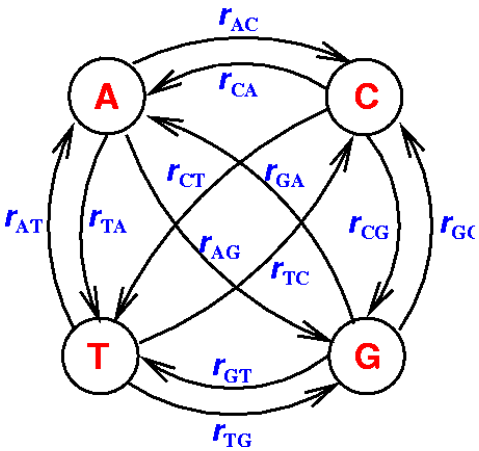
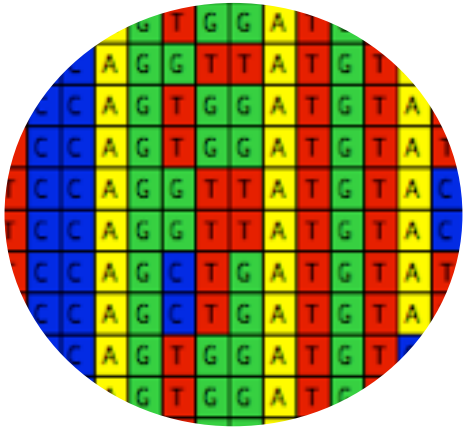
PMB2023

PATHOGEN MULTIOMICS AND BIOINFORMATICS

Rio Grande RS 2023

Module 4: Introduction to Phylogenetics and Public Health

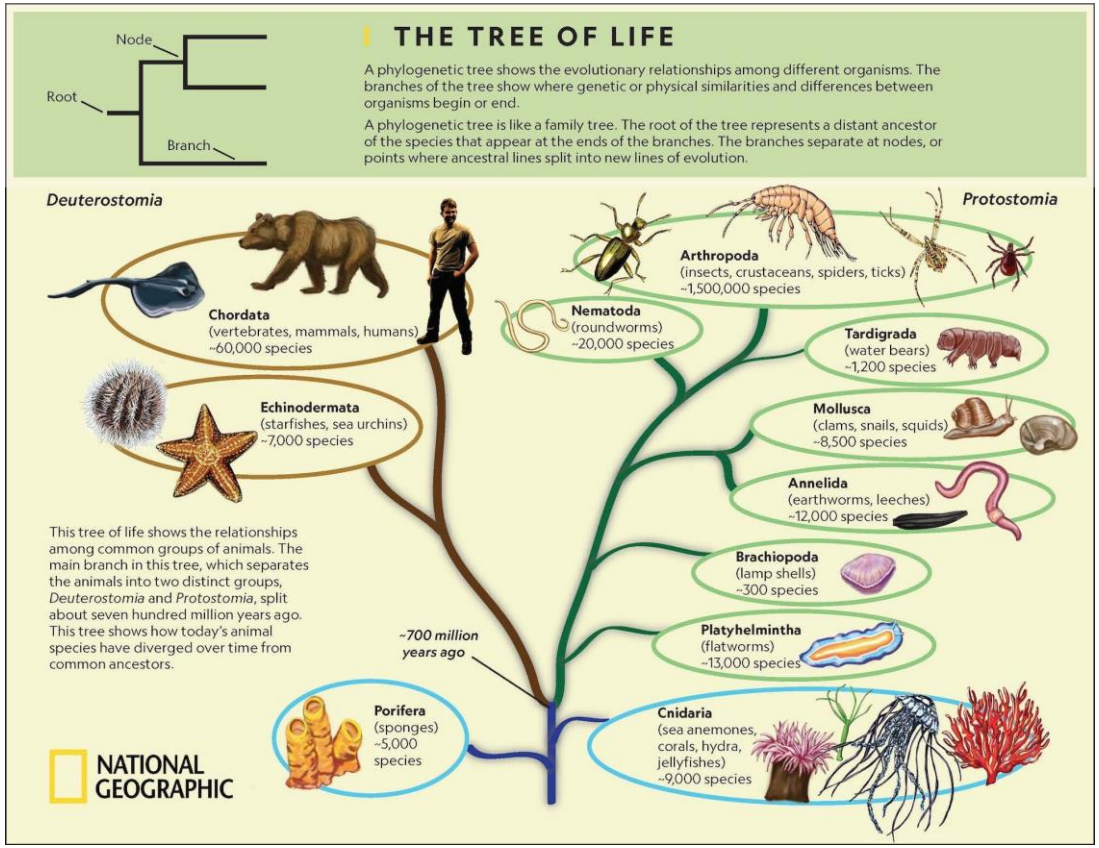




João Perdigão

Phylogenetics

*Phylogenetics pertains to the **study of the evolutionary relationships***



Between what?

- *organisms, e.g., species or strains*
- *genes*
- *genomes*
- *Etc.*

Phylogenetics should refer to how closely the taxa are

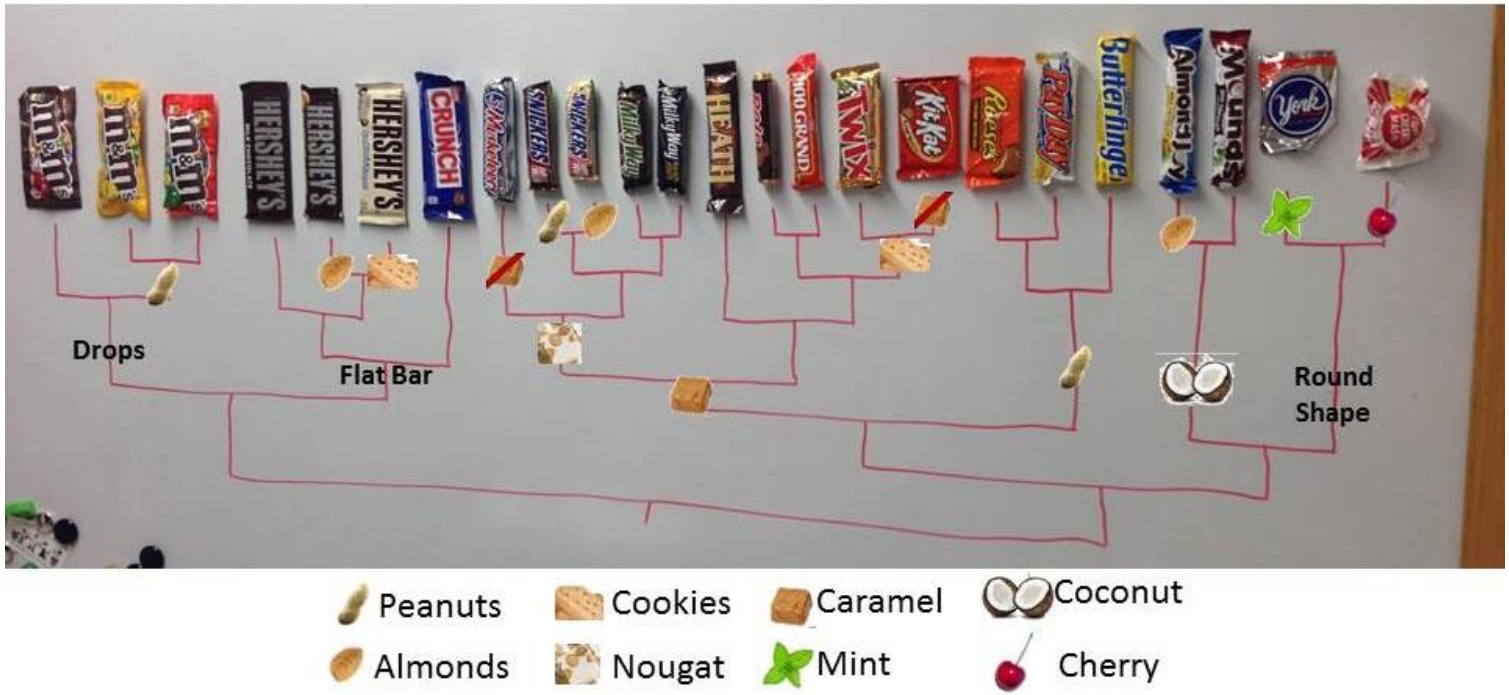
and…

its evolutionary history

Phylogenetics

What characters to use to construct a tree?

Early characters for phylogenetic reconstruction relied upon morphological and physiological characteristics



Example: ability to grow at different temperatures, drug resistance, sugar fermentation

Problems?

Molecular Phylogenetics

Morphological/Physiological characters have two main problems associated:

Proneness to convergent evolution

Limited number of characters – poorly informative

However … molecular data (DNA or Protein sequence),

are less prone to convergent evolution

can provide an increasing number of characters

A T G C T T T G C
A T G T T T T G C
A G G C T T T G C
A G G C T T T A C

But… which characters are these?

A T G C T T T G C
A T G T T T T G C
A G G C T T T G C
A G G C T T T A C

Each homologous position between sequences comprise a character?

