, Carol Churcher^a, David Harris^a, Stephen D. Bentley^a, Christine Burrows^a, Louise Clark^a, Craig Corton^a, Vicky Murray^a, Graham Rose^a, Scott Thurston^a, Andries van Tonder^a, Danielle Walker^a, Brendan W. Wren^a, Gordon Dougan^a, and Julian Parkhill^{a,3}

^aWellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire CB10 1SA, United Kingdom; and ^bDepartment of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom

PNAS

ARTICLES

nature genetics 2012; 52 strains

Whole-genome analysis of diverse *Chlamydia trachomatis* strains identifies phylogenetic relationships masked by current clinical typing

Simon R Harris¹, Ian N Clarke², Helena M B Seth-Smith¹, Anthony W Solomon³, Lesley T Cutcliffe², Peter Marsh⁴, Rachel J Skilton², Martin J Holland³, David Mabey³, Rosanna W Peeling³, David A Lewis^{3,5,6}, Brian G Spratt⁷, Magnus Unemo⁸, Kenneth Persson⁹, Carina Bjartling¹⁰, Robert Brunham¹¹, Henry J C de Vries¹²⁻¹⁴, Servaas A Morre^{15,16}, Arjen Speksnijder¹⁷, Cécile M Bébear^{18,19}, Maité Clerc^{18,19}, Bertille de Barbeyrac^{18,19}, Julian Parkhill¹ & Nicholas R Thomson¹

Dense sampling of bacterial populations

- Population structure
- Response to selective pressures

Staphylococcus aureus

- Widespread Gram positive bacterium
- Associated with natural carriage as well as wide range of diseases
 - Minor wound infections
 - Food poisoning
 - Toxic shock syndrome
 - Endocarditis
 - Haemolytic pneumonia
- Methicillin-resistant *S. aureus* (MRSA)
 - First described in 1960s

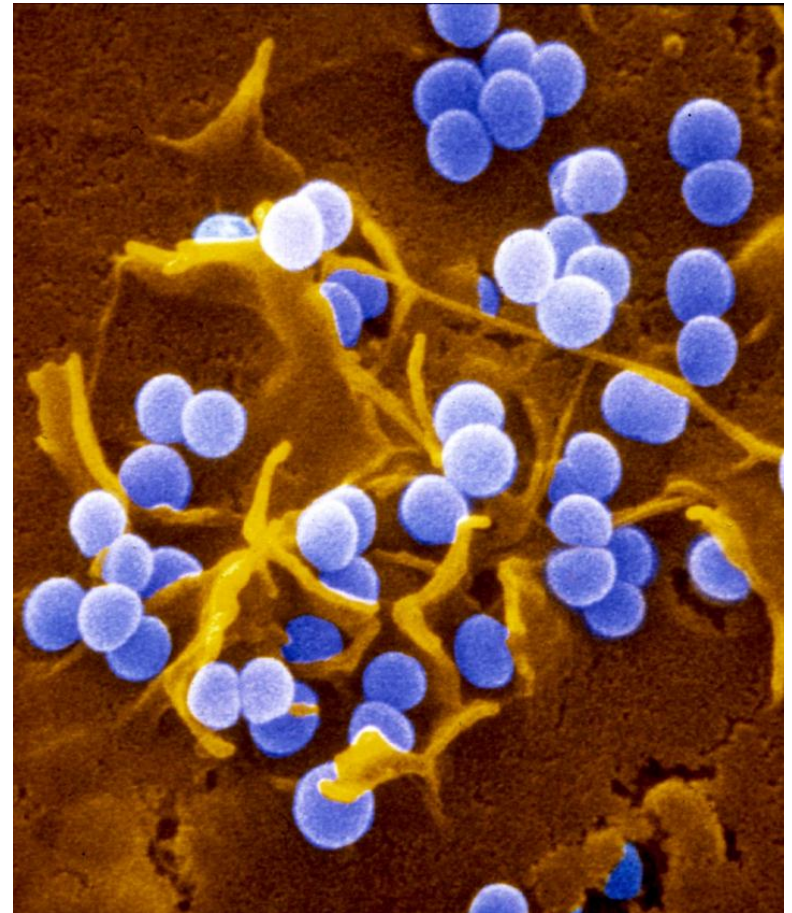
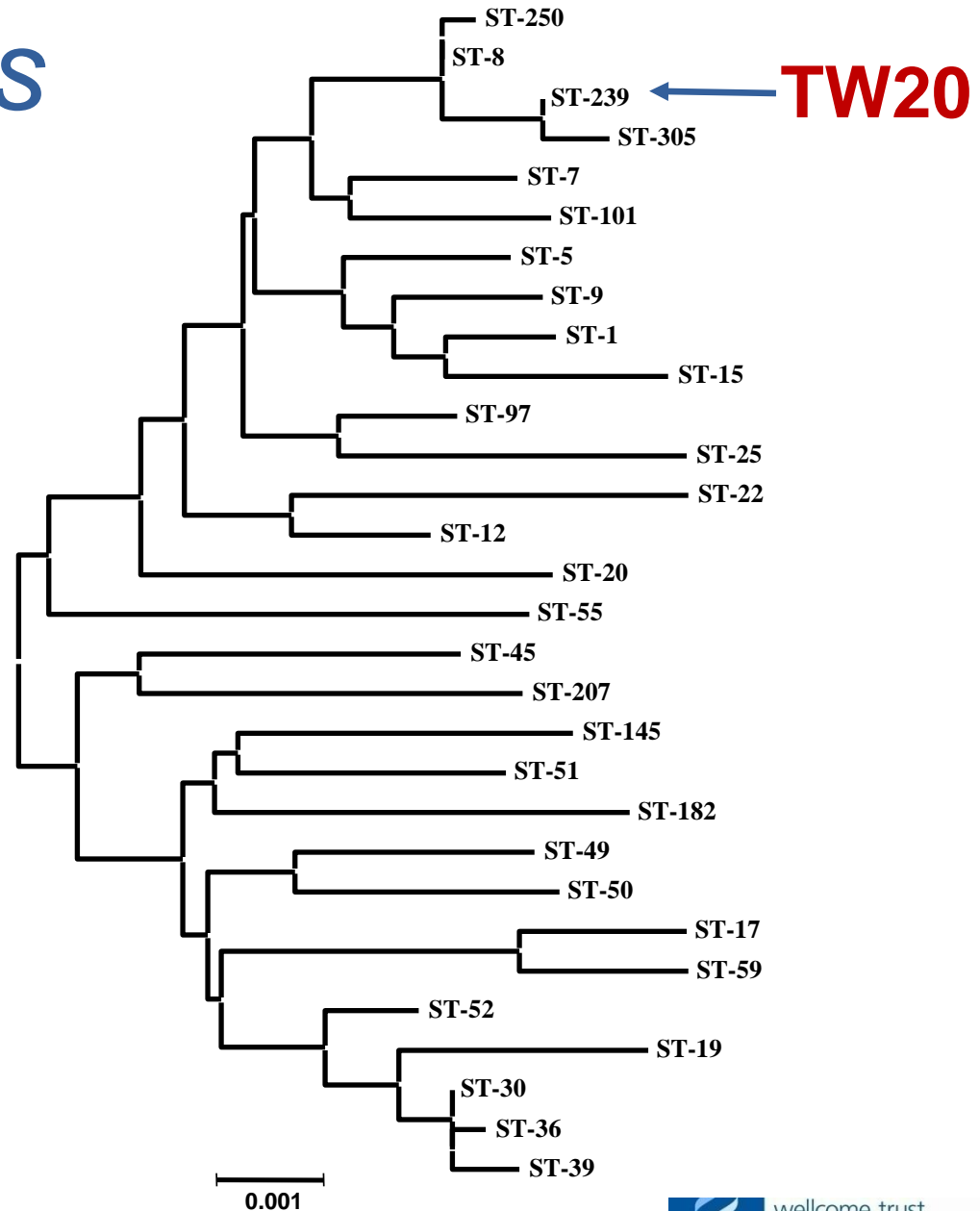


Image kindly provided by Sharon Peacock, Cambridge University

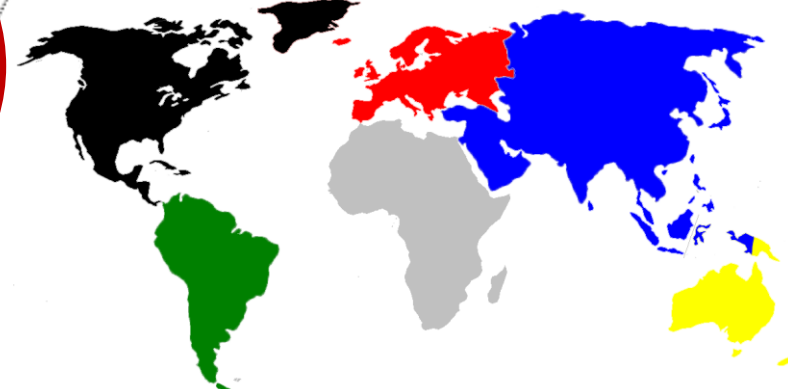
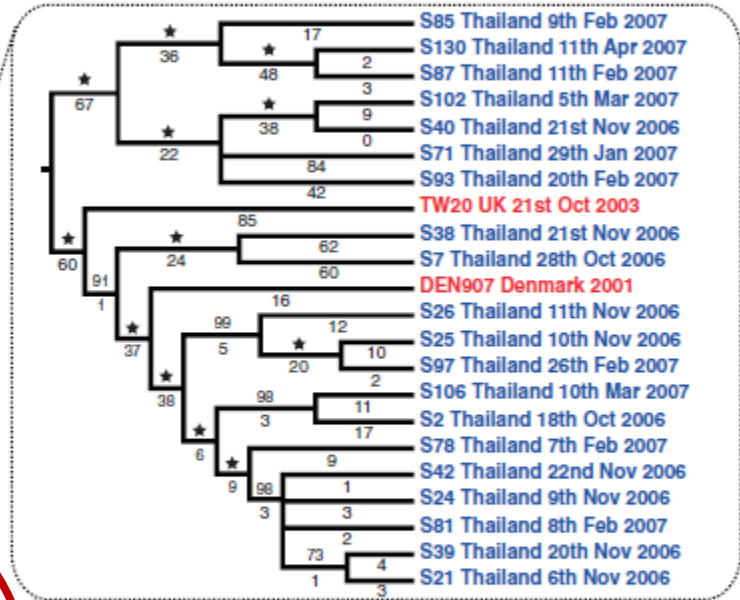
Staphylococcus aureus

