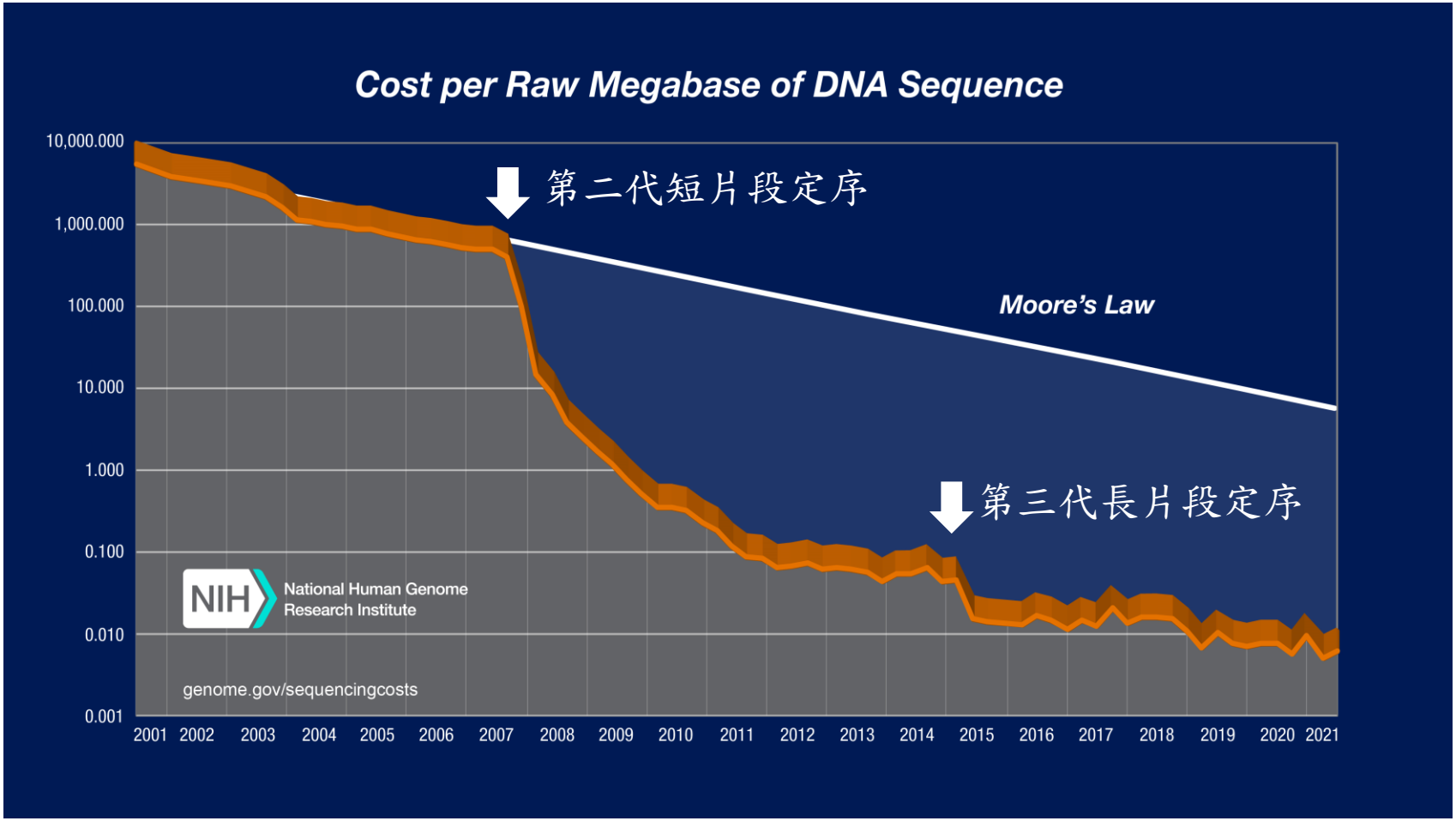
臺北醫學大學
TAIPEI MEDICAL UNIVERSITY

結核分枝桿菌鑑定的最新進展：長片段全基因組定序發展

臺北醫學大學
醫學檢驗暨生物技術學系
林榮俊 教授

基因定序的演進、比較、與原理

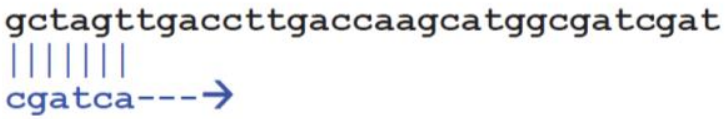
Sequencing Costs in 2021

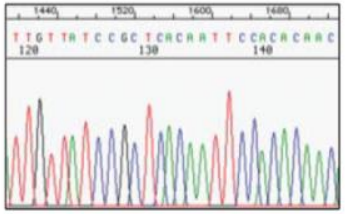


圖片來源：https://www.genome.gov/27541954/dna-sequencing-costs-data/

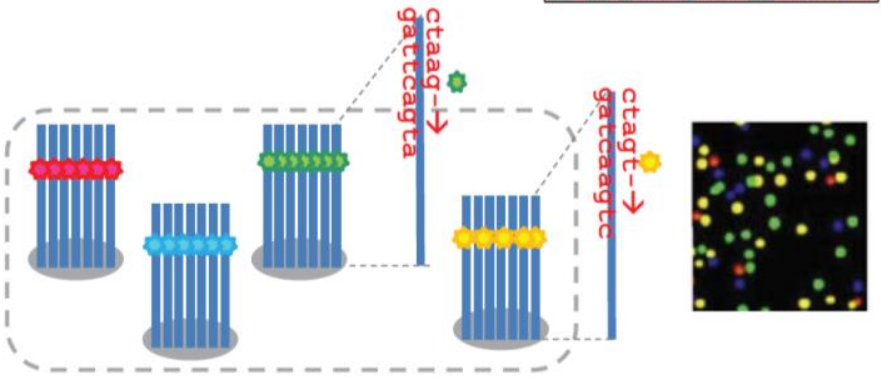
基因定序的演進:

第一代定序:單分子

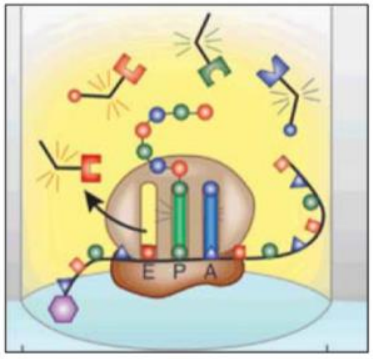


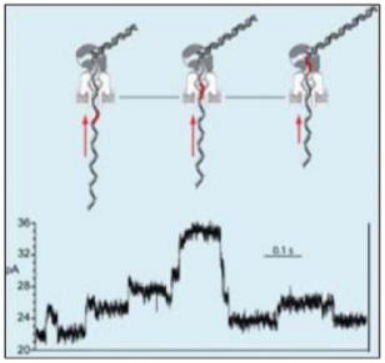


第二代定序:短片段叢聚式放大/定序
(Illumina)



第三代定序:長片段長片段即時定序
(PacBio; ONT)





基因定序平台的比較:

Platform	Roche 454	Illumina	Life Technologies	PacBio

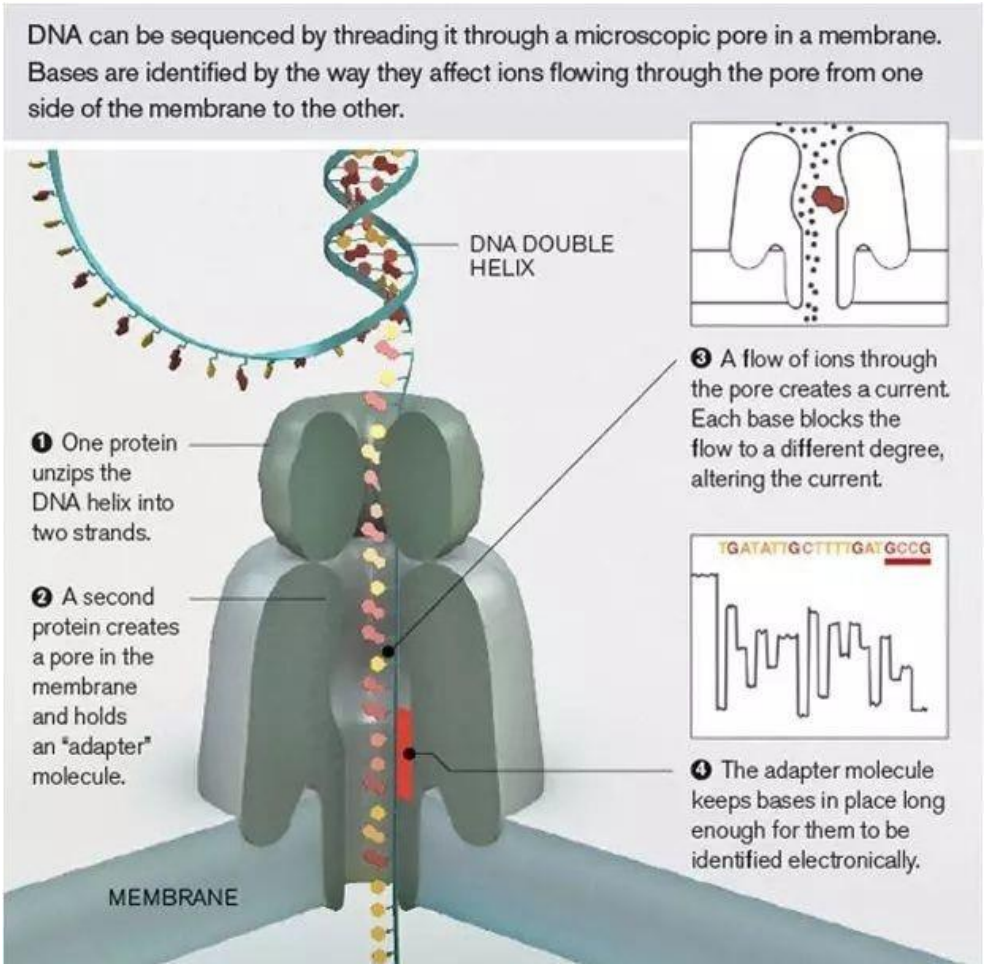
	Sanger sequencing	Next generation sequencing (Illumina etc.)	Third generation sequencing (ONT; PacBio)
Library preparation	Non-essential	Essential	Essential
PCR-amplification	Essential	Essential	Non-essential
Sequencing approach	Dideoxynucleotide termination	Reversible Dye Terminator Pyrophosphate sequencing	Real-time single molecule sequencing

Table 1. Comparison of the output of selected sequencing platforms. Numbers are according to companies or recent publications.

