ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

Méthodes et futures applications du WGS pour les bacilles de la lèpre

Charlotte Avanzi
Unité du Prof. S. Cole, EPFL

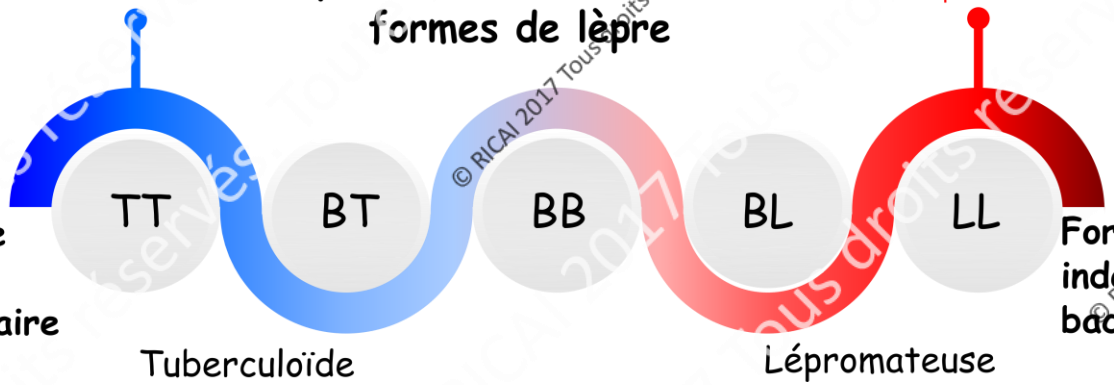
"RICAI 2017"
18/12/2017

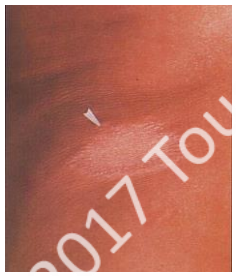
La Lèpre

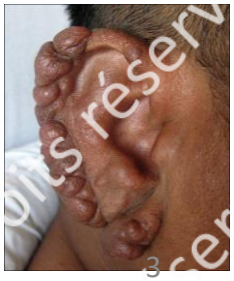
- **Maladie de Hansen**
  - *M. leprae* (1893)
  - *M. lepromatosis* (2008)

La Lèpre

- **Maladie de Hansen**
  - *M. leprae* (1893)
  - *M. lepromatosis* (2008)
- **Multiforme, progressive et invalidante**
  - Incubation de quelques mois à 20 ans



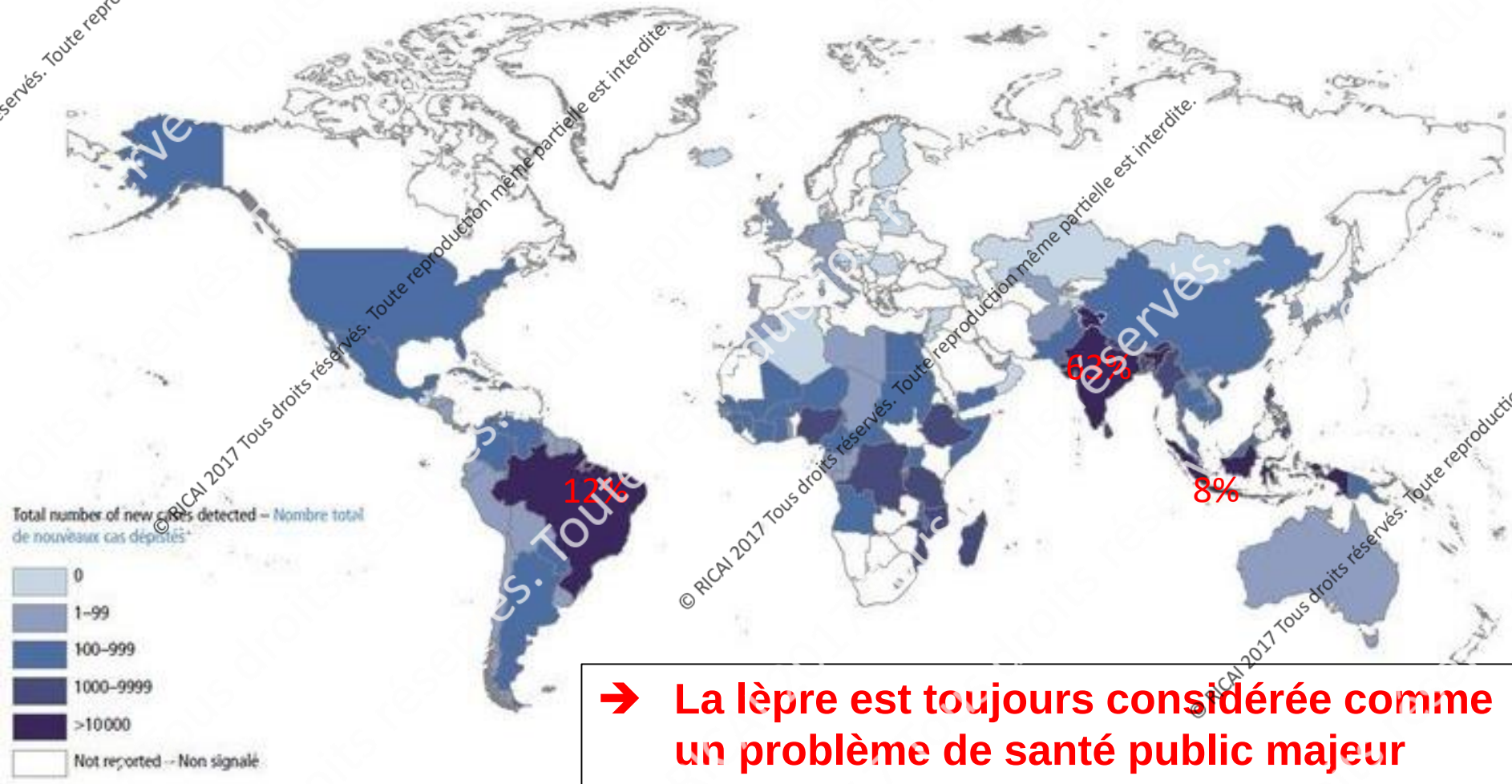




Han & al., 2008 – Singh et al., 2015 – © photos Audrey Delhom, Thèse de Médecine, 2014 / Jere Mammino / Omar Havana / Action Damien

La Lèpre n'est pas éradiquée

200,000 nouveaux cas/an
≈ 8% d'enfants (<15 ans)

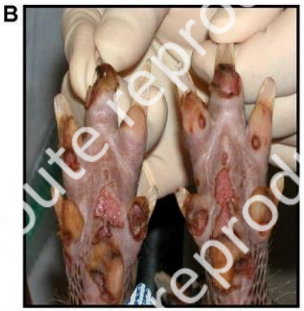


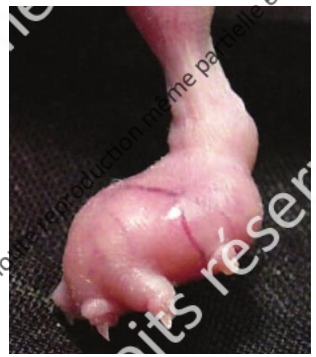
➔ **La lèpre est toujours considérée comme un problème de santé public majeur dans plus de 14 pays**

WER WHO 2017

CARACTÉRISTIQUES DES BACILLES

- Non cultivable *in vitro*
- Modèles *in vivo* (*M. leprae*)





- Temps de division: 12 jours
- Mode de transmission: inconnu

Sharma et al, 2013 - Disease Models & Mechanisms

LA POLYCHIMIOTHERAPIE (PCT)

Paucibacillaire (<5 lésions)
6 mois

DAPSONE
Cible : FolP1
Posologie: 100mg (j)

RIFAMPICINE
Cible : RpoB
Posologie: 600mg (m)

Traitement gratuit financé par l'OMS

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RIFAMPICINE
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CLOFAZIMINE
Cible: inconnue
Posologie: 50mg (j); 300mg (m)

Multibacillaire (>5 lésions)
12 mois

Traitement gratuit financé par l'OMS

LA POLYCHIMIOTHERAPIE (PCT)

Paucibacillaire (<5 lésions)
6 mois

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Cible : FolP1
Posologie: 100mg (j)

RIFAMPICINE
Cible : RpoB
Posologie: 600mg (m)

CLOFAZIMINE
Cible: inconnue
Posologie: 50mg (j); 300mg (m)

Multibacillaire (>5 lésions)
12 mois

DEUXIÈME LIGNE

OFLOXACINE
Cible: GyrA/GyrB
Posologie: 400mg (j)

MINOCYCLINE
Cible: inconnue
Posologie: 100mg (j)

CLARITHROMYCINE
Cible: inconnue
Posologie: 500mg (j)

Traitement gratuit financé par l'OMS

JCM
Journals.ASM.org

Identification of Primary Drug Resistance to Rifampin in *Mycobacterium leprae* Strains from Leprosy Patients in Amazonas State, Brazil

Matilde del Carmen Contreras Mejía,[a] Maísa Porto Dos Santos,[a,e] George Allan Villarouco da Silva,[a,b] Isabella da Motta Passo Felipe Gomes Naveca,[b] Maria da Graça Souza Cunha,[c] Milton Ozório Moraes,[c] Lucia de Paula[a,g]

Clinical Infectious Diseases

BRIEF REPORT

Transmission of Drug-Resistant Leprosy in Guinea-Conakry Detected Using Molecular Epidemiological Approaches

Charlotte Avanzi,[1] Philippe Busso,[1] Andrej Benjak,[1] Chloé Loiseau,[1] Abdoulaye Fomba,[2] Glodia Doumbia,[2] Idrissa Camara,[3] André Lamou,[3] Gouressy Sock,[3] Tiguidanke Drame,[3] Mamadou Kodio,[2] Fatoumata Sakho,[3] Samba O. Sow,[2] Stewart T. Cole,[1] and Roch Christian Johnson[4]

INTERNATIONAL JOURNAL OF LEPROSY

Volume 71, Number 3
Printed in the U.S.A.
(ISSN 0148-916X)

CASE REPORTS

A Second Case of Multidrug-resistant *Mycobacterium leprae* Isolated from a Japanese Patient with Relapsed Lepromatous Leprosy[1]

Masanori Matsuoka, Yoshiko Kashiwabara, Zhang Liangfen, Masamichi Goto, and Shin-ichi Kitajima[2]