- Tree of *S. aureus* sequence types (STs)
- Drug resistance on several branches
- Strain TW20 is reference for outbreak lineage ST239
 - Epidemic in London
 - Highly transmissible
- Genome sequences give finer resolution

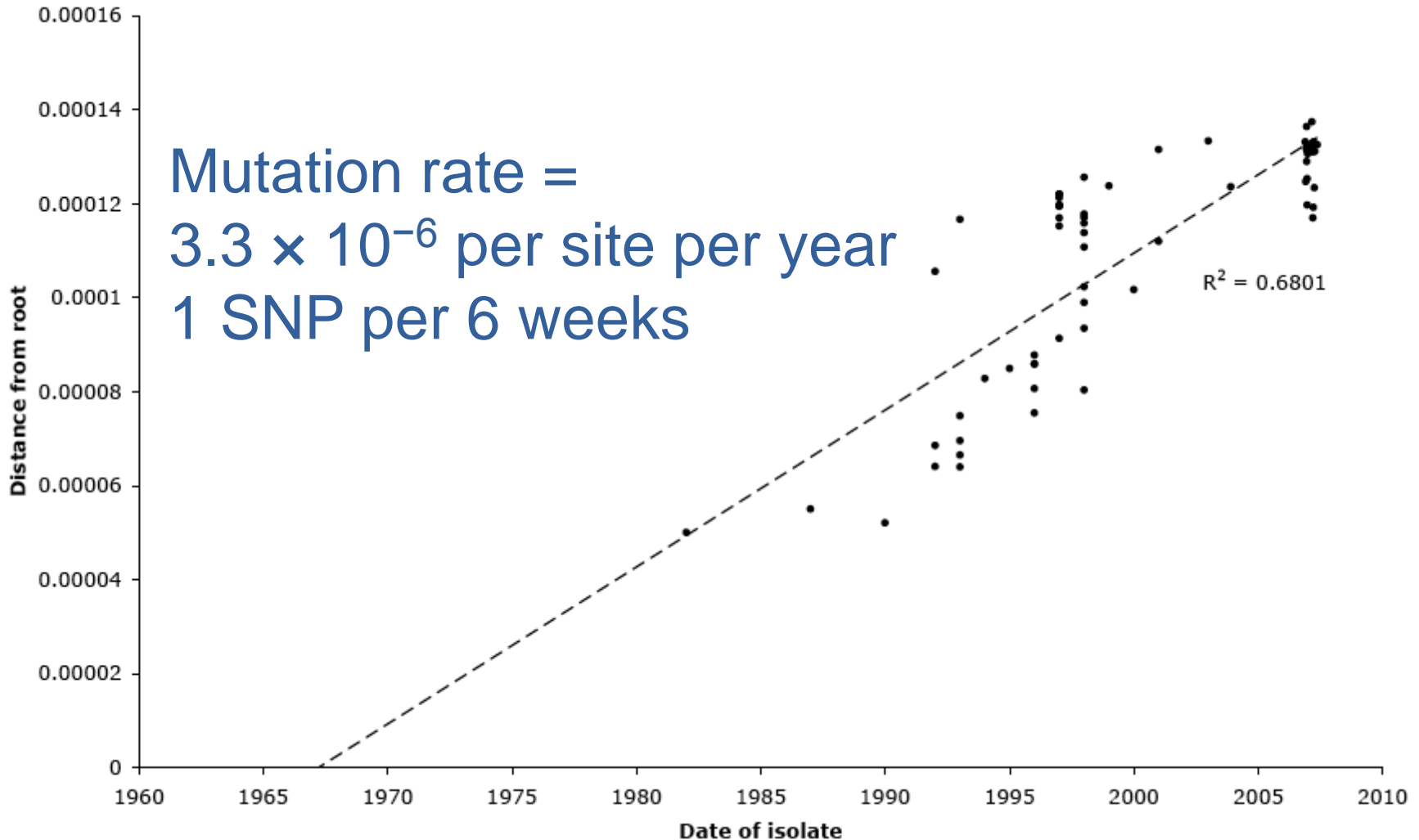


MRSA, ST239

- 63 ST239 strains sequenced
- Global, over 4 decades
- Single Nucleotide Polymorphisms (SNPs) identified
- Used to reconstruct evolution



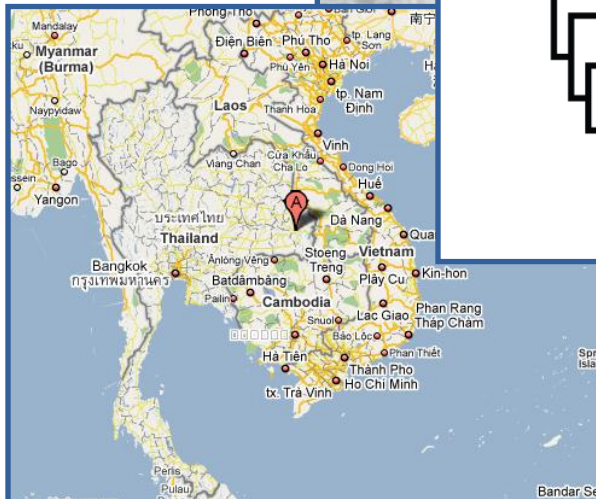
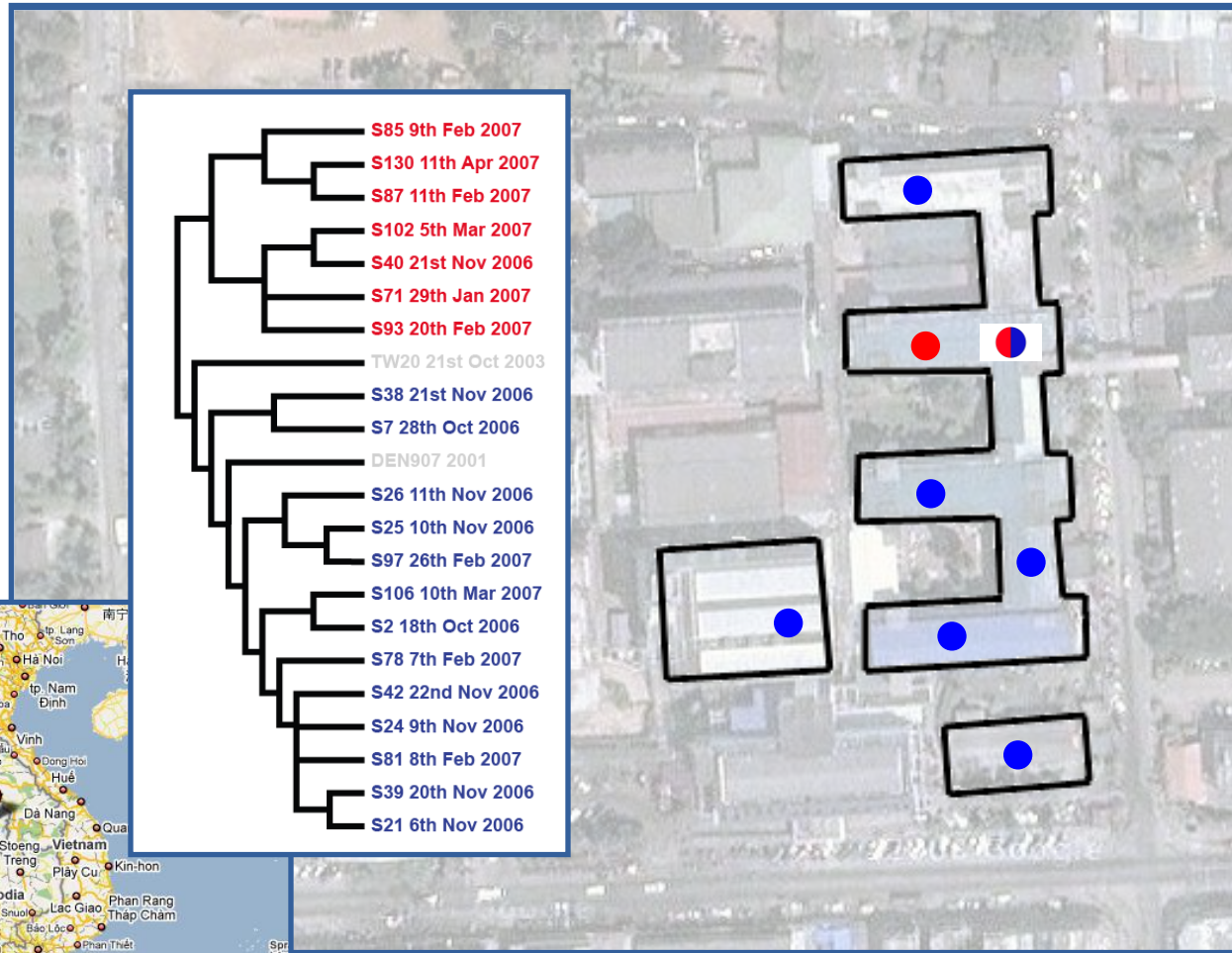
MRSA: Accumulation of SNPs