Platform	Sequencer	Costs sequencing platform	Reads per run/lane	Output per run/lane	Maximal read lengths[1]	Average run duration
Sanger	ABI 3730xl	$100,000	96	100 kbp	1000 bp	2–3 hours
454	GS FLX	$450,000	1,000,000	700 mpb	1000 bp	24 hours
Illumina	HiSeq 3000	$750,000	300,000,000[2]	150 gbp[3]	250 bp	4 days
Illumina	NextSeq500	$250,000	400,000,000	120 gbp[3]	150 bp	30 hours
Illumina	MiSeq	$100,000	25,000,000	15 gbp[3]	300 bp	24 hours
Ion Torrent	Proton II	$224,000	330,000,000	66 gbp	200 bp	4 hours
Ion Torrent	PGM 318	$50,000	5,000,000	2 gbp	400 bp	7 hours
PacBio	RS II	$700,000	50,000	400 mbp	54 kbp	3 hours
Nanopore	MinION	$1,000	80,000[4]	490 mbp[4]	150 kbp	n.a.[4]

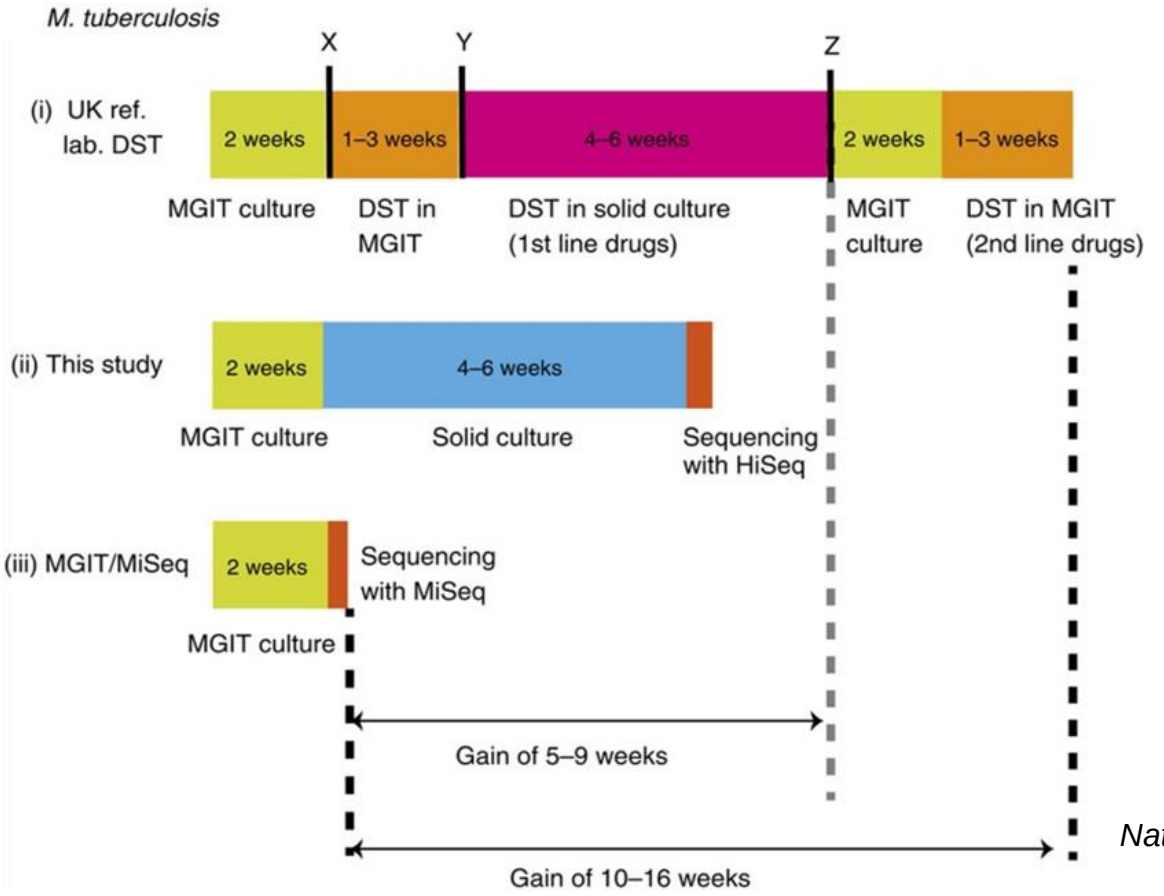
第三代定序–Nanopore sequencing

- 此定序平台使用可產生固定電流的的納米孔，DNA/RNA 鹼基通過納米孔時，根據鹼基大小對遮蔽納米孔的電流強度影響，鑑定所通過的鹼基序列。



基因定序對於結核桿菌之鑑定與診斷

Advantage of Whole genome sequencing on MTB identification: Time-saving



Nat. Commun. 2015;6:10063-10076.

Is it practicable to conduct WGS with original clinical specimen?

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The NEW ENGLAND JOURNAL of MEDICINE

ESTABLISHED IN 1812 OCTOBER 11, 2018 VOL. 379 NO. 15

Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing

The CRyPTIC Consortium and the 100,000 Genomes Project

ABSTRACT

BACKGROUND

The World Health Organization recommends drug-susceptibility testing of *Mycobacterium tuberculosis* complex for all patients with tuberculosis to guide treatment decisions and improve outcomes. Whether DNA sequencing can be used to accurately predict profiles of susceptibility to first-line antituberculosis drugs has not been clear.

METHODS

We obtained whole-genome sequences and associated phenotypes of resistance or susceptibility to the first-line antituberculosis drugs isoniazid, rifampin, ethambutol, and pyrazinamide for isolates from 16 countries across six continents. For each isolate, mutations associated with drug resistance and drug susceptibility were identified across nine genes, and individual phenotypes were predicted unless mutations of unknown association were also present. To identify how whole-genome sequencing might direct first-line drug therapy, complete susceptibility profiles were predicted. These profiles were predicted to be susceptible to all four drugs (i.e., pansusceptible) if they were predicted to be susceptible to isoniazid and to the other drugs or if they contained mutations of unknown association in genes that affect susceptibility to the other drugs. We simulated the way in which the negative predictive value changed with the prevalence of drug resistance.

RESULTS

A total of 10,209 isolates were analyzed. The largest proportion of phenotypes was predicted for rifampin (9660 [95.4%] of 10,130) and the smallest was predicted for ethambutol (8794 [89.8%] of 9794). Resistance to isoniazid, rifampin, ethambutol, and pyrazinamide was correctly predicted with 97.1%, 97.5%, 94.6%, and 91.3% sensitivity, respectively, and susceptibility to these drugs was correctly predicted with 99.0%, 98.8%, 93.6%, and 96.8% specificity. Of the 7516 isolates with complete phenotypic drug-susceptibility profiles, 5865 (78.0%) had complete genotypic predictions, among which 5250 profiles (89.5%) were correctly predicted. Among the 4037 phenotypic profiles that were predicted to be pansusceptible, 3952 (97.9%) were correctly predicted.

CONCLUSIONS

Genotypic predictions of the susceptibility of *M. tuberculosis* to first-line drugs were found to be correlated with phenotypic susceptibility to these drugs. (Funded by the Bill and Melinda Gates Foundation and others.)

The members of the writing group (Timothy M. Walker, D.Phil., A. Sarah Walker, Ph.D., and Tim E.A. Peto, D.Phil.) assume responsibility for the overall content and integrity of this article. The authors' full names and academic degrees are listed in the Appendix. The authors' affiliations are listed in the Supplementary Appendix, available at NEJM.org. Address reprint requests to Dr. Timothy Walker at the Department of Microbiology, Level 7, John Radcliffe Hospital, Headley Way, Headington, Oxford, OX3 9DU, United Kingdom, or at timothy.walker@ndm.ox.ac.uk.

