Le séquençage ciblé : une boite à outils pour la surveillance et le diagnostic de l'antibiorésistance

Nelly Badalato, PhD

Colloque ALBIO
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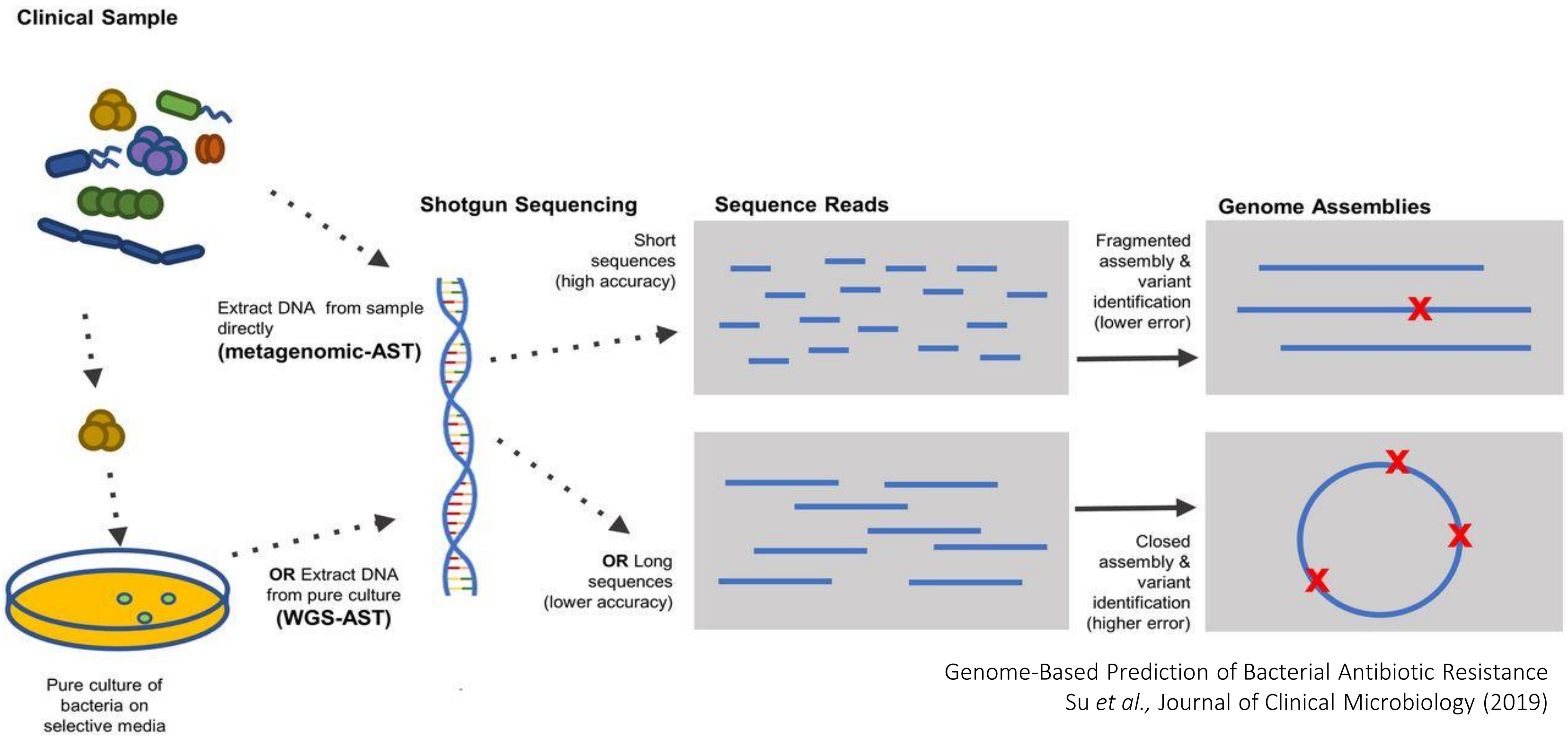
GenoScreen
Innovative Genomics

1, rue du Professeur Calmette 59000 LILLE
Phone: +33 (0)3 20 87 71 53 - Fax : +33 (0)3 3 20 87 72 64
www.genoscreen.com - contact@genoscreen.com

Genome sequencing to detect antimicrobial resistance



Genome-Based Prediction of Bacterial Antibiotic Resistance
Su *et al.*, Journal of Clinical Microbiology (2019)