Based on core genome

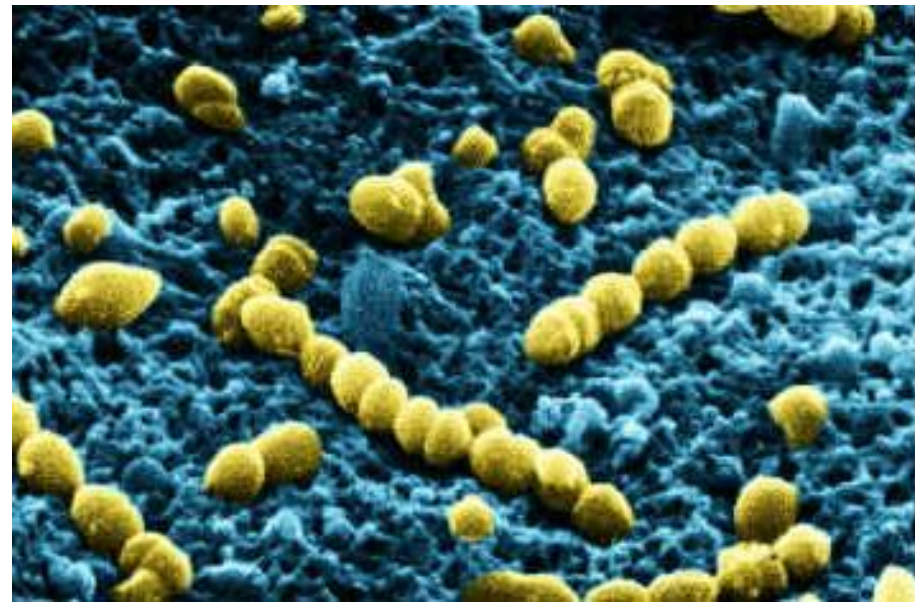
Harris *et al.* (2010) Science 327:469

Sequencing Isolates within Sappasithprasong Hospital



Streptococcus pneumoniae

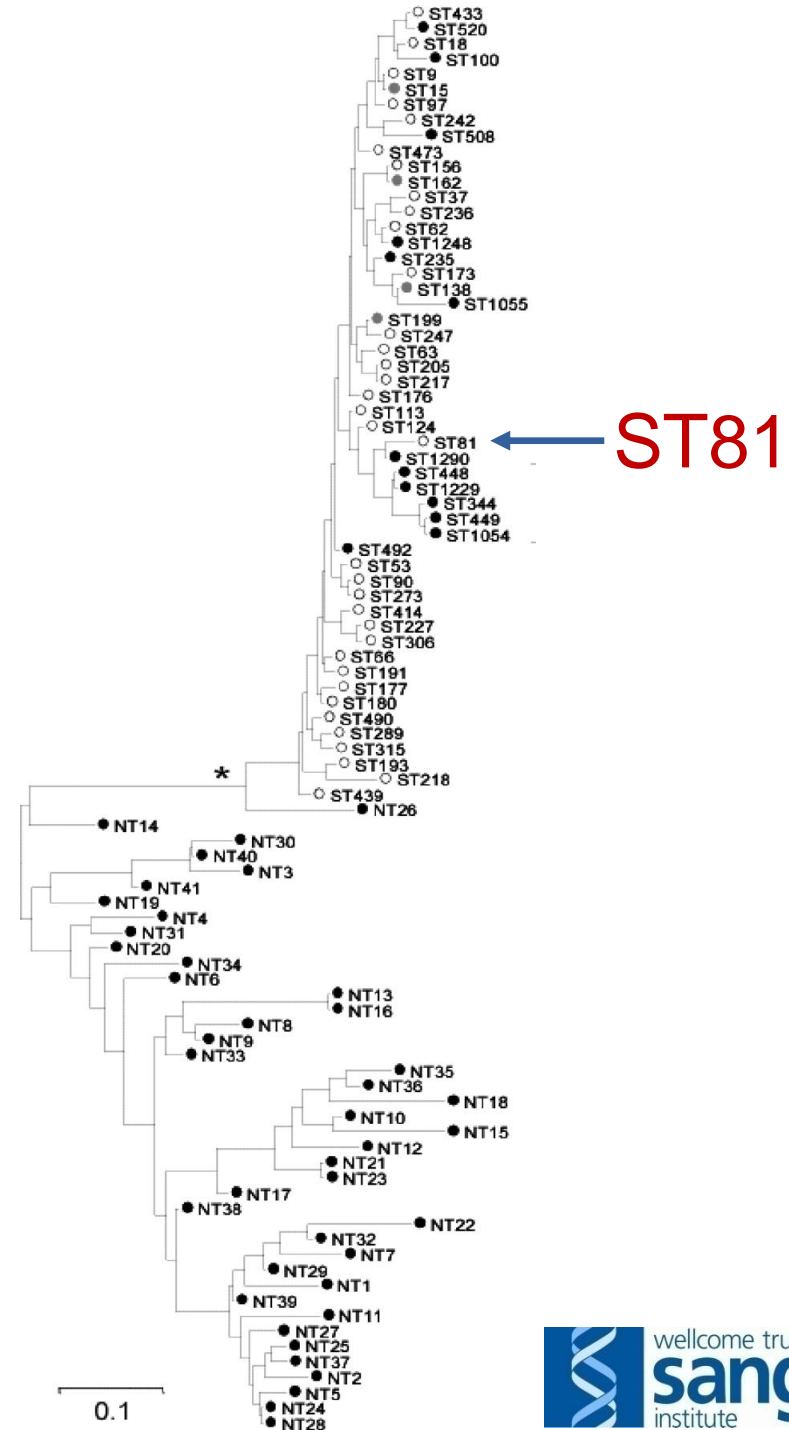
- Widespread Gram positive bacterium
- Associated with natural carriage as well as being complex pathogen
 - Respiratory disease
 - Otitis media
 - Invasive: meningitis
- Complex population structure
 - No simple association with disease
- Increasing drug resistance



- *Strep. pneumo* (pneumococcus) used to establish DNA as the “transforming principle” (Avery, MacLeod & McCarty, 1944)
- Capsule as virulence factor

Streptococcus pneumoniae: MLST tree

- Sequence type (ST)81 associated with capsule (serotype) 23F
- Global lineage causing 40% of penicillin-resistant pneumococcal disease in USA
- Vaccine against 7 capsule types used since 2000 (PCV7)
 - Includes 23F



Initial Analysis

- 240 ST81 strains sequenced
- Europe (7 countries), South Africa, Americas (6 countries), Asia (8 countries)
- 1984-2008
- Odd shaped tree

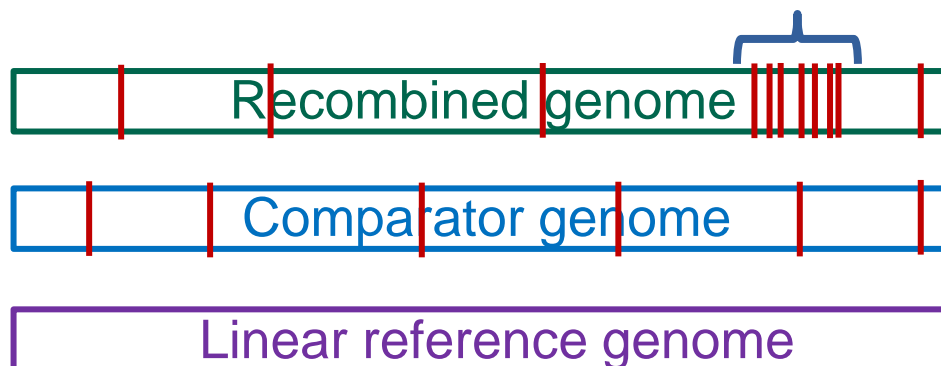


Key:

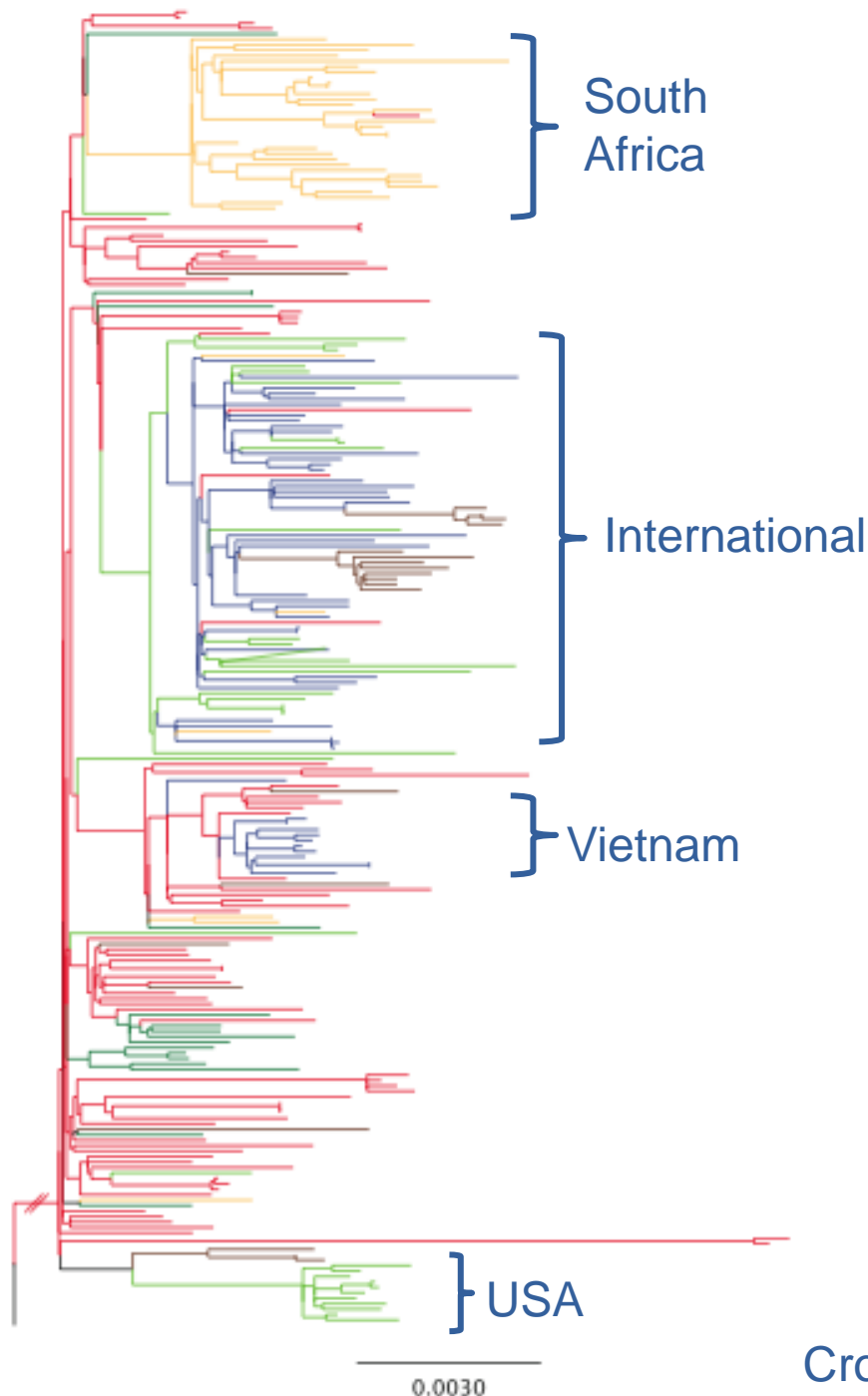
- | | |
|------------------|-----------------------------|
| — Western Europe | — South Africa |
| — Eastern Europe | — South-East Asia |
| — North America | — Central and South America |

Recombination in Bacteria

Mutations (SNPs) often
accumulate randomly
High density of SNPs
implies gain of foreign
DNA



Analysis with Recombinations Removed



Key:

Western Europe

Eastern Europe

North America

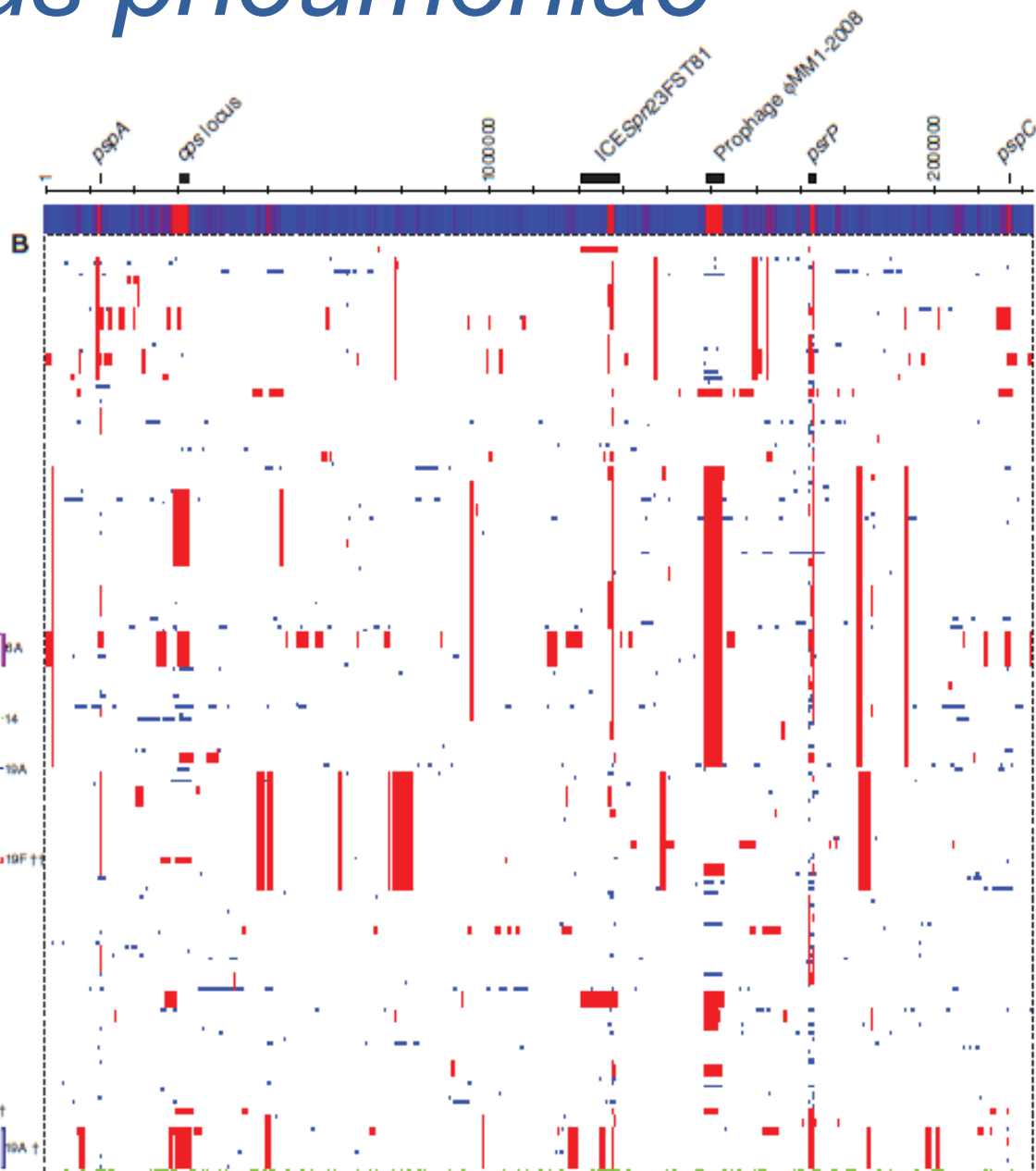
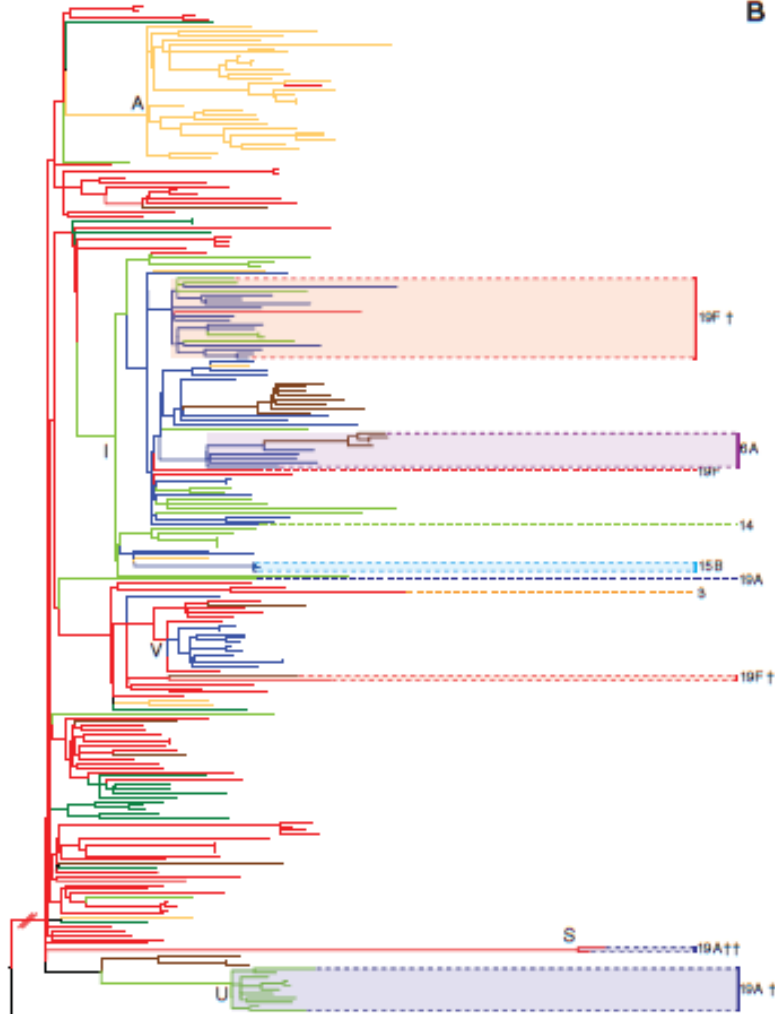
South Africa

