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HIV/TB Agency, Information and Services Activity

At a glance on Genetic Mapping of *Mycobacterium tuberculosis*

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Outlines

1. Tuberculosis disease and structure of bacteria (MTB)
2. Genome of MTB and molecular genotypic methods
3. Evolution of *Mycobacterium tuberculosis* (MTB)
4. Review on the distribution of Major lineages of MTB and associated factors

Tuberculosis disease



L The Lancet
ancient Egyptian m...



NS New Scientist
mummy show TB killed ancient ...



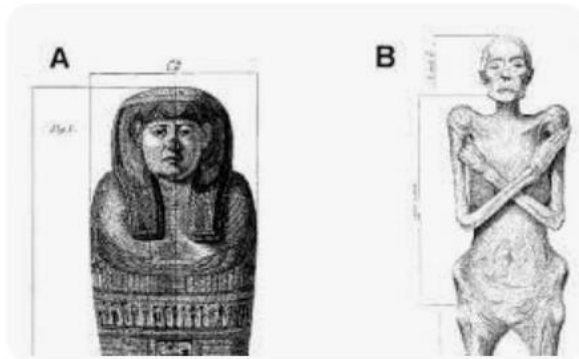
VOA VOA News
Tuberculosis Bacteria Found to Inhabit ...



R ResearchGate
spine of Egyptian mummies ...



NPR NPR
Early Humans Left Africa ...



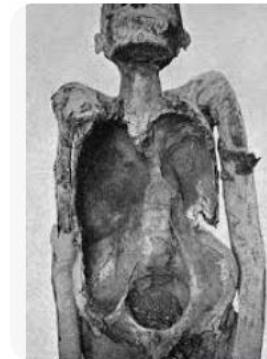
The Guardian
Fresh autopsy of Egyptian mummy shows ...



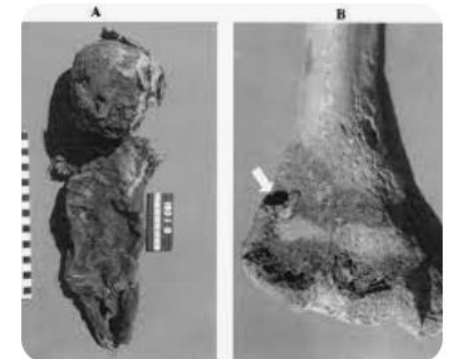
Pavilion Health Today
TB DNA from mummy could shed new li...



SN Science News
Mummies tell tuberculosis tales...



Strange Remains
Tuberculosis bacter...

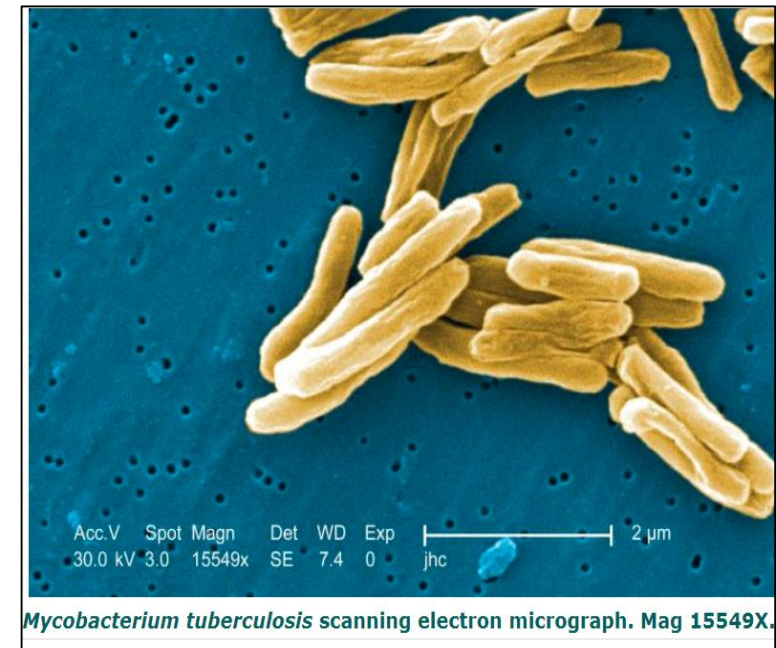
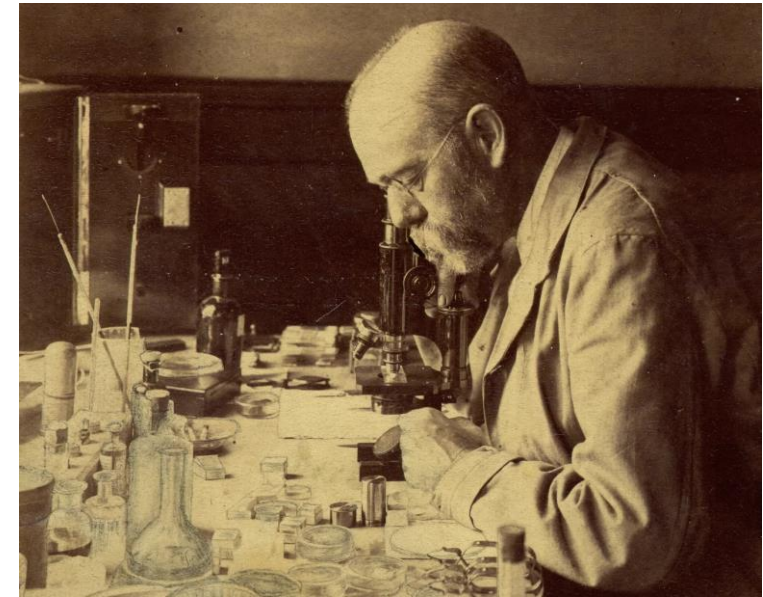


ASM Journals - American Societ...
Egyptian Mummies by Spoligo...

Tuberculosis has long been recognized in Egyptian mummies (Pott's disease), and *Mycobacterium tuberculosis complex* DNA has been detected

Structure of bacteria (MTB)

- ❖ TB is mainly caused by *Mycobacterium tuberculosis* (MTB)
- ❖ A fairly large non-motile rod-shaped bacterium
- ❖ Rods size of 2-4 μm in length and 0.2-0.5 μm in width.
- ❖ Aerobic and non-spore forming
- ❖ Slow generation time, 15-20 hours, a physiological characteristic that may contribute to its chronic nature.
- ❖ Cell walls with a high lipid content includes waxes - mycolic acid, improving survival.
- ❖ This cell wall lipid allows Zeil Nelseen stain to get through and not removed by acid-alcohol --- , Acid-fast bacteria

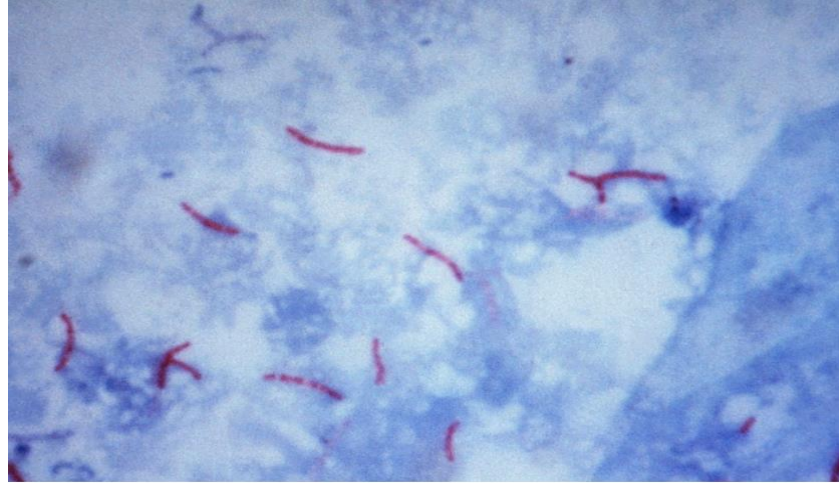


Diagnosis of TB

- ❖ ZN stain microscopy
- ❖ Fluorescence Microscope
- ❖ Solid culture
- ❖ Liquid culture

Limitation/Cannot know:

- Different strains/lineage
- Transmission link
- Relapse/re-infection
- Drug resistance, etc.

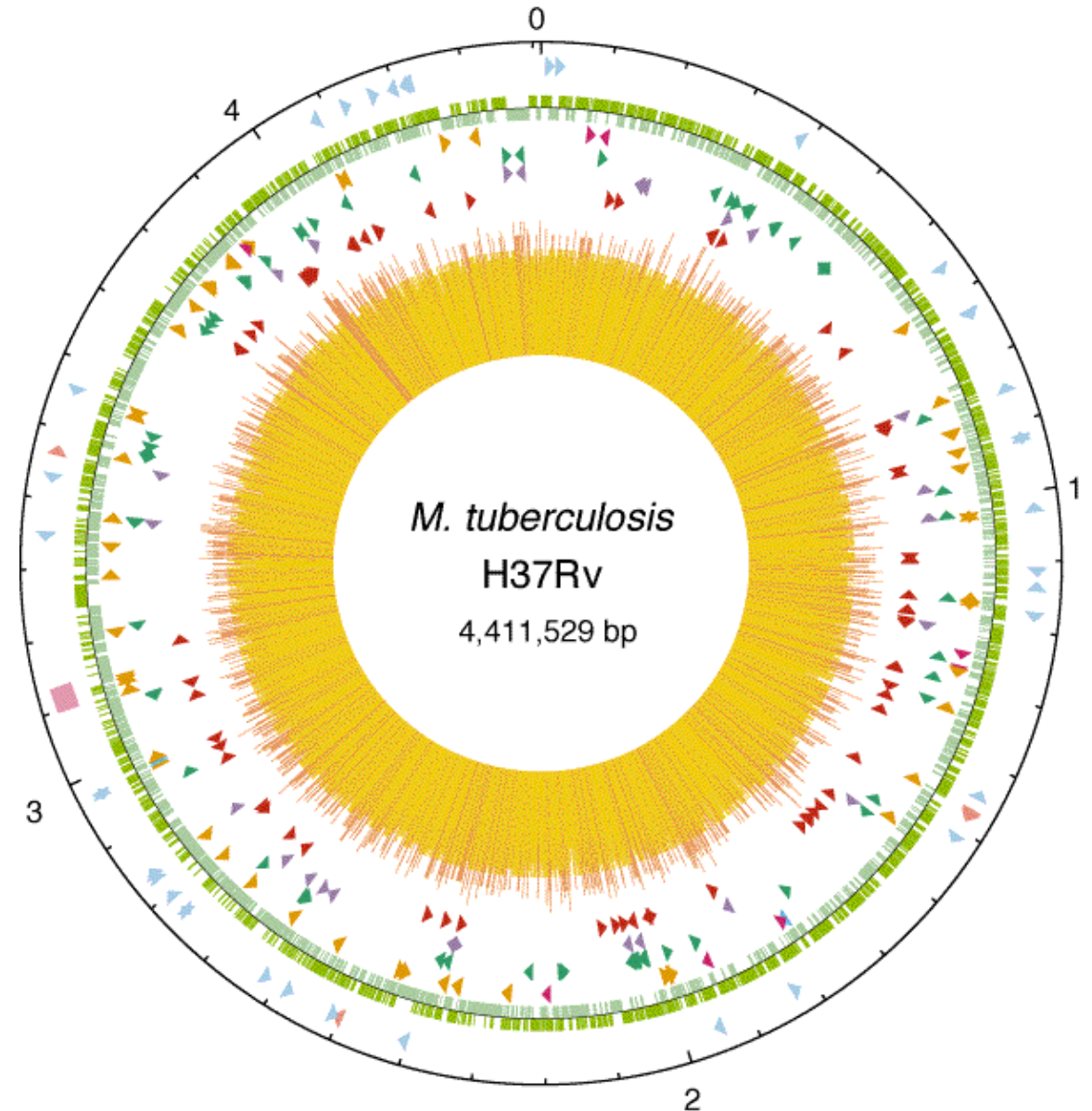


Visualization using the Ziehl–Neelsen stain



Genome of MTB

- ❖ Contain about 4,000 genes.
- ❖ The circular genome comprises of 4,411,529 nucleotides long
- ❖ 4 types of DNA* nucleotides
 - ❖ Thymine (T) binds Adenine (A)
 - ❖ Guanine (G) bind Cytosine (C)