An alignment provides a way to:

- Identify homology – regions of common ancestry
- Contrast regions

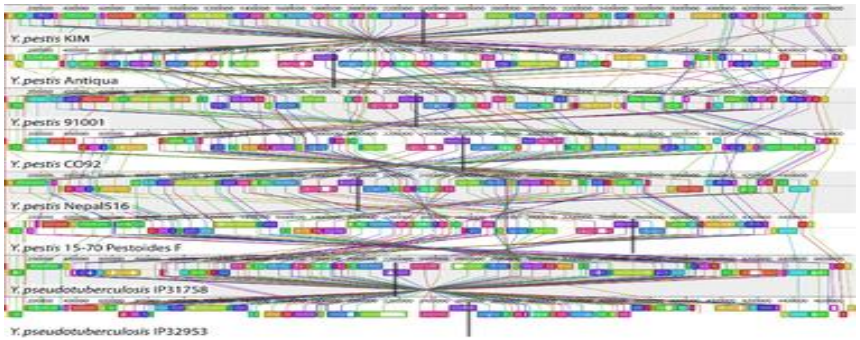
Molecular Phylogenetics

In summary, molecular phylogenetics require a Multiple Sequence Alignment

High amount of information – each column is an evolutionary marker

Possibility of going genome-wide – compare entire genomes

Problem: *alignment of entire genomes consumes large amounts of memory, even more upon tree bulding*



Research Questions & Trade-offs:

- *Sequence length (full length vs gene fragments)*
- *Genetic variation (conserved vs variable regions)*

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Molecular Phylogenetics

A possible approach: construct a DNA pseudo-molecule based on SNPs

In theory (perfect world)

A T G C T T T G C
A T G T T T T G C
A G G C T T T G C
A G G C T T T A C

T C G
T T G
G C G
G C A

Real-life:

A T G C T T T G C
A T G T T T T G -
A G G - - T T G C
A G G C T T T A C

T C G
T T G
G - G
G C A

wgSNP alignment
gapped alignment

T G
T G
G G
G A

coreSNP alignment

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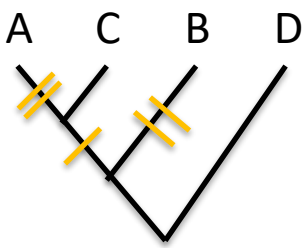
Methods for Phylogenetic Reconstruction

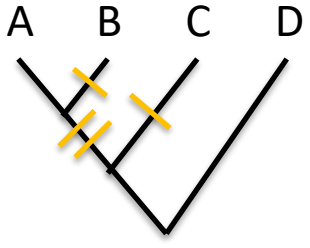
Maximum Parsimony

- *Simplest possible evolution scenario – the best tree is the shortest tree*
- *The rationale is to have the tree with the least homoplasy*

A: GGG
B: GTG
C: TGT
D: TTT



Length: 5

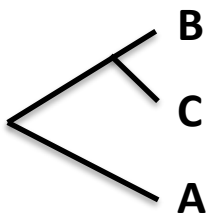


Length: 4

Distance Matrix Methods

- *Start by calculating pairwise distances from sequence data*
- *Tree is constructed from pairwise distances through clustering algorithms (e.g. Neighbour-Joining)*

	A	**B**	**C**
A	-	4	4
B	4	-	2
C	4	2	-



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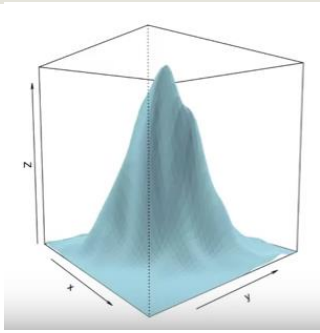
Methods for Phylogenetic Reconstruction



Maximum Likelihood

More robust approach with parameter estimation using a probabilistic model:

- *Tree topology and branch lengths*
- *Nucleotide frequencies*
- *Nucleotide substitution rates*
- *Measure how well the model fits the data*

Calculates the likelihood for every column first and for the entire alignment using different permutations, random starting values.

Likelihood (Model) = Probability (Data | Model)

Maximum Likelihood – Best set of parameter values yielding the highest possible likelihood

Software: PhyML [MEGA,Seaview, etc]

Bayesian Inference

- Based on the calculation of posterior probabilities given a set of prior parameter values
- Requires starting prior value
- Involves millions of iterations and mesasures the convergence to parameter values over the iterations

Software: MrBayes, BEAST

Nucleotide Substitution Models

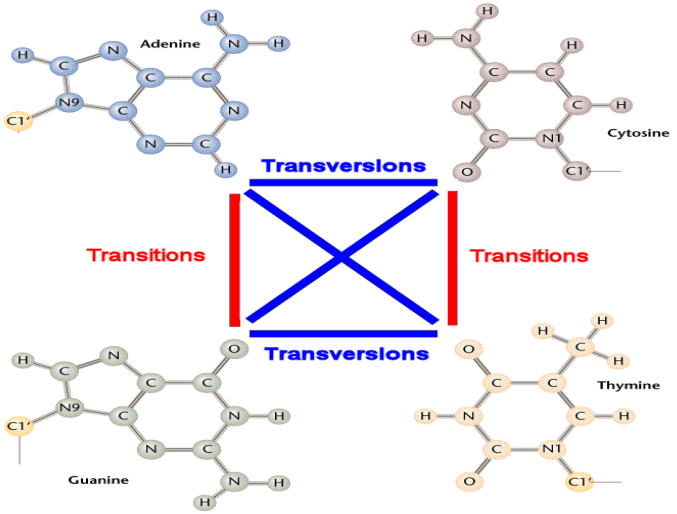
Models of Evolution

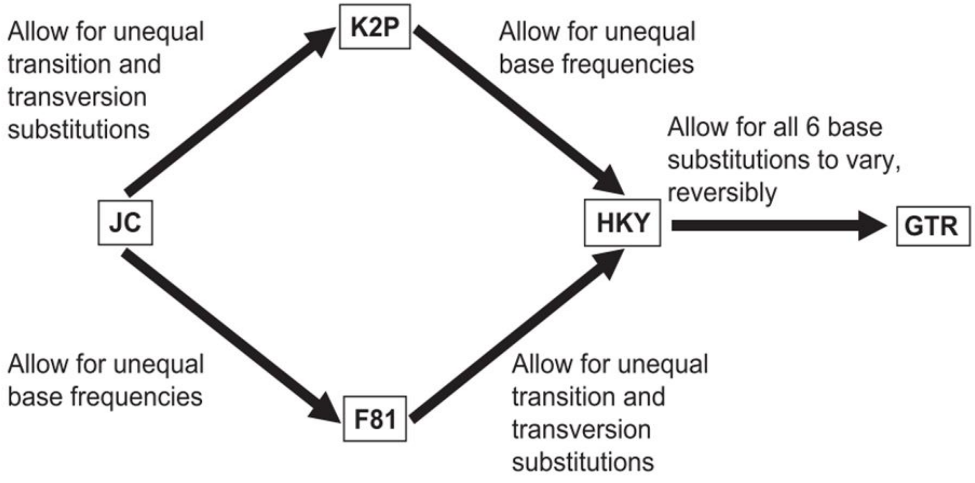
Aim to infer the number of real evolutionary events based on observed events!

Need to know how sequences are evolving – requires a model!!

Possibility of the existence of different susbstitution rates across site (gamma distribution).

The six possible substitution patterns for nucleotide data



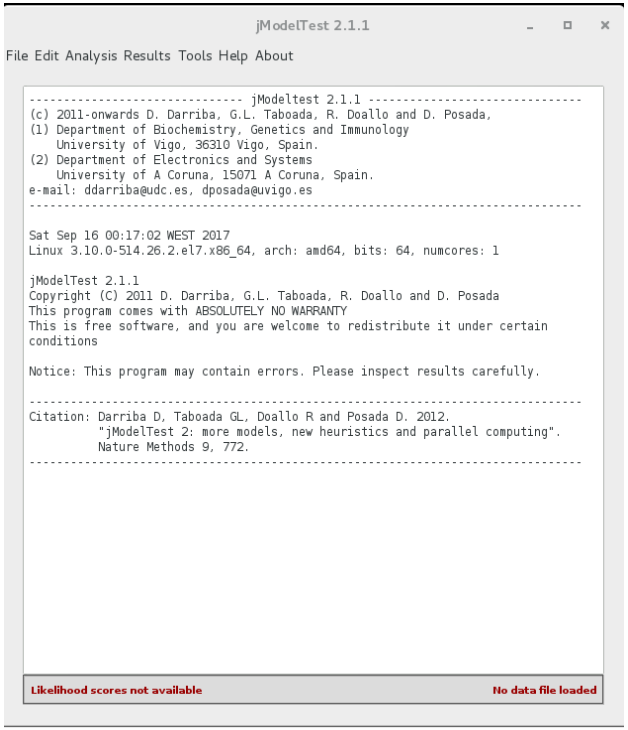


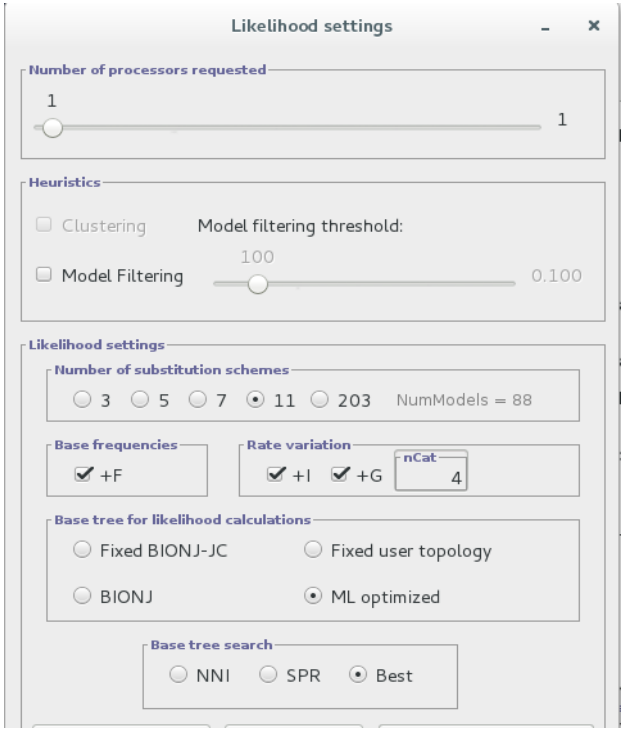
Transitions occur at higher frequency than Transversions!

How to choose the right parameters?

Answer: *test for a set of parameter permutations and choose on the combination that yields the combination that best fits your data*

jModelTest





Also possible: R, phangorn package (model.test function); ModelTest online server
Increasingly integrated in popular Tree building programs: RAxML, IQ-Tree

Tree Searching

Tree space – the number of all possible trees for a given dataset

How many branches are present in a tree with 3 tips? And with 4 tips?