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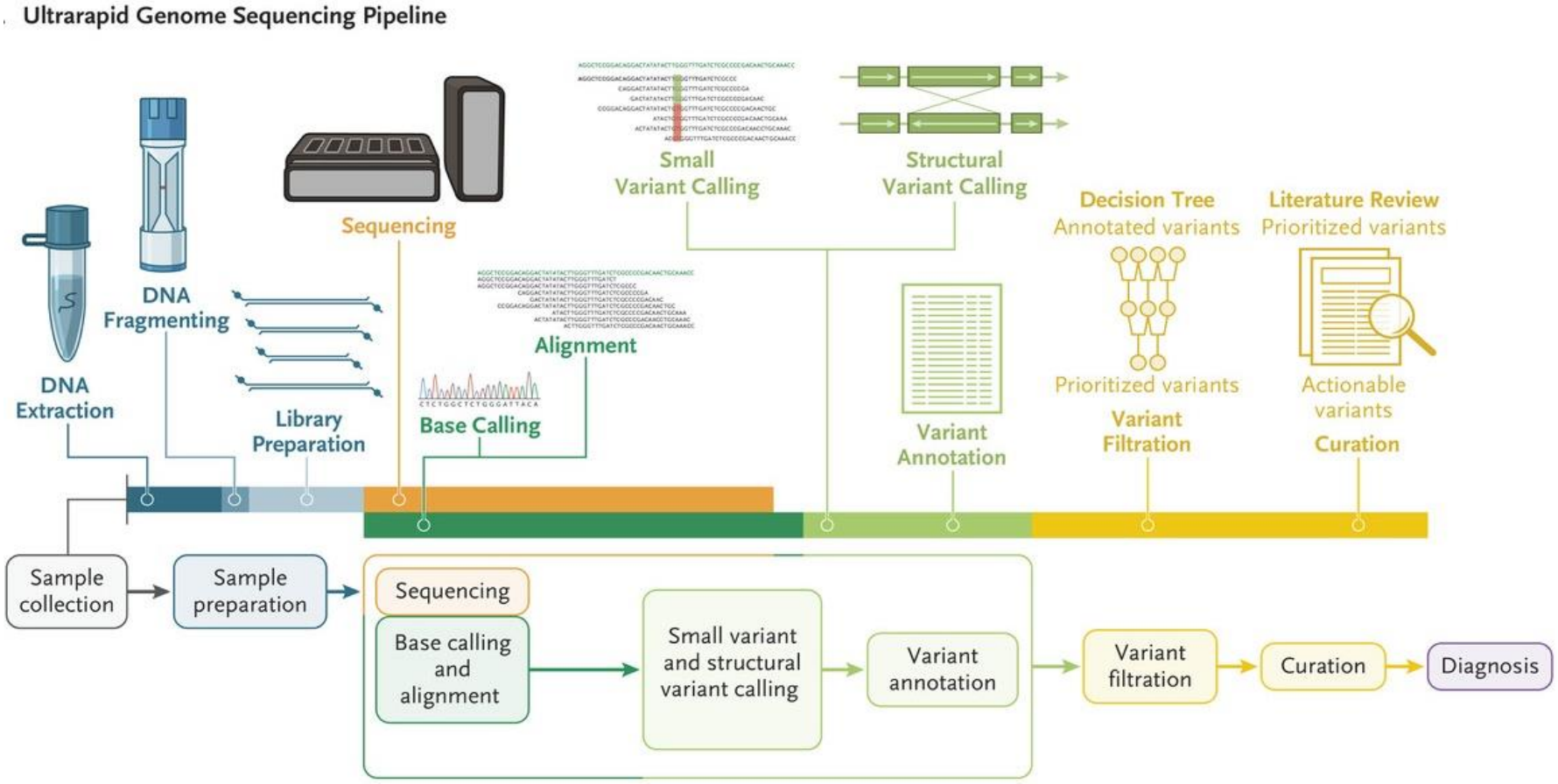
N Engl J Med 2018;379:1403-15.
DOI: 10.1056/NEJMoa1800474

- 10,209 isolates
  - Resistance to isoniazid, rifampin, ethambutol, and pyrazinamide
    - Sensitivity: 97.1%, 97.5%, 94.6%, and 91.3%
    - Specificity: 99.0%, 98.8%, 93.6%, and 96.8%
- 7,516 isolates (with complete phenotypic drug-susceptibility profiles)
  - 5,865 (78.0%) (with complete genotypic predictions)
    - Among which 5,250 profiles (89.5%) were correctly predicted
    - Among the 4,037 phenotypic profiles that were predicted to be pansusceptible
      - 3,952 (97.9%) were correctly predicted