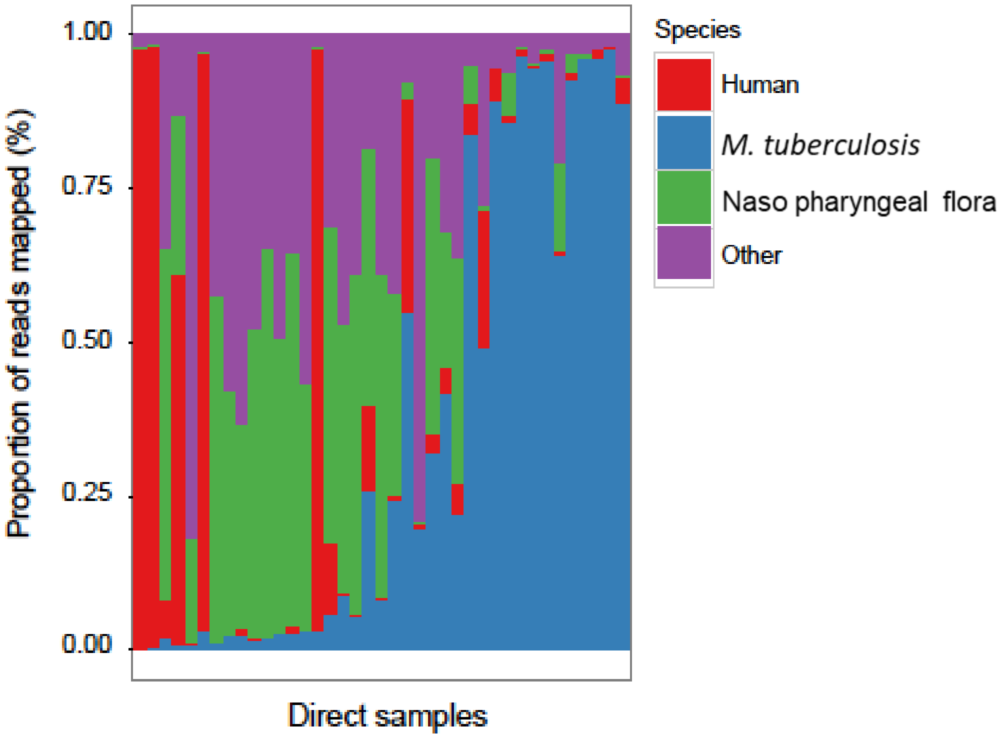
- Helps if culture is long or difficult,
- in case of unreliable phenotypic testing,
- or mixed infections
- Gives information on typing
- Usually Whole Genome Sequencing

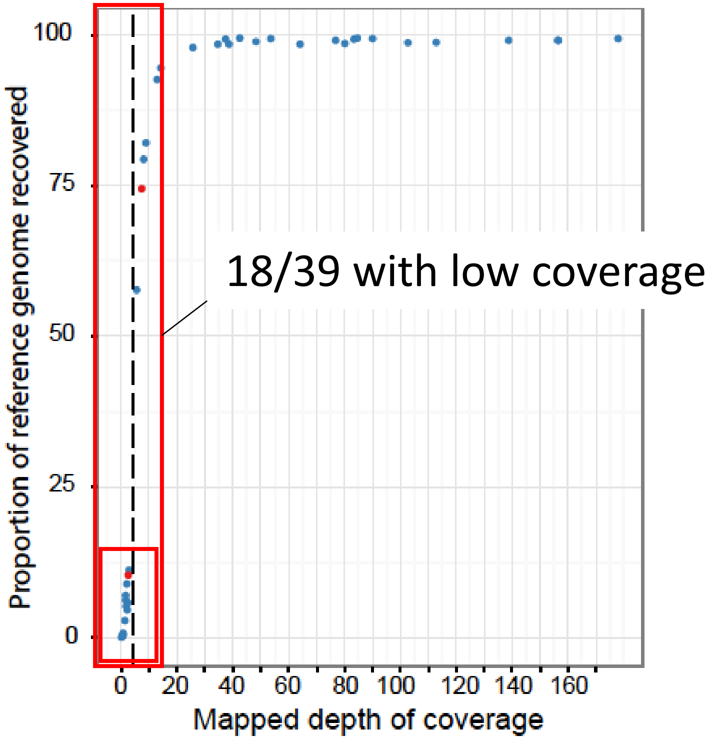
WGS drawbacks in antimicrobial resistance detection

Clinical samples : low amount of bacterial DNA, host DNA, contamination …

➡

Low coverage : inability to detect resistance and potential resistant subpopulations