Number of branches in a tree with x tips = 2x-3

3 tips ->	3 branches
4 tips ->	5 branches
5 tips ->	7 branches
10 tips ->	17 branches
20 tips ->	37 branches
40 tips ->	77 branches
100 tips ->	197 branches

But, how many possible trees:

$$\prod_{i=2}^{n-1}(2i-3)$$

For every tree with x tips it is possible to construct 2x-3 derive trees by adding na extra tip

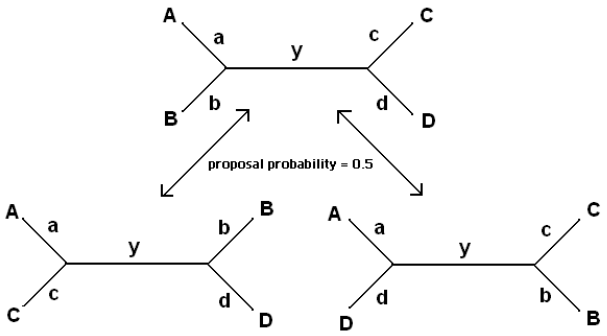
3 tips ->	1 tree
4 tips ->	3 trees
5 tips ->	15 trees
6 tips ->	105 trees
7 tips ->	945 trees
8 tips ->	10395 trees
9 tips ->	135135 trees
10 tips ->	2027025 trees
100 tips ->	1.7×10^{182} trees

It is not possible to exhaustively screen and search all possible trees in present day datasets.

Answer: start with random tree(s) and progress by making alterations and removing less likely pathways

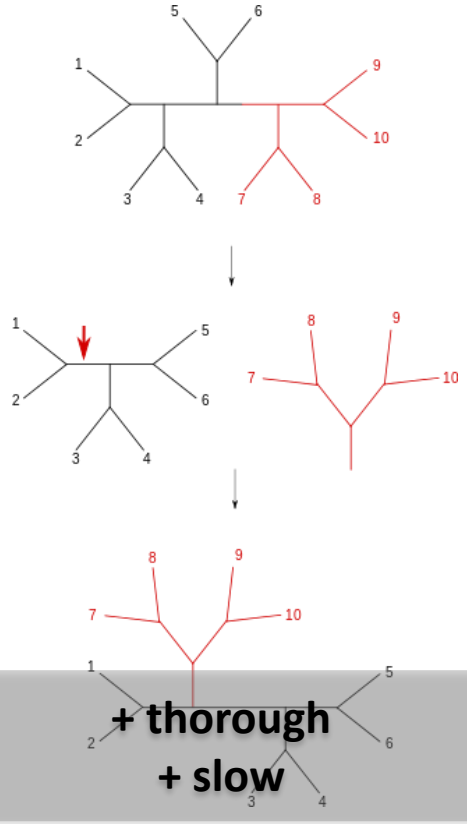
Tree Searching

Nearest Neighbour Interchange (NNI)



Swap neighbors at every internal branch

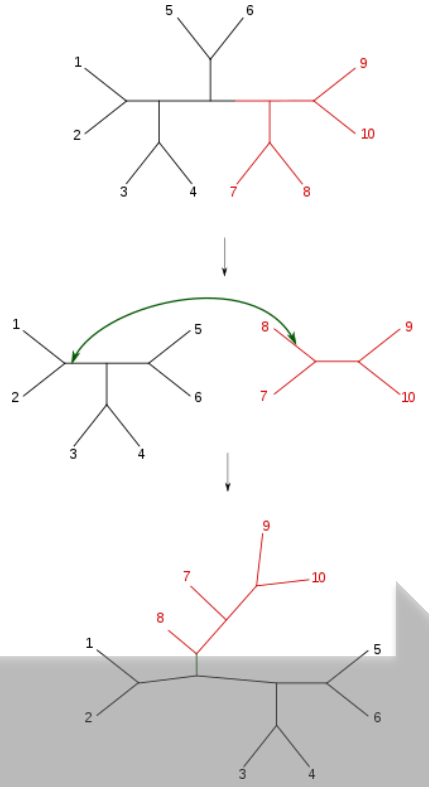
Subtree Prunning and Regrafting (SPR)



+ thorough
+ slow

Cut a subtree at every possible point and regrafts at multiple points

Tree Bisection and Reconnection (TBR)

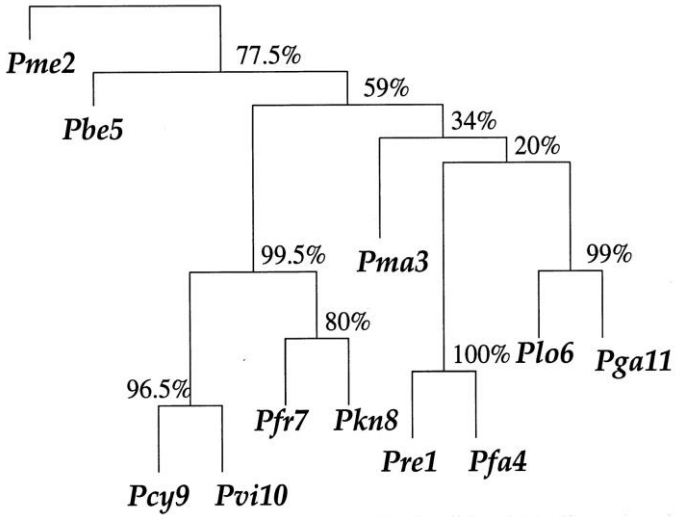


Divides tree in two parts, regrafts using every possible branch of the detached tree at all possible branches of the other tree

Tree Reliability

Bootstrap

- Widely used;
- Random sampling from the alignment (with replacement) until achieving the original length;
- Tree reconstruction n times – for each new alignment;
- Calculation for each branch the occurrence of that same clade in each tree;
- Expressed as a percentage/fraction (0-100%).
- Can be extremely time consuming



Alternatives:

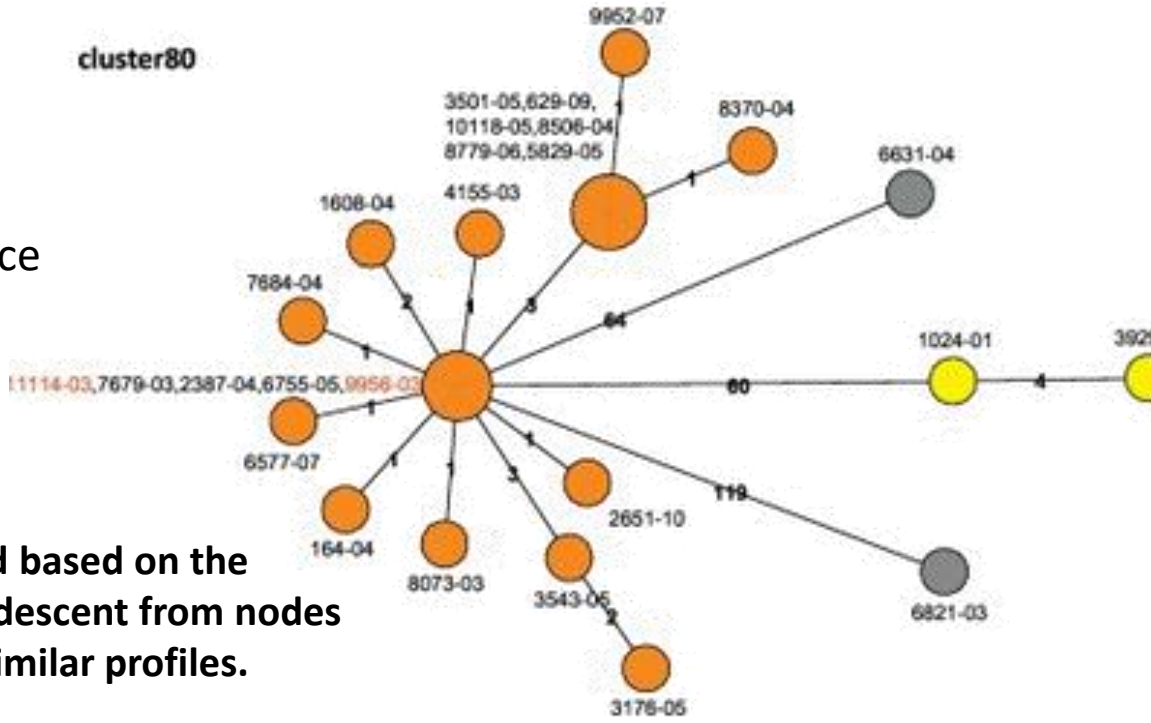
- Jacknife – removes a position from the alignment (leave one out)

- aLRT – aproximate Likelihood Ratio Test – provides a *p*-value – likelihood gains between having a branch and not having a branch

Alternative Approaches

Distance based methods using the eBURST/goeBURST

- core genome Multi Locus Sequence Typing – cgMLST
- multiple sequence alignment



Trees/relationships can be inferred based on the goeBURST/eBURST most parsimonious descent from nodes aggregating isolates/strains with similar profiles.

"eBURST approach subdivides large MLST data sets into nonoverlapping groups of related STs or clonal complexes and then discerns the most parsimonious patterns of descent of isolates within each clonal complex from the predicted founder"
Feil et al 2004