Accepted Manuscript

Title: Molecular detection of multi drug resistant Mycobacterium leprae from Indian leprosy patients

Authors: Mallika Lavania, Itu Singh, Ravindra P Turankar, Madhvi Ahuja, Vinay Pathak, Utpal Sengupta, Loretta Das, Archana Kumar, Joydeepa Darlong, Rajeev Nathan, Asha Maseey

Primary Multidrug-Resistant Leprosy, United States

JCM
Journals.ASM.org

Diana Lynn Williams, Timothy Hagino, Rahul Sharma, and David Scollard

Drug and Multidrug Resistance among *Mycobacterium leprae* Isolates from Brazilian Relapsed Leprosy Patients

Adalgiza da Silva Rocha,[a] Maria das Graças Cunha,[b] Lucia Martins Diniz,[c] Claudio Salgado,[d] Maria Araci P. Aires,[e] José Augusto Nery,[f] Eugênia Novisck Gallo,[f] Alice Miranda,[f] Monica M. F. Magnanini,[g] Masanori Matsuoka,[h] Euzenir Nunes Sarno,[f] Philip Noel Suffys,[a] and Maria Leide W. de Oliveira[g]

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2001, p. 3635–3639
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.12.3635–3639.2001

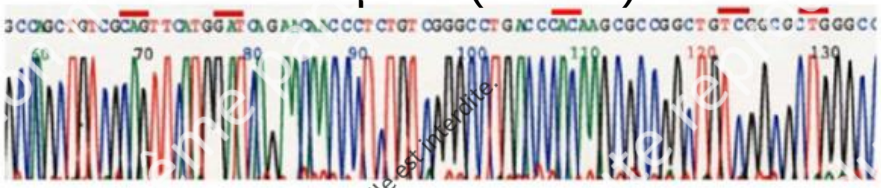
Multidrug Resistant *Mycobacterium leprae* from Patients with Leprosy

SHINJI MAEDA,[1,2] MASANORI MATSUOKA,[1]* NOBORU NAKATA,[1] MASANORI KAI,[1] YUMI MAEDA,[1] KEN HASHIMOTO,[1] HIROAKI KIMURA,[1] KAZUO KOBAYASHI,[2] AND YOSHIKO KASHIWABARA[1]

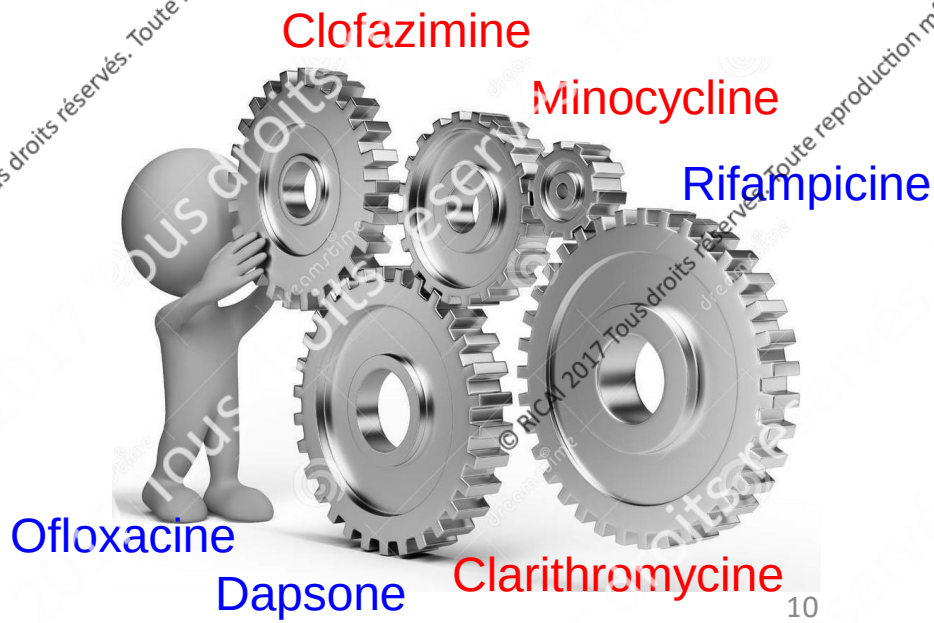
Enjeux de la résistance

- Détection des résistances
  - *rpoB*
  - *folP1*
  - -- *gyrA*

Rifampicin (DRDR)

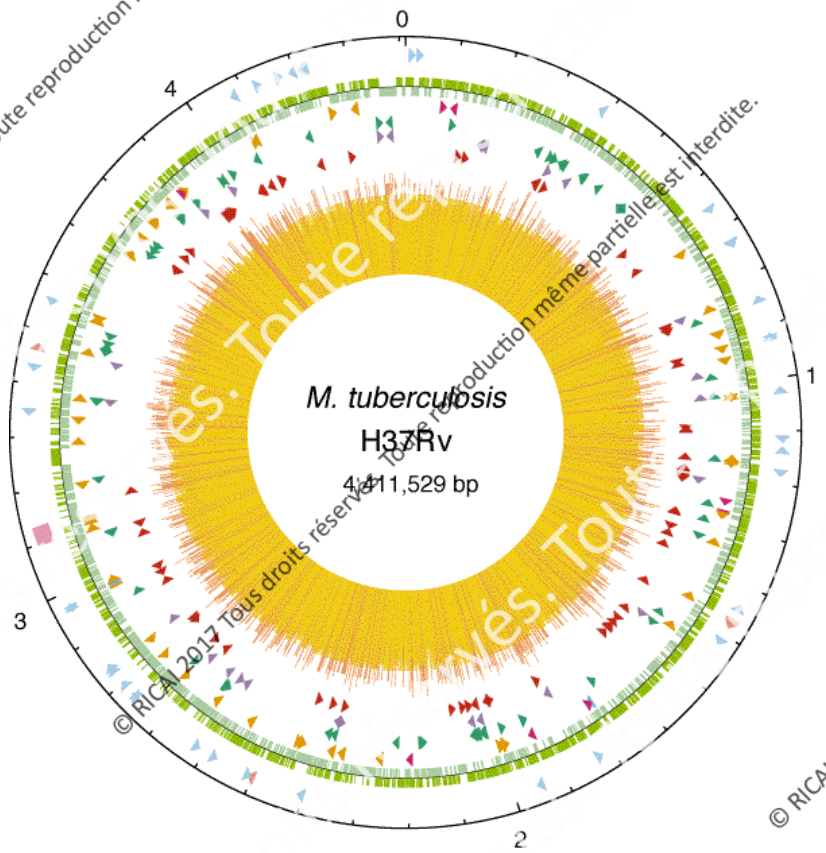


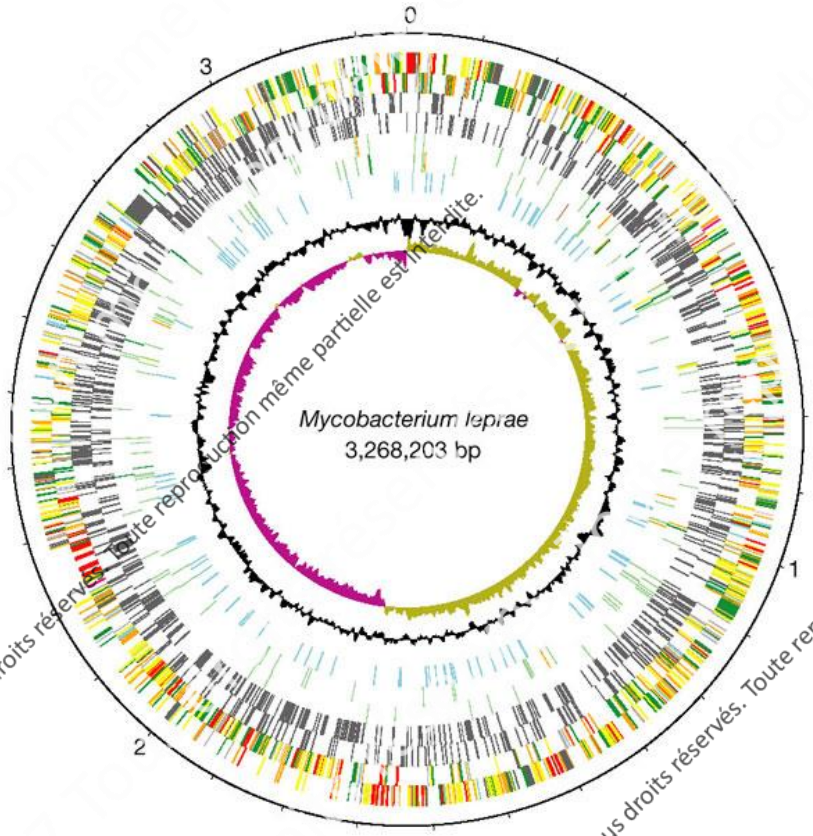
- Mécanismes de résistance



Matsuoka, 2015, Res. Rep. Trop. Med.

SOLUTION: LE WGS?