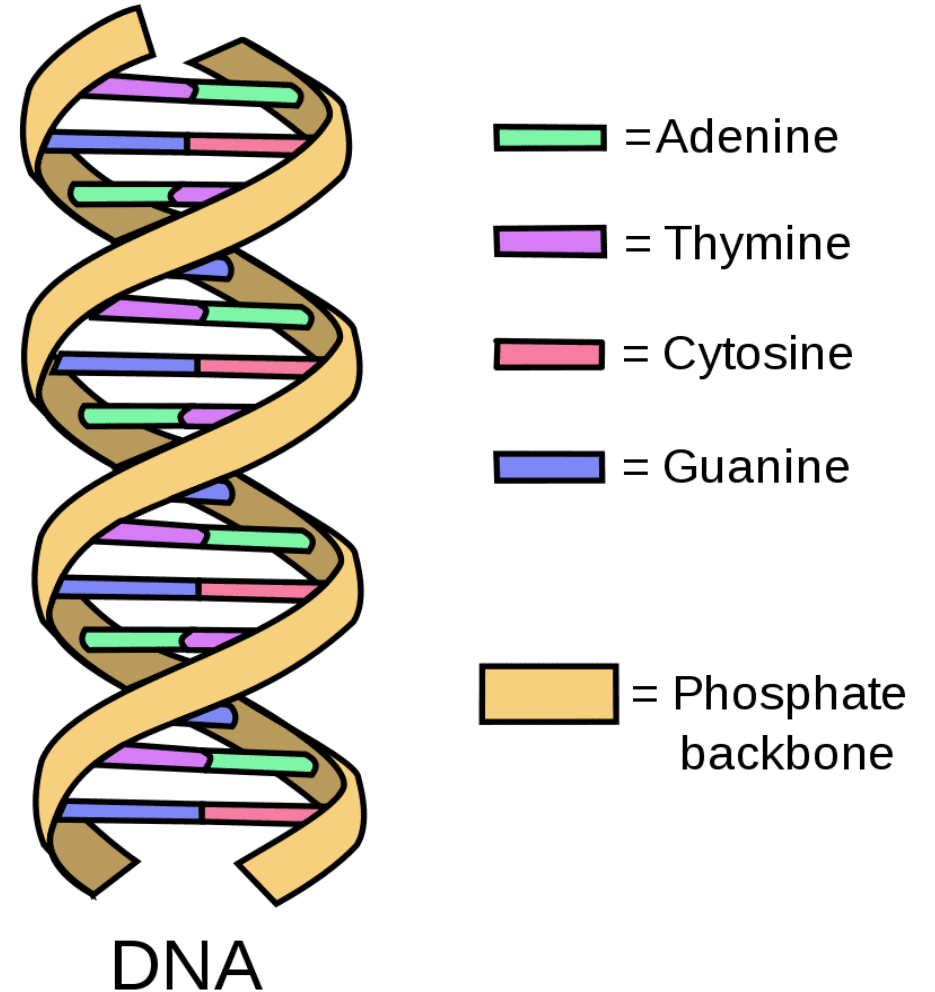
Gene and DNA

Gene

- ❖ A gene is the basic physical and functional unit of heredity
- ❖ Genes are made up of Deoxyribonucleic acid (DNA)

DNA

- ❖ The molecule that carries genetic information for the development and function of an organism
- ❖ DNA is a double helix formed by based pairs attached to a sugar-phosphate backbone