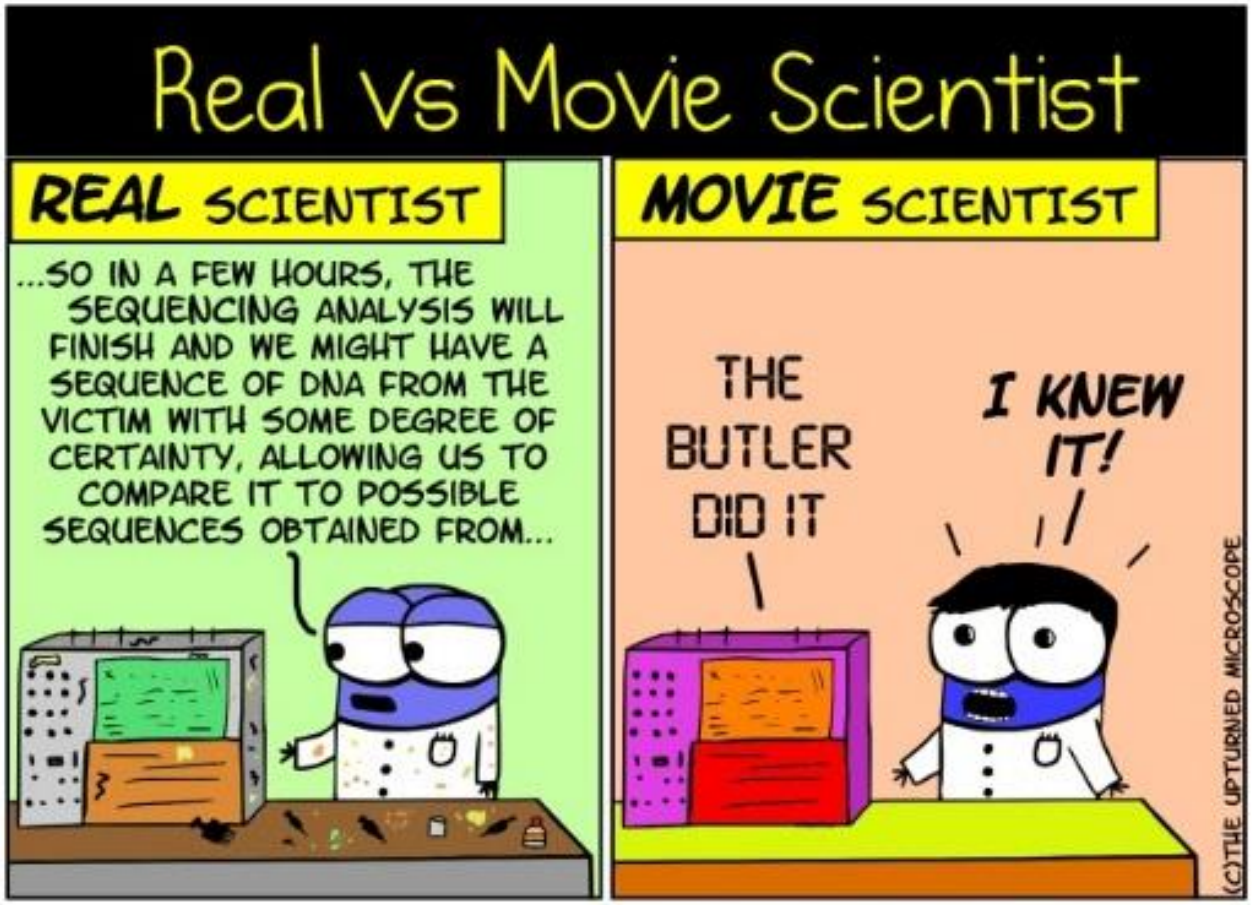
"goeBURST is a globally optimized implementation of the eBURST algorithm, that identifies alternative patterns of descent for several bacterial species. Furthermore, the algorithm can be applied to any multilocus typing data based on the number of differences between numeric profiles."
Francisco et al 2009

Software: Phyloviz, chewBACCA

Applications

DNA sequence analysis

- I
- I
- I
- I
- I



es;

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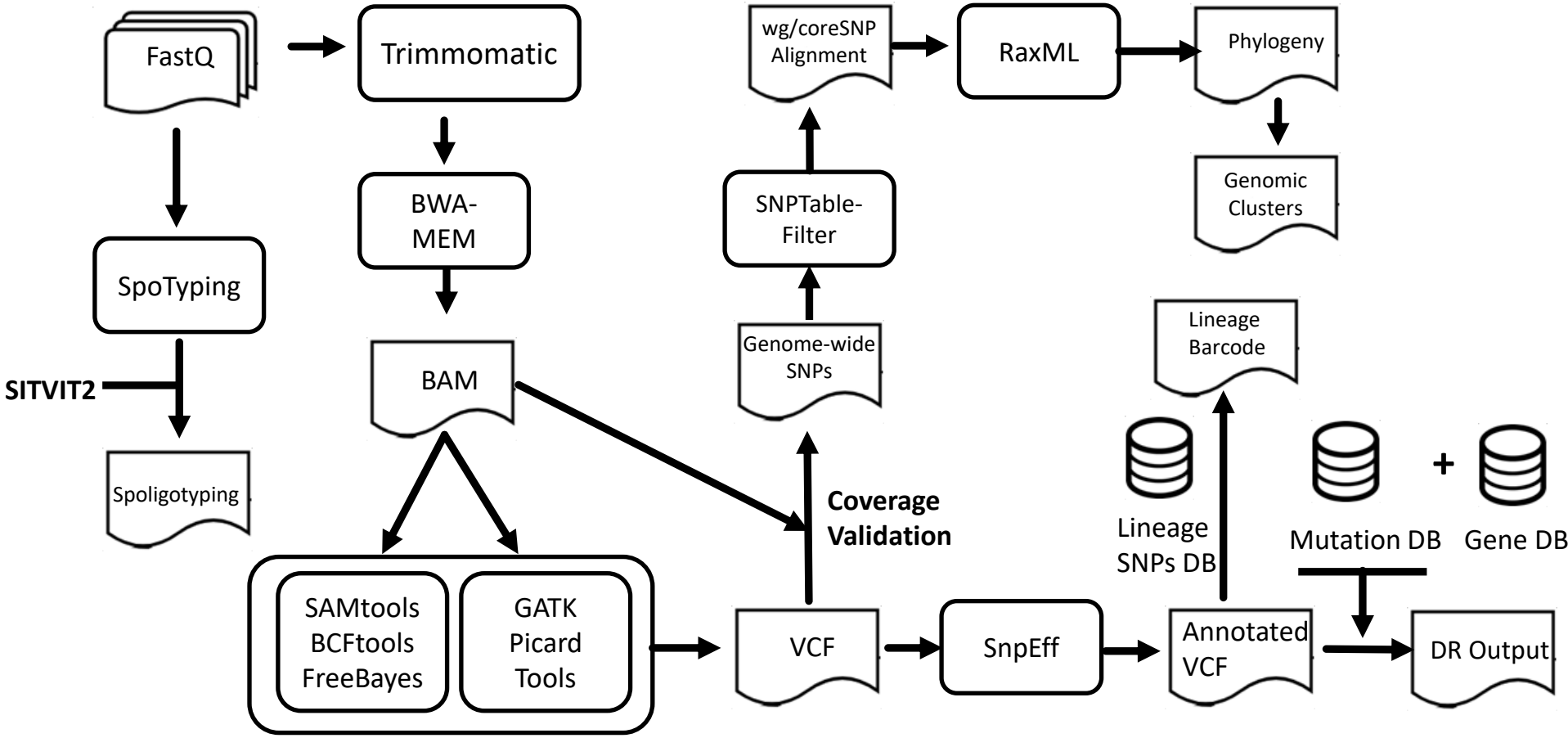
WGS analytic workflow: an overview



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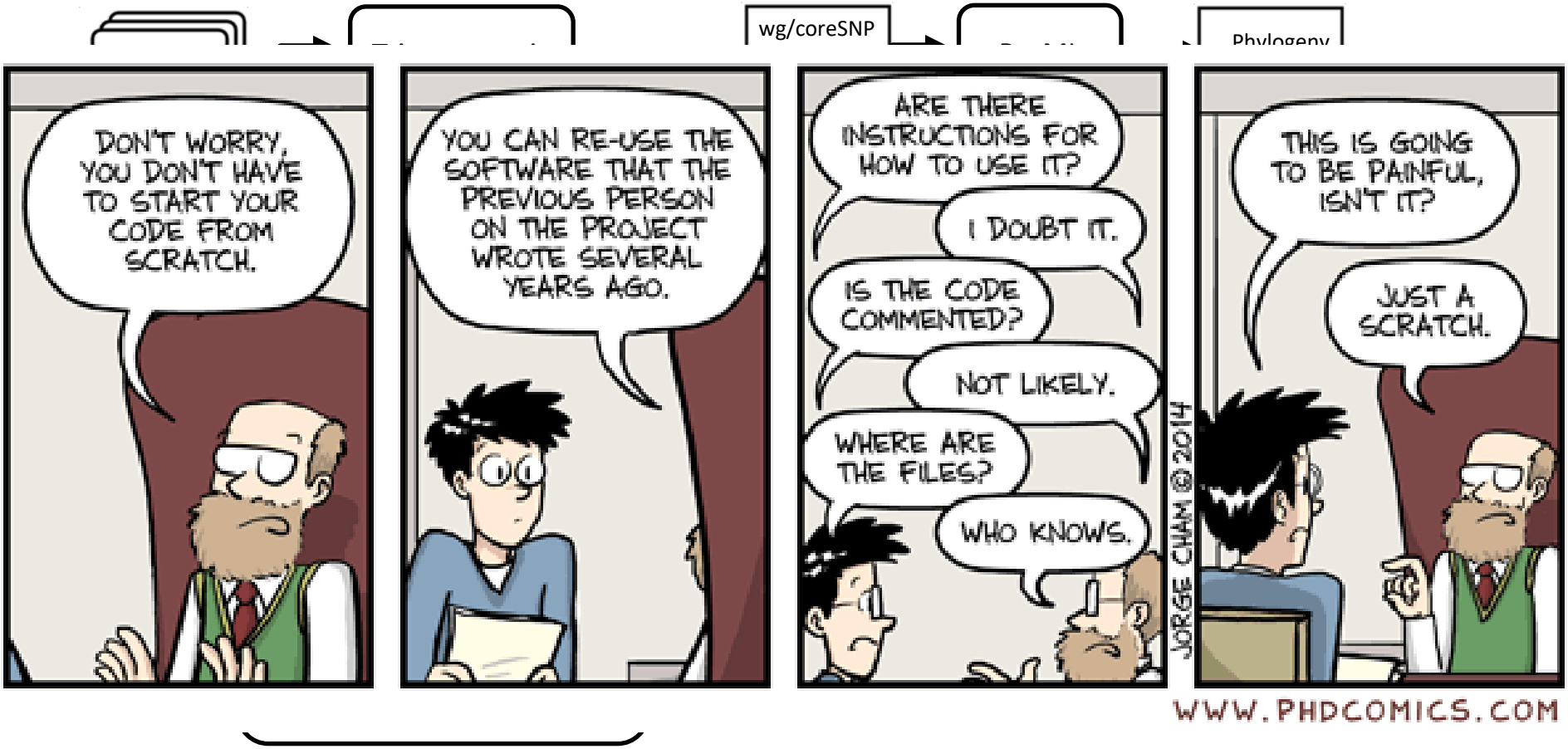
WGS analytic workflow: an overview



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Barcoding *M. tuberculosis*!