>22,000 génomes

~ 20 génomes

Cole et al., Nature, 1998 & 2001

M. LEPRAE EST DIFFICILE À SÉQUENCER!

1- DNA extraction

2- DNA shearing

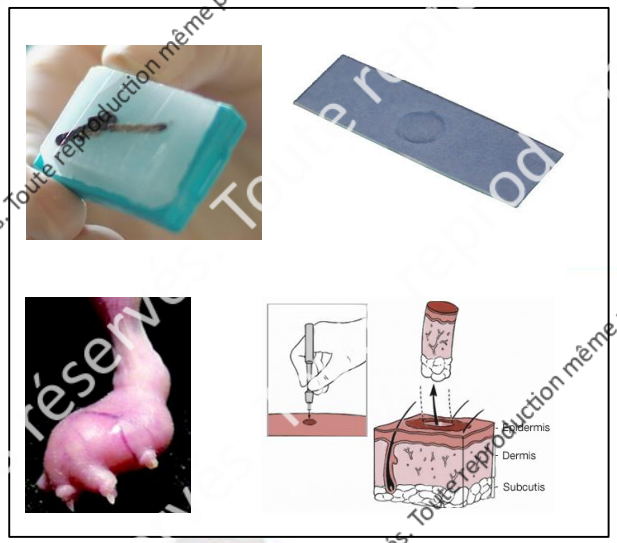
3- DNA lib. preparation

4- DNA lib. sequencing

5- DNA sequence analysis

DNA Reads

M. LEPRAE EST DIFFICILE À SÉQUENCER!



1- DNA extraction

2- DNA shearing

3- DNA lib. preparation

4- DNA lib. sequencing

5- DNA sequence analysis

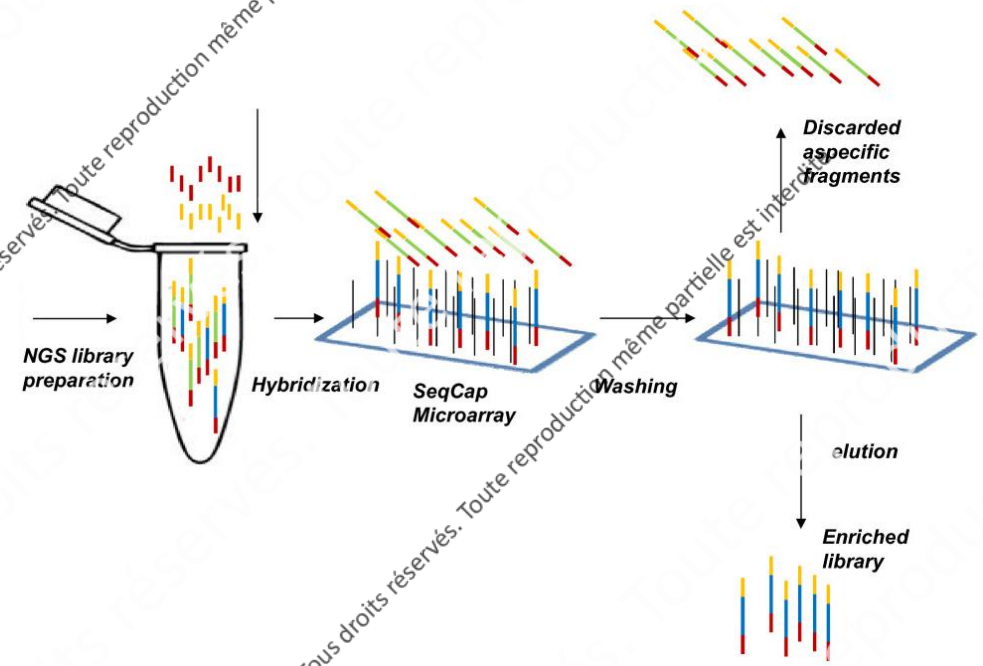
Fragmentation: Covaris
Kit Kapa Hyper prep
HiSeq (SE, 100, 200mo)

% d'alignement
- Humain: 99%
- *M. leprae:* 1%

DNA Reads

ADN de l'hôte

OUTILS N°1: ENRICHISSEMENT DES LIBRAIRIES!

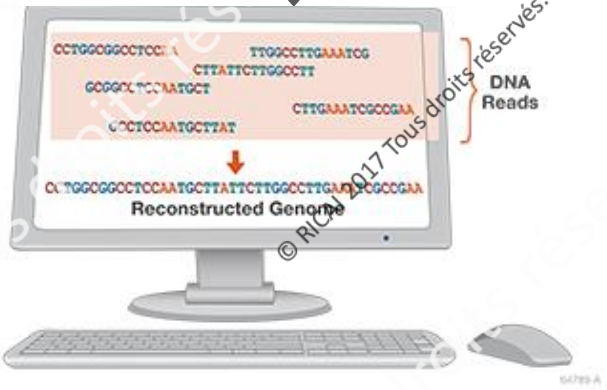


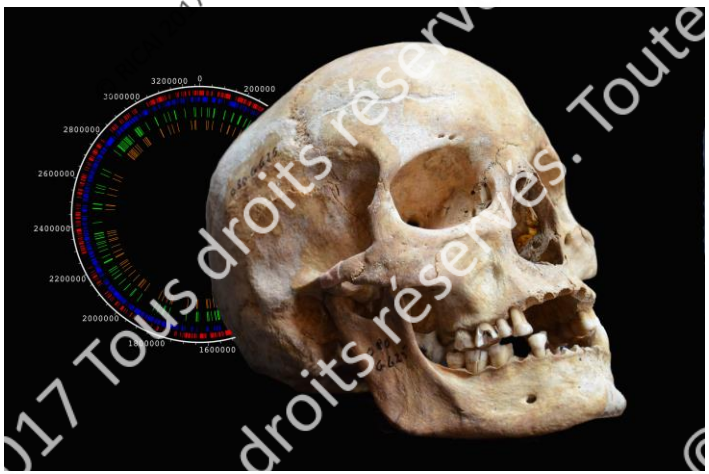
4- DNA lib. sequencing

P. Singh

V. Schuenemann

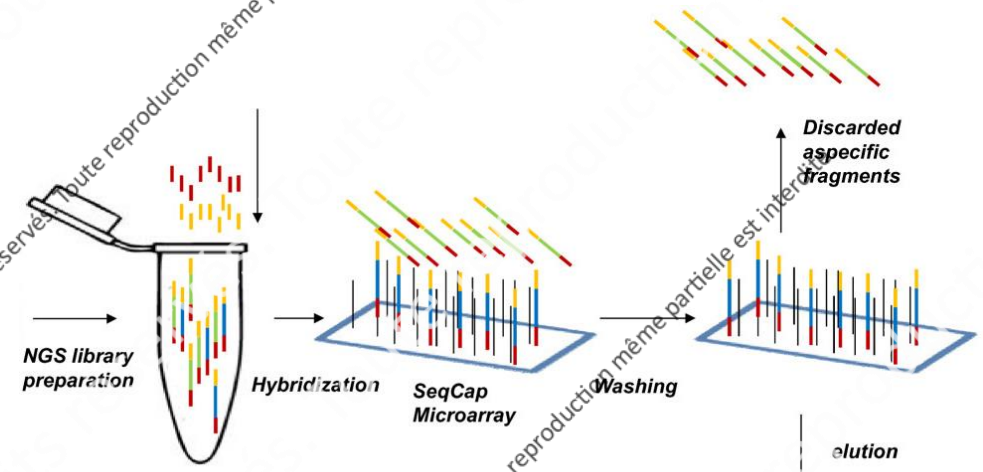
5- DNA sequence analysis





Schuenemann , Singh *et al.,* Science, 2013

OUTILS N°1: ENRICHISSEMENT DES LIBRAIRIES!



4- DNA lib. sequencing

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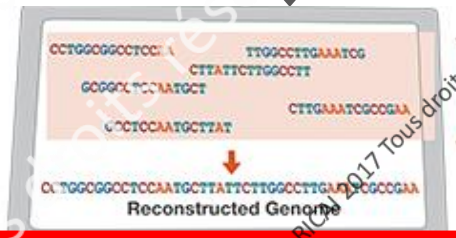
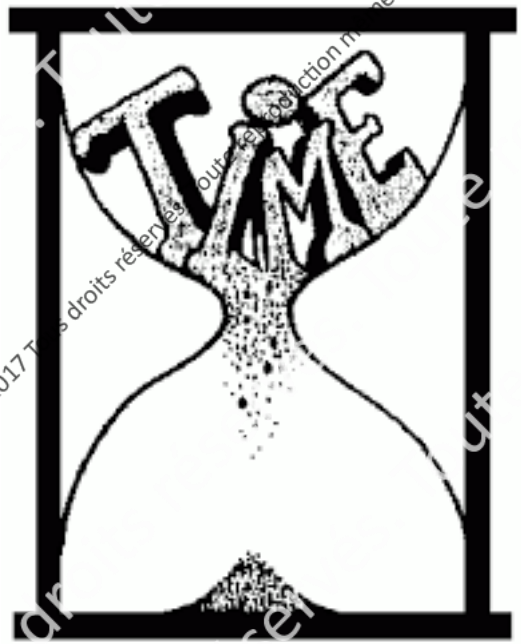
5- DNA sequence analysis

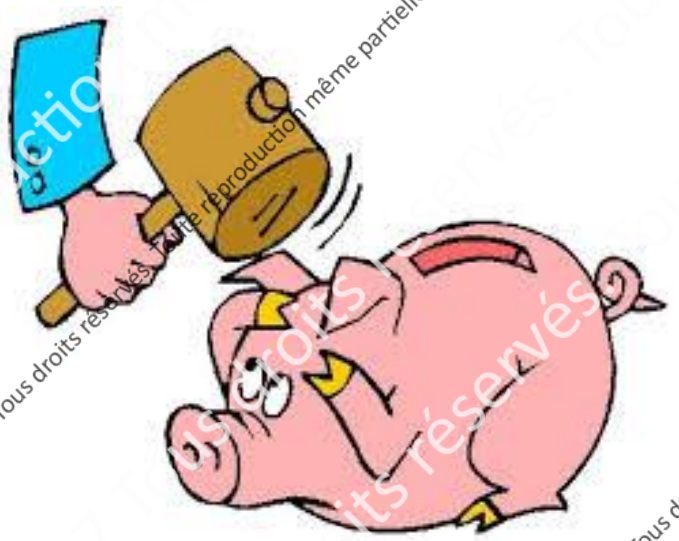


Echantillions avec peu de bacilles (ex: ADN ancient)
Multiplexage de 10 échantillons / ligne)

Schuenemann , Singh *et al.*, Science, 2013

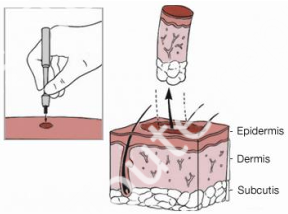
Outils n°1: Inconvenients!

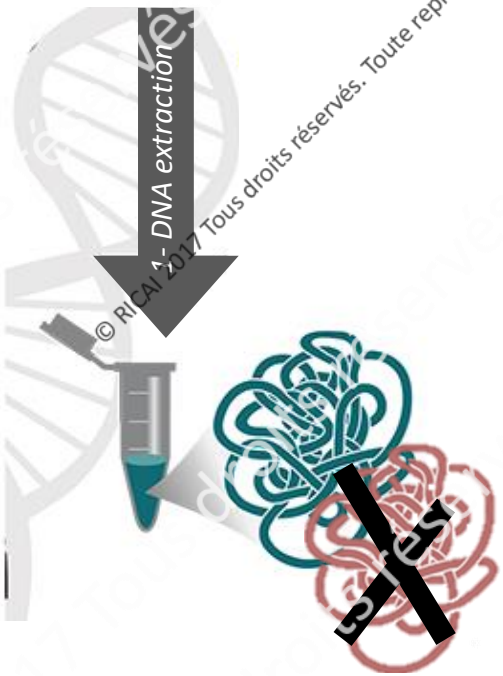




Outils n°2: Evitons la capture !