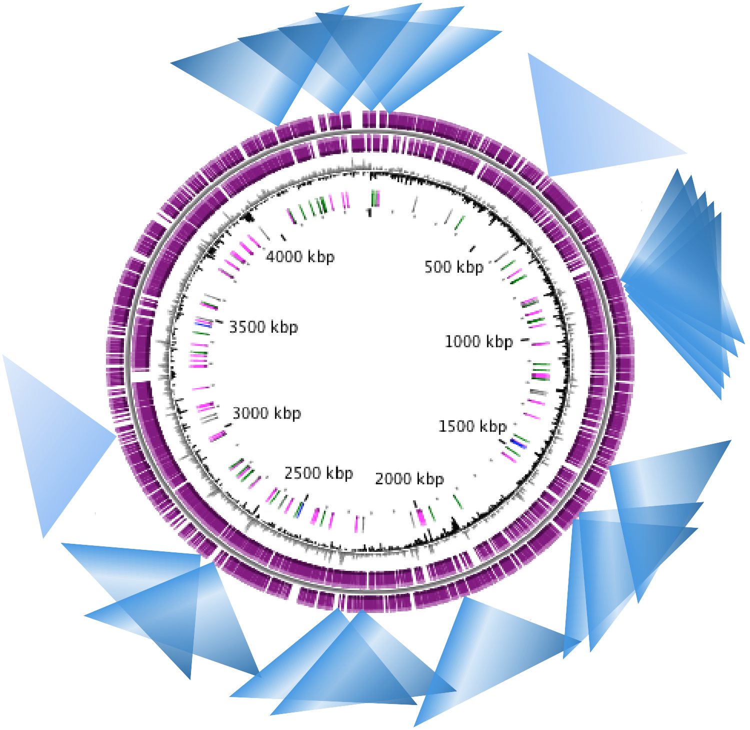




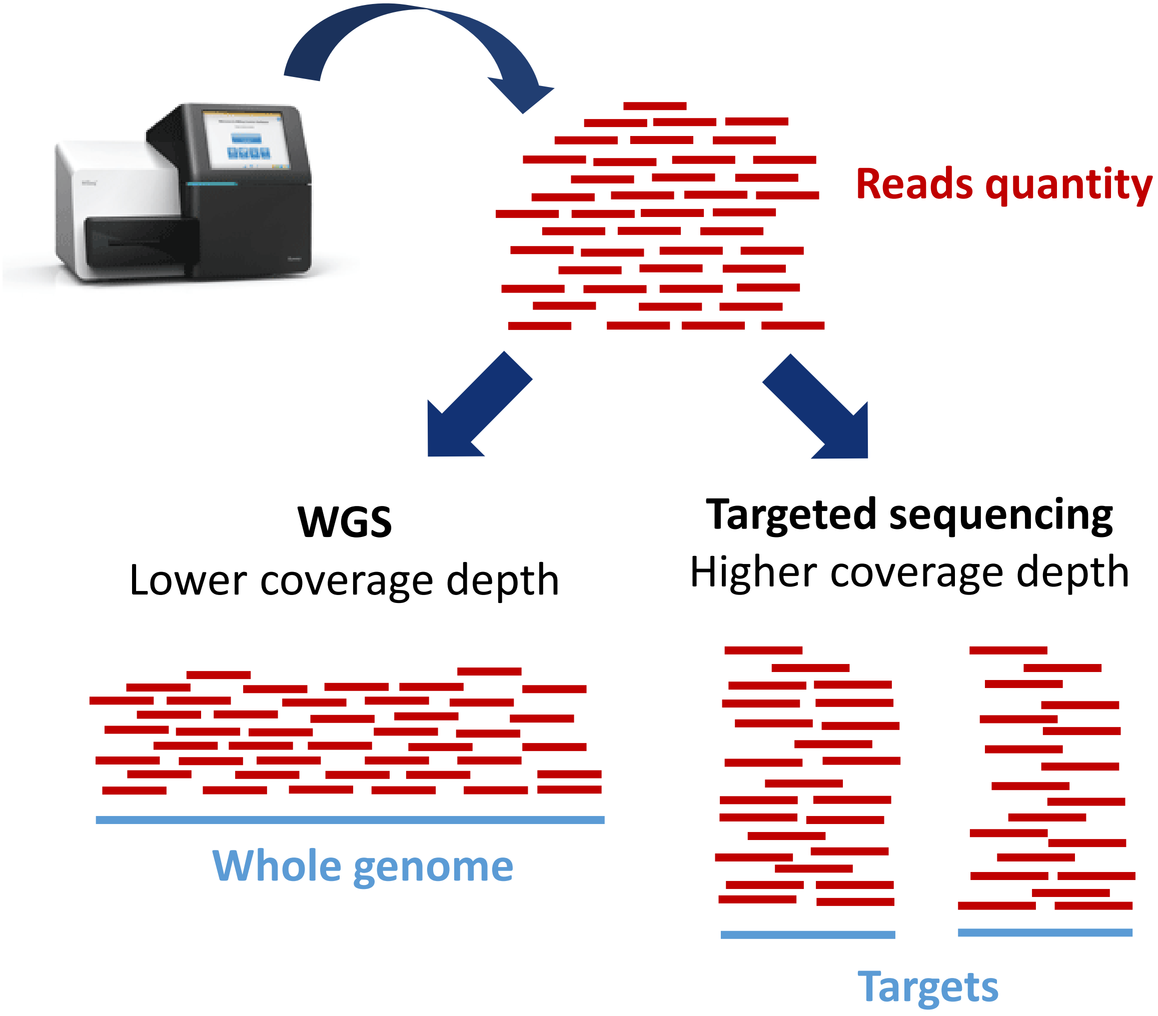
18/39 with low coverage

Same-day diagnostic and surveillance data for tuberculosis via whole-genome sequencing of direct respiratory samples
Votintseva *et al.,* Journal of Clinical Microbiology (2017)

