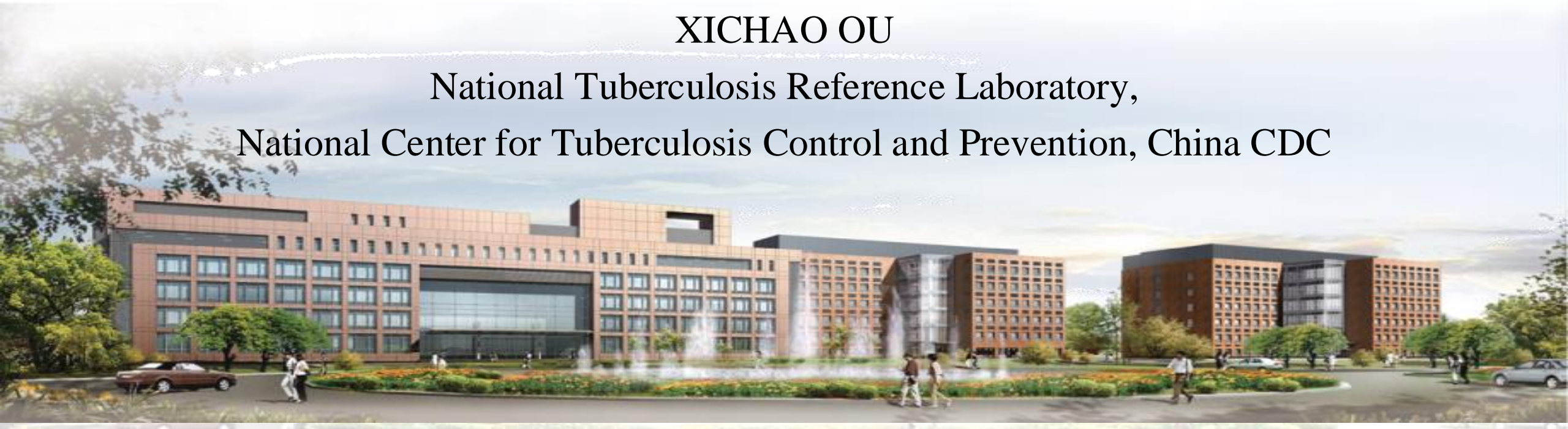




Advances in the Application of Bacteriological Diagnosis of TB

XICHAO OU

National Tuberculosis Reference Laboratory,
National Center for Tuberculosis Control and Prevention, China CDC





Bacteriological diagnostics for TB

TB bacteriological diagnostics

Smear microscopy

Simple and fast,
Low sensitivity,
Unable to distinguish
NTM from MTBC

Culture

Gold Standard,
Time-consuming,
Cumbersome operation,
Unable to distinguish NTM
from MTB

Nucleic acid amplification test (NAAT)

Sensitive and fast,
High costs

Detection of drug resistance

Mycobacterium speciation



Nucleic acid amplification tests for TB

- **Diagnosis of TB:** Detection of MTB-specific conserved housekeeping genes
 - IS6110、IS1081
 - 16S rRNA、gyrB、rpoB、MPT64、Rv0069c、Rv3133c、hsp65
- **Diagnosis of DR-TB:** Detecting mutations in drug resistance relevant genes
 - Rifampicin (RFP) : rpoB
 - Isoniazide (INH) : KatG, inhA, ahpC, fabG1
- **Species identification:** Housekeeping gene sequencing
 - 16S rRNA
 - 16S ~ 23S spacer region, rpoB, hsp65