... Sur des biospies de peau

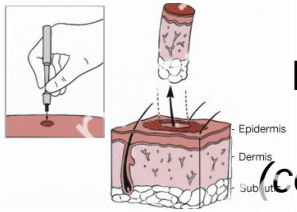




ADN humain

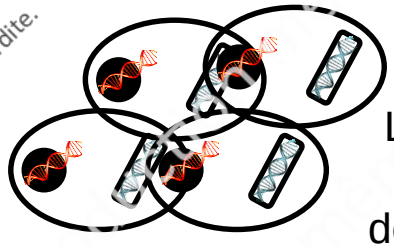
OUTILS N°2: DÉPLÉTION DE L'ADN DE L'HÔTE

... Sur des biospies de peau



Digestion du tissue

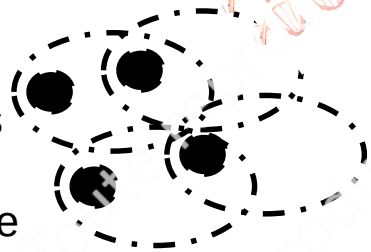
(collagenase, trypsine, prot. K)

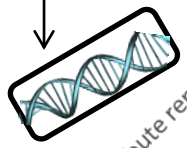


Cellules de l'hôte

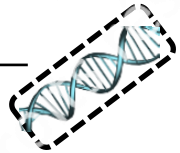
Lyse des cellules

de l'hôte et DNAse





Lyse bactérienne (chimique & mécanique)



Purification de l'ADN

sur colonne

PCR & Lib. prep

Shotgun sequencing (*ML* Pipeline)

↓

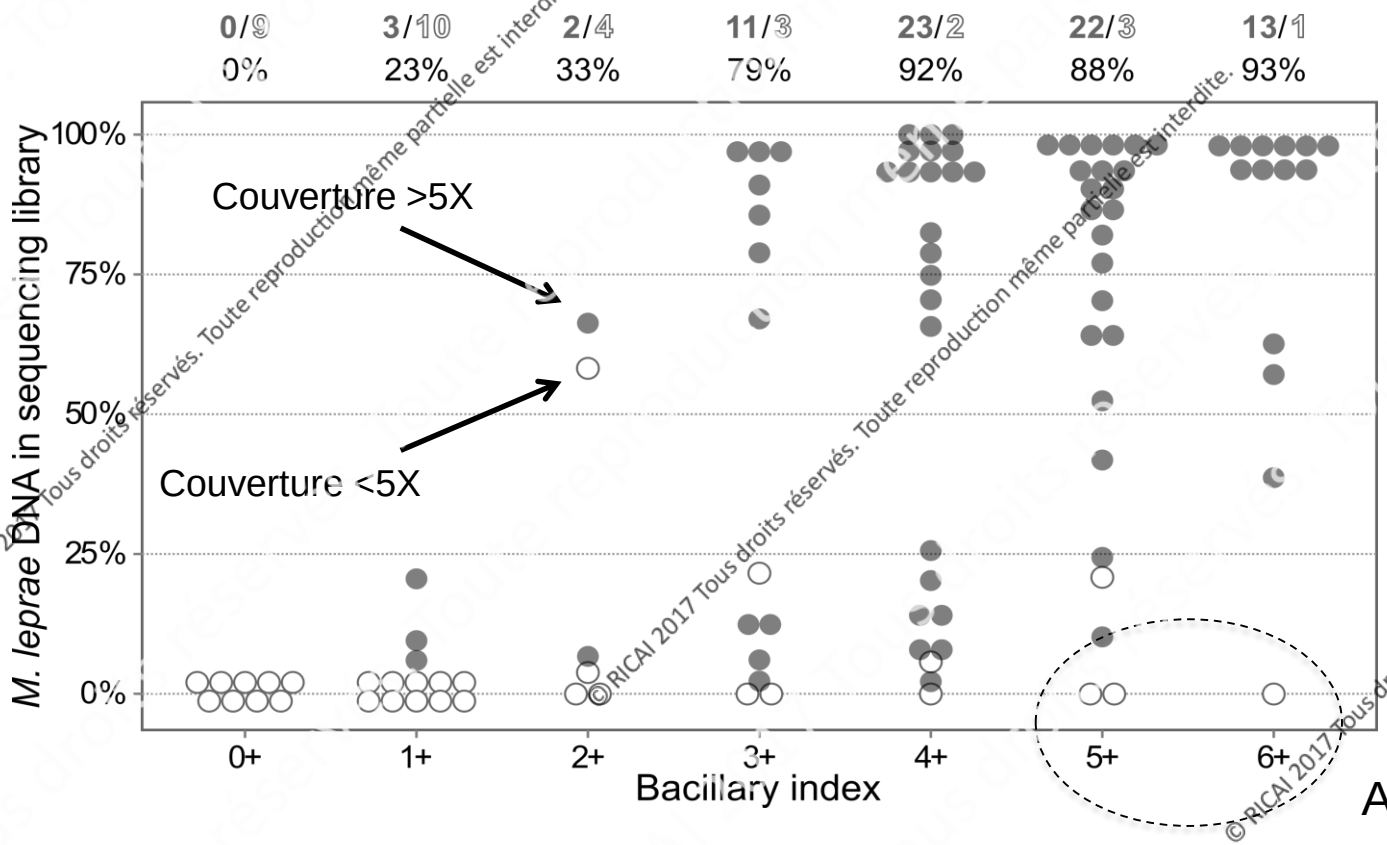
M. leprae %?

QIAamp DNA Microbiome Kit

Avanzi, Del Pozo, Benjak *et al.* Science, 2016

EFFICACITÉ DE LA MÉTHODE

106 biopsies cutanées



Ancienne méthode

Benjak, Avanzi *et al. Nat. Com accepted*

EFFICACITÉ DE LA MÉTHODE

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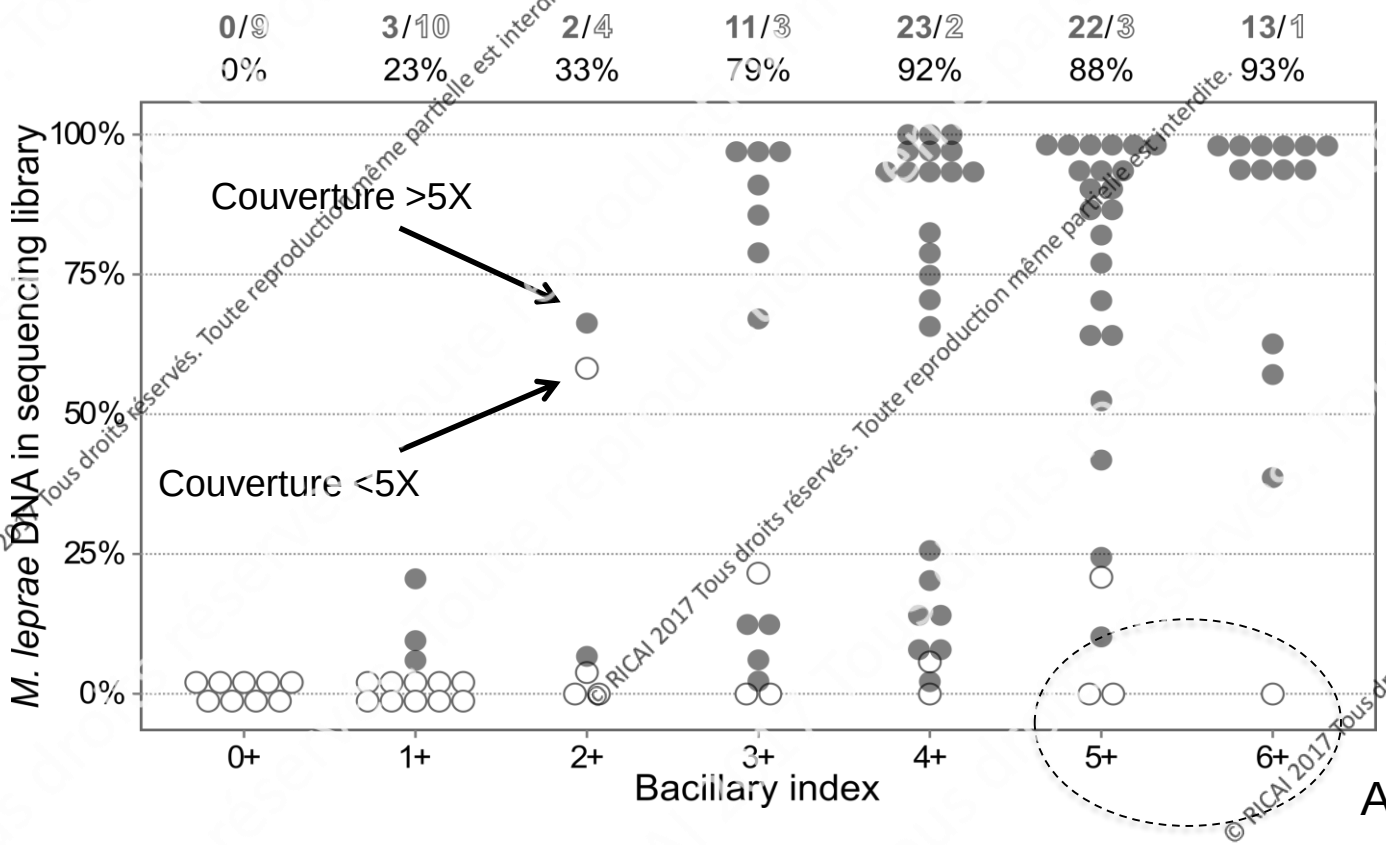