Targeted sequencing to detect antimicrobial resistance



- ➡ **Custom panels of genes/targets**



Reads quantity

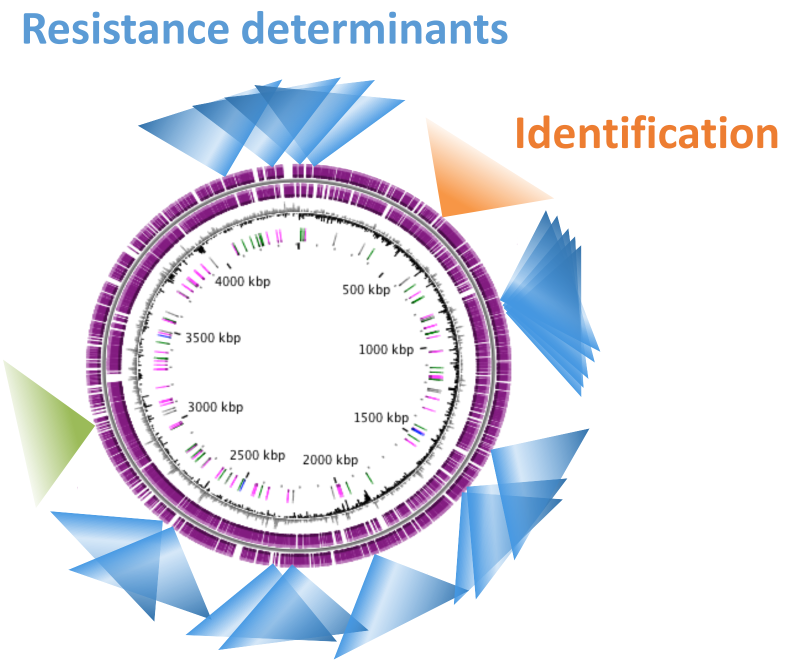
WGS
Lower coverage depth

Targeted sequencing
Higher coverage depth

Whole genome

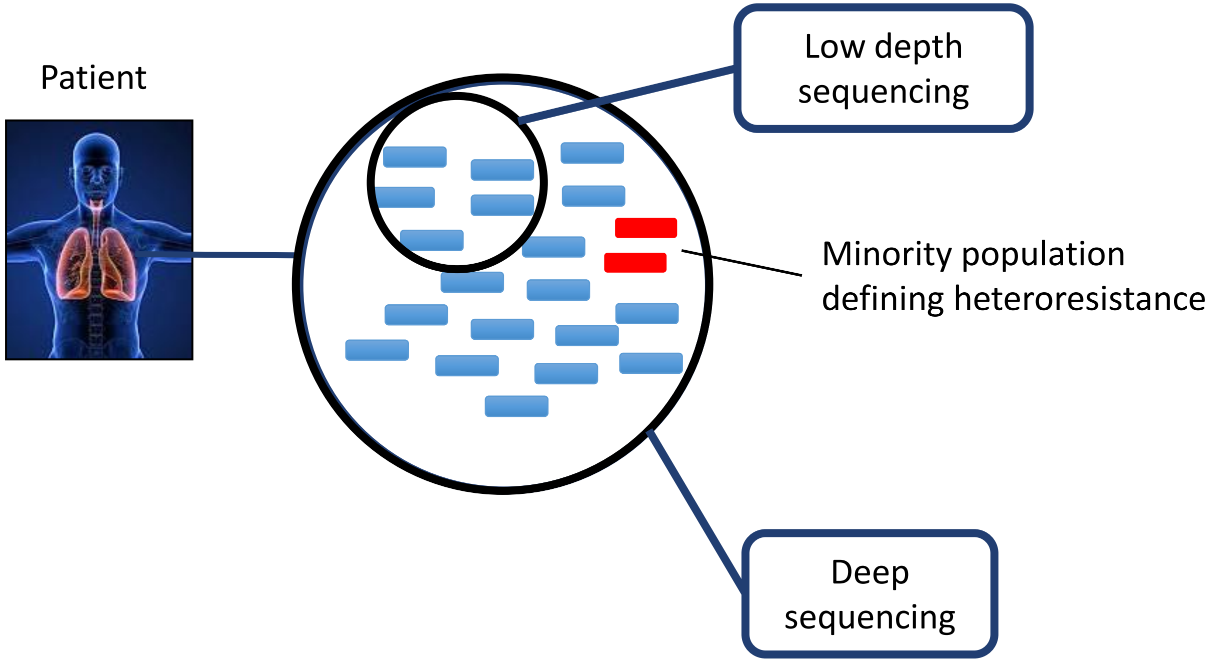
Targets

A toolbox for drug resistance diagnosis



Resistance determinants

Identification



Patient

Low depth sequencing

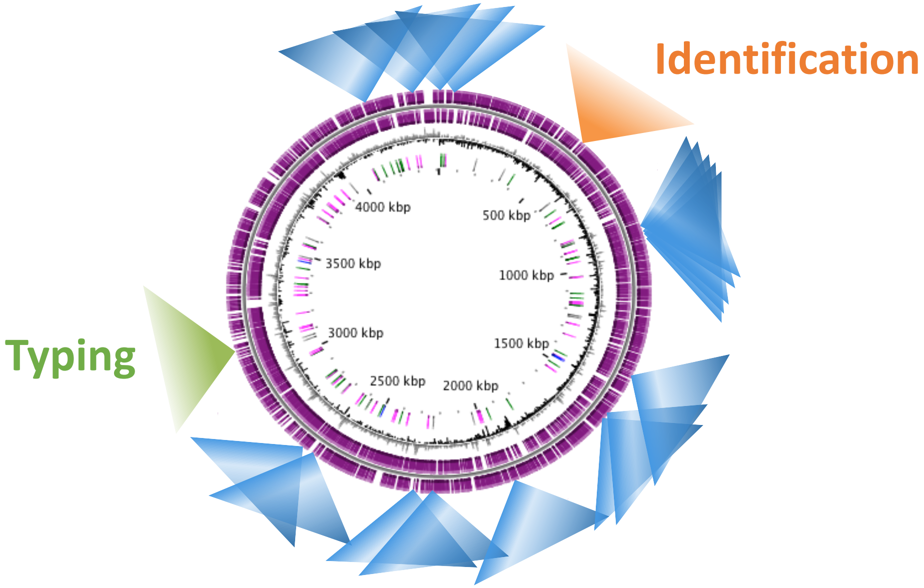
Minority population defining heteroresistance