South-East Asia

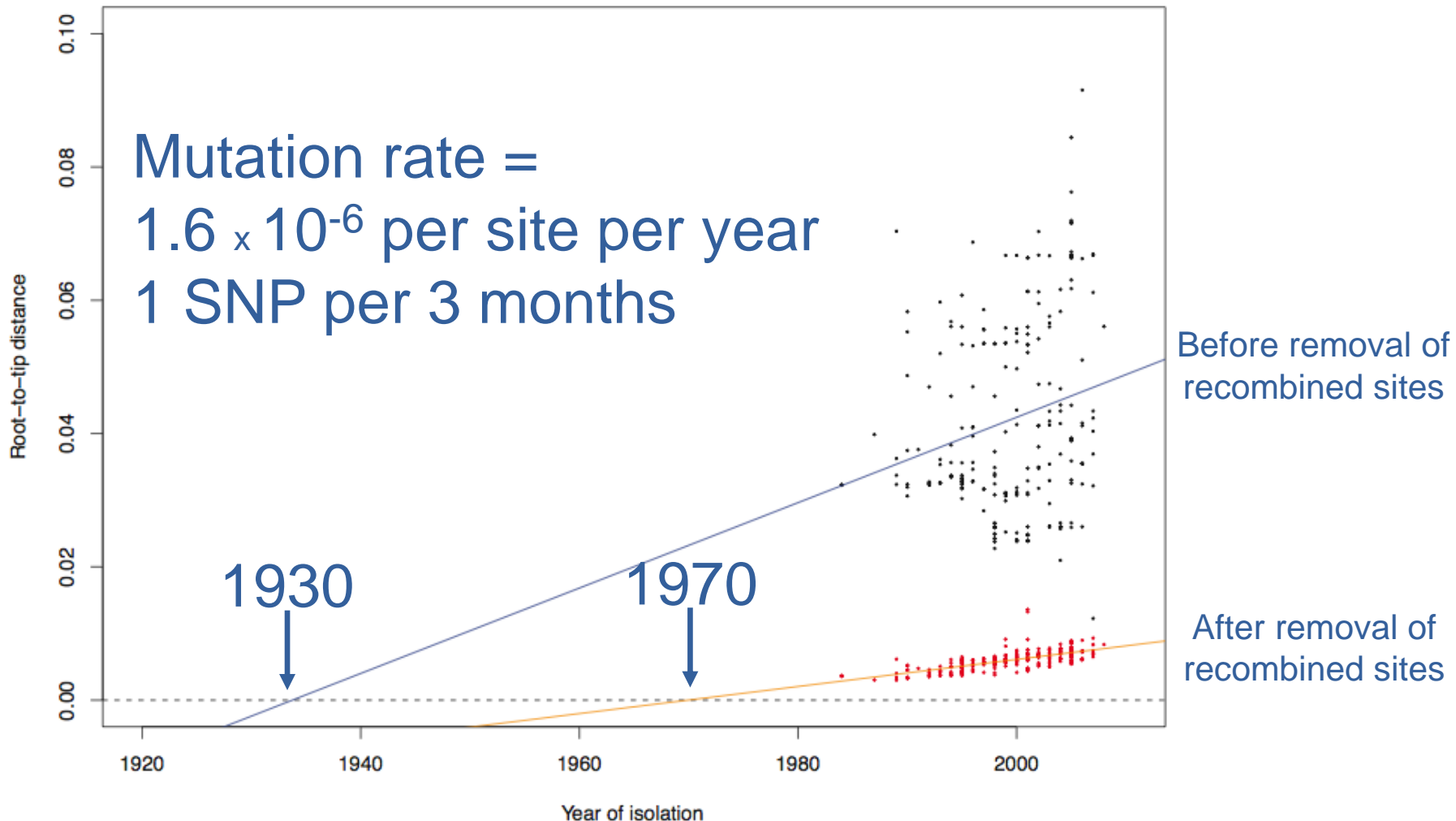
Central and South America

Streptococcus pneumoniae

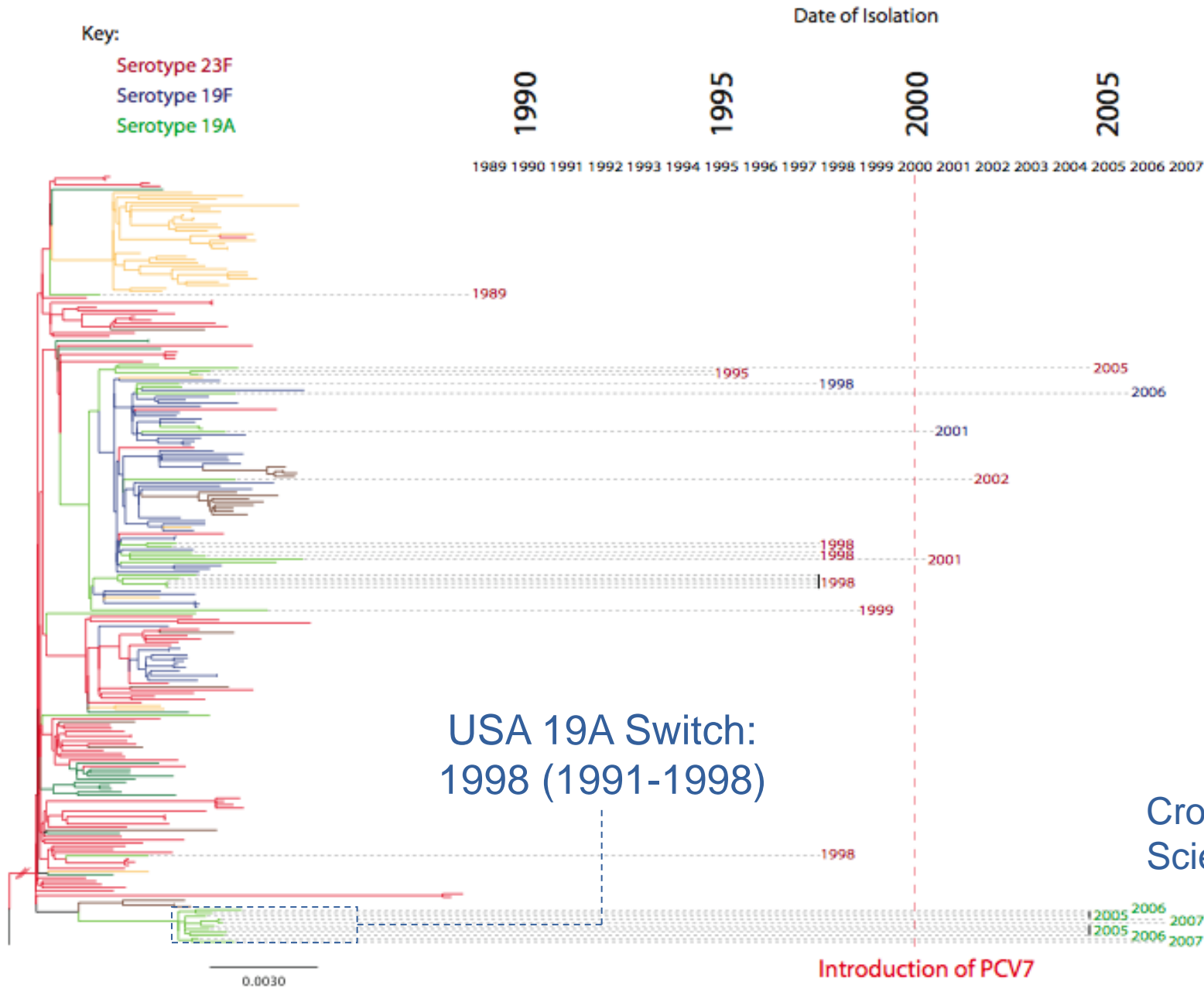
ST81



MRSA: Accumulation of SNPs



S. pneumoniae 23F: Capsule Replacement



Croucher *et al.*
Science 2011

Vibrio cholerae

- Common marine Gram negative bacterium
- Infects small intestine causing profuse watery diarrhoea
- Endemic in some areas but also pandemics / epidemics
- How do outbreaks occur?