N. Engl. J. Med. 2018;379:1403-1415.

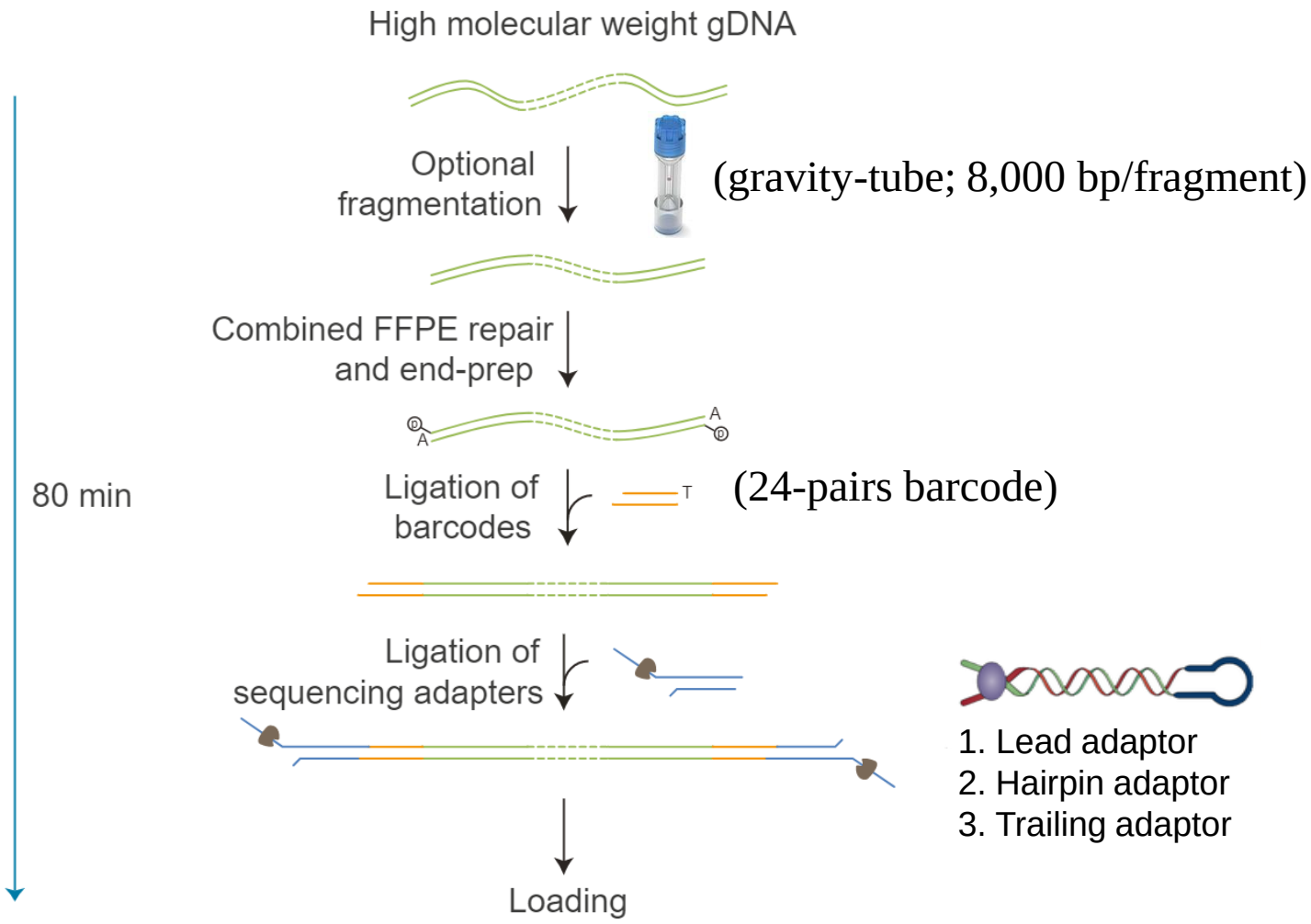
長片段定序進行全基因組定序的步驟

Workflow of long-read sequencing for whole genome of MTB

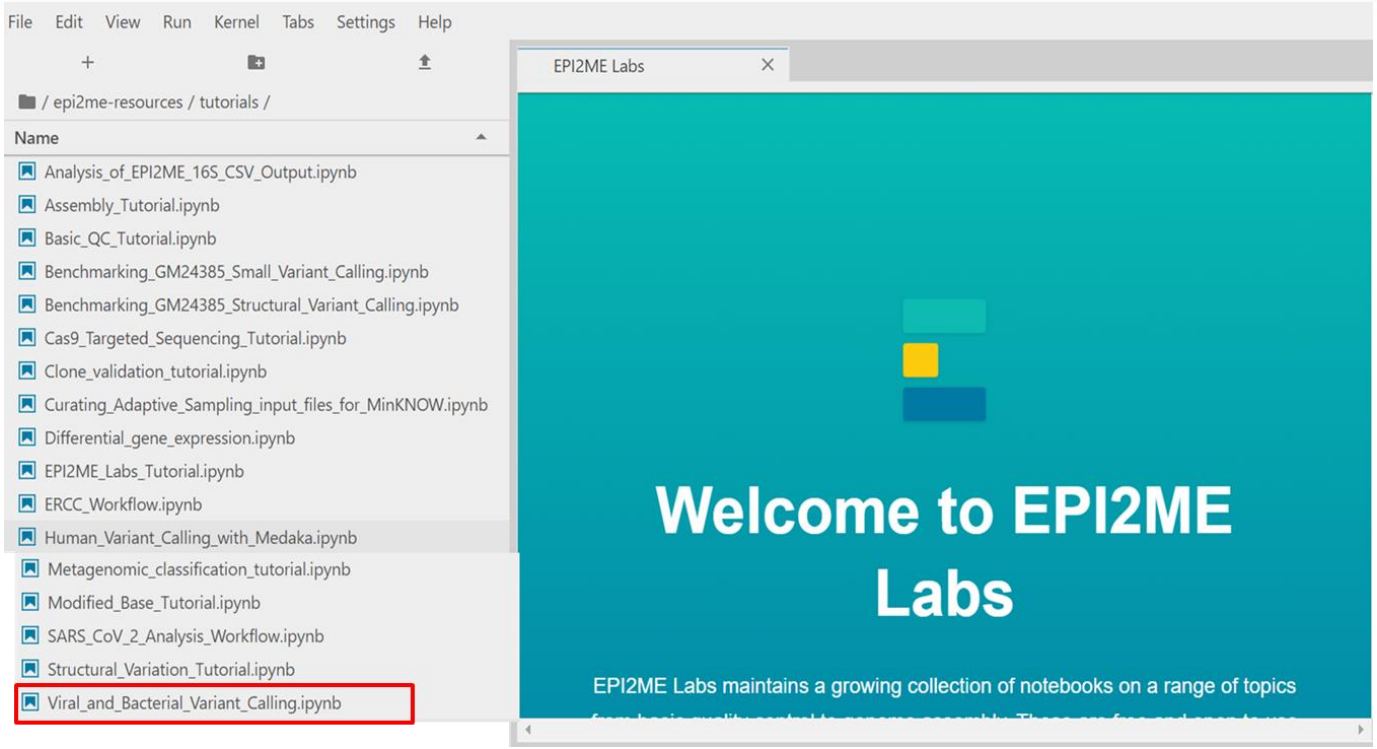


N Engl J Med. 2022;doi: 10.1056/NEJMc2112090.

Overview of library construction for ONT sequencing



EPI2ME Labs Notebook is applied for analysis of MTB WGS



- Minimap2 for reads alignment to reference sequence (H37Rv)
- `medaka` for variant calling and annotation.
- `pomoxis` for basic data QC.(Reads No, Reads length, Q-score, Frequency…)
- `pysam` for iterating through VCF files.
- `pandas` for manipulating VCF files as a table.
- `bcftools` for filtering VCF files on the command-line.

Non-synonymous variant within drug resistance gene of *MTB* is annotated using CLC genomics workbench

In CLC Workbenches you can..

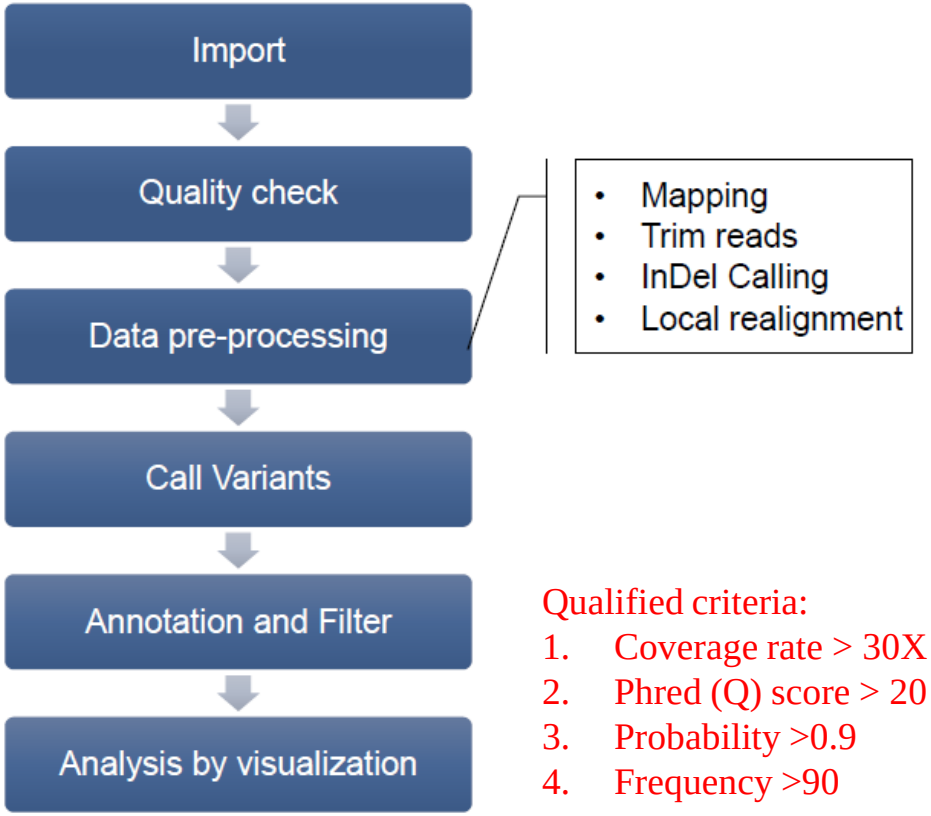
Ready-to-Use Workflows
- Preparing Raw Data
- QIAseq Panel Analysis
- Whole Genome Sequencing
- Whole Exome Sequencing
- Targeted Amplicon Sequencing
- Whole Transcriptome Sequencing
- Small RNA Sequencing

Tools
- Genome Finishing Module
- Microbial Genomics Module
- Long Read Support (beta)
- Classical Sequence Analysis
- Molecular Biology Tools
- BLAST
- Track Tools
- Prepare Sequencing Data
- Quality Control
- Resequencing Analysis
- RNA-Seq and Small RNA Analysis
- Microarray Analysis
- Epigenomics Analysis
- De Novo Sequencing
- Installed Workflows
- Utility Tools
- QIAseq Panel Expert Tools
- Legacy Tools

CLC Genomics Workbench	QC & Reads Processing	●
	Resequencing (whole genome, exome, targeted)	●
	Transcriptomics (RNA-Seq)	●
	De novo assembly	●
	Epigenomics	●
	Long Reads Supports (Oxford Nanopore & Pacbio)	●
	QIAseq Panel Analysis – TMB & MSI	●
	Workflow (Pipeline)	●
	Microarray Analysis	●
	Phylogeny Tools	●
	Blast, Sanger Sequencing, Cloning, Primer Design, …	●
Extended Modules	Microbiome Analysis (Microbial Genomics Module)	●
	Contigs Assembly (Genome Finishing Module)	●

Analytic workflow regarding variant calling of *MTB* genome using CLC genomics workbench

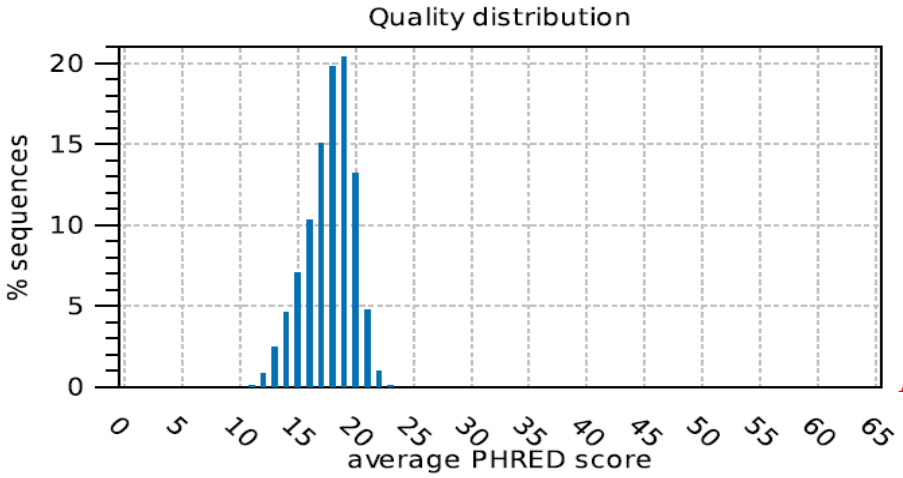
Call Variants from NGS data



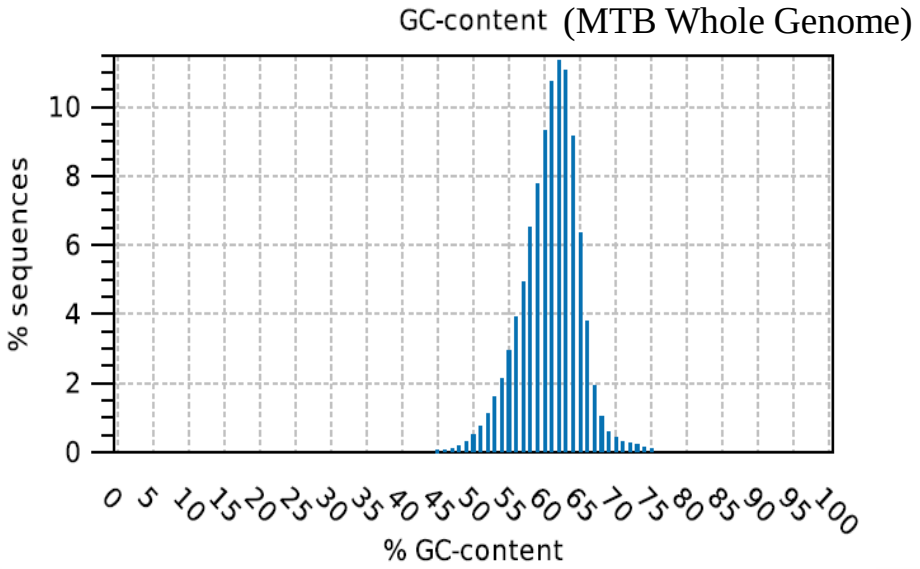
Qualified criteria:

1. Coverage rate > 30X
2. Phred (Q) score > 20 (Accuracy >99%)
3. Probability >0.9
4. Frequency >90

Quality control of sequencing results: Quality score and GC content

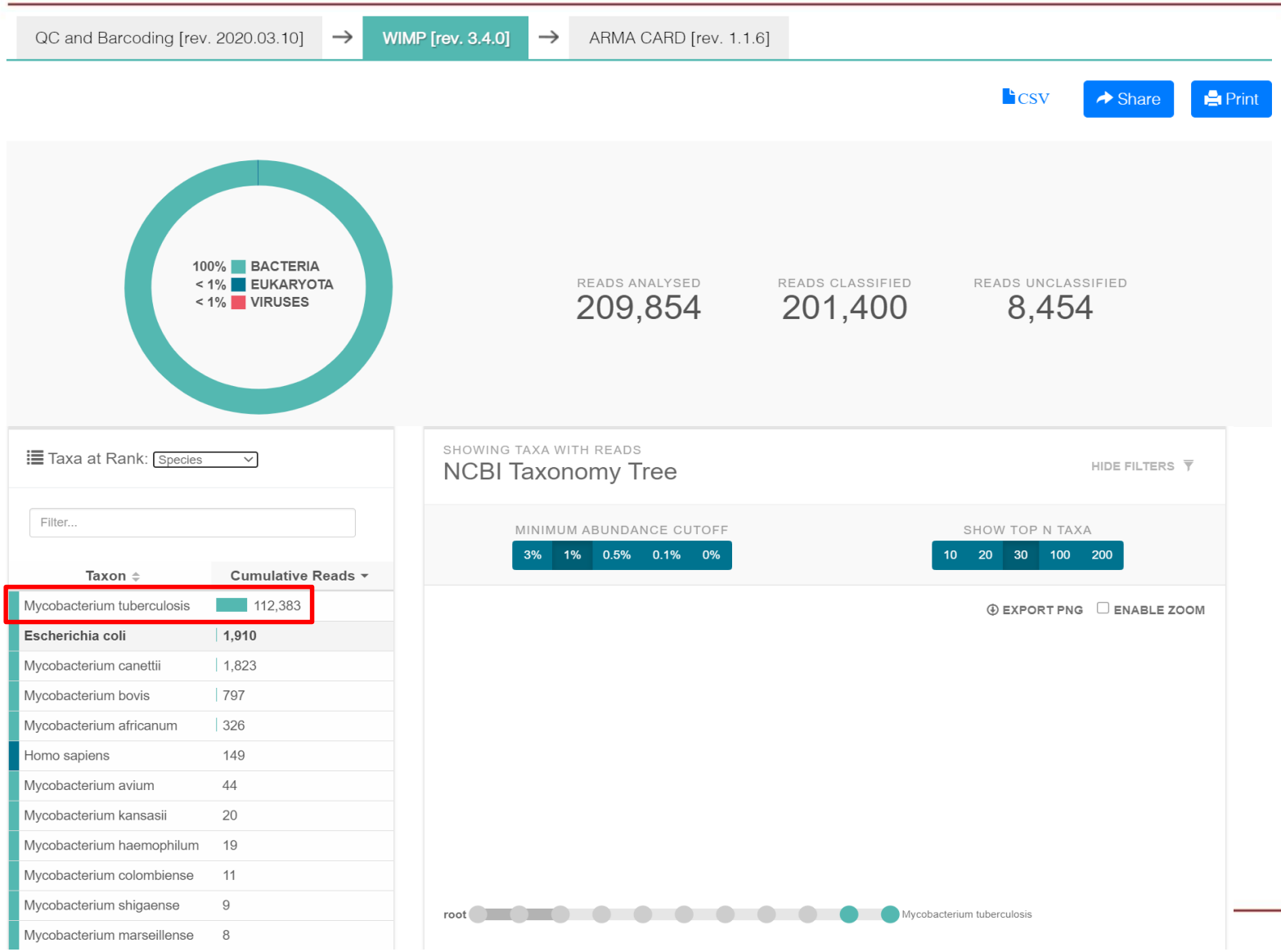


Accuracy:> 98.75%



CLC Genomics Workbench

Species identification with the reads alignment to reference sequence



Preliminary results of long-read sequencing toward variant calling within *MTB* genome

Rows: 819 / 823,278 Table view: Genome Filter to Selection... Match any Match all

gene (Mycobacterium tuberculosis H37Rv_Gene) contains rpoB Filter

Chromosome	Region	Type	Reference	Allele	Average q...	Amino acid change	Non-synon...	gene (Myc...
NC_000962.3	761069^761070	Insertion	-	-	12.11		No	rpoB
NC_000962.3	761073^761074	Insertion	-	A	8.67	NP_215181.1:p.Phe424fs	Yes	rpoB
NC_000962.3	761073^761074	Insertion	-	-	12.27		No	rpoB
NC_000962.3	761095^761096	Insertion	-	G	13.20	NP_215181.1:p.Ser431fs	Yes	rpoB
NC_000962.3	761095^761096	Insertion	-	-	8.38		No	rpoB
NC_000962.3	761097	SNV	A	G	10.86	NP_215181.1:p.Ser431Gly	Yes	rpoB
NC_000962.3	761097	SNV	A	A	13.67		No	rpoB
NC_000962.3	761104^761105	Insertion	-	C	12.33	NP_215181.1:p.Met434fs	Yes	rpoB
NC_000962.3	761104^761105	Insertion	-	-	14.06		No	rpoB
NC_000962.3	761132	SNV	G	T	17.50		No	rpoB
NC_000962.3	761132	SNV	G	G	20.82		No	rpoB
NC_000962.3	761139	SNV	C	T	4.55	NP_215181.1:p.His445Tyr	Yes	rpoB
NC_000962.3	761139..761140	Deletion	CA	-	19.54	NP_215181.1:p.His445fs	Yes	rpoB
NC_000962.3	761139..761140	MNV	CA	CA	8.88		No	rpoB
NC_000962.3	761141^761142	Insertion	-	A	13.00	NP_215181.1:p.Arg447fs	Yes	rpoB
NC_000962.3	761141^761142	Insertion	-	-	9.06		No	rpoB
NC_000962.3	761152^761153	Insertion	-	G	14.67	NP_215181.1:p.Ser450fs	Yes	rpoB
NC_000962.3	761152^761153	Insertion	-	-	13.14		No	rpoB
NC_000962.3	761162	SNV	G	C	12.00		No	rpoB
NC_000962.3	761162	Deletion	G	-	9.89	NP_215181.1:p.Gly455fs	Yes	rpoB
NC_000962.3	761162	SNV	G	G	13.87		No	rpoB
NC_000962.3	761166	Deletion	C	-	13.00	NP_215181.1:p.Gly455fs	Yes	rpoB
NC_000962.3	761166	SNV	C	C	16.77		No	rpoB
NC_000962.3	761168	SNV	C	T	4.61		No	rpoB
NC_000962.3	761168..761170	MNV	CGG	TAA	11.55	NP_215181.1:p.Gly455Asn	Yes	rpoB
NC_000962.3	761168..761170	MNV	CGG	CGG	14.41		No	rpoB
NC_000962.3	761169	SNV	G	A	8.17	NP_215181.1:p.Gly455Ser	Yes	rpoB
NC_000962.3	761169..761170	MNV	GG	AA	3.78	NP_215181.1:p.Gly455Asn	Yes	rpoB
NC_000962.3	761170	SNV	G	A	7.74	NP_215181.1:p.Gly455Asp	Yes	rpoB
NC_000962.3	761180	Deletion	A	-	22.33	NP_215181.1:p.Arg459fs	Yes	rpoB
NC_000962.3	761180	SNV	A	A	19.09		No	rpoB
NC_000962.3	761191	Deletion	C	-	15.83	NP_215181.1:p.Leu464fs	Yes	rpoB
NC_000962.3	761191	SNV	C	C	20.93		No	rpoB
NC_000962.3	761194	SNV	G	T	17.00	NP_215181.1:p.Gly463Val	Yes	rpoB
NC_000962.3	761194	SNV	G	G	17.38		No	rpoB
NC_000962.3	761196	SNV	C	T	9.33		No	rpoB
NC_000962.3	761196	SNV	C	C	20.25		No	rpoB
NC_000962.3	761198	Deletion	G	-	8.50	NP_215181.1:p.Glu465fs	Yes	rpoB
NC_000962.3	761198	SNV	G	G	21.55		No	rpoB
NC_000962.3	761199^761200	Insertion	-	A	22.33	NP_215181.1:p.Val466fs	Yes	rpoB
NC_000962.3	761199^761200	Insertion	-	-	16.06		No	rpoB
NC_000962.3	761204	Deletion	C	-	13.12	NP_215181.1:p.Arg467fs	Yes	rpoB
NC_000962.3	761204	SNV	C	C	26.50		No	rpoB
NC_000962.3	761211..761212	Deletion	GT	-	12.41	NP_215181.1:p.Val469fs	Yes	rpoB
NC_000962.3	761211..761212	MNV	GT	GT	16.80		No	rpoB
NC_000962.3	761211^761212	Insertion	-	A	8.53	NP_215181.1:p.Val469fs	Yes	rpoB
NC_000962.3	761242	SNV	C	T	7.21	NP_215181.1:p.Pro479Leu	Yes	rpoB

Table Settings

- [ ] Reverse read count
- [ ] Forward read coverage
- [ ] Reverse read coverage
- [ ] Forward/reverse balance
- [x] Average quality
- [ ] Read count
- [ ] Read coverage
- [ ] # unique start positions
- [ ] # unique end positions
- [ ] BaseQRankSum
- [ ] Read position test probability
- [ ] Read direction test probability
- [ ] Homopolymer length
- [ ] Homopolymer
- [ ] QUAL
- [ ] Coding region change
- [x] Amino acid change
- [ ] Amino acid change in longest transcript
- [ ] Coding region change in longest trans...
- [ ] Other variants within codon
- [x] Non-synonymous
- [ ] Start by 3' rule
- [ ] Symbols by 3' rule
- [ ] Mycobacterium tuberculosis H37Rv_Gene
- [ ] type (Mycobacterium tuberculosis H37R...
- [ ] source (Mycobacterium tuberculosis H3...
- [ ] ID (Mycobacterium tuberculosis H37Rv_...
- [ ] experiment (Mycobacterium tuberculosi...
- [ ] gbkey (Mycobacterium tuberculosis H37...
- [x] gene (Mycobacterium tuberculosis H37R...
- [ ] gene_biotype (Mycobacterium tuberculo...
- [ ] locus_tag (Mycobacterium tuberculosis...
- [ ] gene_synonym (Mycobacterium tubercul...
- [ ] GeneID
- [ ] GeneID (Mycobacterium tuberculosis H3...