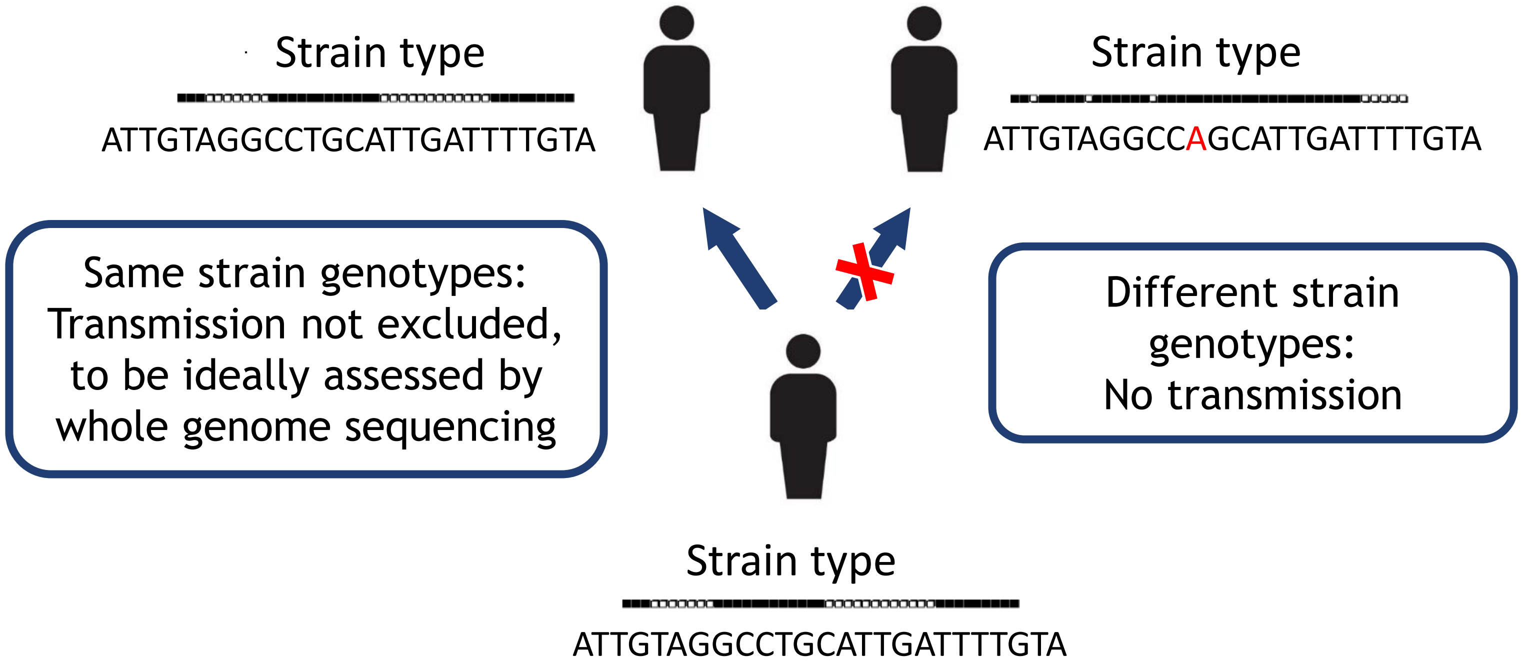
Deep sequencing

➧ **Detection of minority variants (heteroresistance) and mixed infection for personalized treatment**

Wild type, drug sensitive 🟦
Mutant, drug resistant 🟥

A toolbox for molecular-guided surveillance





Strain type

ATTGTAGGCCTGCATTGATTTTGTA

Strain type

ATTGTAGGCCAGCATTGATTTTGTA

Same strain genotypes:
Transmission not excluded,
to be ideally assessed by
whole genome sequencing

Different strain
genotypes:
No transmission

Strain type

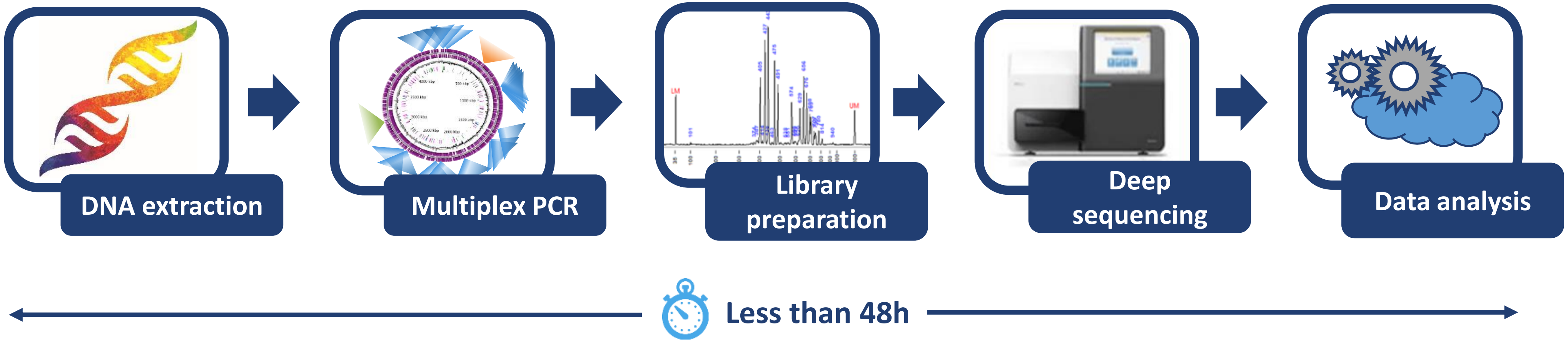
ATTGTAGGCCTGCATTGATTTTGTA

➡ **Detection of strain genotypes to guide information on transmission, laboratory cross-contamination, distinguish re-infection from treatment failure and true relapse**