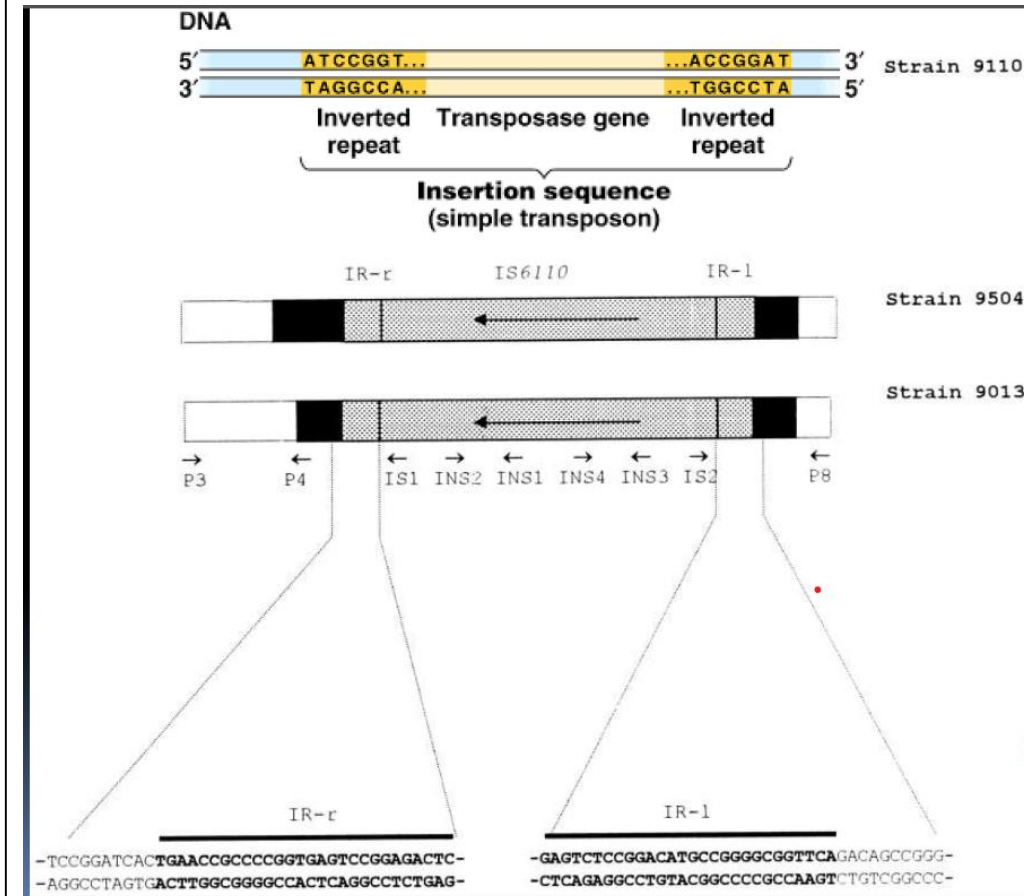


Main methods of MTB molecular genotyping

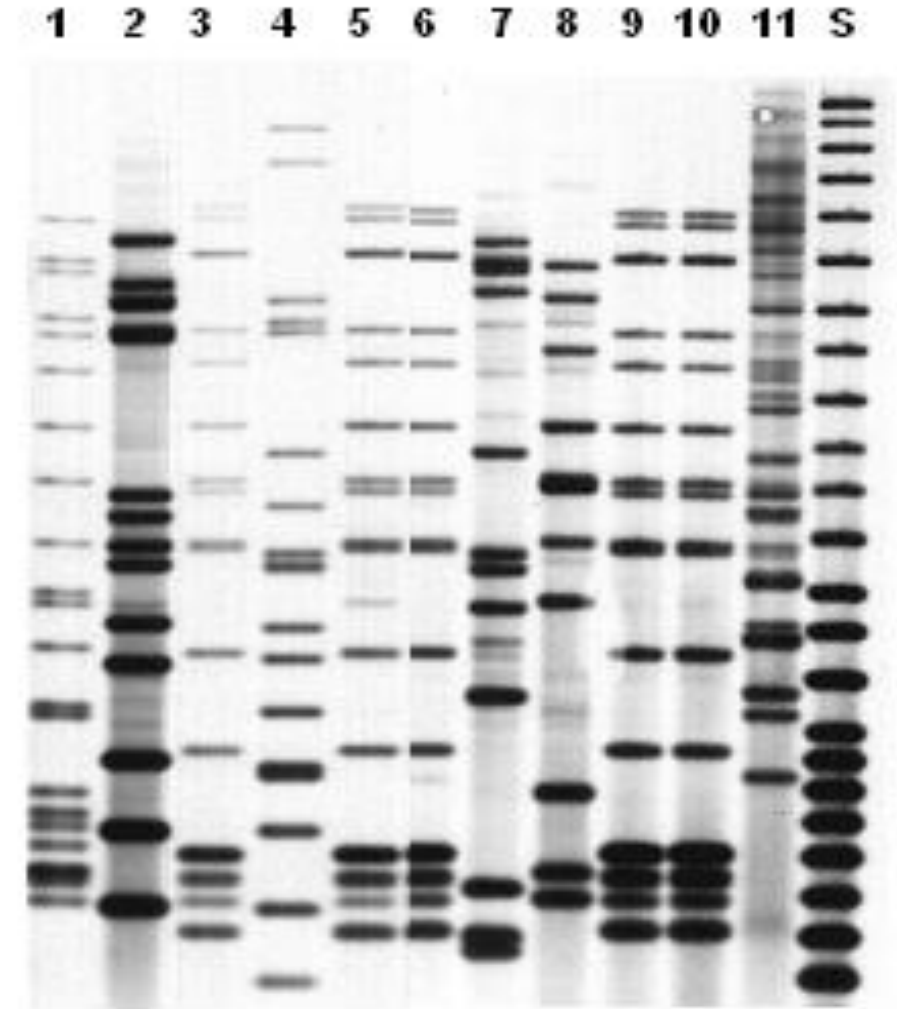
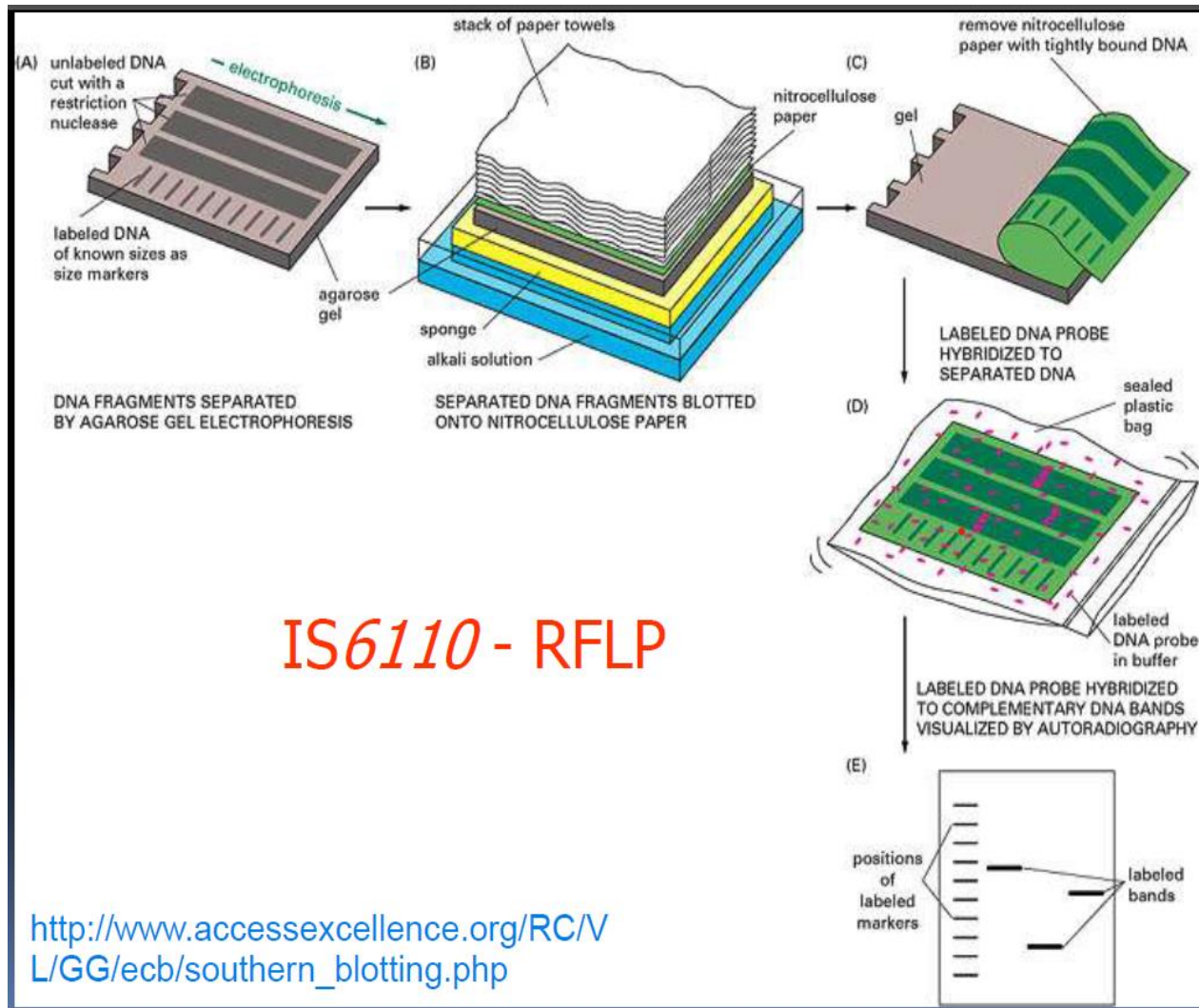
- ❖ **Molecular TB genotyping** is a laboratory-based approach used to analyze the genetic material (e.g., DNA) of Mtb
- ❖ **Genotyping** determines differences in genetic complement by comparing a DNA sequence to that of another sample or a reference sequence.
- ❖ It identifies small variations in genetic sequence within populations, such as single-nucleotide polymorphisms (SNPs).
- ❖ Availability of DNA and especially PCR-based methods, genotyping became the new gold standard for species and strain differentiation.
- ❖ These chronologically developed methods are;
 - ❖ **IS6110 RFLP** (Restriction Fragment Length Polymorphism)
 - ❖ **Spoligotyping**
 - ❖ **MIRU-VNTR** (Mycobacterial Interspersed Repetitive Units - Multiple Loci Variable Number of Tandem Repeats)
 - ❖ **WGS** (Whole Genome sequencing).

IS6110 RFLP (Restriction Fragment Length Polymorphism)

- ❖ Based on a repetitive DNA element called insertion sequence 6110 (IS6110)
- ❖ IS6110 is an insertion element containing only a transposase gene.
- ❖ Can identify outbreaks and chains of transmission of TB
- ❖ labor-intensive, time consuming and needs a lot of high-quality DNA (4.5µg).
- ❖ Not ideally suited for strain classification
- ❖ Produces a complex pattern and cannot translate into a specific numbered designation
- ❖ lacks a format to be easily exchanged between laboratories (Comparison across multiple labs are difficult)



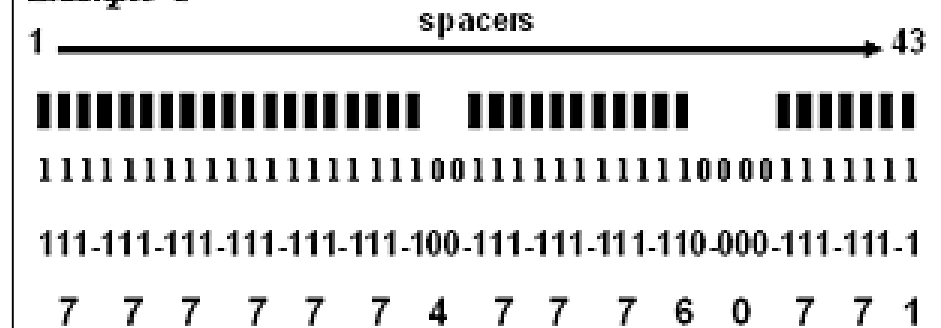
IS6110 RFLP (Restriction Fragment Length Polymorphism)



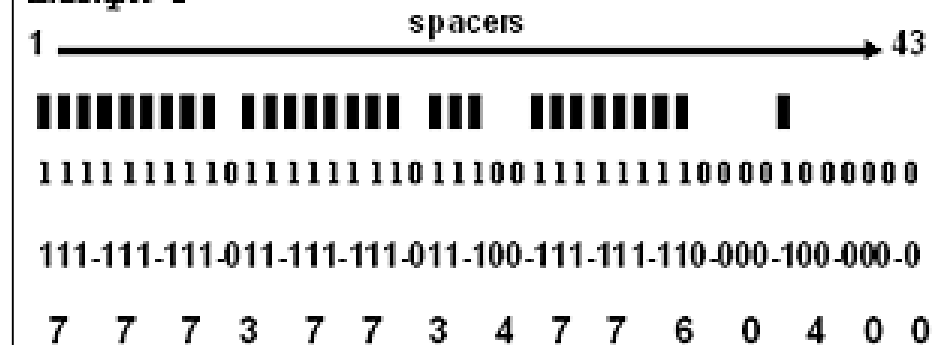
Spoligotyping

- ❖ Spoligotyping takes advantage of the **Direct Repeat (DR)** locus of the **MTB** genome
- ❖ Testing for the presence or absence of each of the **43 spacers**
- ❖ It can translate into a specific numbered designation
- ❖ The method is easy and required low **DNA (10ng)**
- ❖ Discriminating power is poorest
- ❖ Constructing phylogenies are limited value

Example 1



Example 2



Octal Code Key

000= 0
001= 1
010= 2
011= 3
100= 4
101= 5
110= 6
111= 7

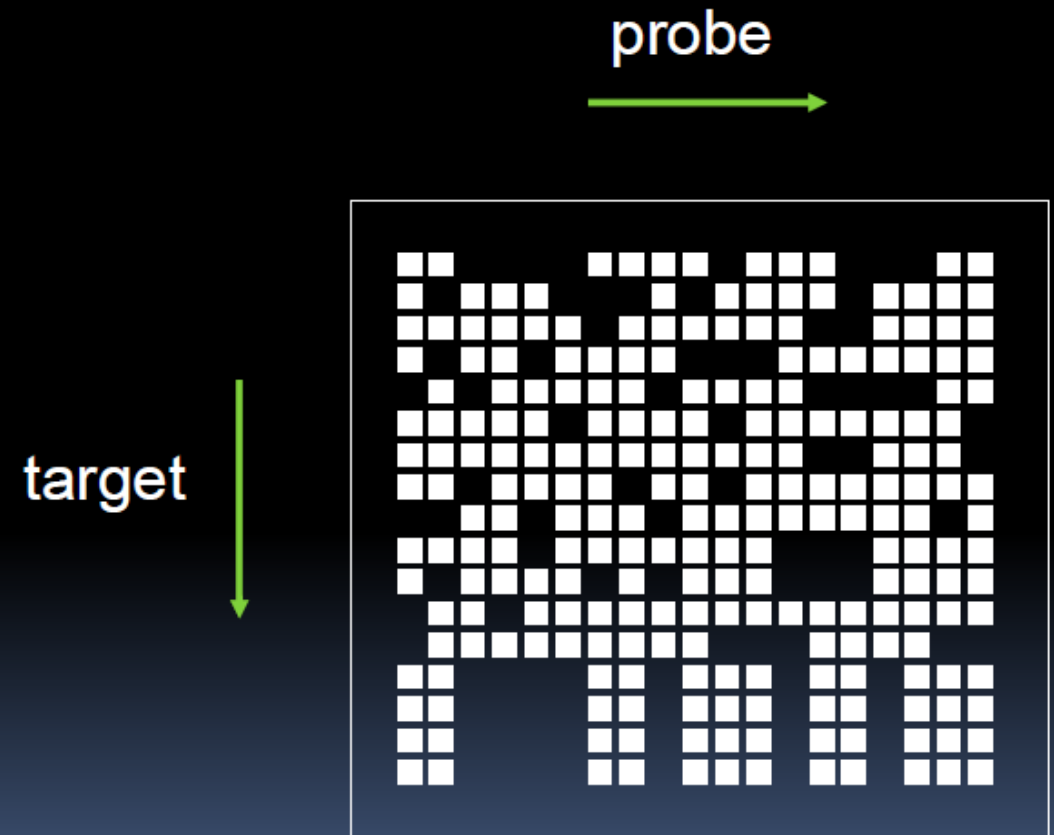
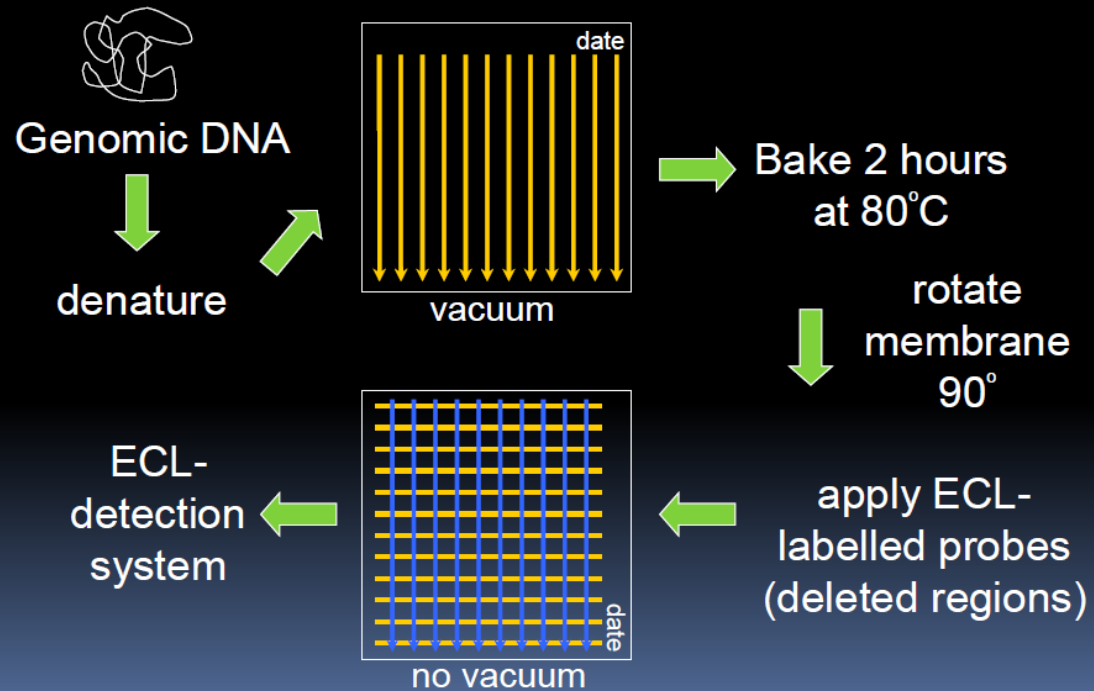
Spacer 43