Ancienne méthode

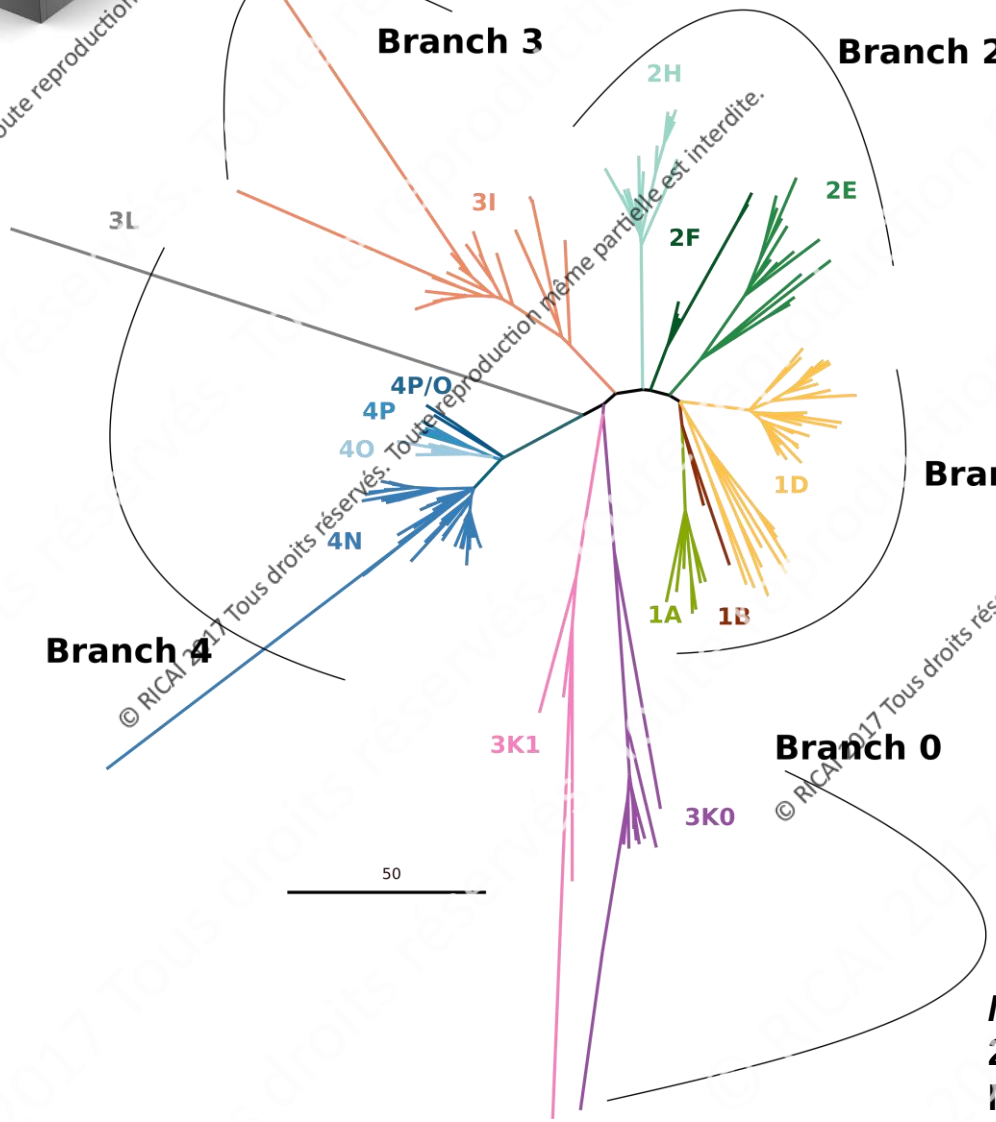
Limitations: pour les faibles bacilloscopies (<3+)

Solution: plus de profondeur ou capture sur puce

~ 20-30 échantillons/ligne

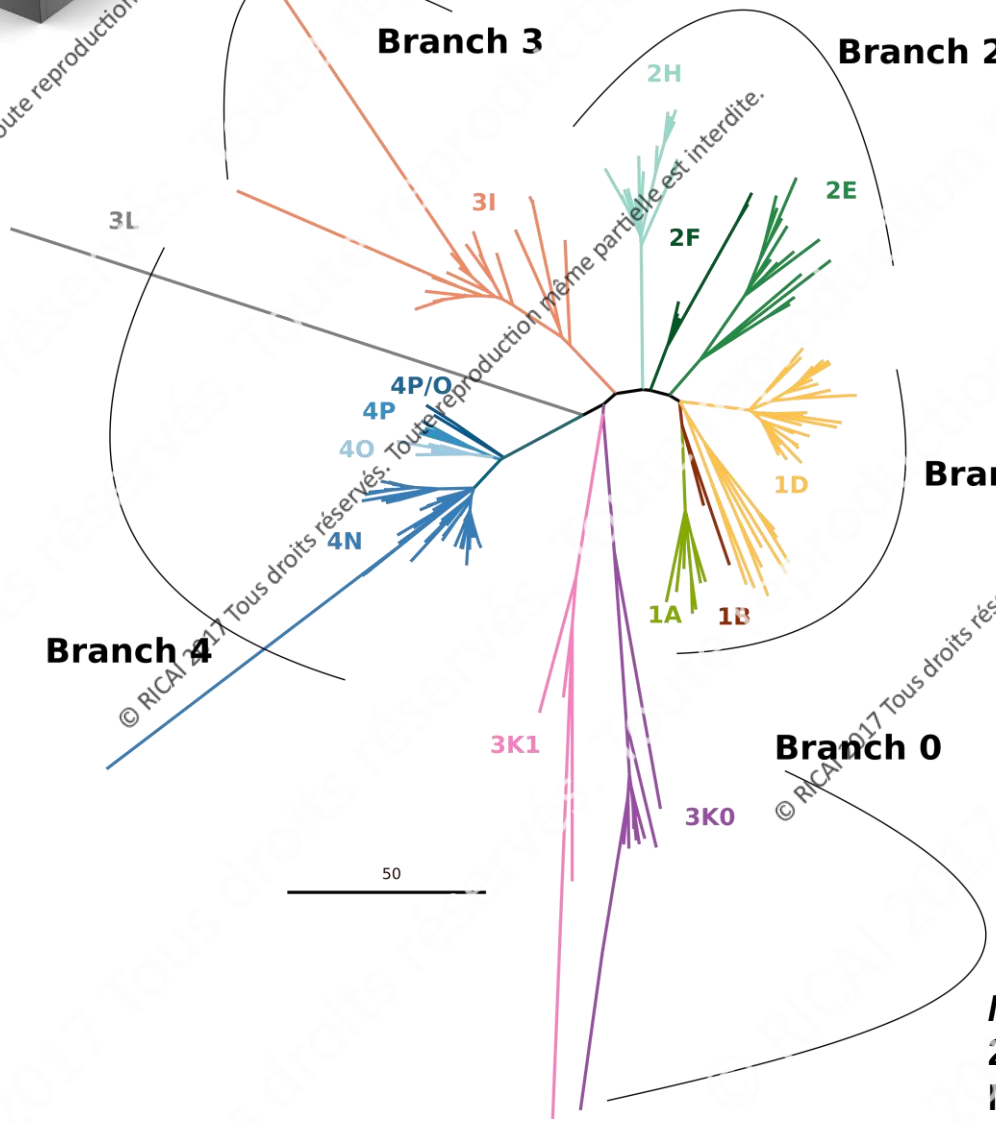
Benjak, Avanzi *et al. Nat. Com accepted*

De 20 à +100 génomes



M. leprae* - 185 génomes (ACLP: *M. lepromatosis
20 pays
MP (500 boostraps)

De 20 à +100 génomes



Phylogénie

Transmission

Résistance

M. leprae* - 185 génomes (ACLP: *M. lepromatosis
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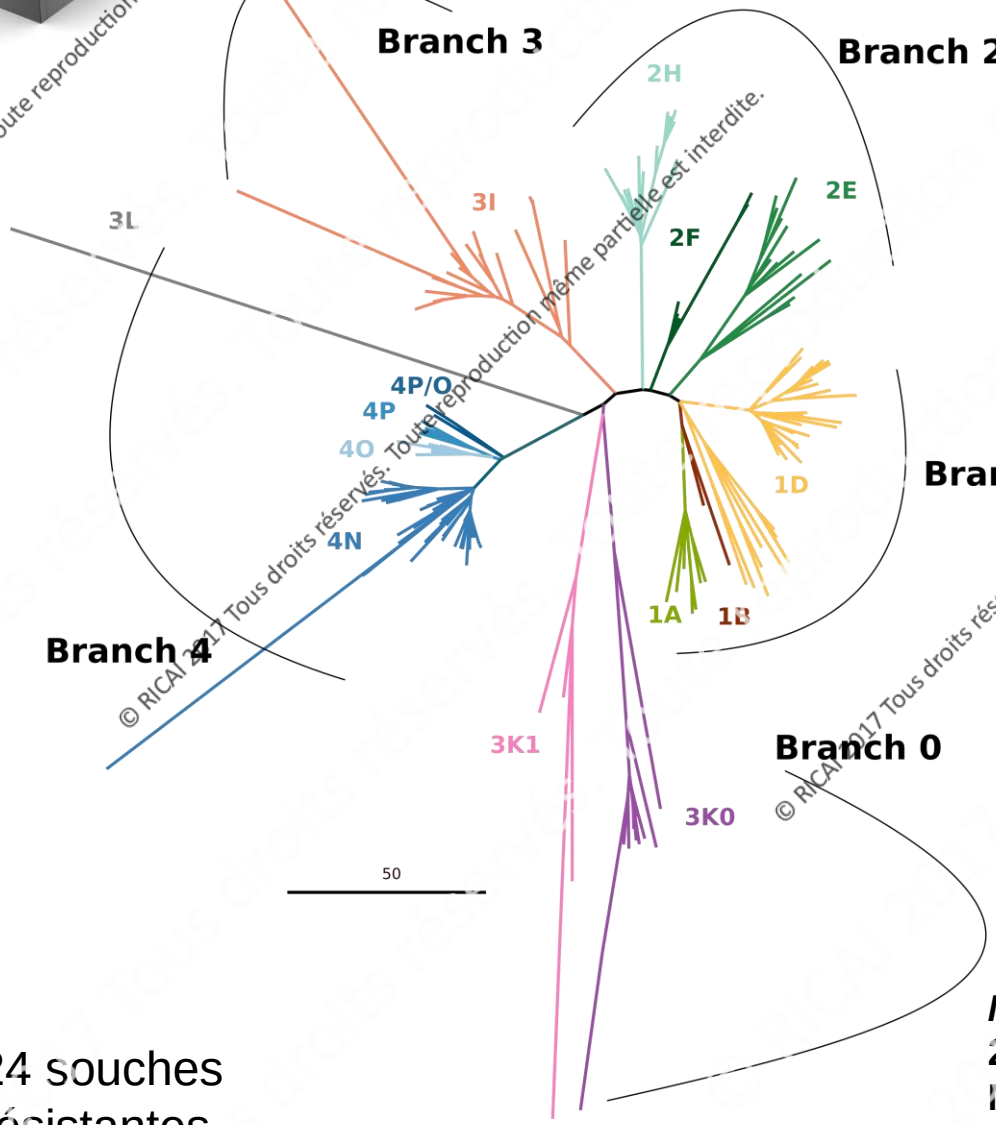
De 20 à +100 génomes