Likelihood of epidemiological links in a molecular cluster depends on :

- Resolution power of molecular marker used
- Local prevalence of molecular type

A targeted sequencing workflow



DNA extraction → Multiplex PCR → Library preparation → Deep sequencing → Data analysis

Less than 48h

Low genomic DNA input
Directly applicable on clinical samples

Flexible throughput
To fit the need

Deep sequencing
For heteroresistance and mixed infection detection

Standardized softwares
For analysis and interpretation

Diagnostic and surveillance of drug resistant tuberculosis as an example

rpoB	Rifampicin	1st line antibiotics
ahpC, fabG1, katG, inhA	Isoniazid	
pncA	Pyrazinamide	
embB	Ethambutol	
hsp65	Species ID	Identification
CRISPR/DR	Spoligotyping	
phyloSNPS	Genotyping	

2nd line antibiotics	Streptomycin	*gidB, rrs, rpsL*
	Fluoroquinolones	*gyrA, gyrB*
	Kanamycin	*eis, rrs*
	Amikacin	*rrs*
	Capreomycin	*tlyA, rrs*
	Ethionamide	*ethA, inhA, fabG1*
New antibiotics	Bedaquiline, Clofazimine	*rv0678*
	Linezolid	*rrl, rplC*

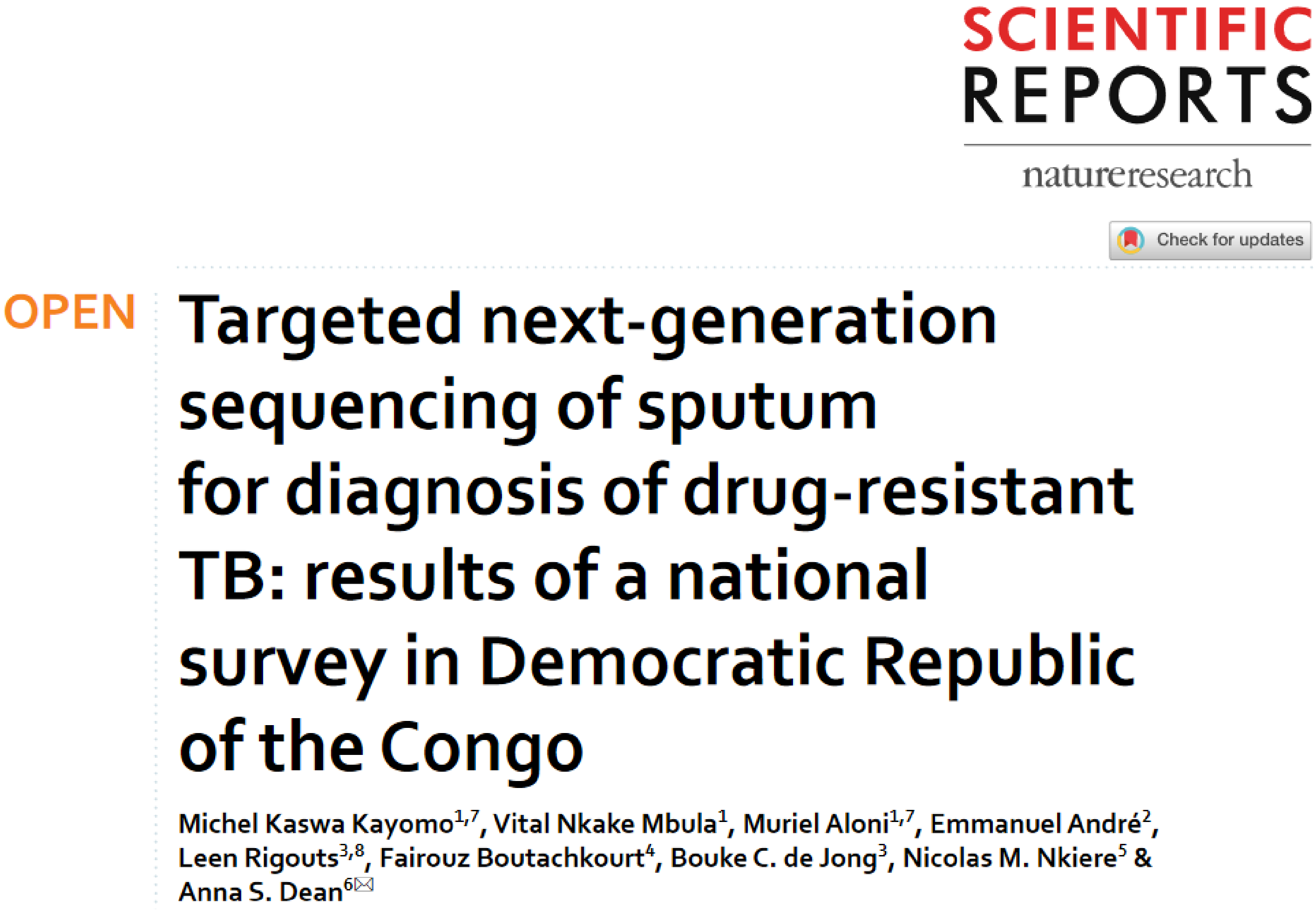
- 20 main genes or gene regions of the *M. tuberculosis* genome amplified and sequenced

- *In silico* evaluation on > 3600 MTB genomes *vs* WGS: prediction of >97-99% phenotypes (2 subsets)