0= 0
1= 1

Spoligotyping

Spoligotyping can be done by
“macroarray”

Slot-blot procedure (cont.)



Spoligotyping

- Hybridization patterns (spots)
- Binary pattern (43-digit) : 0 (deletion), 1 (presence)
- Octal code (15-digit) : 14 sets [0-7] + 1 digit [0/1]

0 = 000

1 = 001

2 = 010

3 = 011

4 = 100

5 = 101

6 = 110

7 = 111



1111111000011111111111111111111111000010111111111 = 774177777413771

Spoligo.Pu30
mem.3
29-11-08

Spoligotyping

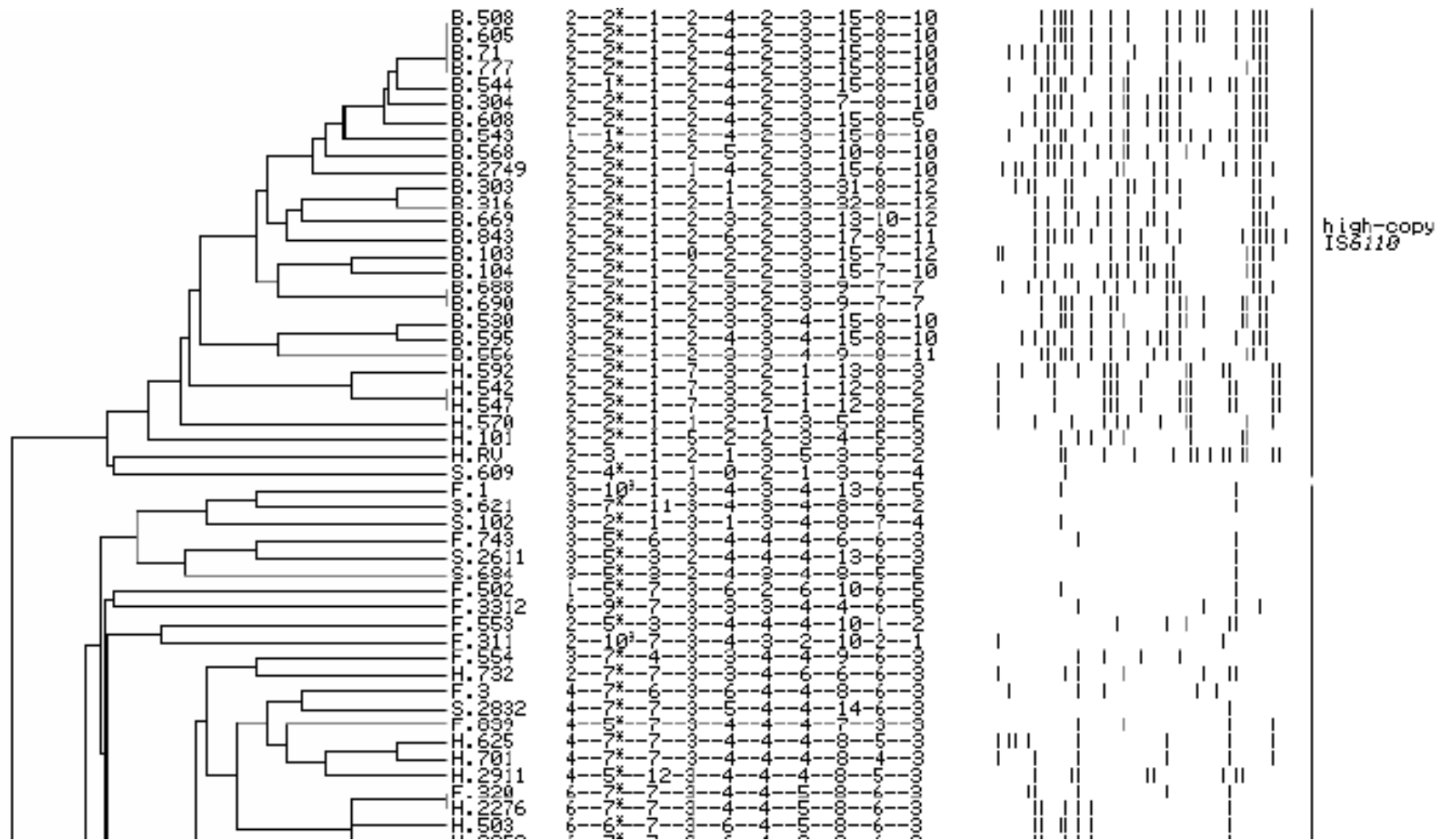
Sub Lineage*	SIT ^b	Spoligotype Description ^c	Octal Number	No. of isolates	% of isolates
Lineage 1 (Indo-Oceanic Lineage)					
EAI3_IND	11		477777777413071	16	18.8
EAI1_SOM	48		777777777413731	2	1.2
EAI3_IND	355		477777777413031	1	1.2
EAI5	126		477777777413771	1	1.2
EAI5	962		777777777413031	1	1.2
EAI5	1957		477777777013771	1	1.2
EAI2_MANILLA	19		677777477413771	1	1.2
EAI6_BGD1	2908		777777757413671	1	1.2
New type 5			777775747413671	1	1.2
Lineage 2 (East-Asian Lineage)					
Beijing	1		000000000003771	19	22.4
Beijing	190		000000000003731	1	1.2
Lineage 3 (Central-Asian Lineage)					
CAS1-Delhi	26		703777740003771	1	1.2
Lineage 4 (Euro-American Lineage)					
Undesignated	124		77777777700771	8	9.4
Undesignated	3234		777777777600371	8	9.4
Undesignated	1952		7777777774000771	4	4.7
H2	2		000000004020771	3	3.5
H3	50		77777777720771	3	3.5
T1	53		77777777760771	2	2.4
New type 1			77777777700671	2	2.4
X2	478		61777677760601	2	2.4
New type 2	orphan		7777777774000731	2	2.4
H3	49		77777777720731	1	1.2
T1	823		776000003760771	1	1.2
T1	519		77777777740371	1	1.2
New type 3			77770377760700	1	1.2
New type 4			7777777774100751	1	1.2
^a Sub lineages were annotated using the SITVITWEB database. ^b SIT (Spoligo International Types) were assigned by SITVITWEB database. ^c Closed squares represent positive hybridization (presence of spacer) and open squares represent no hybridization (absence of spacer). Undesignated: Spoligotype pattern is available in SITVIT 2 database with SIT number but the sub lineage is not assigned; New type: Spoligotype pattern found in our study.					

MIRU-VNTR (Mycobacterial Interspersed Repetitive Units - Multiple Loci Variable)

- ❖ **MIRUs are tandemly repeated DNA elements which are dispersed in inter- and intragenic regions of the genome.**
- ❖ **Genotyping using a standardized set of 24 loci**
- ❖ **The number of tandem repeats per locus varies from strain to strain, and can be presented in a numeric way**
- ❖ **In rare instances, the number of repeats is greater than 9.**
- ❖ **The method is still labor-intensive**