nature COMMUNICATIONS

ARTICLE

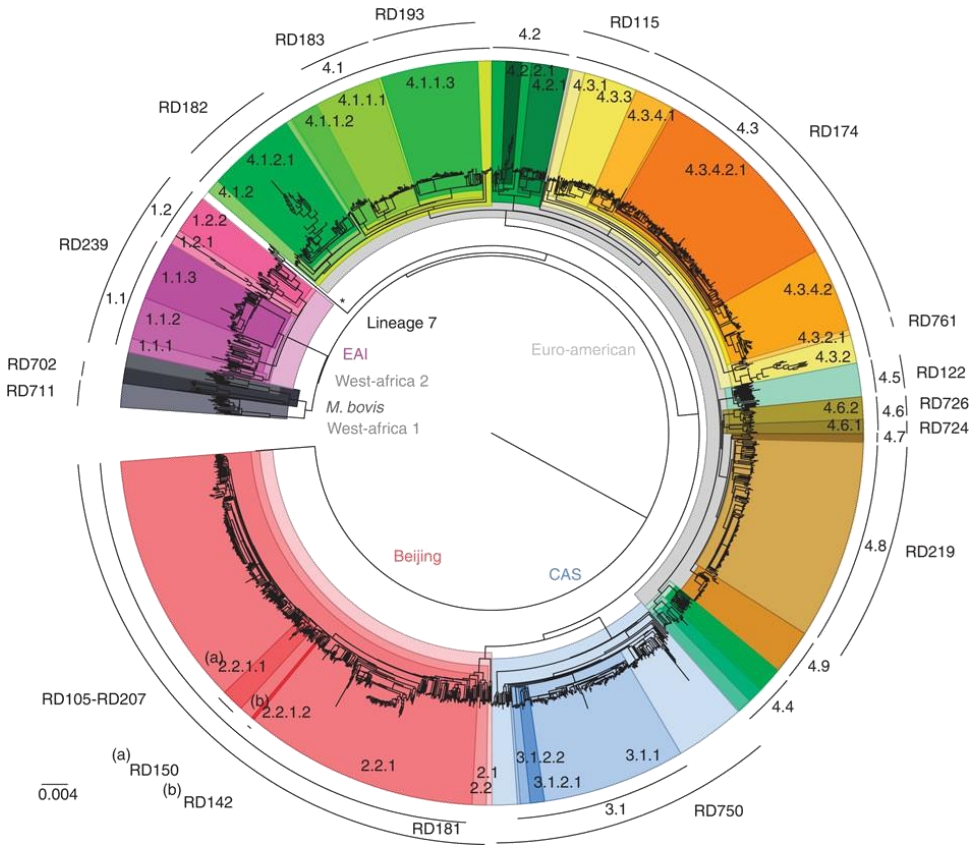
Received 11 Apr 2014 | Accepted 25 Jul 2014 | Published 1 Sep 2014 DOI: 10.1038/ncomms5812 OPEN

A robust SNP barcode for typing *Mycobacterium tuberculosis* complex strains

Francesc Coll[1], Ruth McNerney[1], José Afonso Guerra-Assunção[2], Judith R. Glynn[2], João Perdigão[3], Miguel Viveiros[4], Isabel Portugal[3], Arnab Pain[5], Nigel Martin[6] & Taane G. Clark[1,2]

:: 1601 *M. tuberculosis* genomes

:: 62 SNP markers for strain barcoding



Coll *et al*, 2014

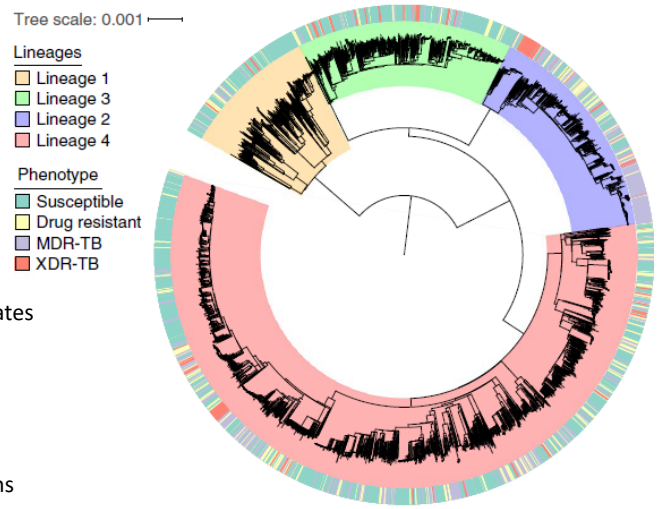
GWAS –Tuberculosis and Drug resistance

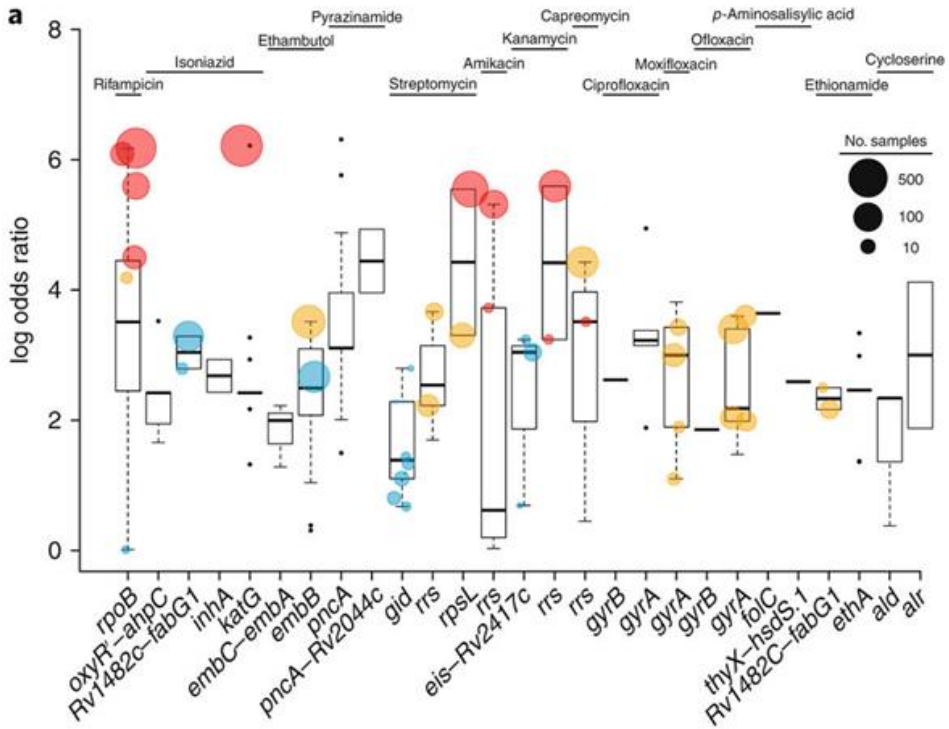
A Framework for Genome-Wide Association Studies (GWAS):

nature genetics ARTICLES
https://doi.org/10.1038/s41588-017-0029-0

Genome-wide analysis of multi- and extensively drug-resistant *Mycobacterium tuberculosis*

Francesc Coll[1], Jody Phelan[1], Grant A. Hill-Cawthorne[2,3], Mridul B. Nair[2], Kim Mallard[1], Shahjahan Ali[2], Abdallah M. Abdallah[2], Saad Alghamdi[4], Mona Alsomali[2], Abdallah O. Ahmed[5], Stephanie Portelli[1,6], Yaa Oppong[1], Adriana Alves[7], Theolis Barbosa Bessa[8], Susana Campino[1], Maxine Caws[9,10], Anirvan Chatterjee[11], Amelia C. Crampin[12,13], Keertan Dheda[14], Nicholas Furnham[1], Judith R. Glynn[12,13], Louis Grandjean[15], Dang Minh Ha[10], Rumina Hasan[16], Zahra Hasan[16], Martin L. Hibberd[1], Moses Joloba[17], Edward C. Jones-López[18], Tomoshige Matsumoto[19], Anabela Miranda[7], David J. Moore[13,15], Nora Mocillo[20], Stefan Panaiotov[21], Julian Parkhill[22], Carlos Penha[23], João Perdigão[24], Isabel Portugal[24], Zineb Rchiad[5], Jaime Robledo[25], Patricia Sheen[14], Nashwa Talaat Shesha[26], Frik A. Sirgel[27], Christophe Sola[28], Erivelton Oliveira Sousa[8,29], Elizabeth M. Streicher[27], Paul Van Helden[27], Miguel Viveiros[30], Robert M. Warren[27], Ruth McNerney[1,14]*, Arnab Pain[2,31]* and Taane G. Clark[1,12]*





:: 6 465 clinical isolates

:: > 35 countries

:: 102 106 SNPs

:: 11 122 Indels

:: 284 large deletions

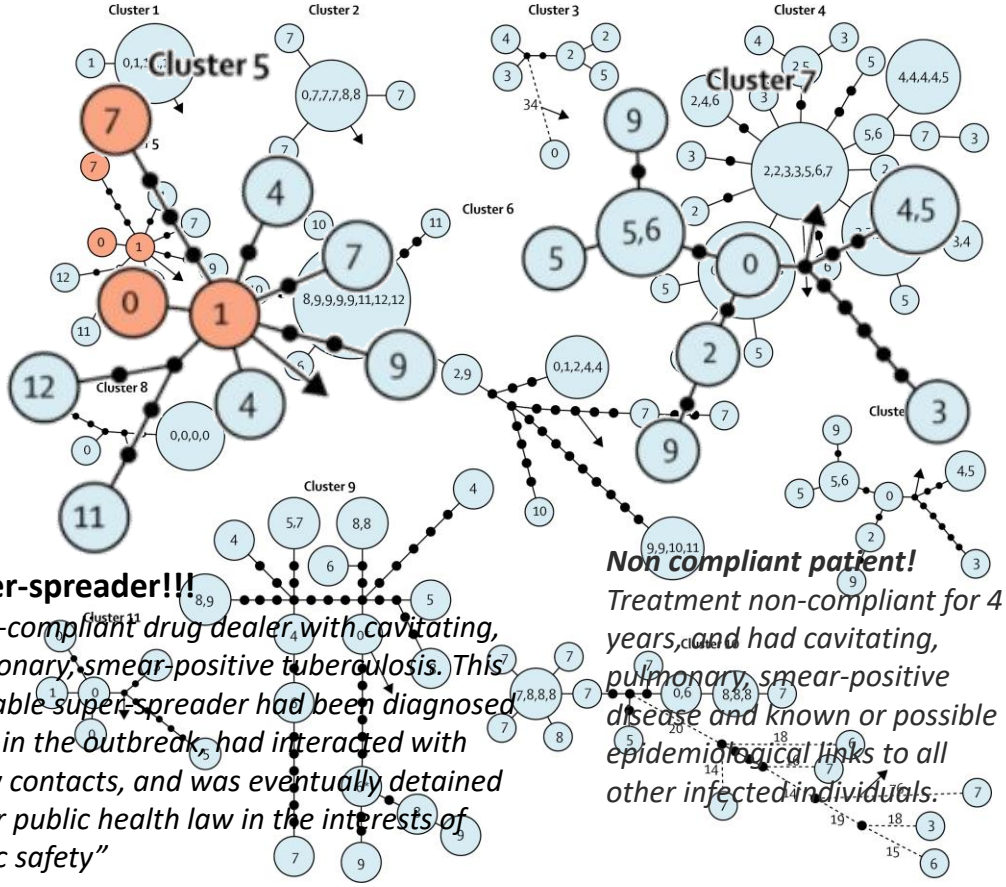
WGS as a Public Health Tool

Whole-genome sequencing can delineate outbreaks of tuberculosis and allows inference about direction of transmission between cases.