Select All

Qualified criteria:
1. Coverage rate > 30X
2. Phred (Q) score > 20 (Accuracy >99%)
3. Probability > 0.9

Discriminative efficacy of variant calling by using short-read and long-read sequencing

Illumina; MiSeq platform

Type	Reference	Allele	Coverage	Probability	Amino acid change	gene (Myc...
SNV	C	A	145	1.00	NP_216424.1:p.Arg463Leu	katG

ONT; MinION platform

Type	Reference	Allele	Coverage	Probability	Amino acid change	gene (Myc...
SNV	T	A	38	0.64	NP_216424.1:p.Tyr678Phe	katG
SNV	C	G	34	0.91	NP_216424.1:p.Gly599Arg	katG
SNV	G	T	38	0.94	NP_216424.1:p.Thr579Asn	katG
SNV	G	C	35	0.99	NP_216424.1:p.Ala551Gly	katG
SNV	C	A	39	1.00	NP_216424.1:p.Arg463Leu	katG
SNV	G	T	37	0.70	NP_216424.1:p.Asp448Glu	katG
SNV	T	C	37	0.70	NP_216424.1:p.Gln439Arg	katG
SNV	G	A	32	0.77	NP_216424.1:p.Pro388Leu	katG
SNV	T	G	24	0.98	NP_216424.1:p.Lys310Thr	katG
SNV	T	C	21	1.00	NP_216424.1:p.Glu287Gly	katG

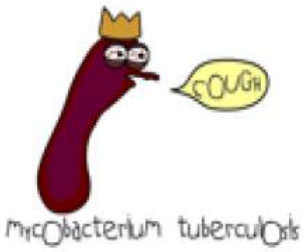
驗證長片段定序結果與結核桿菌抗藥性特性:

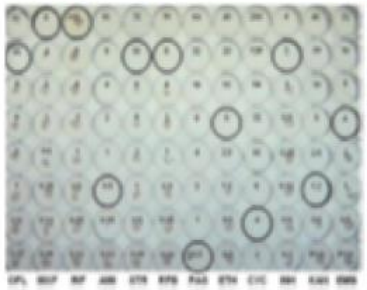
最小抑菌濃度試驗
Minimal inhibitory concentration (MIC) assay

MTBC MIC Panel

Sensititre - MTBC MIC Panel

- First dry microbroth dilution plate
- Test First and Second line drugs on a single plate
  - **12** antimicrobics
- MIC results in **10-21** days





MYCOTBI	
Antimicrobial Agent	Dilution Range (µg/mL)
Ethambutol	0.5-32
Isoniazid	0.03-4
Rifampin	0.12-16
Streptomycin	0.25-32
Moxifloxacin	0.06-8
Amikacin	0.12-16
Kanamycin	0.6-40
Cycloserine	2-256
Ethionamide	0.3-40
Ofloxacin	0.25-32
Para-aminosalicylic acid	0.5-64
Rifabutin	0.12-16

MTBC MIC Worksheet

SENSITITRE ® MYCOTB		英美醫療器材有限公司 服務電話0809091689
Date:	Patient Name:	Lot#:
Isolate #:	Technician:	Signature:

	1	2	3	4	5	6	7	8	9	10	11	12
A	OFL 32	MXF 8	RIF 16	AMI 16	STR 32	RFB 16	PAS 64	ETH 40	CYC 256	INH 4	KAN 40	EMB 32
B	OFL 16	MXF 4	RIF 8	AMI 8	STR 16	RFB 8	PAS 32	ETH 20	CYC 128	INH 2	KAN 20	EMB 16
C	OFL 8	MXF 2	RIF 4	AMI 4	STR 8	RFB 4	PAS 16	ETH 10	CYC 64	INH 1	KAN 10	EMB 8
D	OFL 4	MXF 1	RIF 2	AMI 2	STR 4	RFB 2	PAS 8	ETH 5	CYC 32	INH 0.5	KAN 5	EMB 4
E	OFL 2	MXF 0.5	RIF 1	AMI 1	STR 2	RFB 1	PAS 4	ETH 2.5	CYC 16	INH 0.25	KAN 2.5	EMB 2
F	OFL 1	MXF 0.25	RIF 0.5	AMI 0.5	STR 1	RFB 0.5	PAS 2	ETH 1.2	CYC 8	INH 0.12	KAN 1.2	EMB 1
G	OFL 0.5	MXF 0.12	RIF 0.25	AMI 0.25	STR 0.5	RFB 0.25	PAS 1	ETH 0.6	CYC 4	INH 0.06	KAN 0.6	EMB 0.5
H	OFL 0.25	MXF 0.06	RIF 0.12	AMI 0.12	STR 0.25	RFB 0.12	PAS 0.5	ETH 0.3	CYC 2	INH 0.03	POS	POS

Antimicrobics

OFL	Ofloxacin
MXF	Moxifloxacin
RIF	Rifampin
AMI	Amikacin
STR	Streptomycin
RFB	Rifabutin
PAS	Para-aminosalicylic acid
ETH	Ethionamide
CYC	Cycloserine
INH	Isoniazid
KAN	Kanamycin
POS	Positive Control
EMB	Ethambutol

長片段定序應用於鑑別結核桿菌抗藥性基因變異位點

Integrative utility of long read sequencing-based whole genome analysis and phenotypic assay on differentiating isoniazid-resistant signature of Mycobacterium tuberculosis

J Biomed Sci. 2021 Dec 18;28(1):86.

Ming-Chih Yu, Ching-Sheng Hung, Chun-Kai Huang, Cheng-Hui Wang, Yu-Chih, Liang, Jung-Chun Lin

Drug susceptibility test of recruited isolates

No.	INH 0.2	INH 1.0	RIF 1.0	EM 5.0	EM 10.0	S 2.0	S 10.0
1	R	S	S	S	S	S	S
2	R	S	S	S	S	S	S
3	R	S	S	R	S	S	S
4	R	S	R	R	S	R	R
5	R	S	R	R	S	S	S
6	R	S	S	R	S	S	S
7	R	S	S	S	S	S	S
8	R	S	S	S	S	S	S
9	R	S	S	S	S	S	S
10	R	S	S	S	S	R	R
11	R	S	R	S	S	S	S
12	R	S	S	S	S	S	S
13	R	S	R	S	S	S	S
14	R	S	S	S	S	S	S
15	R	S	R	S	S	R	R
16	R	S	R	R	S	S	S
17	R	S	R	S	S	S	S
18	R	S	S	S	S	S	S
19	R	S	S	S	S	S	S
20	R	S	S	S	S	S	S

Low INH-resistance MTB

No.	INH 0.2	INH 1.0	RIF 1.0	EM 5.0	EM 10.0	S 2.0	S 10.0 (μg/mL)
21	R	R	S	S	S	S	S
22	R	R	R	R	S	R	S
23	R	R	S	S	S	R	S
24	R	R	S	S	S	R	R
25	R	R	S	S	S	R	R
26	R	R	R	R	S	R	R
27	R	R	S	S	S	R	R
28	R	R	S	S	S	S	S
29	R	R	R	S	S	S	S
30	R	R	S	R	S	S	S
31	R	R	S	S	S	S	S
32	R	R	S	S	S	S	S
33	R	R	R	R	S	R	R
34	R	R	S	S	S	R	S
35	R	R	S	S	S	R	S
36	R	R	S	S	S	R	S
37	R	R	S	S	S	S	S
38	R	R	S	R	S	S	S
39	R	R	S	S	S	R	S
40	R	R	S	S	S	R	R
41	R	R	S	R	S	S	S
42	R	R	R	R	S	R	S