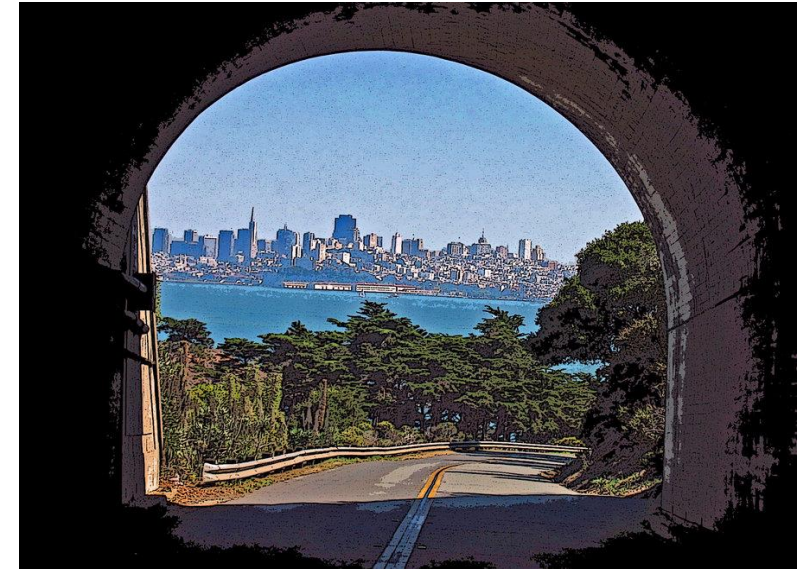
MIRU 02	MIRU 04	MIRU 10	MIRU 16	MIRU 20	MIRU 23	MIRU 24	MIRU 26	MIRU 27	MIRU 31	MIRU 39	MIRU 40	424	577	1955	2163	2165	2347	2401	2461	3171	3690	4052	4156
2	2	5	3	2	5	1	5	3	3	2	3	2	3	4	5	3	4	4	2	3	4	6	3
2	2	5	3	2	5	1	5	3	3	2	3	2	3	4	4	3	4	4	2	3	4	7	3
2	2	5	3	2	5	1	5	3	3	2	3	2	3	3	5	3	4	4	2	3	3	5	3
2	2	5	3	2	5	1	5	3	3	2	3	1	3	3	5	3	4	4	2	3	3	5	3
2	2	5	3	2	5	1	5	3	3	2	3	1	3	3	6	3	4	4	2	3	3	5	3
2	2	5	3	2	5	1	5	3	3	2	3	2	3	3	3	3	4	4	2	3	3	5	3
2	2	5	3	2	5	1	5	3	3	2	3	2	3	3	3	3	4	4	2	3	3	5	3
2	2	5	3	2	5	1	5	3	3	2	3	2	3	3	3	3	4	4	2	3	3	5	3
2	2	5	3	2	5	1	5	3	3	2	3	2	3	3	3	3	4	4	2	3	3	5	3
2	2	5	3	2	5	1	5	3	3	2	3	2	3	3	3	3	4	4	2	3	3	5	3
2	2	5	3	2	5	1	5	3	3	2	3	2	3	3	3	3	4	4	2	3	3	5	3

MIRU-VNTR (Mycobacterial Interspersed Repetitive Units - Multiple Loci Variable)



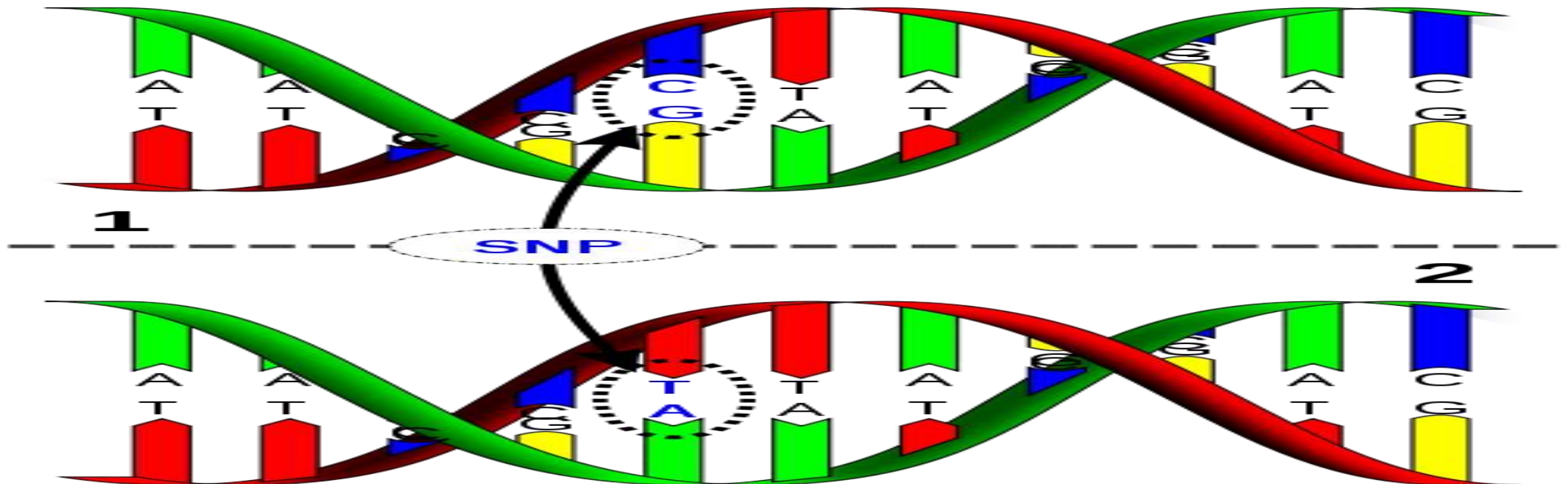
Whole Genome Sequencing (WGS)

- ❖ Also known as full genome sequencing, complete genome sequencing, or entire genome sequencing
- ❖ **Single nucleotide polymorphisms (SNPs)** are discovered by sequencing the complete genome of MTB isolates.
- ❖ Existing MTB molecular genotyping methods can detect just a small part of a microbial genome and have low discrimination power.
- ❖ **WGS** has the highest discrimination power
- ❖ **WGS** can give the information of virulence factors, clonal outbreak and genotypic drug resistance testing.



Single nucleotide polymorphism (SNPs)

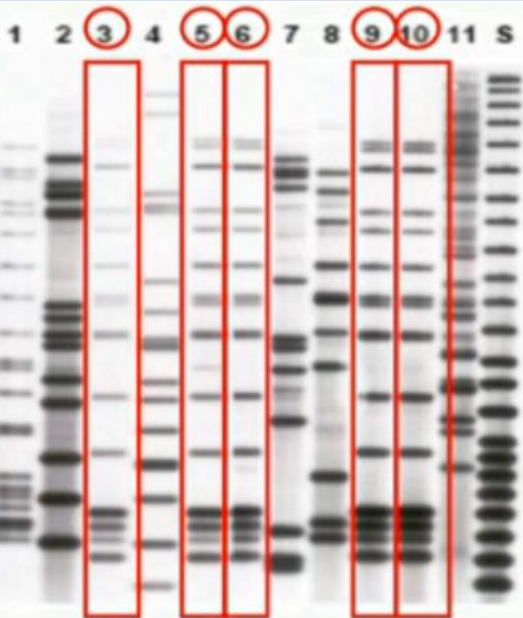
- ❖ SNPs are the most common type of genetic variation,
- ❖ SNP is a DNA sequence variation occurring when a single nucleotide adenine (A), thymine (T), cytosine (C), or guanine (G) in the genome differs between paired chromosomes in an individual.



Transmission link cluster or genetic cluster

- ❖ A transmission link cluster was defined as two or more patients infected by strains:
- ❖ having identical IS6110 RFLP, Spoligotypes and 24-locus-MIRU-VNTR patterns
- ❖ less than twelve single nucleotide polymorphisms (< 12 SNPs differences)

IS6110-RFLP



	A	B
spoligotype	000000000003771	777776777760601
MIRU1	224225173533	324325153323
MIRU2	112341156711	2463376589a2

	C	D
spoligotype	000000000003771	000000000003771
MIRU1	224225173533	224225173533
MIRU2	112341156711	112341156711

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

Moving usual molecular genotyping methods to WGS

	IS6110 RFLP	Spoligotyping	MIRU-VNTR	WGS
Coverage of genome	Small fraction of the genome	Small fraction of the genome	Small fraction of the genome	Nearly to complete the whole genome
The technique	- Strain specific banding pattern - Cannot change to numerical patterns	- Bar-code liked signals - Can change to numerical patterns	Numerical patterns (24 locus)	SNP are discovered by sequencing for the whole genome
Discrimination between two strains	- The two patterns are compared visually - Can compare between strains in the same laboratory only.	- Can compare with 15 digits code - Can compare between strains across the different laboratories	- Can compare with 24 digits code - Can compare between strains across the different laboratories	- Can compare with SNP difference in very long nucleotides - Can compare between strains across the different laboratories
Discrimination power	***	**	***	*****
Weakness	Labor intensive Requires large amounts of DNA well-grown cultures	Should be used in combination with another, high-resolution method	Labor-intensive	Too costly High technical challenges

Generation of DNA sequencing machine

A

2000
1st Generation
(Sanger sequencing)



Scientists – Hundreds
Machines - Hundreds
Cost \$3 billion
Time - 10 years

B

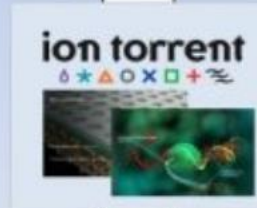
2006-2010
2nd Generation
("Next Generation")



Scientists – 1-2
Machines - 1
Cost \$5-10,000
Time - 2 weeks

C

2010-2015
3rd Generation
("Next-Next Generation")



Scientists – 1-2
Machines - 1
Cost \$1000?
Time - Hours ?