Phylogénie

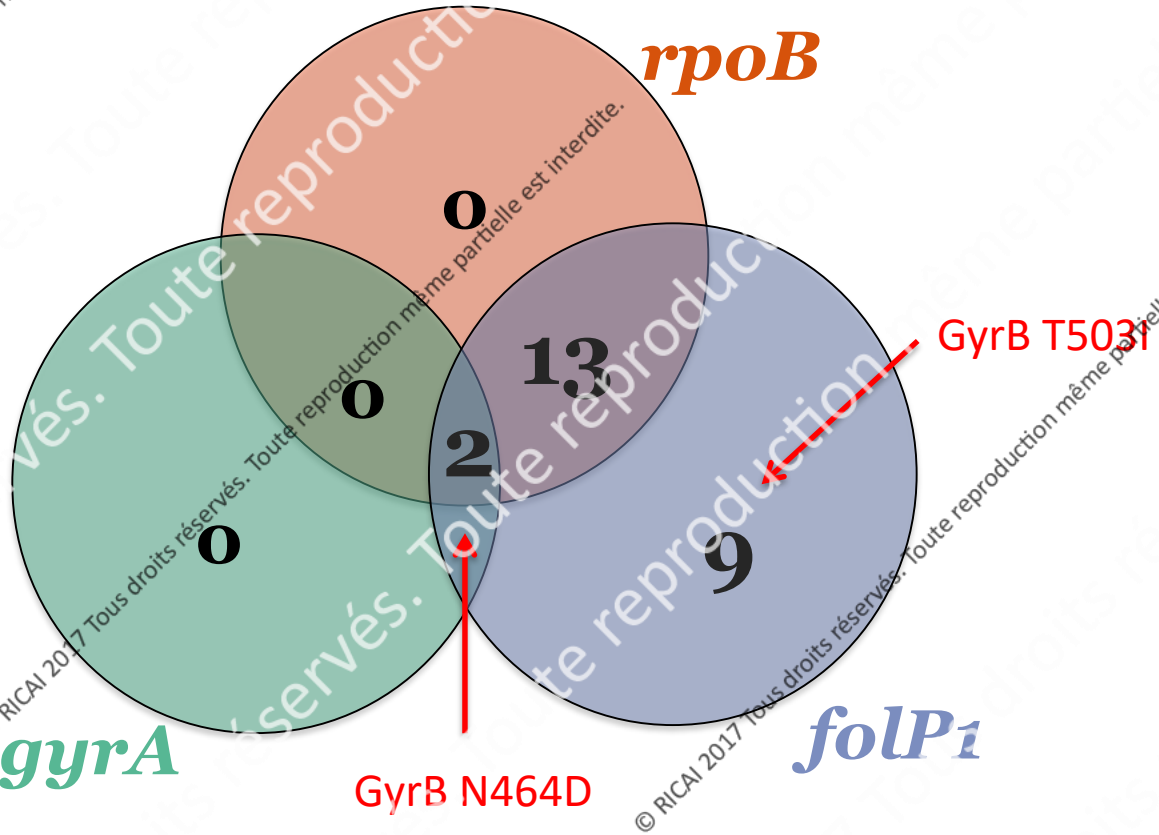
Transmission

Résistance

24 souches résistantes

M. leprae* - 185 génomes (ACLP: *M. lepromatosis
20 pays
MP (500 boostraps)

24 SOUCHES RÉSISTANTES



Possibles mutations compensatrices dans *rpoB*, *rpoC* et *rpoA* (**T187P**)

Benjak, Avanzi *et al. Nat. Com accepted,*
Comas et al, *Nat. Gen*, 2012

GÈNES HYPERMUTÉS (TOP 10)

Gene or region	Description	Non-synonymous mutations (multi-allele loci)	Synonymous mutations	Homo-plasy	Frameshift	STOP
ML0411	Serine-rich antigen	23 (4)	1	4		
ML1040c	Putative ATP-dependent helicase	18 (1)	0	0		
fadD9	Probable fatty-acid-CoA ligase	9	1	1	6(1)	2
ML1750c	Putative nucleotide cyclase	17	1	0		
ML1512c	Putative ribonuclease J	17	0	0		
ribD	Bifunctional enzyme riboflavin biosynthesis protein	13 (1)	0	2	2	1
rpoB	DNA-directed RNA polymerase (beta chain)	12 (1)	0	3		
ML1753c	Probable transcriptional regulatory protein	9	0	0		
gyrA	DNA gyrase (subunit A)	8	8	1		
ML1300	Conserved hypothetical protein	8	0	0		

fadD9: 16/18 mutations dans des souches résisantes
ribD: 15/18 mutations dans des souches résistantes

Benjak, Avanzi *et al. Nat. Com accepted*

GÈNES HYPERMUTÉS (TOP 10)

Gene or region	Description	Non-synonymous mutations (multi-allele loci)	Synonymous mutations	Homo-plasy	Frameshift	STOP
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ML1750c	Putative nucleotide cyclase	17	1	0		
ML1512c	Putative ribonuclease J	17	0	0		
ribD	Bifunctional enzyme riboflavin biosynthesis protein	13 (1)	0	2	2	1
rpoB	DNA-directed RNA polymerase (beta chain)	12 (1)	0	3		
ML1753c	Probable transcriptional regulatory protein	9	0	0		
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fadD9: 16/18 mutations dans des souches résisantes
ribD: 15/18 mutations dans des souches résistantes

Benjak, Avanzi *et al. Nat. Com accepted*

RibD A DES ORTHOLOGUES CHEZ….

- *M. tb: Rv2671 (ribD)*
- *M. smegmatis: MSMEG_2787*

RibD est un analogue de DHFR

- *M. tb: Rv2671 (ribD) – pas essentiel*
- *M. smegmatis: MSMEG_2787*

Structural Insights into *Mycobacterium tuberculosis* Rv2671 Protein as a Dihydrofolate Reductase Functional Analogue Contributing to *para*-Aminosalicylic Acid Resistance

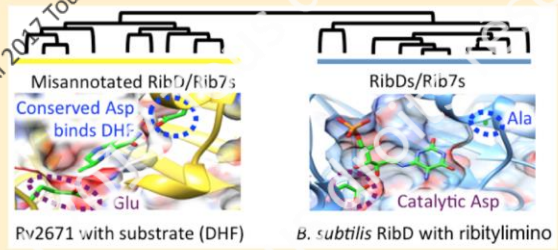
Yu-Shan Cheng[†] and James C. Sacchettini[*,†,‡]

[†]Department of Chemistry, Texas A&M University, College Station, Texas 77842, United States

[‡]Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, United States

Supporting Information

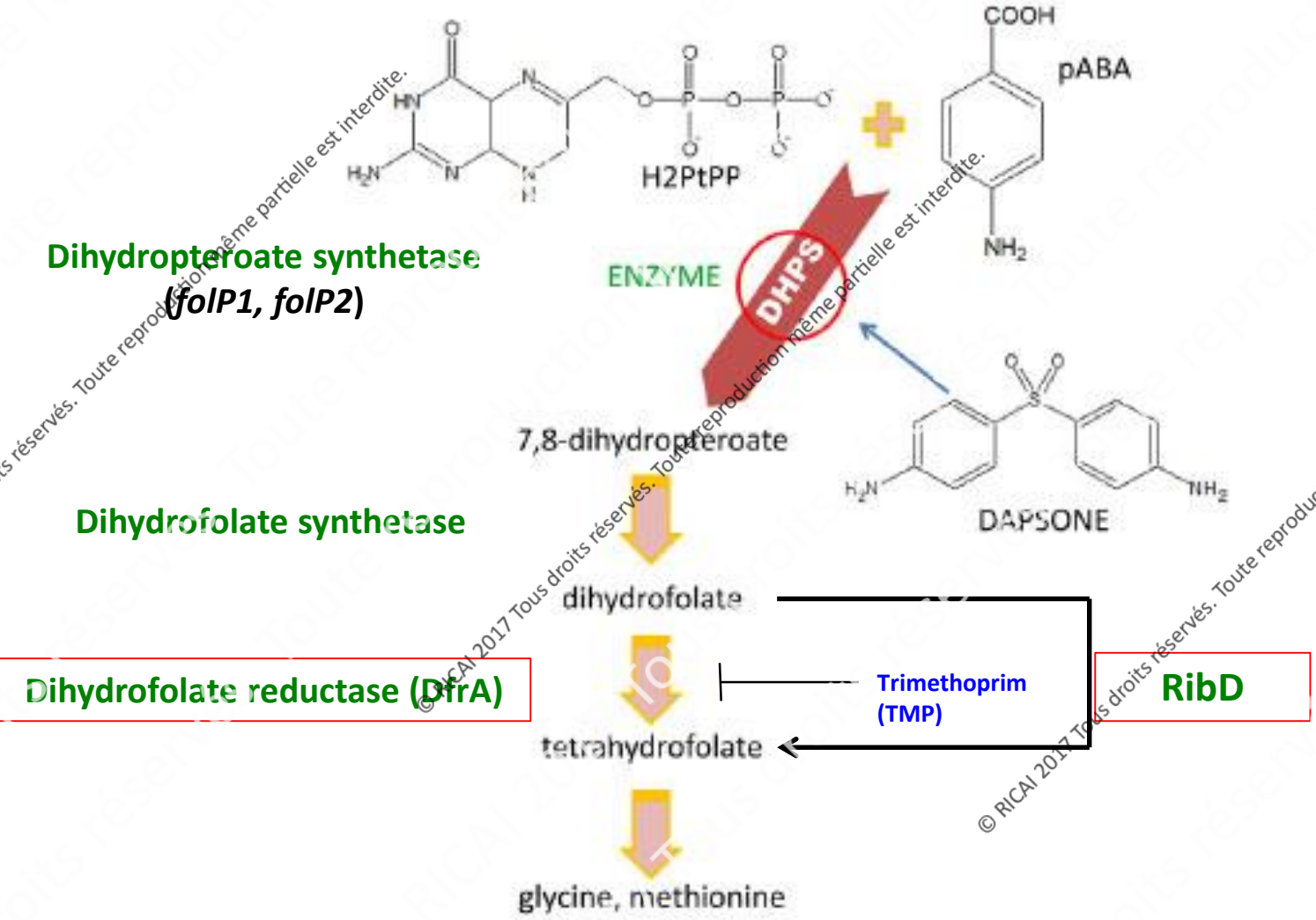
ABSTRACT: *Mycobacterium tuberculosis* (*Mtb*) Rv2671 is annotated as a 5-amino-6-ribitylamino-2,4(1*H*,3*H*)-pyrimidinedione 5′-phosphate (AROPP) reductase (RibD) in the riboflavin biosynthetic pathway. Recently, a strain of *Mtb* with a mutation in the 5′ untranslated region of *Rv2671*, which resulted in its overexpression, was found to be resistant to dihydrofolate reductase (DHFR) inhibitors including the anti-*Mtb* drug para-aminosalicylic acid (PAS). In this study, a biochemical analysis of Rv2671 showed that it was able to catalyze the reduction of dihydrofolate (DHF) to tetrahydrofolate (THF), which explained why the overexpression of Rv2671 was sufficient to confer PAS resistance. We solved the structure of Rv2671 in complex with the $NADP^+$ and tetrahydrofolate (THF), which revealed the structural basis for the DHFR activity. The structures of Rv2671 complexed with two DHFR inhibitors, trimethoprim and trimetrexate, provided additional details of the substrate binding pocket and elucidated the differences between their inhibitory activities. Finally, Rv2671 was unable to catalyze the reduction of AROPP, which indicated that Rv2671 and its closely related orthologues are not involved in riboflavin biosynthesis.