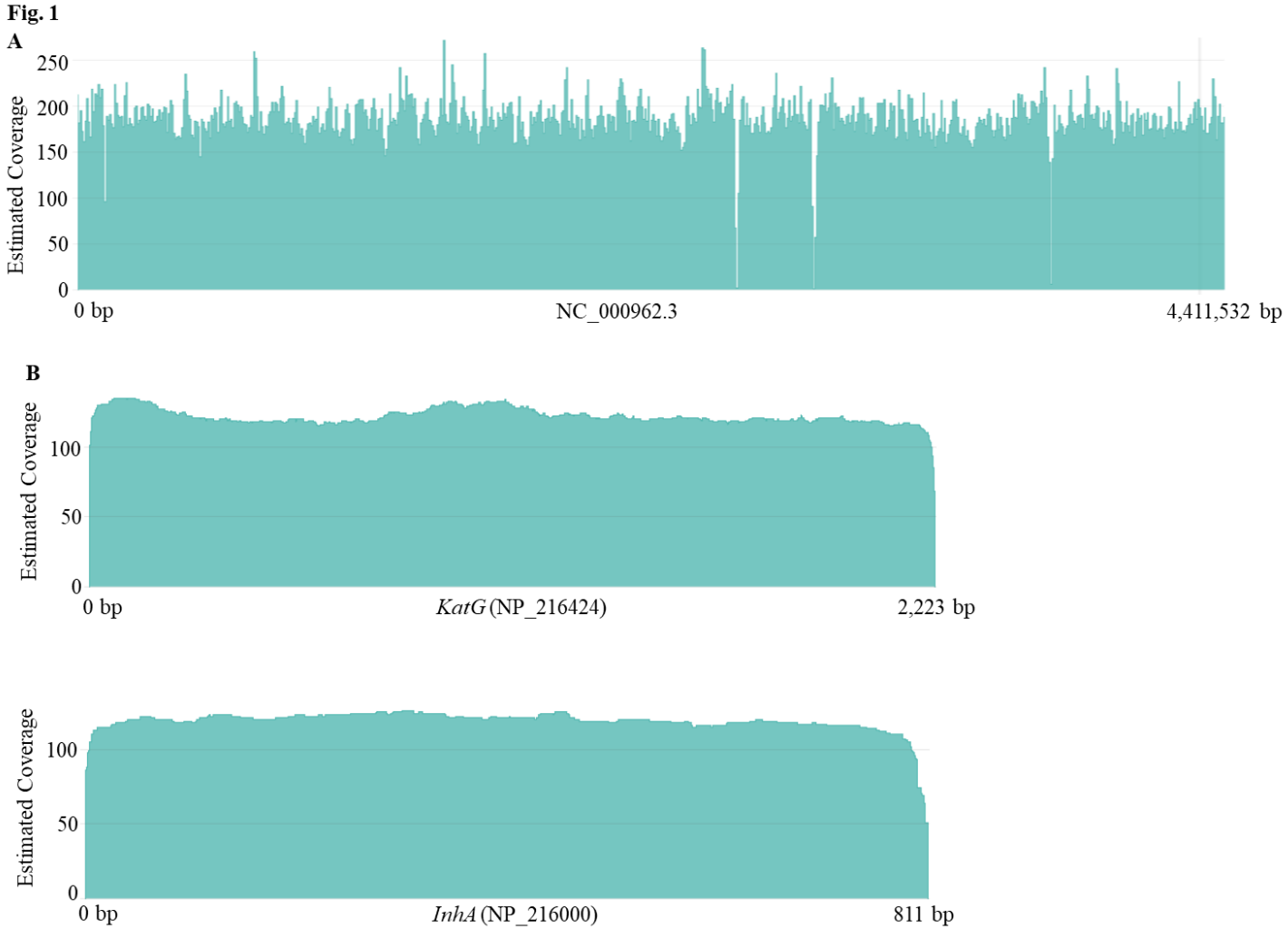
High INH-resistance MTB

Summary statistics of sequencing results with the HMW gDNA prepared from the clinical isolates

Group	Susceptible isolates *(n*=10*)*	Low Isoniazid-resistance *(n*=20*)*	High Isoniazid-resistance *(n*=22*)*	*p* value
Number of Raw reads (Mean; (SD))	382,196 (±17,321)	333,823 (±15,702)	356,660 (±14,407)	>0.1
Number of aligned reads (Mean; (SD))	366,547 (±10,121)	322,075 (±8,332)	339,176 (±11,540)	>0.1
Correctly classified (% (SD))	95.91 (5.64)	96.48 (5.97)	95.09 (5.26)	>0.1

No statistical difference in read number is noted among any of the groups.

The coverage rate of sequenced reads to the whole MTB genome or individual gene



EPI2ME Fastq custom alignment algorithm

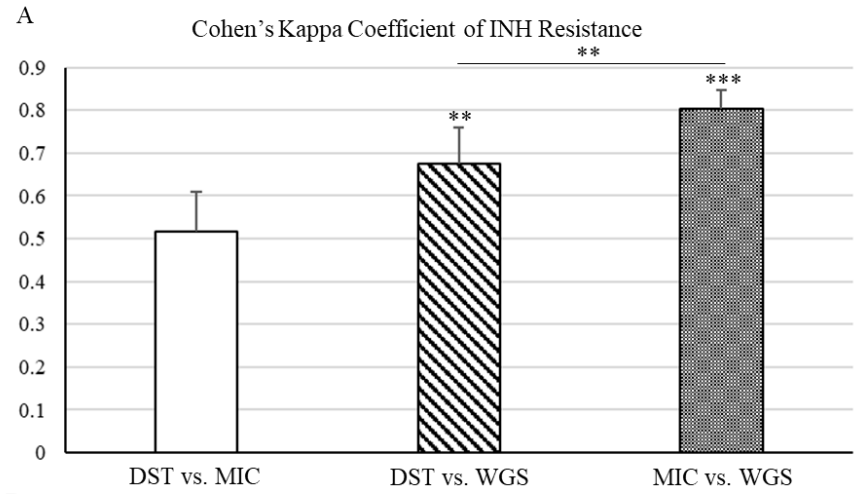
Emerging variants within *katG* gene are characterized using long-read sequencing

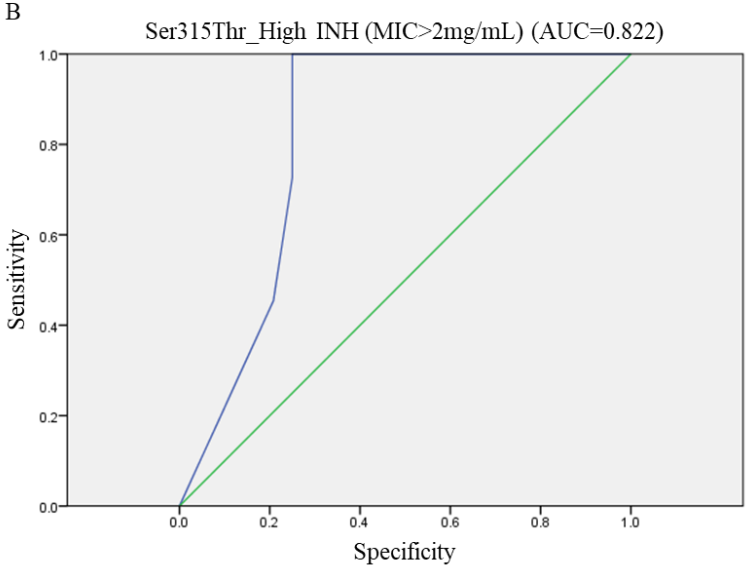
	Variants in *katG*	Isolate No.	Variant No.
Susceptible only	Leu101Arg/Ile462Asn/Ala478Val/Pro566Thr/Leu631Pro	10	5
Susceptible and Resistant	Gln50Lys/Asn51Thr/Glu81Gln/Met84Thr/Pro92Ala/Gly123Arg/Lys158Asn/Tyr304Asp/Gly307Glu /Asp311Asn/Pro325Arg/Lys327Asn/Lys356Met/Tyr390Asp/Glu399Lys/Pro443His/Arg463Leu /Leu546Pro/Thr564Pro/Leu611Pro/Val659Leu	52	21
	Variants (Identified copy) in *katG*	**Isolate No.**	**Variant No.**
Low INH resistant isolate	Gly14Arg(2)/ Gly124Ala(2)/ Leu141Phe(2)/ Leu141Met(2)/ Val68Ala(2)/ Pro235Gln(2) Ser374Phe(2)/ Asp509Gly(3)/ Gly570Ala(2)/ Gly665Val(2)/ Leu704Val(2)/ Phe737Leu(2)	20	12
High INH resistant isolate	Tyr95Phe(2)/ Pro136Ala(2)/ Ser160Ala(2)/ Val188Leu(5)/ Glu287Asp(2)/ Ser315Thr(13)/ Ser315Gly(1)/ Ser315Asn(1)/ Thr354Ile(2)/ Ser376Met(3)/ Gly560Val(2)/ Thr579Asn(2)/Lys600Glu/Arg(2)/ Asn602Lys/Asp(3)/ Met664Leu/Ile(2)	22	15
Low and High INH resistant isolate	Pro7Thr(2)/ Trp38Cys(5)/ Asp142Asn(4)/ Arg146Gln(2)/ Arg146Trp(2)/ Val166Phe(2)/ Val196Ala(3) Pro235Leu(5)/ Glu289Gly(2)/ Glu318Gln(3)/ Glu334Gln(2)/ Pro375Arg(4)/ Ser383Ala(2)/ Trp412Cys(3) Val442Ala(2)/ Pro432Ser(3)/ Ser457Asn(3)/ Arg463Trp(6)/ Ile552Arg(4)/ Ile552Thr(2)/ Gly599Glu(5) Gly599Arg(8)/ Glu588Gly(2)/ Ala621Gly(2)/ Ans637Thr(6)/ Tyr638Phe(2)/ Ser671Pro(2)/ Gly676Arg(2)	42	28

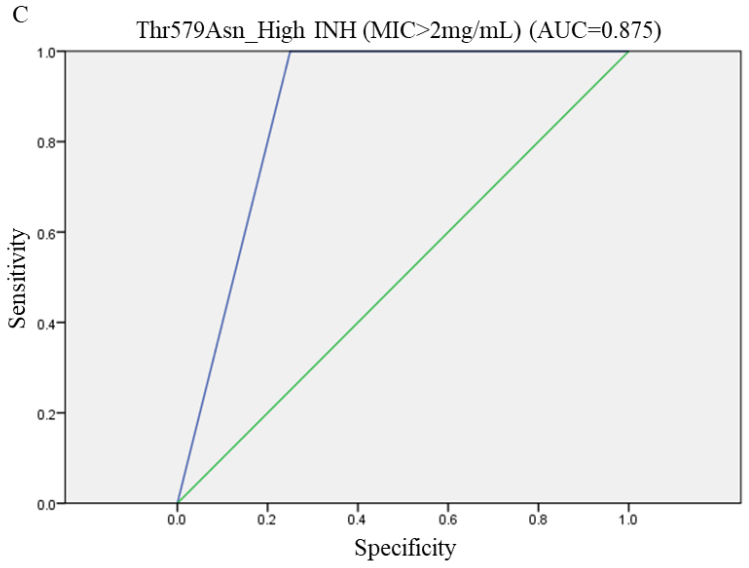
Emerging variants within *katG* gene is relevant to high -INH resistance of MTB isolates