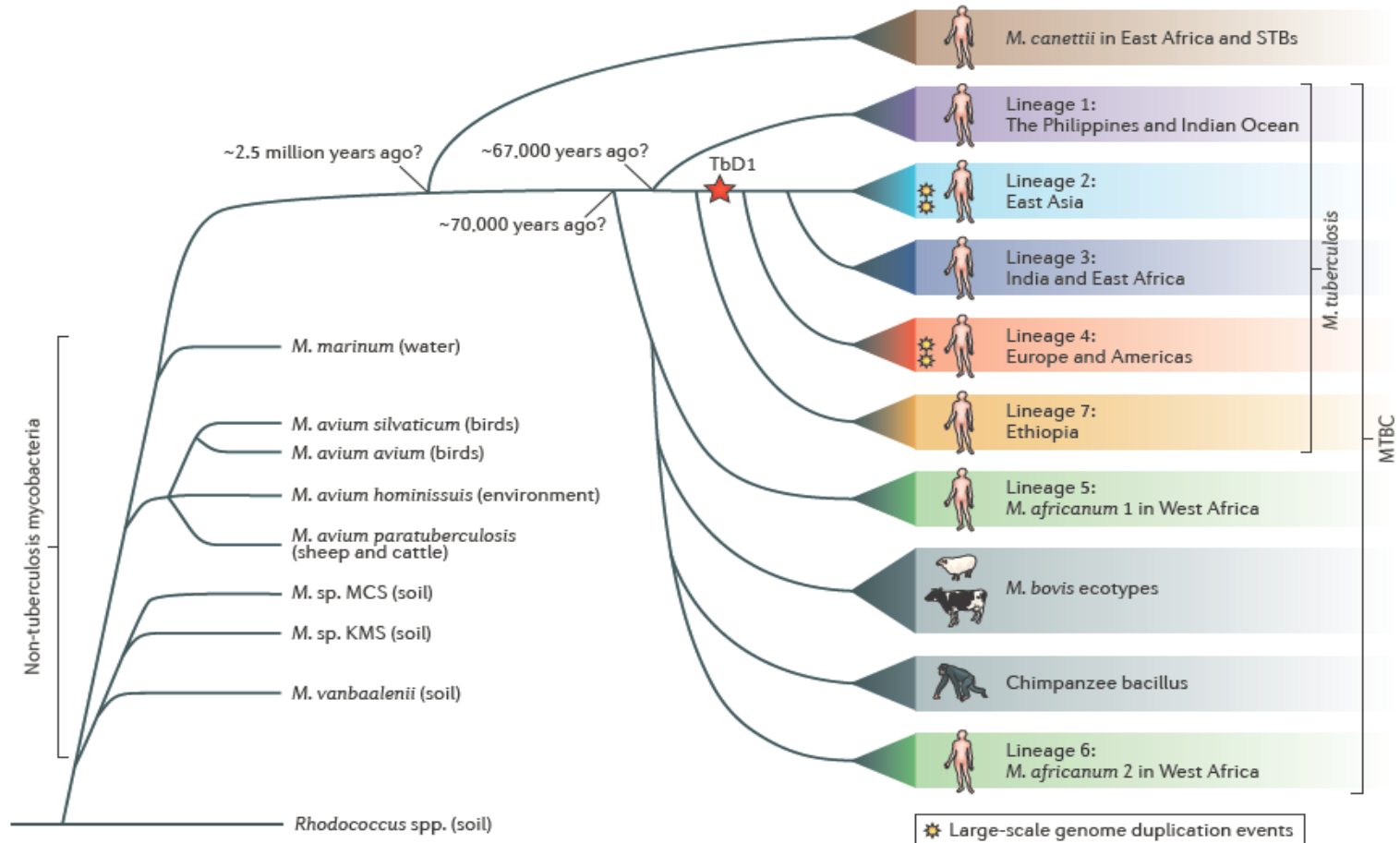
? 4th Generation



Evolution of MTB

A story of how what was most likely a soil bacterium evolved to become one of the most infectious human pathogens in history

- ❖ Mycobacteria are predominantly environmental organisms
- ❖ Several transitions from the environment to pathogenicity have occurred
- ❖ The MTB was thought to arise as a clonal expansion from *M. Canettii* (a smooth tubercle bacillus (STB)/progenitor population.)
- ❖ *M. bovis* ecotypes branch from a lineage of *M. africanum* that is currently restricted to West Africa



Genomic insights into tuberculosis (James E. Galagan. et al.,)

Evolution of MTB

TB in human

M. tuberculosis

M. africanum

M. bovis

- Not easily transmit between humans

TB in animal

M. bovis (a pathogen of cattle)

M. caprae (sheep and goats)

M. microti (voles)

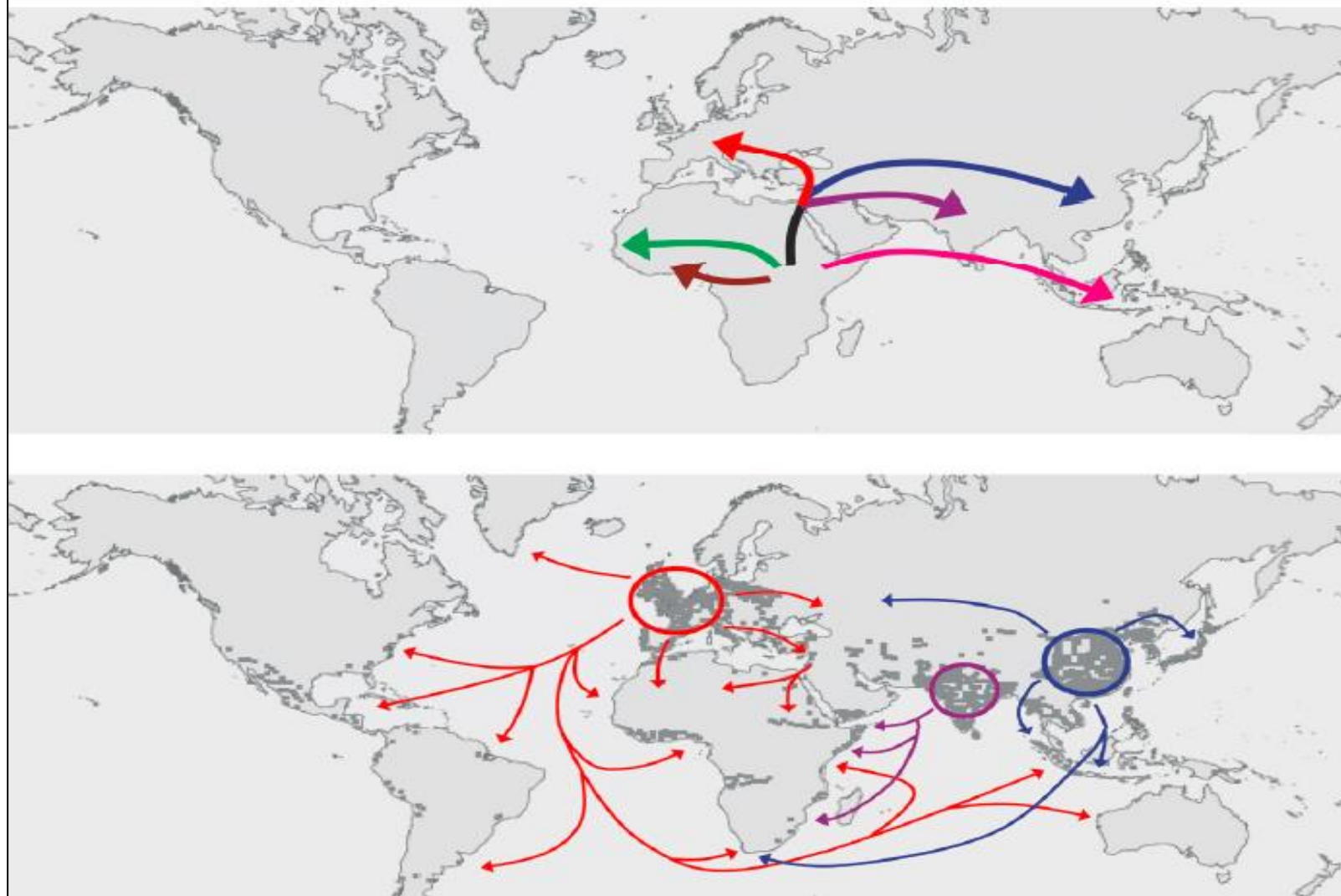
M. pinnipedii (seals and sea lions)

***M. canettii* / 'smooth TB bacilli'**

- lack of evidence for human-to-human transmission of *M. canettii*
- Therefore, they are rare

Evolution of MTB

- ❖ Human TB originated in Africa
- ❖ Africa is the only region of the world that harbours all seven main human-adapted MTB lineages
- ❖ Two 'ancient' MTB lineages remained in Africa
- ❖ Three 'modern' lineages seeded in Europe, India and China
- ❖ Spread globally through waves of human exploration, trade and conquest



Distribution of the major lineages of MTB in the previous studies

Lineage 1 (L1) = East-African-Indian (EAI)

Lineage 2 (L2) = East Asia (Beijing)