≤ 5 SNPs – strong likelihood for epidemiological link

5-12 SNPs – uncertain!

≥ 12 SNPs - Epidemiological link unlikely



Super-spreader!!!

"Non-compliant drug dealer with cavitating, pulmonary, smear-positive tuberculosis. This probable super-spreader had been diagnosed early in the outbreak, had interacted with many contacts, and was eventually detained under public health law in the interests of public safety"

Non compliant patient!

Treatment non-compliant for 4 years, and had cavitating, pulmonary, smear-positive disease and known or possible epidemiological links to all other infected individuals.

Walker *et al* 2013

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Integrating Molecular Epidemiology in Clinical Management and Public Health

Investigate cross-contamination, exogenous reinfection, relapse and outbreaks.

- Negative acid-fast smears;
- Only one specimen culture positive;
- Clinical findings not supportive of TB;

Genotype concurrently handled strains in the labopratory

Contamination?

If yes,

Discontinuation of therapy!

- Same patient;
- Different patterns of drug susceptibility (after or during treatment);
- Resistance development or reinfection or cross-contamination

- Second TB episode;
- Relapse or reinfection?

Resistance Development

Non-adherence?
Low drug concentration?
(malabsorption,drug interactions)

Reinfection

Identify Source!!

Highly important in drug trials!

Relapse

Evaluate treatment failure
Susceptibility of original isolate, etc.

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Case Study II: Recurrence, Relapse or Reinfection?

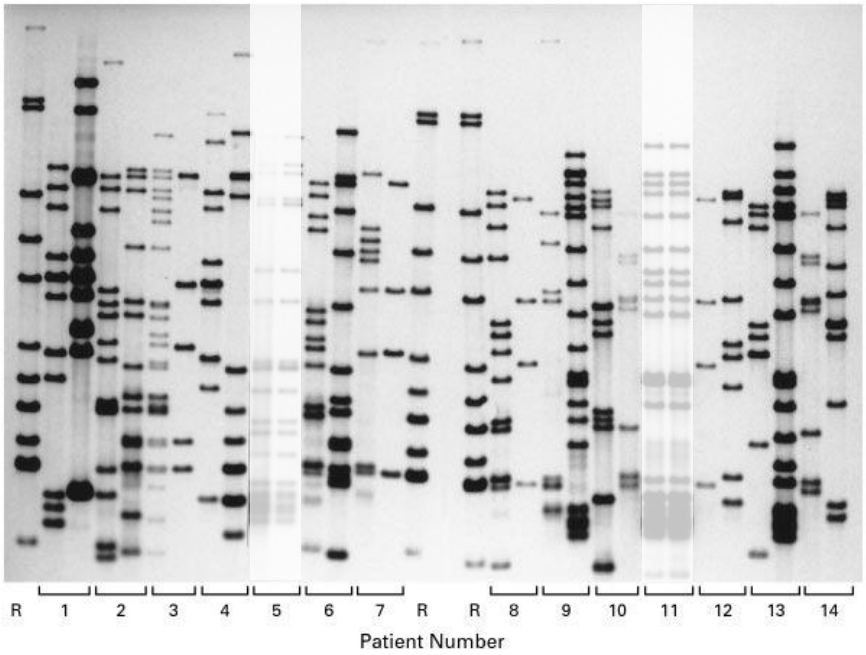
ORIGINAL ARTICLE

Exogenous Reinfection as a Cause of Recurrent Tuberculosis after Curative Treatment

Annelies van Rie, M.D., Robin Warren, Ph.D., Madeleine Richardson, M.Sc., Thomas C. Victor, Ph.D., Robert P. Gie, M.D., Donald A. Enarson, M.D., Nulda Beyers, Ph.D., and Paul D. van Helden, Ph.D.

We performed DNA fingerprinting with restriction-fragment–length polymorphism analysis on pairs of isolates of Mycobacterium tuberculosis from 16 compliant patients who had a relapse of pulmonary tuberculosis after curative treatment of postprimary tuberculosis.

*For **12 of the 16 patients**, the restriction-fragment–length polymorphism banding patterns for the isolates obtained after the relapse were different from those for the isolates from the initial tuberculous disease. This finding indicates that reinfection was the cause of the recurrence of tuberculosis after curative treatment.*



Case Study II: Recurrence, Relapse or Reinfection?

Patients were examined 3 and 6 months after cure, and then were monitored by the routine tuberculosis surveillance system until December, 1998. IS6110 DNA fingerprints from initial and subsequent episodes of tuberculosis were compared to determine whether recurrence was due to relapse or reinfection

Paired DNA fingerprints were available in 39 of 65 recurrences: 25 pairs were identical (relapse) and 14 were different (reinfection). 93% (13/14) of recurrences within the first 6 months were attributable to relapse compared with 48% (12/25) of later recurrences.

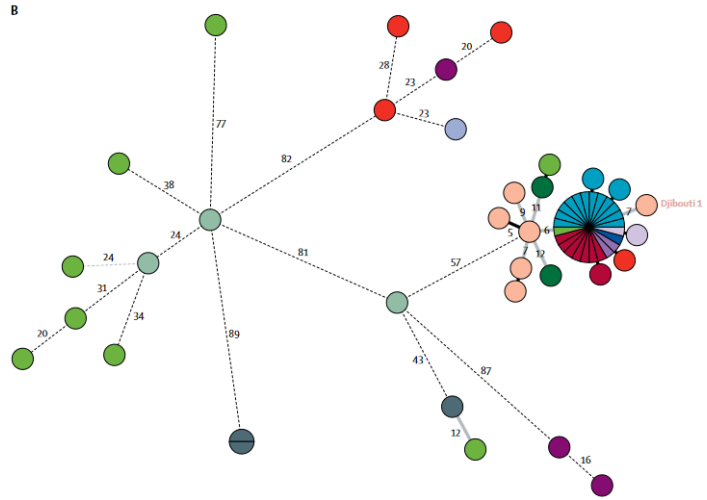
Case Study III: International, Migration-Driven Clone Dissemination

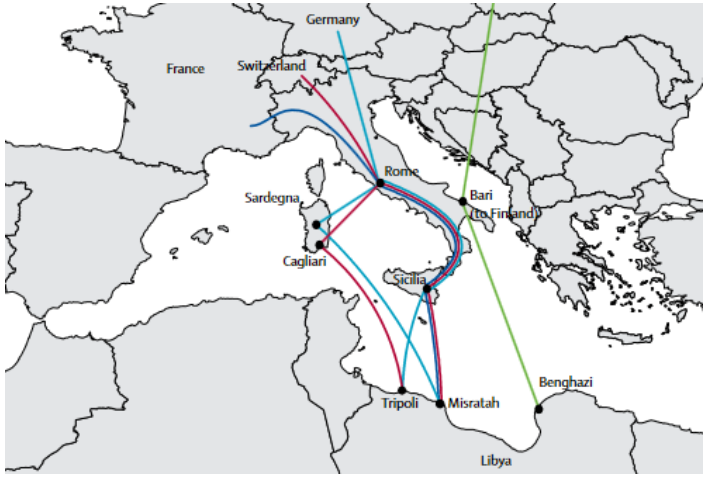
A cluster of multidrug-resistant *Mycobacterium tuberculosis* among patients arriving in Europe from the Horn of Africa: a molecular epidemiological study

Timothy M Walker, Matthias Merker*, Astrid M Knoblauch*, Peter Helbling, Otto D Schoch, Marieke J van der Werf, Katharina Kranzer, Lena Fiebig, Stefan Kröger, Walter Haas, Harald Hoffmann, Alexander Indra, Adrian Egli, Daniela M Cirillo, Jérôme Robert, Thomas R Rogers, Ramona Groenheit, Anne T Mengshoel, Vanessa Mathys, Marjo Haanperä, Dick van Soolingen, Stefan Niemann†, Erik C Böttger†, Peter M Keller†, and the MDR-TB Cluster Consortium‡*