- With subset of 1,552 genomes : 89.2 % of phenotypes predicted with a specificity and sensitivity >90%

Deep amplicon sequencing for culture-free prediction of susceptibility or resistance to 13 anti-tuberculous drugs
Jouet et al., Eur Resp J (2020)

Diagnostic and surveillance of drug resistant tuberculosis as an example



SCIENTIFIC
REPORTS
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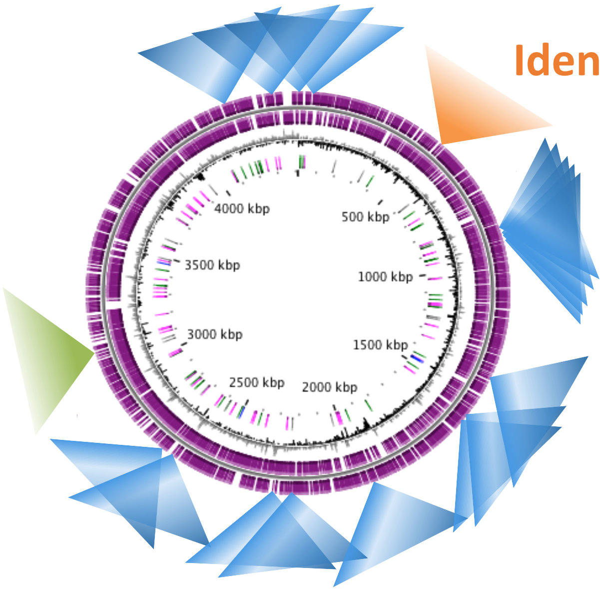
Targeted next-generation sequencing of sputum for diagnosis of drug-resistant TB: results of a national survey in Democratic Republic of the Congo

Michel Kaswa Kayomo[1,7], Vital Nkake Mbula[1], Muriel Aloni[1,7], Emmanuel André[2], Leen Rigouts[3,8], Fairouz Boutachkourt[4], Bouke C. de Jong[3], Nicolas M. Nkiere[5] & Anna S. Dean[6] ✉

- First nationwide survey based only on NGS directly from sputum supported by WHO
- 1, 326 samples sequenced among 1,661 positive MTB samples
  - 70 cases of mixed infections detected
  - 3 cases of infections with non-tuberculous mycobacteria
- Identification of INH resistant infection not detected by the WHO recommended rapid molecular test