TB bacteriological diagnostics endorsed by WHO



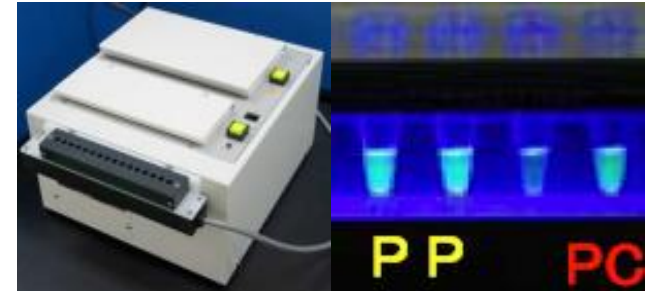
Light microscopy



liquid culture



Xpert MTB/RIF, MTB/RIF
Ultra and MTB/XDR



TB LAMP



Roche cobas MTB and MTB-RIF/INH



Abbott RealTime m2000 MTB and MTB
RIF/INH



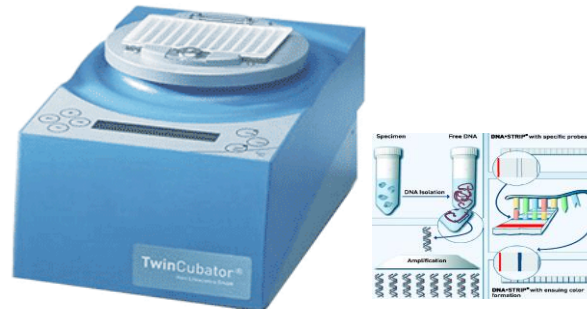
BD Max MDR-TB



TB bacteriological diagnostics endorsed by WHO



Truenat MTB, MTB Plus and MTB-RIF



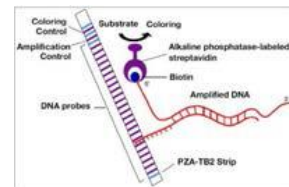
**GenoType MTBDRplus,
GenoType® MTBDRsl,**



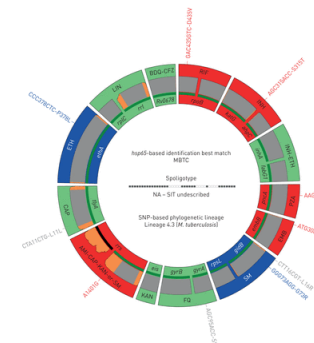
FluoroType MTB and MTBDR



Genoscholar NTM+MDR TB II; Genoscholar PZA TB II



Deeplex Myc-TB, NanoTB, TBseq





Automated smear microscopy



Automatic high-speed separation of smears



Filtered Mezzanine Cups



Automatic membrane filtration tablet machine



Automatic centrifugal immersion dyeing



Microscanning system



Microscopy pipeline for smear staining



Liquid culture test of mycobacterium



BACTEC MGIT960



TBK1200



BS-1000



BS-408





Progress in the development of TB diagnostics

	Technologies planned for WHO policy review	Technologies undergoing WHO policy review
Point of Care TB tests	<ul style="list-style-type: none">• mfloDx MDR-TB, EMPE Diagnostics, India	None planned for review
Near Point of Care NAATs	<ul style="list-style-type: none">• MultNAT® MTB/RIF, Ustar Biotechnologies, China• PortNAT®MTB , Ustar Biotechnologies, China• EasyNAT TB Diagnostic kit, Ustar Biotechnologies, China• RAPID Tuberculosis Nucleic Acid Detection Kit, Nanjing Dinfectome Medical Device and Diagnostic Inc, China• LumiraDx, UK	None planned for review



Progress in the development of TB diagnostics

	Technologies planned for WHO policy review	Technologies undergoing WHO policy review
Low complexity automated NAATs	<ul style="list-style-type: none">• SD Biosensor M10 XDR assay – reflex test , SD Biosensor, Republic of Korea• Compact Dx TB, RiF, INH – Mylab, India• TB Ultima, Molbio, India• Cepheid TB/INH/RIF	<ul style="list-style-type: none">• SD Biosensor standard M10 – TB/RiF/INH, SD Biosensor, Republic of Korea• IRON qPCR RFIA kit, Bioneer Corporation, Republic of Korea
Moderate complexity automated NAATs	Prodiag Xiamen / meltpro and sanity 2, China	<ul style="list-style-type: none">• LiquidArray MTB-XDR, Hain Lifescience, Germany (TB, XDR (FQ, LZD, EMB, SLIDs)
Targeted next generation sequencing		<ul style="list-style-type: none">• DeepChek® DST, ABL, France