Rv2671 with substrate (DHF) — *B. subtilis* RibD with ribitylimino

Cheng et Sacchettini, 2016, Biochemistry

VOIE DES FOLATES ET INHIBITEURS



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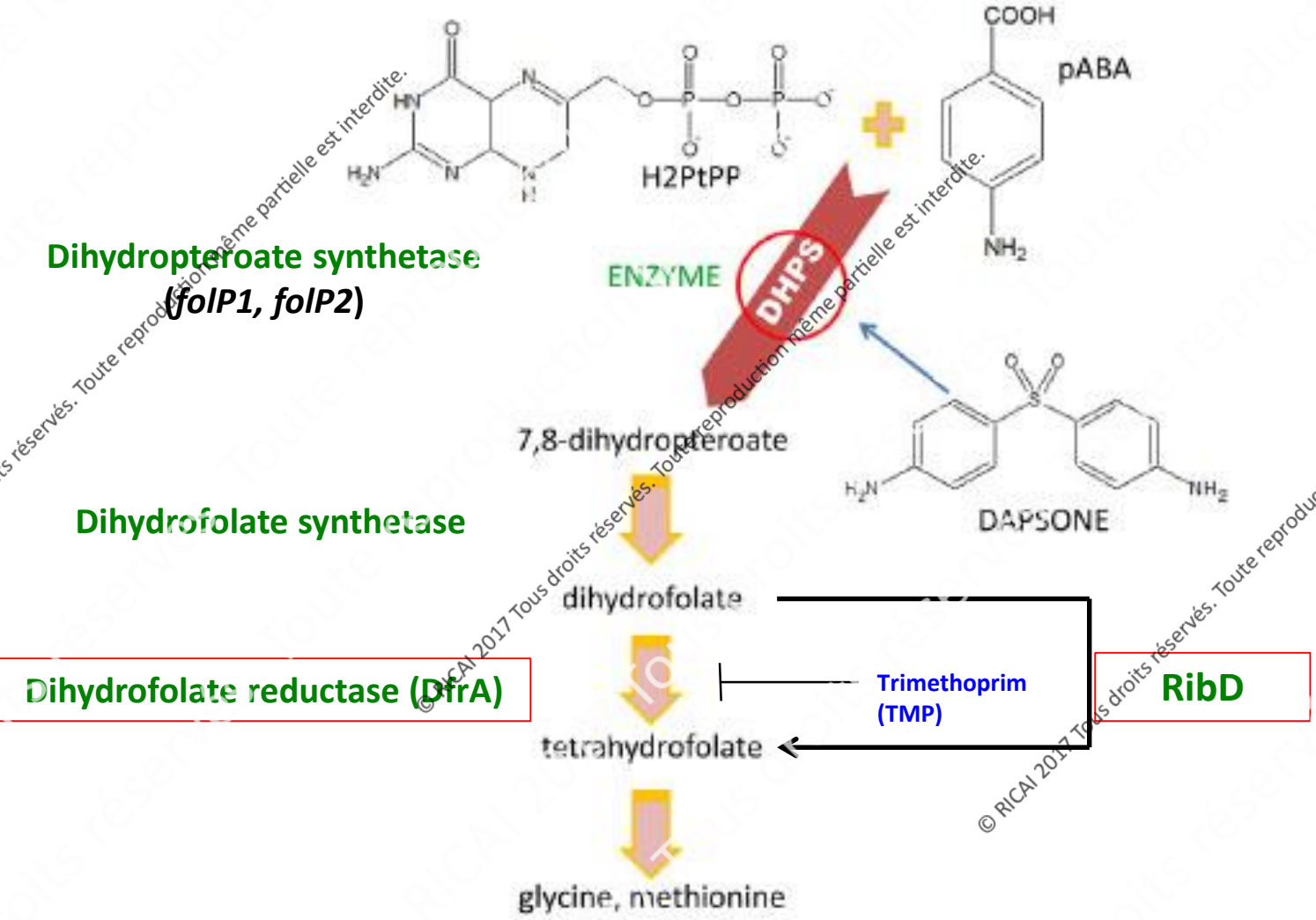
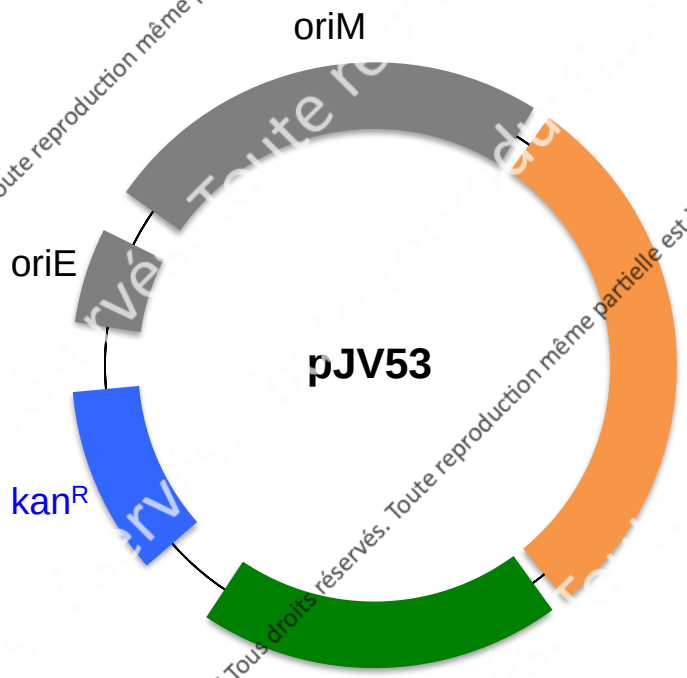
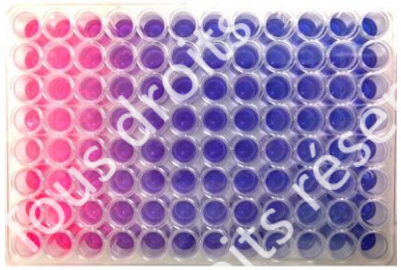


Hypothèse 1: autre mécanisme de résistance à la dapsone

RECOMBINAISON CHEZ *M. SMEGMATIS*

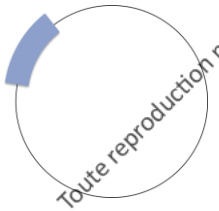




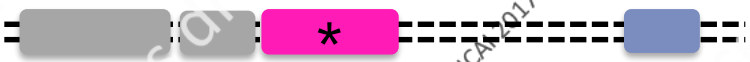
*Mutation ponctuelle

Synthétique sbADN

Plasmide + Hyg^R



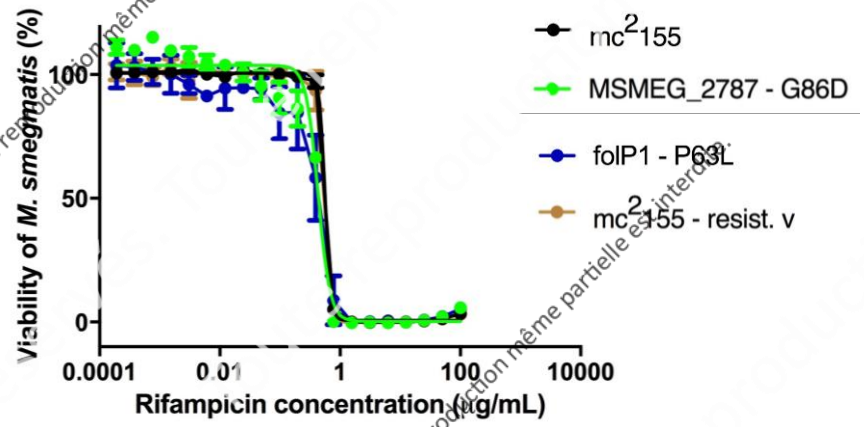
sbADN recombiné dans le chromosome



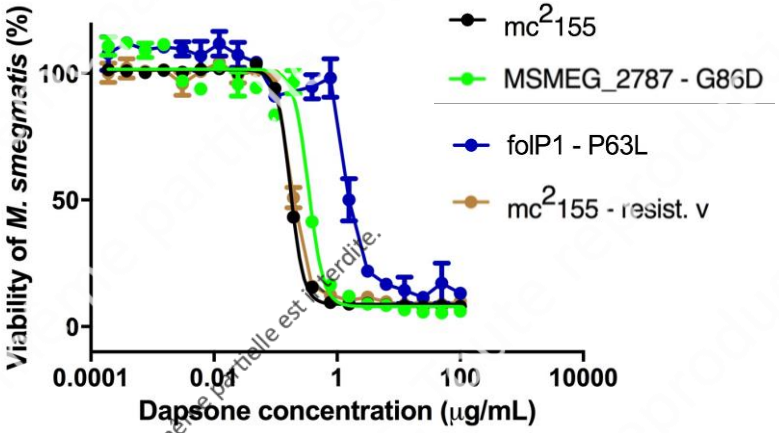
MSMEG_2787 chez M. smegmati
folp1 chez M. smegmatis

Van Kessel et Hatfull 2007, Nat. Meth.