Lineage 3 (L3) = Delhi/Central Asian, Indo-Oceania

Lineage 4 (L4) = European American

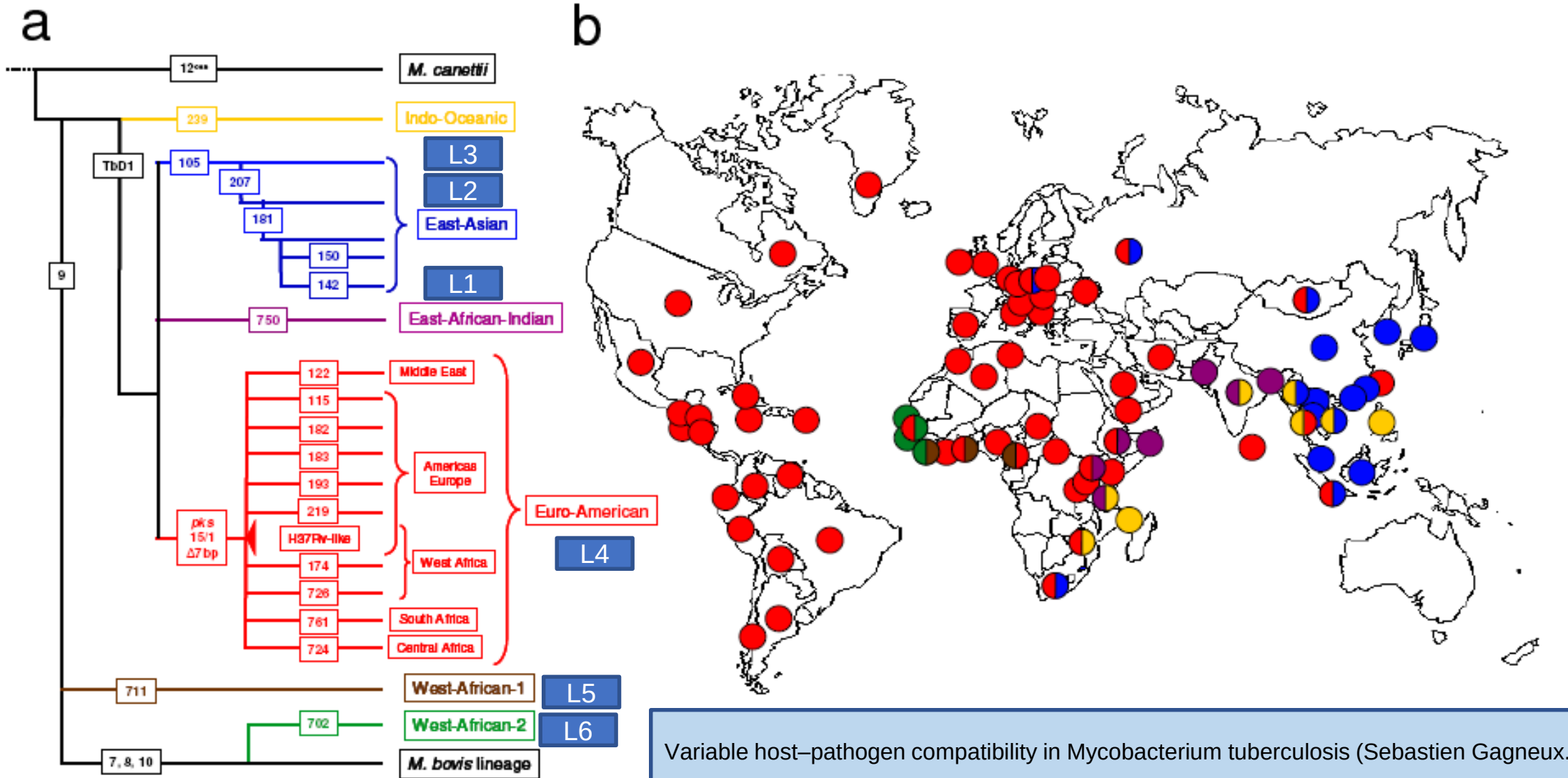
Lineage 5 (L5) = West African I

Lineage 6 (L6) = West African 2

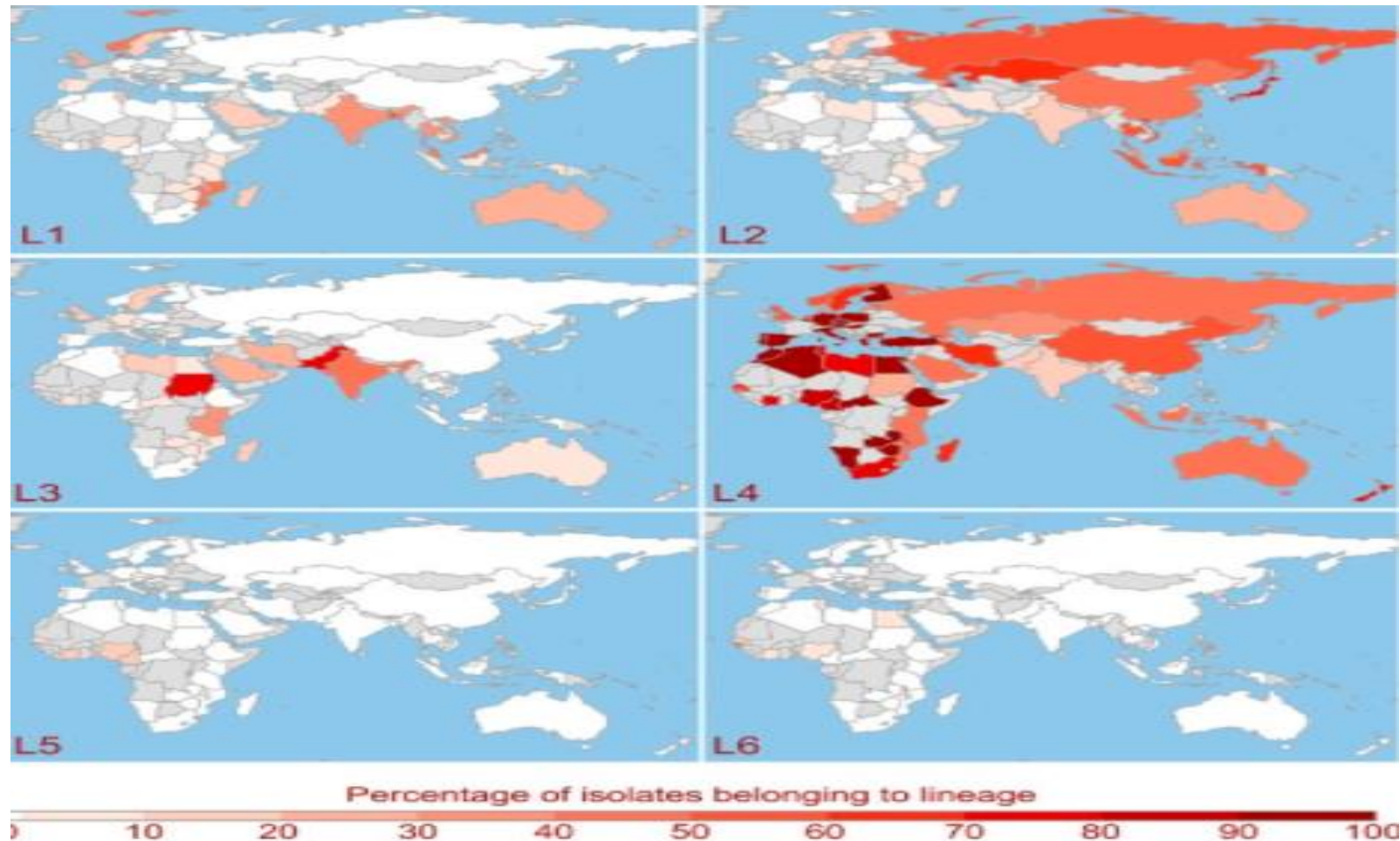
Lineage 7 (L4) = Ethiopia

- ❖ **Myanmar and Thailand showed L1 and L2 equally dominant.**
- ❖ **The L1 was dominant in India and Philippines**
- ❖ **L2 was dominant in China**
- ❖ **L4 was dominant in Europe, America and Africa countries.**
- ❖ **L5 and L6 were found only in Africa**
- ❖ **L7 was only found in Ethiopia country.**

Distribution of Major lineages of MTB



Distribution of Major lineages of MTB



Lineage specific histories of *Mycobacterium tuberculosis* dispersal in Africa and Eurasia (Mary B. O'Neill. et al.)

Previous MTB molecular genotyping studies in Myanmar

Predominance of Mycobacterium tuberculosis EAI and Beijing Lineages in Yangon, Myanmar

- ❖ Spoligotyping and IS6110 study in Yangon
- ❖ 310 isolates from pulmonary TB patients from Yangon
- ❖ The most frequent lineages observed were the East African-Indian (EAI; 48.4%; n 150) and Beijing (31.9%; n 99) lineages.
- ❖ The TB epidemic in Yangon is driven by clonal expansion of the Beijing genotype.

[Sabai Phyu, Ti Ti, et al., 2002]

Whole-genome sequencing of multidrug-resistant Mycobacterium tuberculosis isolates from Myanmar.

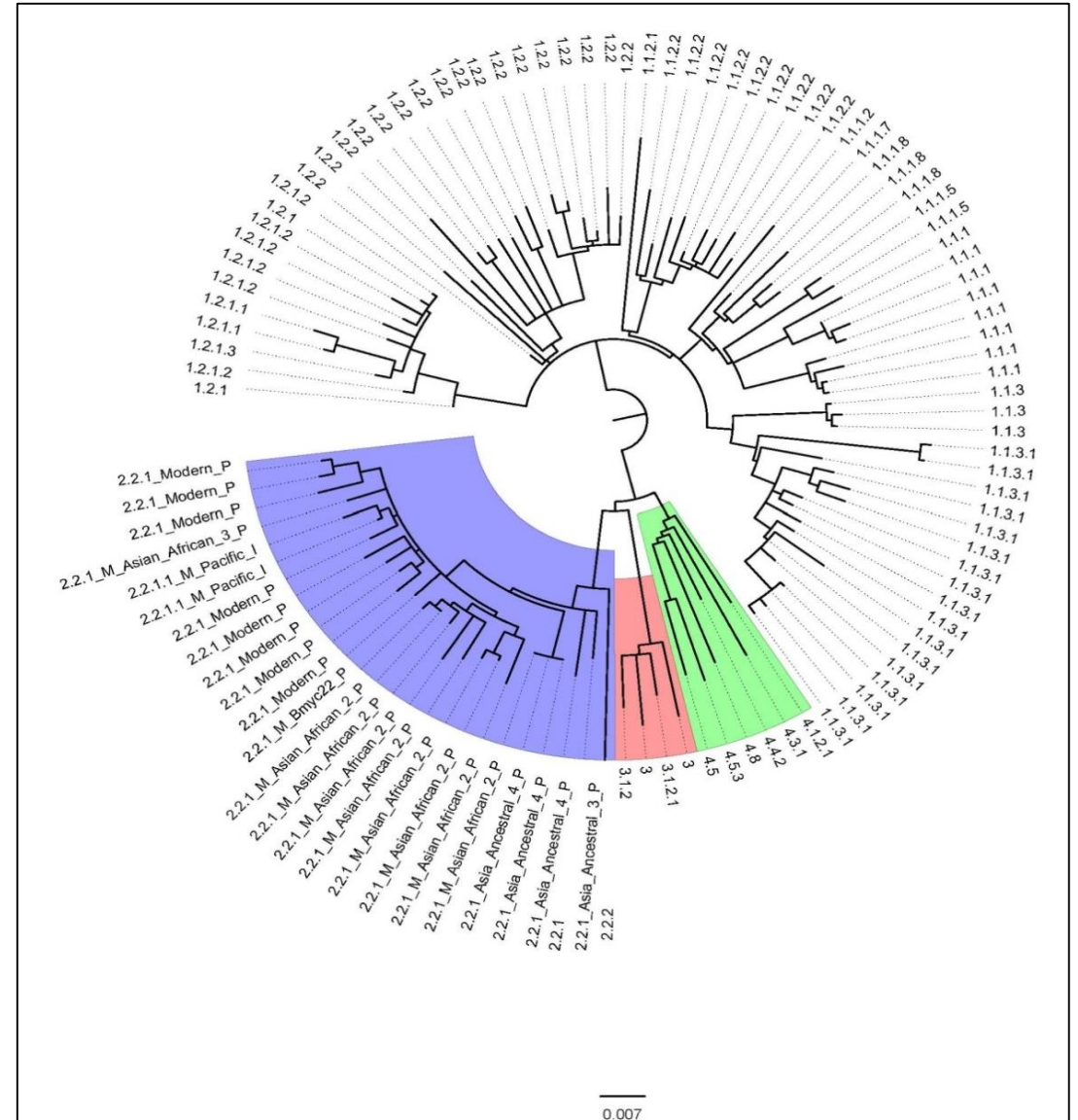
- ❖ WGS study with 14 MDRTB isolates
- ❖ Three lineages were identified (EAI, Beijing and Euro-American)
- ❖ Beijing Lineage was dominant
- ❖ They found other drugs mutation points

(Htin Lin Aung et al., 2016)

Geno-Spatial Distribution of Mycobacterium Tuberculosis and Drug Resistance Profiles in Myanmar-Thai Border Area

- ❖ 109 samples: The average coverage of the genome was 99.56%
- ❖ Four major lineages were identified
 - ❖ L1/East African India (EAI) (n = 73, 67%)
 - ❖ L2/Beijing (n = 26, 23%)
 - ❖ L3/Delhi-Central Asian (n = 4, 4%)
 - ❖ L4/European American (n = 6, 6%)
- ❖ L2 has association with Drug resistance, relapse, delayed smear conversion
- ❖ Simpson's index was 0.07 and very close to zero value. It indicated a very high level of biodiversity in this area.