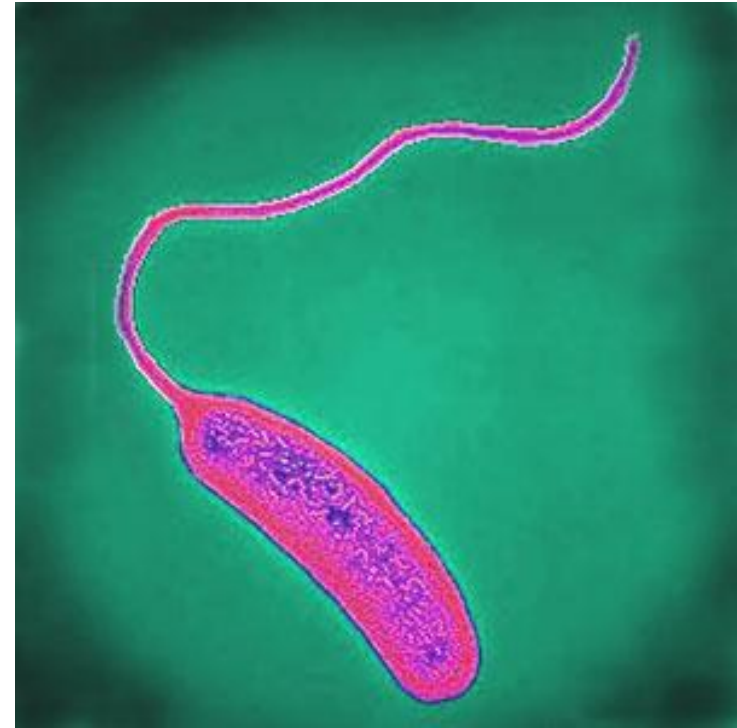
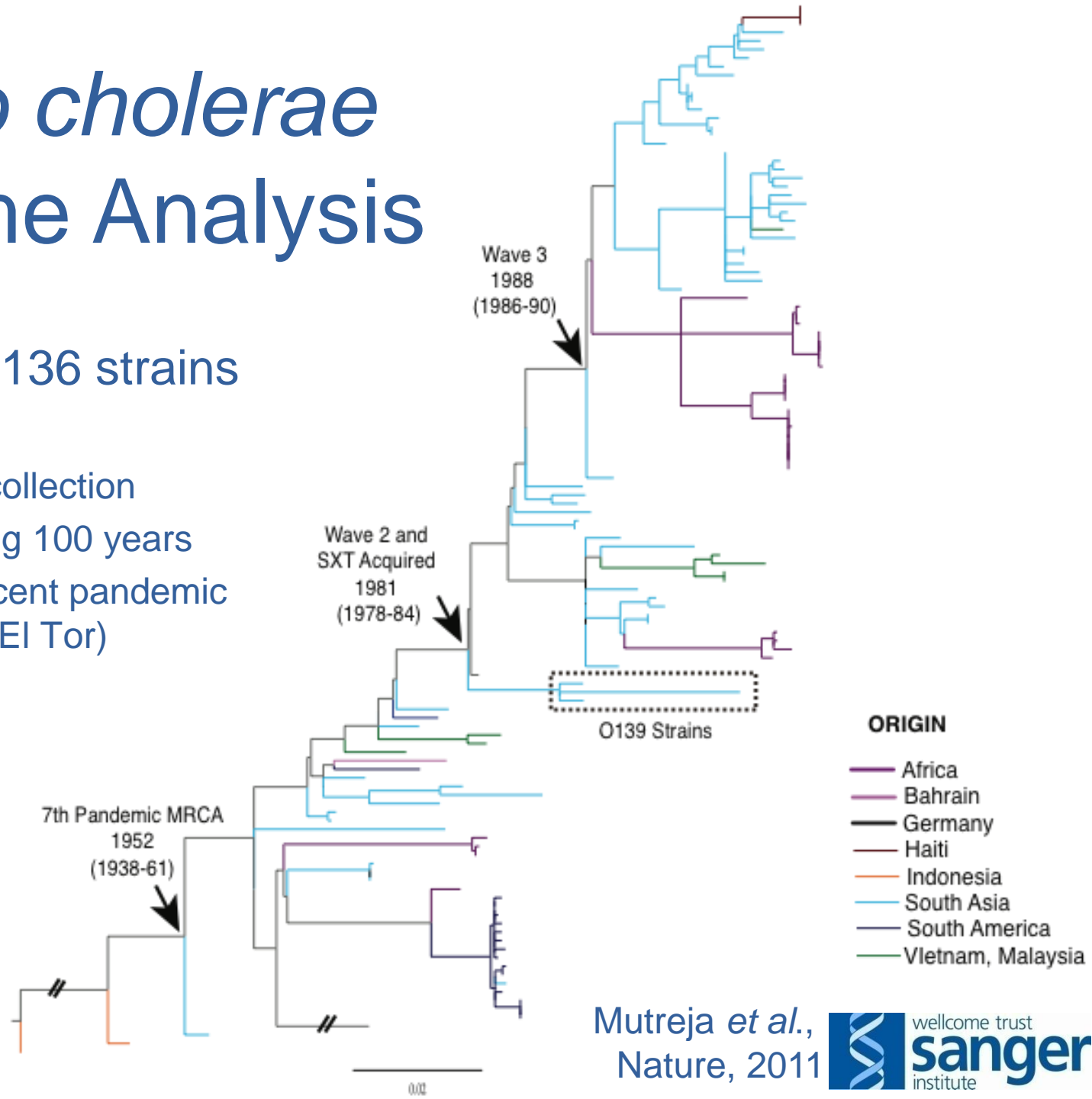


Image: www.microbiologybytes.com

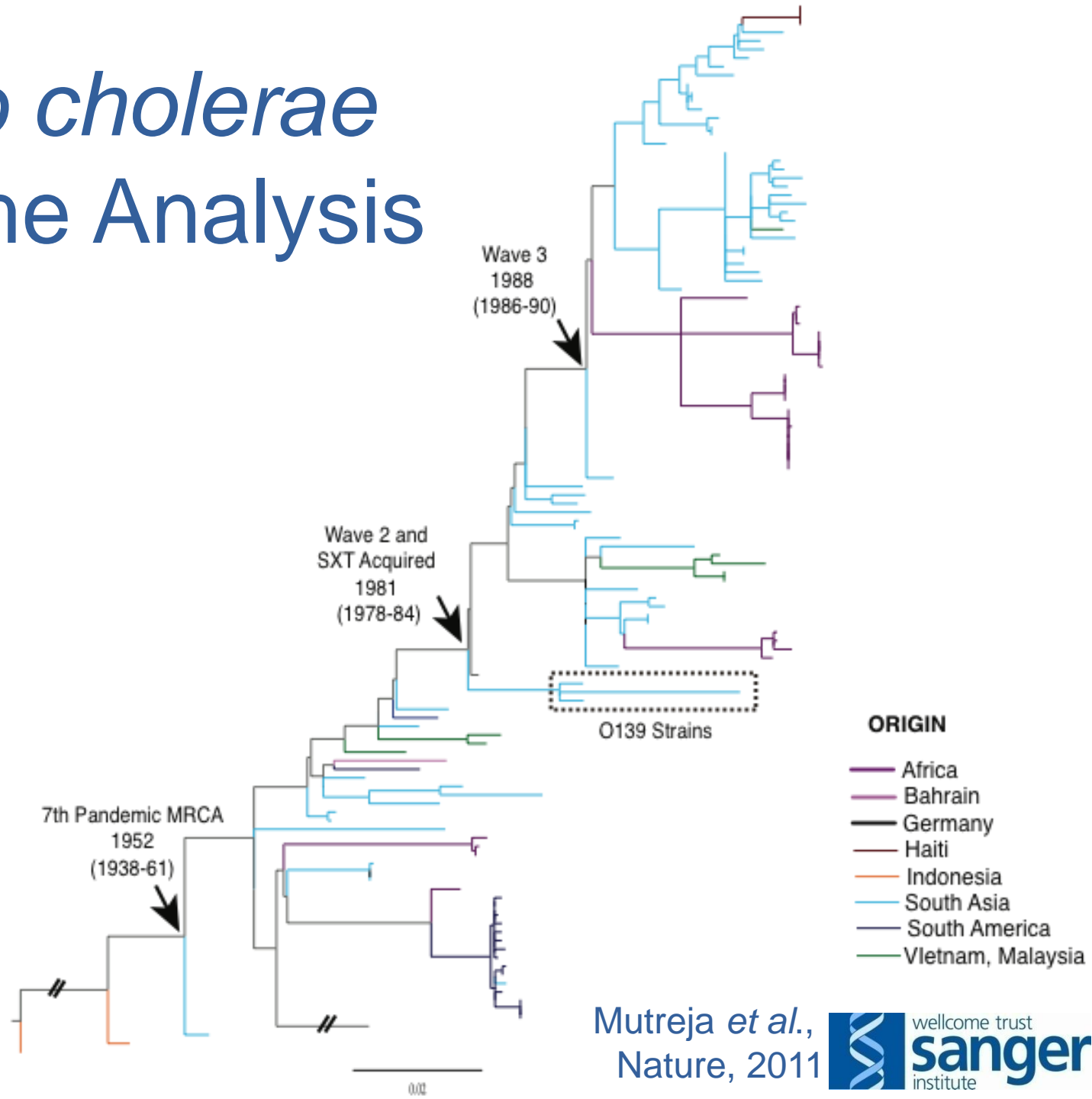
Vibrio cholerae Genome Analysis

Sequenced 136 strains
(total 154)