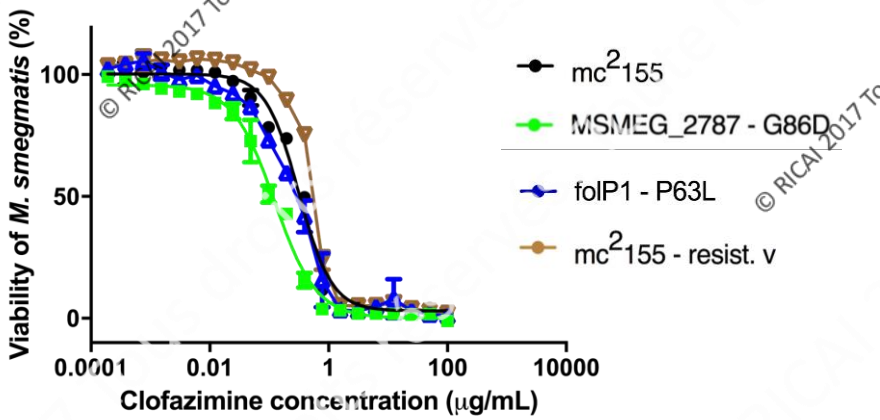
RÉSULTATS PRÉLIMINAIRES



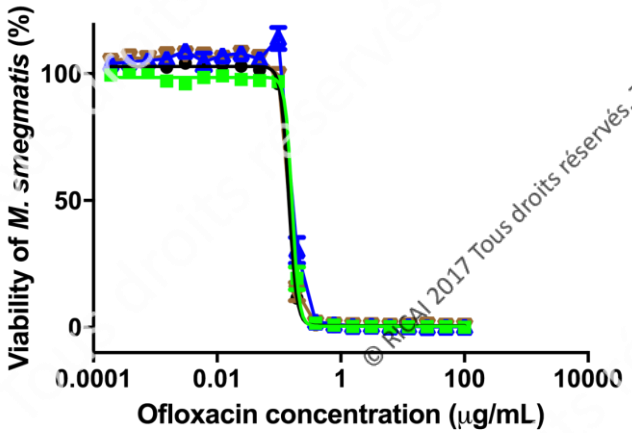
	mc²155	MSMEG_2787 - G86D	folP1-P63L	mc²155 - resist.
MIC	0.7652	0.7999	0.8656	0.6576



	mc²155	MSMEG_2787 - G86D	folP1-P63L	mc²155 - resist.
MIC	0.2561	0.7523	2.499	0.2437

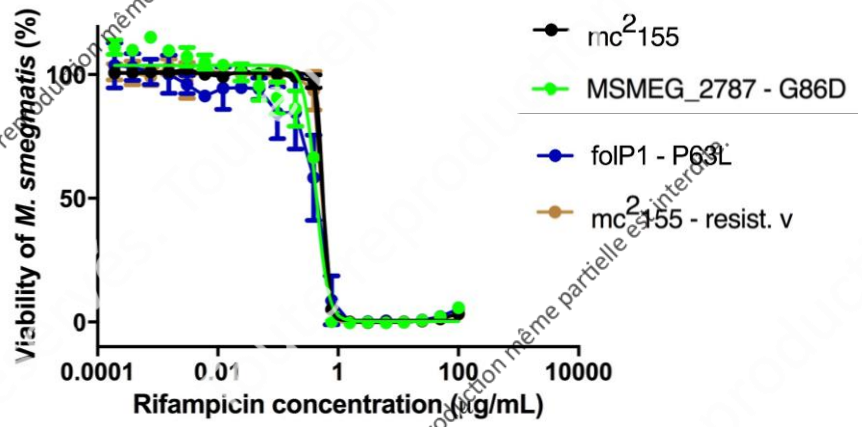


	mc²155	MSMEG_2787 - G86D	folP1-P63L	mc²155 - resist
MIC	1.076	0.6453	1.204	1.052



	mc²155	MSMEG_2787 - G86D	folP1-P63L	mc²155 - resist. v
MIC	0.2029	0.2109	~ 0.2025	0.2067

RÉSULTATS PRÉLIMINAIRES



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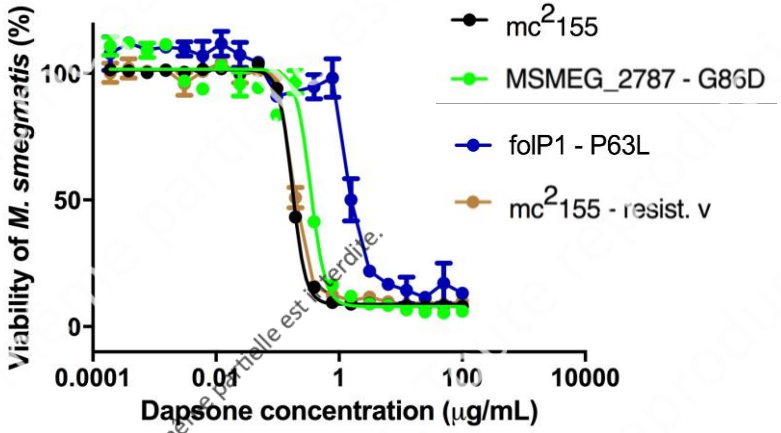

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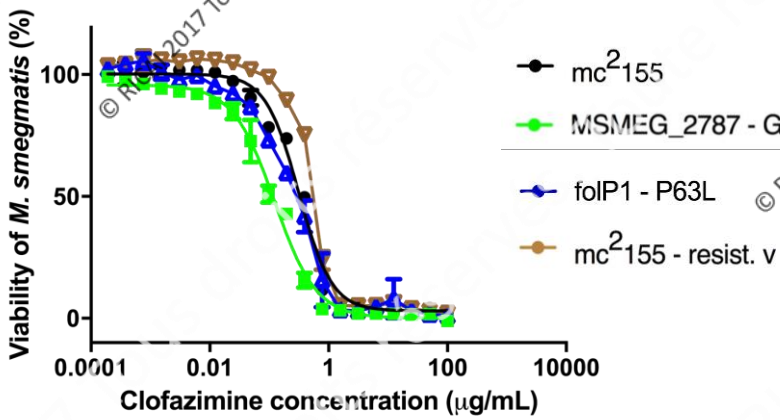

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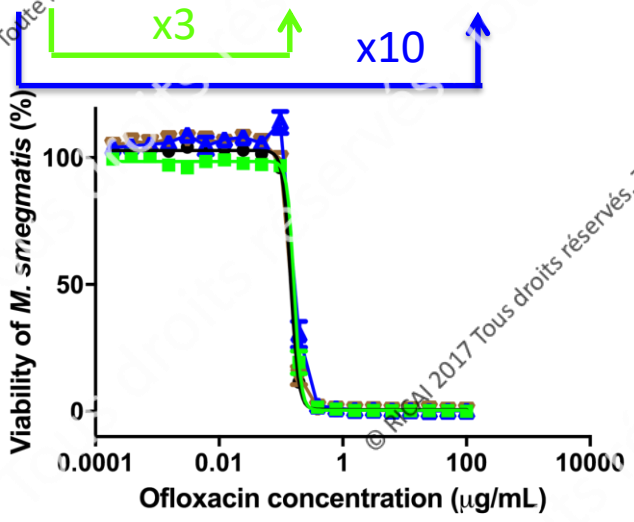

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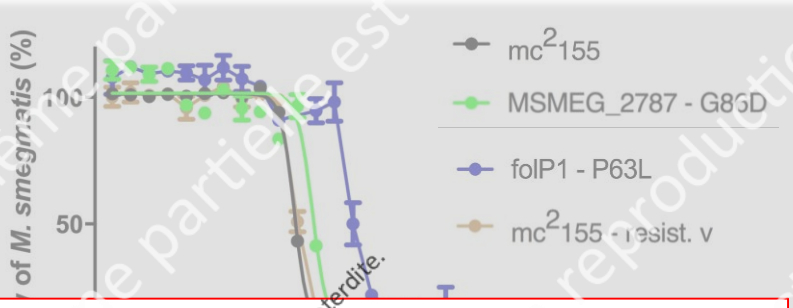
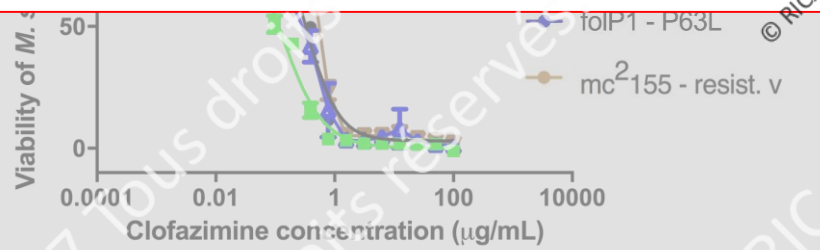
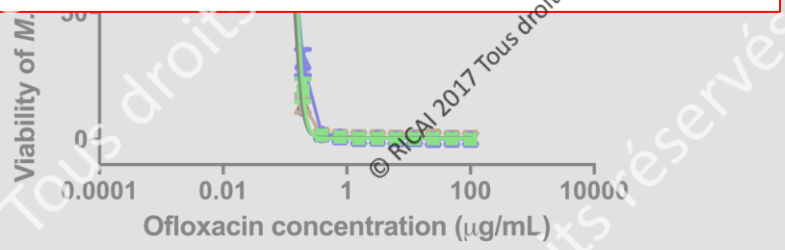


- Faible résistance pour *folP1*
- Résultats non concluants pour le mutant *ribD* (dapsone)
- Autres mutations?
- Résistance à un autre médicament?
- Mécanismes de compensation?
- *M. smegmatis* n'est pas le bon modèle?





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CONCLUSIONS & FUTURES APPLICATIONS

- Outils performant pour le séquençage des génomes de *M. leprae* à partir des échantillons cliniques

- Le WGS est une approche performante pour l'étude des résistances:
  - Identification de nouveaux mécanismes
    - Autres mutations à tester: *fadD9* par exemple
  - Mais…pas facile à démontrer ensuite!

- Futures applications
  - Augmenter le nombre de souches résistantes séquençées
  - Accès à l'historique des patients est primordial!
  - Etude avec plus de souches résistantes provenant de patients ayant rechutés ou ré-infectés: résistance & persistence *vs.* virulence

REMERCIEMENTS