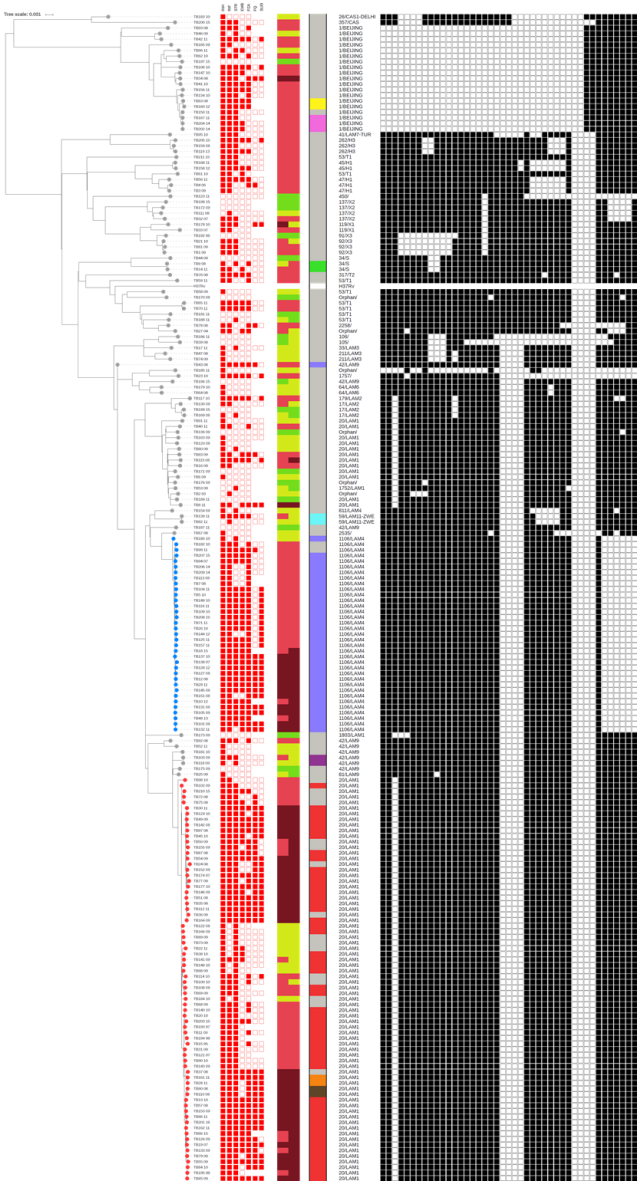
On April 29 and May 30, 2016, the Swiss and German National Mycobacterial Reference Laboratories independently triggered an outbreak investigation after four patients were diagnosed with multidrug-resistant tuberculosis. In this molecular epidemiological study, we prospectively defined outbreak cases with 24-locus mycobacterial interspersed repetitive unit-variable number tandem repeat (MIRU-VNTR) profiles

Between Feb 12, 2016, and April 19, 2017, 29 patients were diagnosed with multidrug-resistant tuberculosis in seven European countries. All originated from the Horn of Africa or Sudan, with all isolates two SNPs or fewer apart. 22 (76%) patients reported their travel routes, with clear spatiotemporal overlap between routes. We identified a further 29 MIRU-VNTR-linked cases from the Horn of Africa that predated the outbreak, but all were more than five SNPs from the outbreak.





Genomic Population Structure in Lisbon, Portugal: an Overview



Genome-wide Phylogenetic Scenario based on 28 051 SNPs

:: N= 207 Clinical Isolates

:: Analysis Period: 1995-2016

:: Two main clades: Lisboa3 (n=72) and Q1 (n=35)

:: 16 Genomic Clusters (≤ 5 SNPs): 92 (44.4%) isolates

GC67/Q1
N=26
SIT1106/LAM4

GC8 Lisboa3
N=17
SIT20/LAM1

GC5/ Lisboa3
N=13
SIT20/LAM1

GC3/Lisboa3
N=6
SIT20/LAM1

GC160/Q1
N=4
SIT1106/LAM4

GC7/Lisboa3
N=4
SIT20/LAM1

Drug Resistance:
101 MDR-TB
49 XDR-TB
38 Other Resistance
19 Susceptible

WGS and Microevolution towards Drug Resistance

Microevolutionary trajectory of the Lisboa3 clade shows multiple development of M/XDR-TB

Lisboa3

INH/STR
inhA C-15T/S94A
rpsL K43R

1973
RIF
rpoB S531L

1990
EMB/FQ/KAN
embB P397T
gyrA S91P
eis G-10A

FQ
gyrA D94G

CAP
tlyA Ins755GT

PZA
pncA
various mutations

RIF
rpoB S531L

RIF
rpoB S531L

EMB
embB M306I

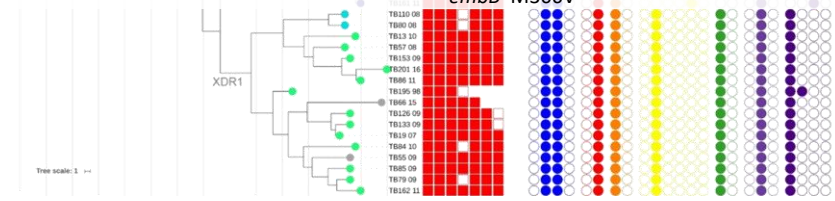
RIF 1981
rpoB D516V

RIF 1975
rpoB S531L

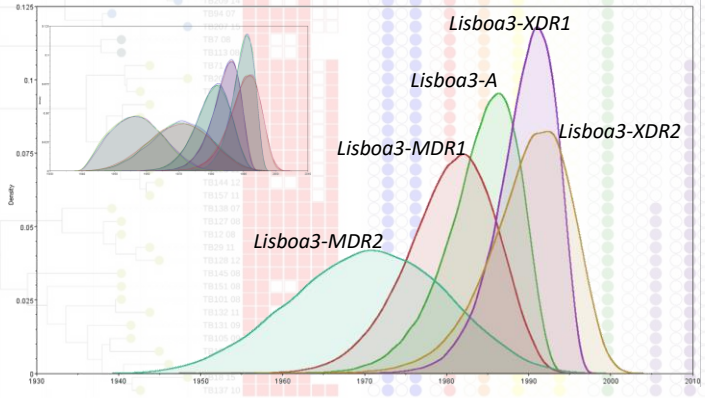
FQ
gyrA D94G

1989
EMB/PZA/KAN
embB M306V
pncA L120P
eis G-10A

EMB
embB M306V



Microevolutionary trajectory of the Q1 clade shows sequential development of M/XDR-TB



Bayesian Dating Estimates (BEAST)
Uncorrelated lognormal clock
Four 50 million MCMC Runs

Q1

1968
INH/STR
inhA C-15T/I194T
gidB A80P

1977
RIF/EMB/PZA
rpoB S531L
embB M306V
pncA V125G

1994
FQ
gyrA D94A

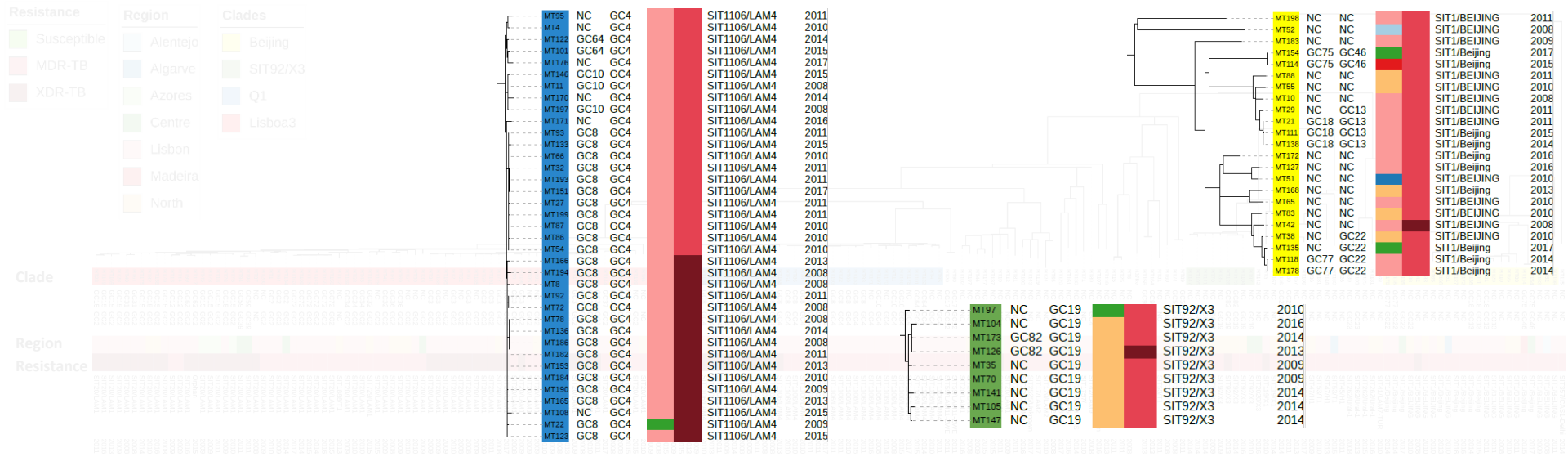
SLID
rrs A1401G

MDR-TB Trends in Portugal: comparing two time periods…

Two time-periods: 2008-2011 and 2013-2017

Whole-genome sequence-based genotyping for 193 MDR-TB isolates nationwide (*115.6% [+26] of notified isolates****)***

Four main clades of interest: Lisboa3, Q1, SIT92/X3 and Beijing strains (*Total: 141/193 [73.1%]****)***