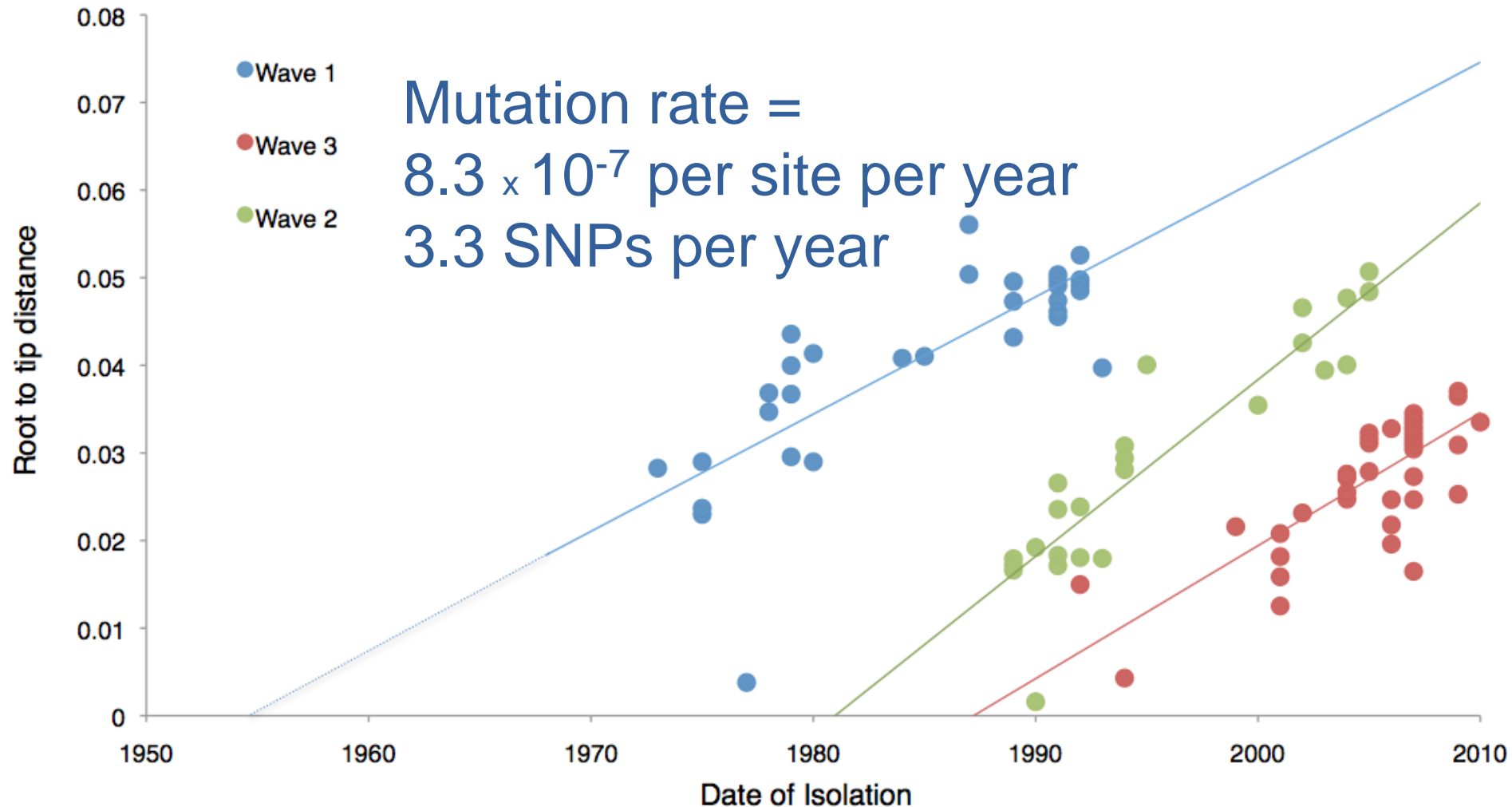
- Global collection
- Spanning 100 years
- Most recent pandemic cluster (El Tor)