Genotyping No.	High confidence variant	Novel variant	MIC of INH (μg/mL)	DST of INH (μg/mL)	Frequency (%)
1	Ser315Thr	N/A	2	1	25% (5/20)
2	Ser315Thr	Thr376Met Pro136Ala	2	1	10% (2/20)
3	Ser315Thr	Val188Leu Ser160Ala	2	1	15% (3/20)
4	Ser315Thr	Val188Leu Thr579Asn	4	1	10% (2/20)
5	Ser315Thr	Thr579Asn	>4	1	5% (1/20)
6	Ser315Thr	Lys600Glu	>4	1	10% (2/20)
7	N/A	Glu287Asp Lys310Thr Thr579Asn	>4	1	10% (2/20)
8	N/A	Glu318Gln Ile552Arg Gly599Arg	0.5	>0.2	5% (1/20)
9	N/A	Glu287Asp Thr376Met Lys600Arg Ser315Asn	0.03	>0.2	5% (1/20)
10	N/A	Met664Ile Ser315Gly	0.06	>0.2	5% (1/20)

Integrative utility of long read and phenotypic assay on differentiating drug-resistant signature of MTB

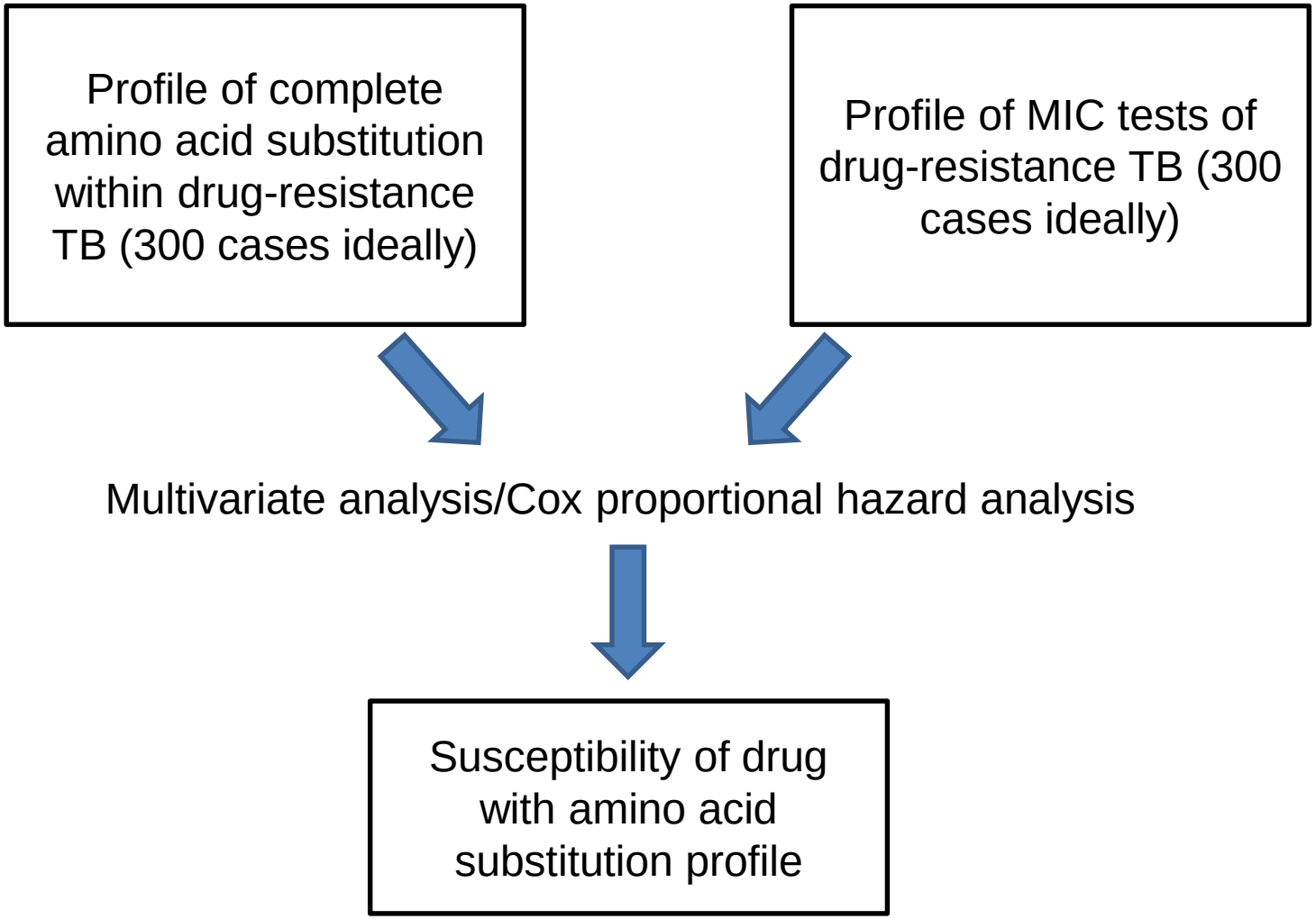




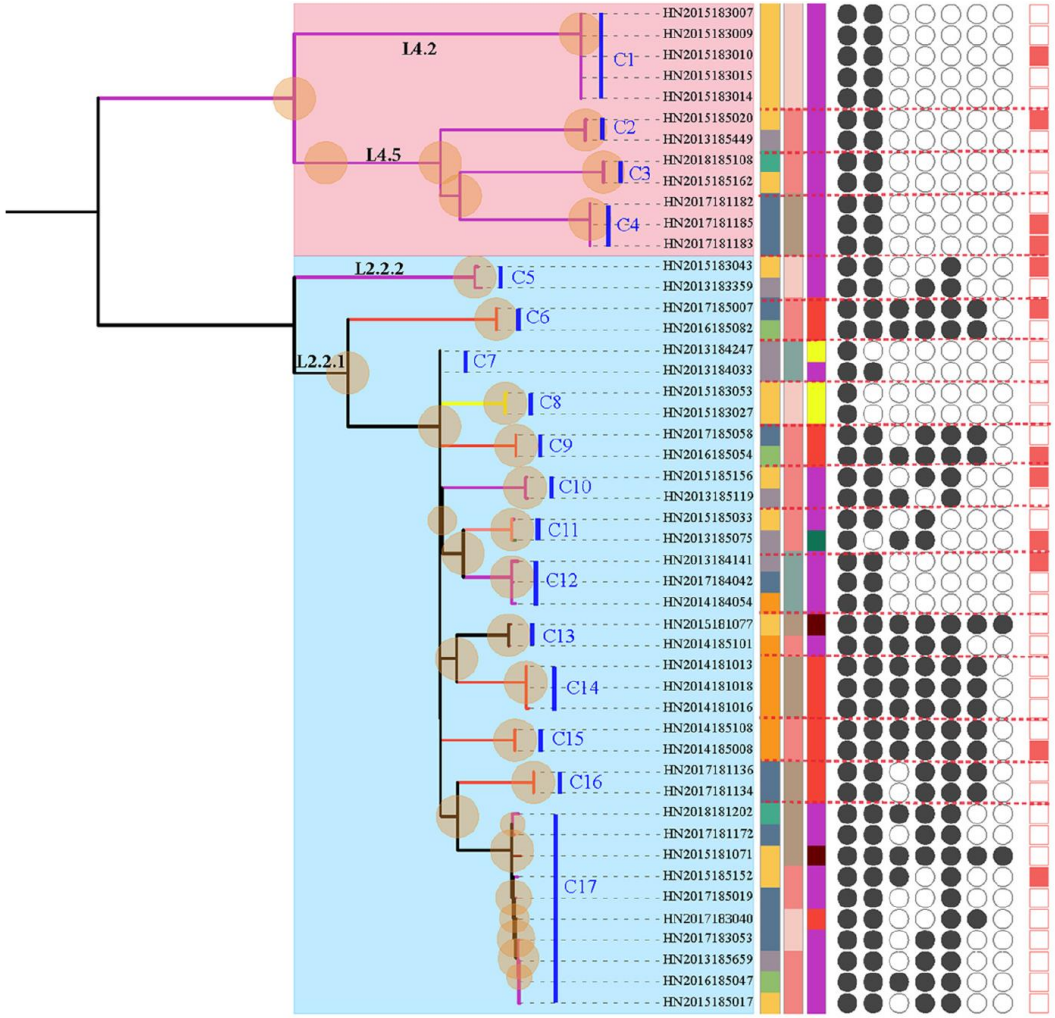


Application of WGS toward precise diagnosis of MTB

Application of deep machine learning on TB diagnosis



Correlation of phenotypic drug-resistant signatures with *MTB* classified with SNP profile using WGS.



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臺北市立萬芳醫院
余明治 執行長
(MTB WGS)

實驗室成員:
博士班四年級　洪經勝
(臺北市立萬芳醫院 檢驗科主任)
碩士班二年級 黃俊凱
(臺北市立萬芳醫院檢驗科 醫檢師)