(Htet et al, 2020)



Frequency distribution of major lineages and sublineages of MTB

Major Lineage	Sublineage	n	Total (109)
L1 (EAI)	L1.1.1	8	73
	L1.1.1.2	1	
	L1.1.1.5	2	
	L1.1.1.7	1	
	L1.1.1.8	3	
	L1.1.2.1	1	
	L1.1.2.2	10	
	L1.1.3	3	
	L1.1.3.1	17	
	L1.2.1	1	
	L1.2.1.1	2	
	L1.2.1.2	6	
	L1.2.1.3	1	
	L1.2.2	17	
L2 (Beijing)	L2.2.1.1_M_Pacific	2	26
	L2.2.1_Asia_Ancestral_2	1	
	L2.2.1_Asia_Ancestral_3	1	
	L2.2.1_Asia_Ancestral_4	3	
	L2.2.1_M_Asian_African_2	8	
	L2.2.1_M_Asian_African_3	1	
	L2.2.1_M_Bmyc22	1	
	L2.2.1_Modern	8	
	L2.2.2	1	
L3 (Delhi/CAS)	L3	2	4
	L3.1.2	1	
	L3.1.2.1	1	
L4 (Euro-American)	L4.1.2	1	6
	L4.3	1	
	L4.4	1	
	L4.5	1	
	L4.5.3	1	
	L4.8	1	

Biodiversity of sublineages of MTB

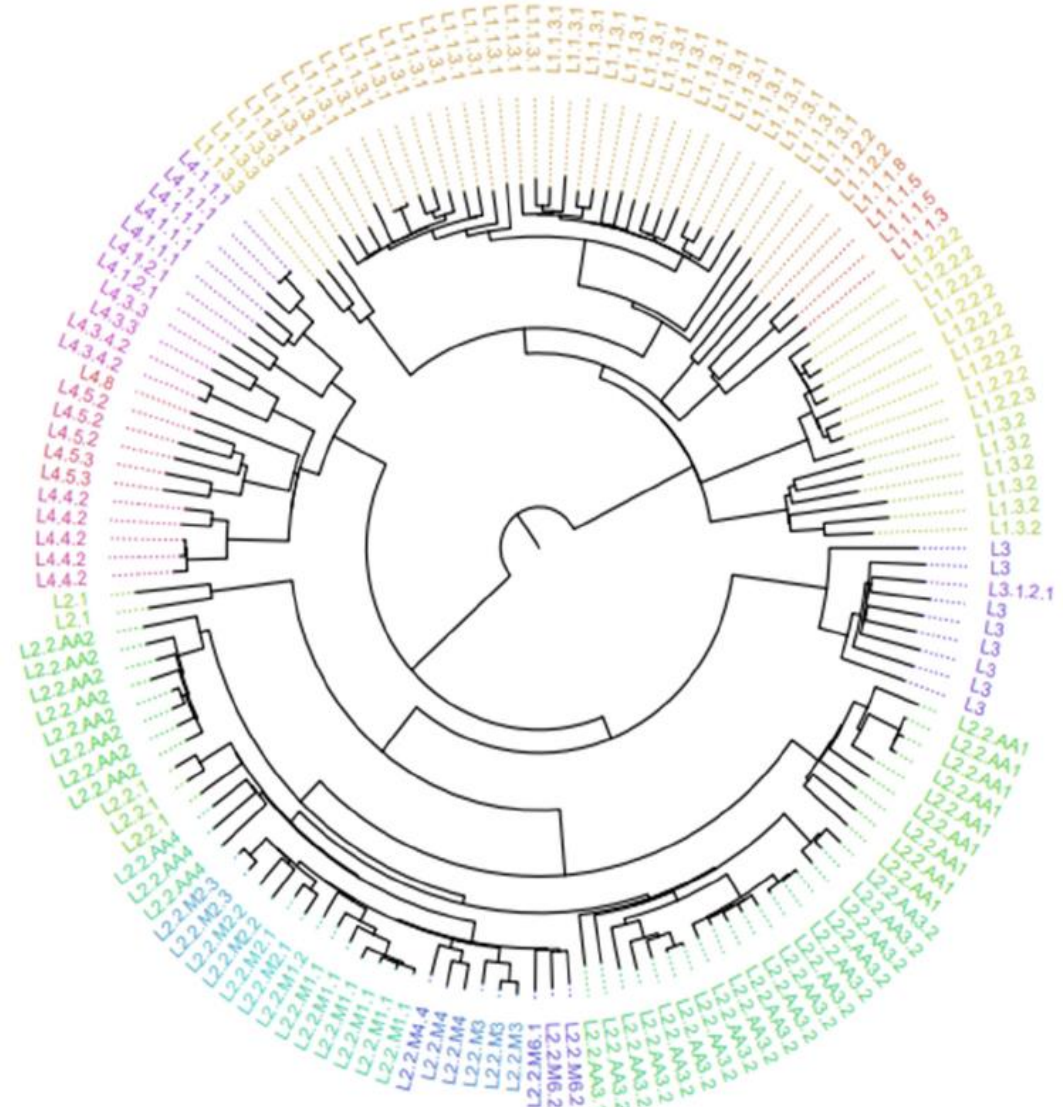
	n	n-1	n(n-1)
L1.1.1	8	7	56
L1.1.1.2	1	0	0
L1.1.1.5	2	1	2
L1.1.1.7	1	0	0
L1.1.1.8	3	2	6
L1.1.2.1	1	0	0
L1.1.2.2	10	9	90
L1.1.3	3	2	6
L1.1.3.1	17	16	272
L1.2.1	1	0	0
L1.2.1.1	2	1	2
L1.2.1.2	6	5	30
L1.2.1.3	1	0	0
L1.2.2	17	16	272
L2.2.1.1_M_Pacific	2	1	2
L2.2.1_Asia_Ancestral_2	1	0	0
L2.2.1_Asia_Ancestral_3	1	0	0
L2.2.1_Asia_Ancestral_4	3	2	6
L2.2.1_M_Asian_African_2	8	7	56
L2.2.1_M_Asian_African_3	1	0	0
L2.2.1_M_Bmyc22	1	0	0
L2.2.1_Modern	8	7	56
L2.2.2	1	0	0
L3	2	1	2
L3.1.2	1	0	0
L3.1.2.1	1	0	0
L4.1.2	1	0	0
L4.3	1	0	0
L4.4	1	0	0
L4.5	1	0	0
L4.5.3	1	0	0
L4.8	1	0	0
N(N-1)			11778
$\sum n(n-1)$			858
$\sum n(n-1)/N(N-1)$			0.072885

- There were 32 sublineages
- There was no significant dominant sublineage
- The biodiversity index was 0.07 : very close to zero value
- It indicates a very high level of biodiversity in this area.

Genomic Sequencing Profiles of Mycobacterium tuberculosis in Mandalay Region, Myanmar

- ❖ **WGS with 151 MTB isolates**
- ❖ **Four major lineages were identified**
 - ❖ **L1/East African India (EAI) (n = 55, 36%)**
 - ❖ **L2/Beijing (n = 65, 43%)**
 - ❖ **L3/Delhi-Central Asian (n = 9, 6%)**
 - ❖ **L4/European American (n = 22, 15%)**
- ❖ **Simpson's index for sublineages was 0.0709. Such high diversity suggests that the area probably had imported Mtb from many geographical sources.**

(Aye Nyein Phyu, Htet, et al, 2023)



Distribution of Mycobacterium tuberculosis Lineages and Drug Resistance in Upper Myanmar

- ❖ **WGS study with 506 sequenced isolates.**
- ❖ **The most common lineage was lineage 2 (n = 223, 44.1%).**
- ❖ **Lineage 2 showed a higher number of MDR-TB compared to other lineages.**
- ❖ **There were significant associations between lineages of Mtb and drug resistance patterns, and between lineages and geographical locations of Upper Myanmar (p value < 0.001). (Aye Nyein Phyu, et al, 2022)**

Table 5. Association between lineages and drug resistance pattern.

Drug Resistance Pattern	Lineages				Fisher's Exact Test, p Value
	1 (N = 201)	2 (N = 223)	3 (N = 20)	4 (N = 62)	
Drug resistance patterns					<0.001
H ₀ R ₀ Z ₀ E ₀ S ₀ Lfx ₀ Eto ₀	193	181	18	54	
H ₀ R ₀ Z ₀ E ₀ S _R Lfx ₀ Eto ₀	3	22	0	1	
H _R R ₀ Z ₀ E ₀ S ₀ Lfx ₀ Eto ₀	2	2	0	4	
H _R R ₀ Z ₀ E ₀ S _R Lfx ₀ Eto ₀	1	6	1	0	
H _R R ₀ Z ₀ E ₀ S ₀ Lfx ₀ Eto _R	0	2	1	1	
H _R R _R Z ₀ E ₀ S _R Lfx ₀ Eto ₀	0	3	0	0	
Others including H _R R _R	1	4	0	1	
Others not including H _R R _R	1	3	0	1	

Subscript 0 denotes sensitive. Subscript R denotes resistant. E = Ethambutol; Eto = Ethionamide; H = Isoniazid; Lfx = Levofloxacin; R = Rifampicin; S = Streptomycin; Z = Pyrazinamide.

What are the benefit to study the different genotypes of MTB?

- ❖ **Understand the changing nature of microbes (evolution of microbes)**
- ❖ **Understand the pathogenicity of microbes**
 - ❖ **Virulence factors**
 - ❖ **Drug resistance**
 - ❖ **To develop the new drug design**
 - ❖ **To develop the new vaccine**
- ❖ **Transmission analysis study**
 - ❖ **Contact tracing**
 - ❖ **Cause of recurrence/relapse (reactivation vs. re-infections)**
 - ❖ **Clonal outbreak transmission**
 - ❖ **Biodiversity**

What are the benefit to study the different genotypes of MTB? (Cont:)

❖ **MTB lineages and sublineages usually associated with several parameters**

- ❖ **Geography**
- ❖ **Ages**
- ❖ **Ethnicity**
- ❖ **Drug resistance**
- ❖ **Transmission rates**
- ❖ **Extrapulmonary form**
- ❖ **HIV infection**
- ❖ **Clinical pulmonary form**
- ❖ **Treatment outcomes**

Revised nomenclature and SNP barcode for *Mycobacterium tuberculosis* lineage 2

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Abstract

Mycobacterium tuberculosis (Mtb) lineage 2 (L2) strains are present globally, contributing to a widespread tuberculosis (TB) burden, particularly in Asia where both prevalence of TB and numbers of drug resistant TB are highest. The increasing avail-

scientific reports



OPEN Whole-genome single nucleotide variant phylogenetic analysis of *Mycobacterium tuberculosis* Lineage 1 in endemic regions of Asia and Africa

Thidarat Netikul^{1,2}, Yuttapong Thawornwattana^{2,3}, Surakameth Mahasirimongkol⁴, Hideki Yanai⁵, Htet Myat Win Maung^{6,7}, Virasakdi Chongsuvivatwong⁷ & Prasit Palittapongarnpim^{2,8,*}

Mycobacterium tuberculosis (Mtb) lineage 1 (L1) contributes considerably to the disease morbidity. While whole genome sequencing (WGS) is increasingly used for studying Mtb, our understanding of genetic diversity of L1 remains limited. Using phylogenetic analysis of WGS data from endemic range

Everyday he shouts "Op op op..." resound around the well, making other small animals are frightened. Frog thought the tiny sky like a lid and he is imposing as a Lord.





HIV/TB Agency, Information and Services Activity

**THANK
YOU.**