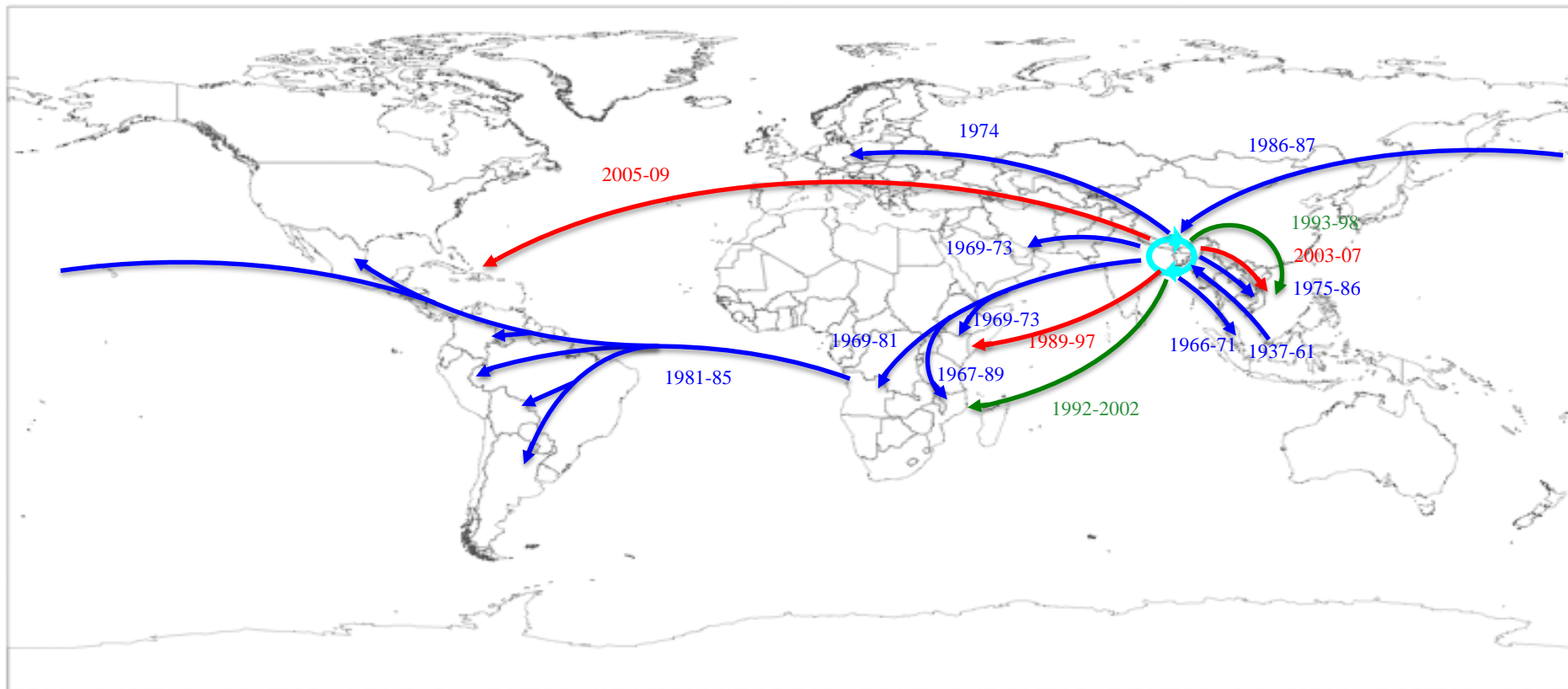
Vibrio cholerae Genome Analysis



Vibrio cholerae: Accumulation of SNPs



Vibrio cholerae: Repeated Global Transmission



→ Wave 1

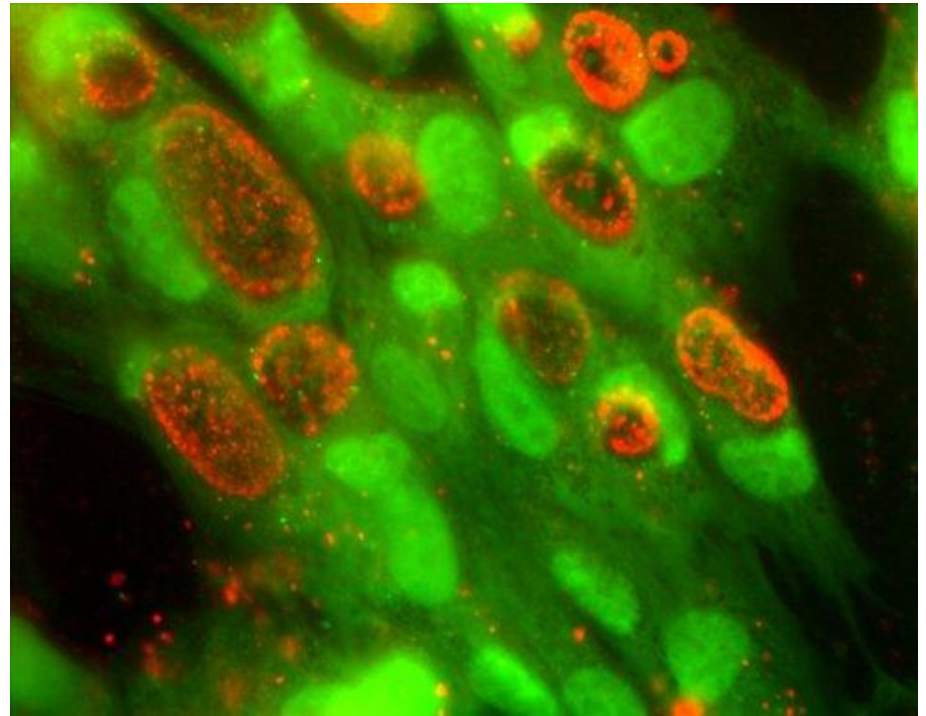
→ Wave 2

→ Wave 3

Mutreja *et al.*, Nature, 2011

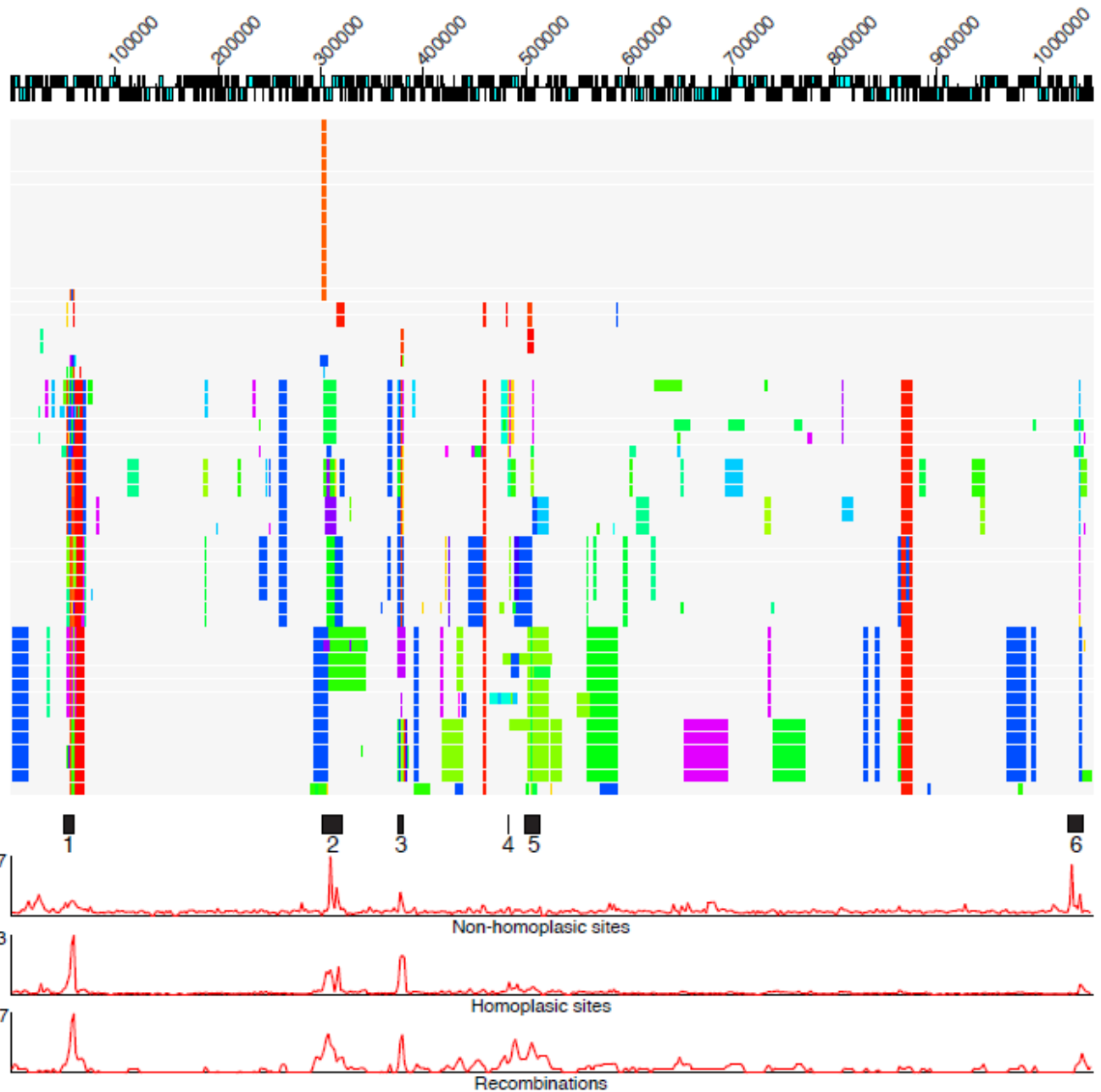
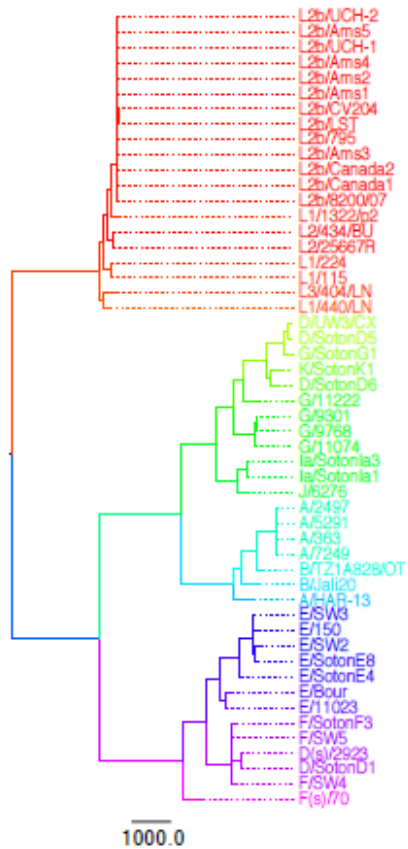
Chlamydia trachomatis

- Gram negative pathogen
- Intracellular
 - Specialised lifecycle
 - Genetically intractable
- STI, infectious blindness, invasive form
- Easily treated
- 36 strains sequenced (52 total) across whole species



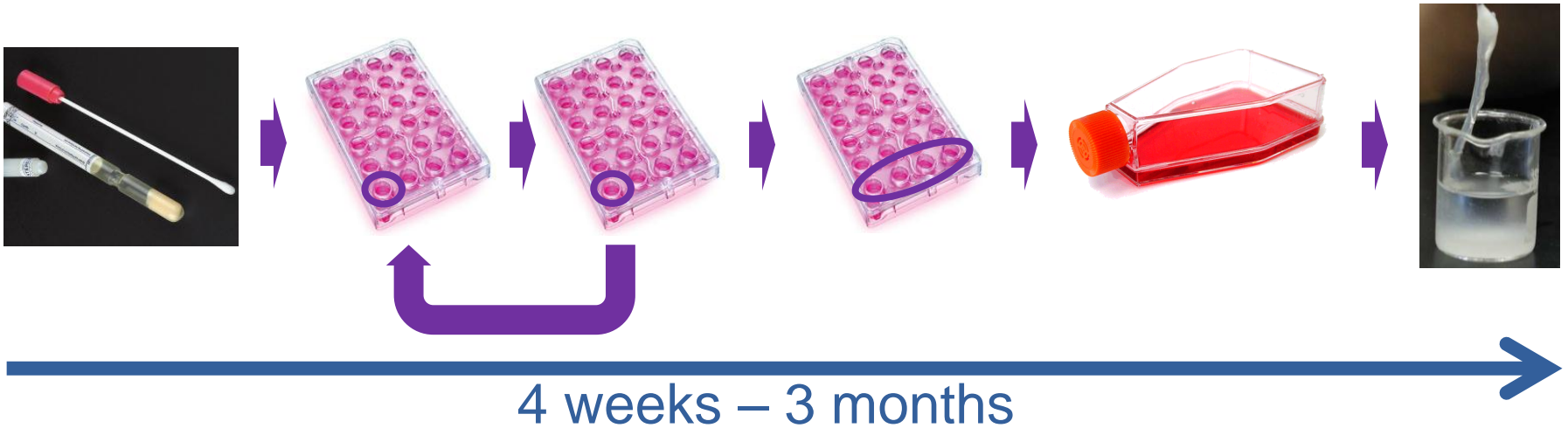
<http://www.ispub.com/journal/the-internet-journal-of-microbiology/volume-2-number-2/infectivity-assays-for-chlamydia-trachomatis.html>

Chlamydia trachomatis



Difficulties with *C. trachomatis*

- Strains derive from swab samples
- Successful strain growth technically challenging
- Highly time consuming & labour intensive



- Can we generate genomes directly from swabs?
- Swabs carry low levels of *Chlamydia*, high levels of human cells / DNA and other bacterial cells / DNA

What Has DNA Done For Us?

- Genome sequencing has given us insights into bacterial pathogen lifestyles
- Next Generation Sequencing has revolutionised bacterial genomics
 - Throughput
 - Speed
 - Quality
- Bacterial population dynamics: global / local
- Response to selective pressures:
 - Antibiotics, Vaccines
- Further investigations required:
 - Epidemiology, outbreak control, antibiotic resistance
- Only genome sequencing gives the required resolution

WTSI:

Julian Parkhill
Gordon Dougan
Nick Thomson
Stephen Bentley
Matthew Holden
Simon Harris
Nick Croucher
Ankur Mutreja
Thomas Connor
Mike Quail
Carol Churcher

Chlamydia:

Southampton
Ian Clarke
Lesley Cutcliffe
Addenbrooke's
Hamid Jalal
Surendra Parmar

MRSA:

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Ed Feil
Mahidol/Oxford
Sharon Peacock
Emma Nickerson
Narisara Chantratita
Nick Day
St George's
Jodi Lindsay
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Nova de Lisboa
Hermínia de Lencastre
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