Lisboa3
73 isolates
39 XDR-TB cases

Q1
37 isolates
16 XDR-TB cases

SIT92/X3
9 isolates
1 XDR-TB case

Beijing
22 isolates
1 XDR-TB case

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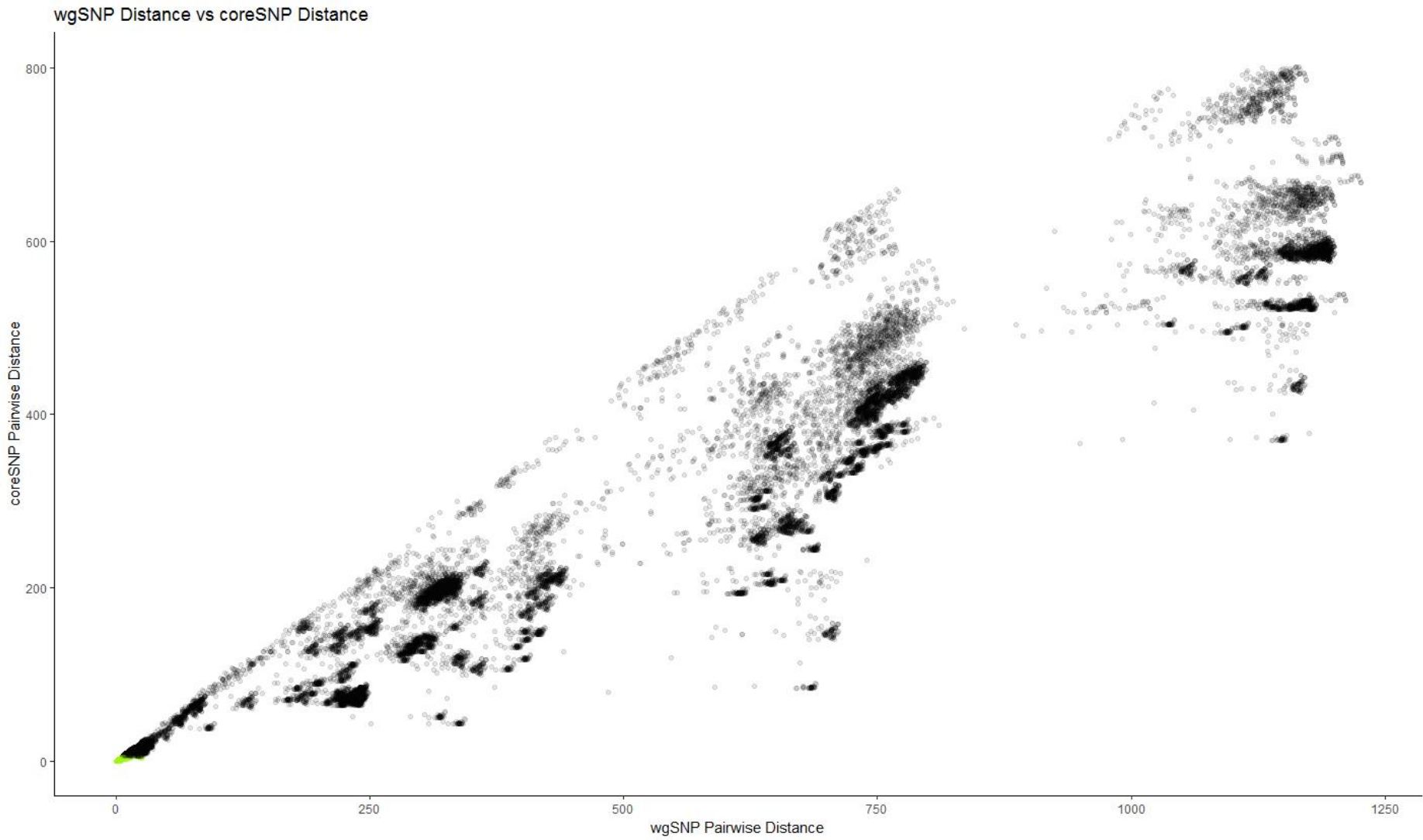
wgSNP Distance vs coreSNP distance: global view



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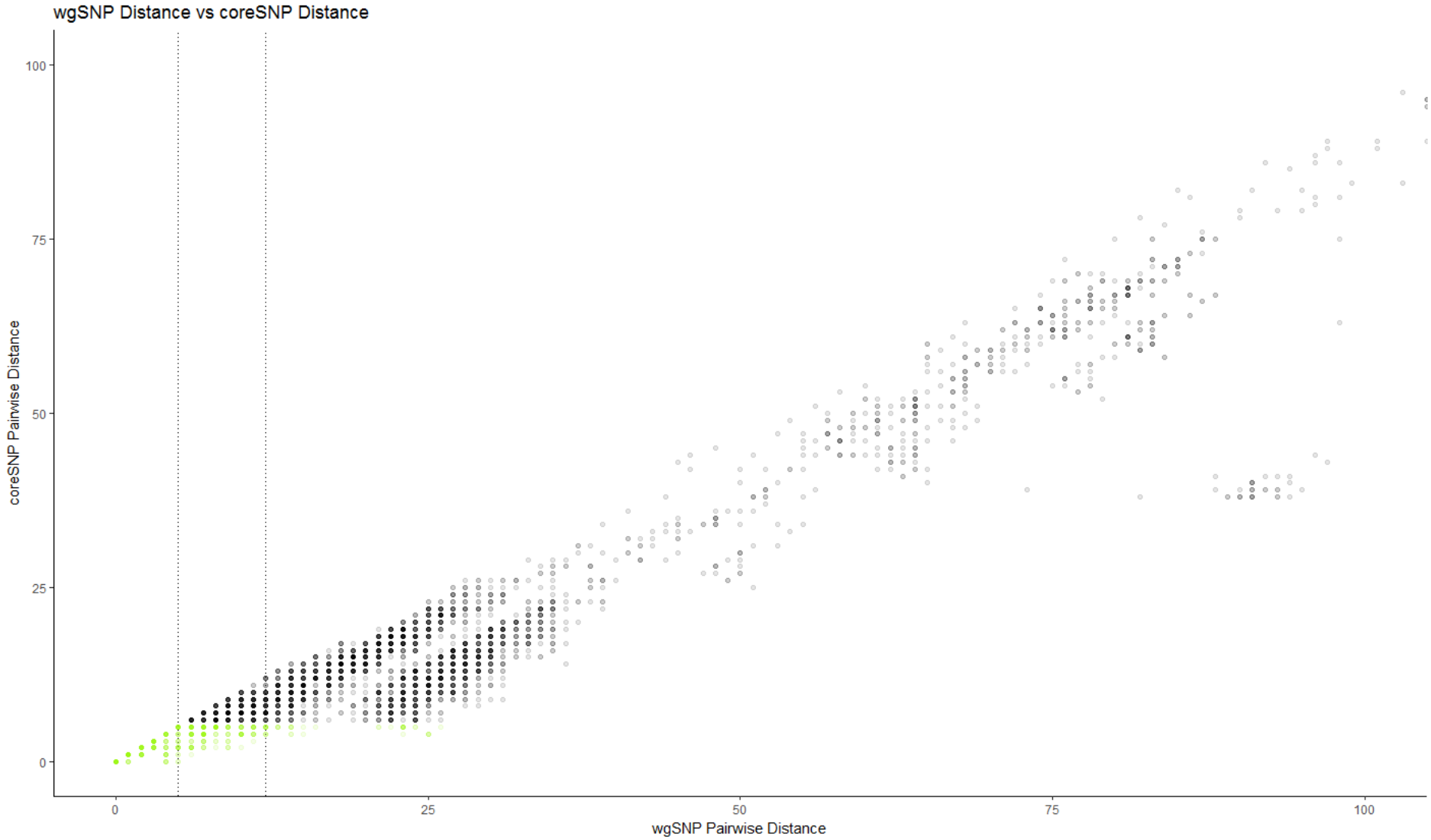
wgSNP Distance vs coreSNP distance: closer look



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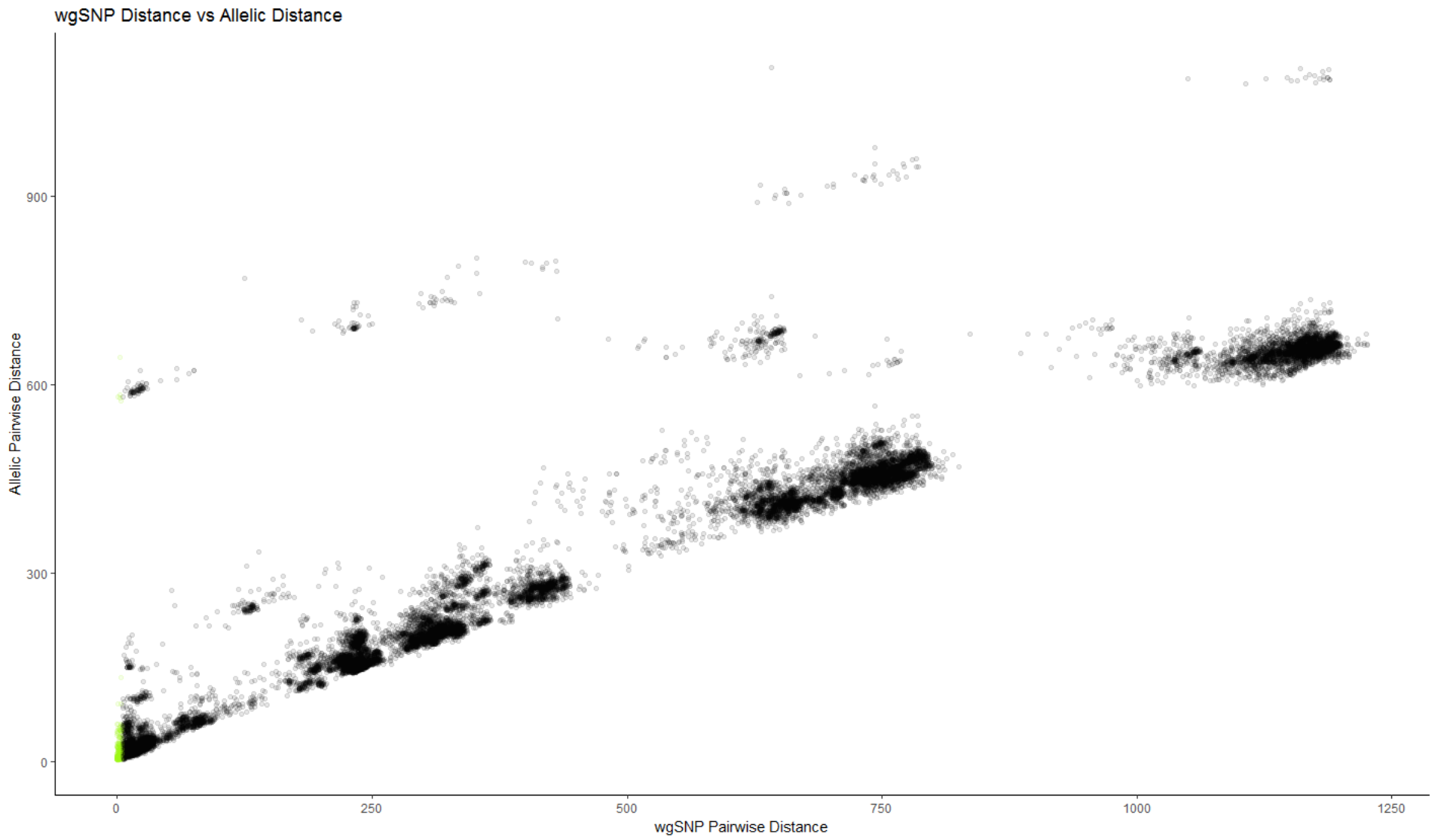
wgSNP Distance vs Allelic distance: global view



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wgSNP Distance vs Allelic distance: closer look