New pathogen, new toolbox

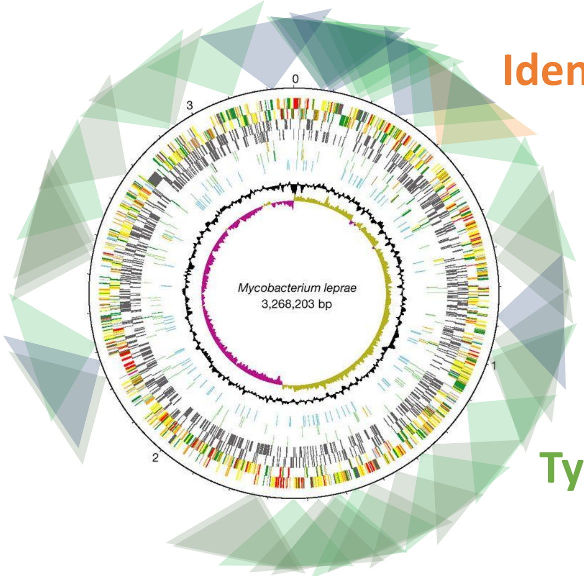


Resistance determinants

Identification

Typing

M. tuberculosis



Resistance determinants

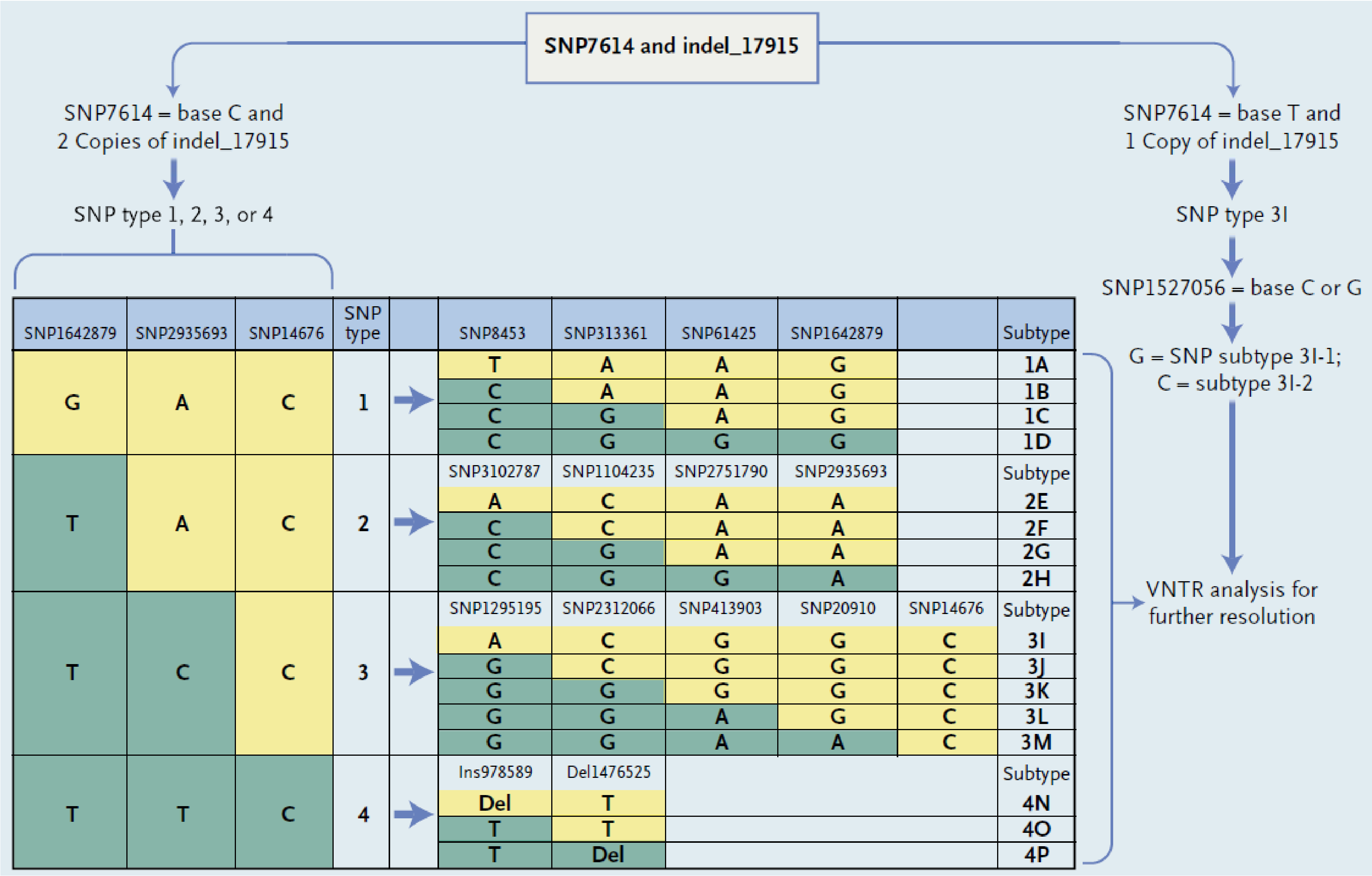
Identification

Typing

M. leprae

➡ **Spoligotypes are specific of *M. tuberculosis* strains ? For *M. leprae* use VNTR and SNPs …**

New pathogen, new toolbox



SNP7614 and indel_17915

SNP7614 = base C and
2 Copies of indel_17915

SNP type 1, 2, 3, or 4

SNP1642879	SNP2935693	SNP14676	SNP type		SNP8453	SNP313361	SNP61425	SNP1642879		Subtype
G	A	C	1	→	T	A	A	G		1A
					C	A	A	G		1B
					C	G	A	G		1C
					C	G	G	G		1D
T	A	C	2	→	SNP3102787	SNP1104235	SNP2751790	SNP2935693		Subtype
					A	C	A	A		2E
					C	C	A	A		2F
					C	G	A	A		2G
					C	G	G	A		2H
T	C	C	3	→	SNP1295195	SNP2312066	SNP413903	SNP20910	SNP14676	Subtype
					A	C	G	G	C	3I
					G	C	G	G	C	3J
					G	G	G	G	C	3K
					G	G	A	G	C	3L
					G	G	A	A	C	3M
T	T	C	4	→	Ins978589	Del1476525				Subtype
					Del	T				4N
					T	T				4O
					T	Del				4P

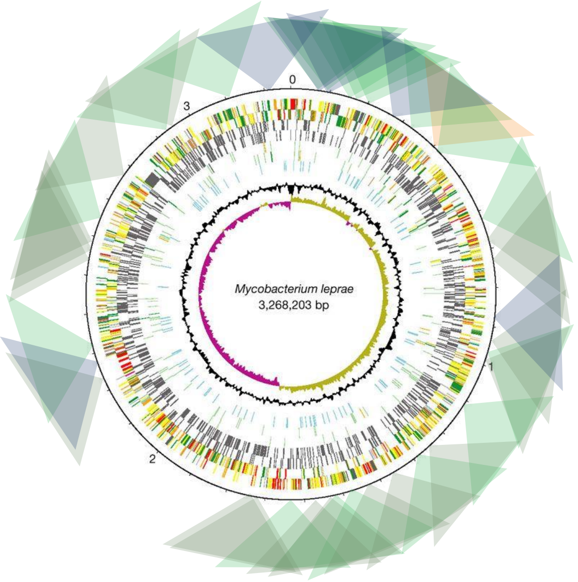
SNP7614 = base T and
1 Copy of indel_17915

SNP type 3I

SNP1527056 = base C or G

G = SNP subtype 3I-1;
C = subtype 3I-2

VNTR analysis for
further resolution



Probable Zoonotic Leprosy in the Southern United States
Truman et al. NEJM (2011)

A nice tool but not perfect

- Limited amount of information/less flexible
  - specially for epidemiological studies
  - for re-analysis
- Need for re-design to include new targets
- As WGS:
  - Depends on available information about drug resistance conferring mechanisms
  - Not yet a near-patient diagnostics
  - Still expensive and labour intensive compared to other rapid molecular tests

Conclusions

- Culture-free method
- Detection of heteroresistance and mixed infections with simultaneous typing
- Versatility for basic research questions and diagnosis
- Will benefit from rapidly-growing information about genotypic antimicrobial resistance …
- … and from sequencing technology evolution for more global adoption

Thank you

Mastering genomic information at the benefit of man and his environment