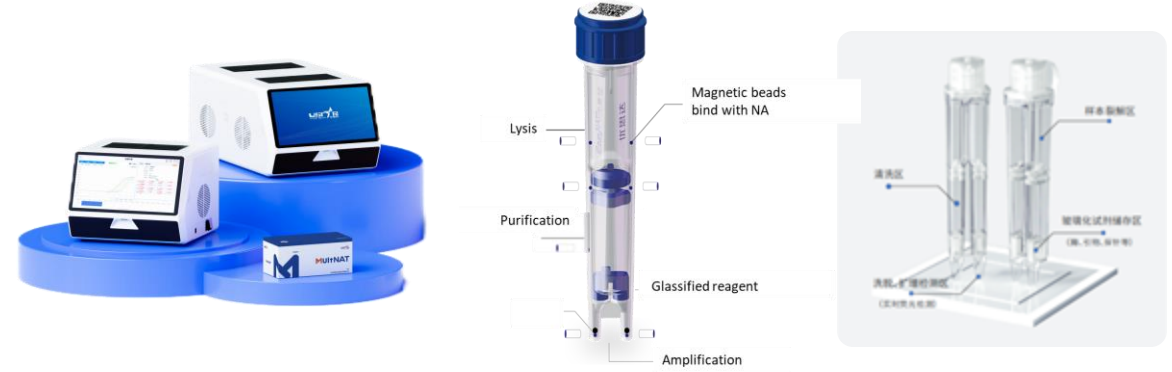


Point/Near Point of Care NAATs

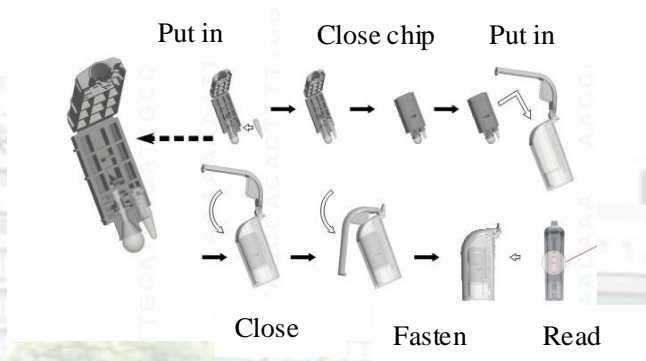
mfloDx MDR-TB



PortNAT、MultNAT MTB/RIF



EasyNAT TB POC



LumiraDx





Low complexity automated NAATs

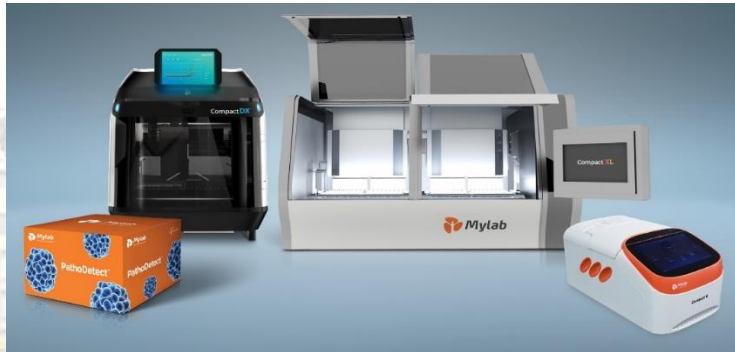
SD Biosensor M10 XDR



IRON qPCR RFIA



MyLAB, Compact Dx TB, RiF, INH



Xpert XDR TB





Low complexity automated NAATs

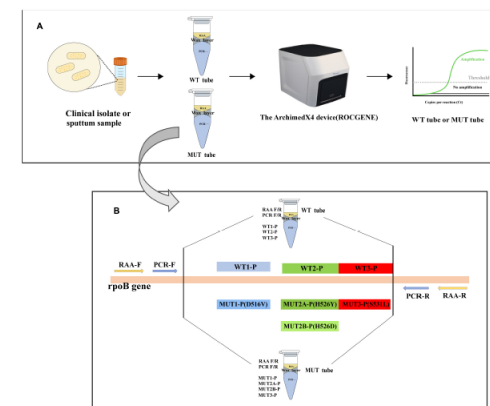
ROCGENE I-FIND



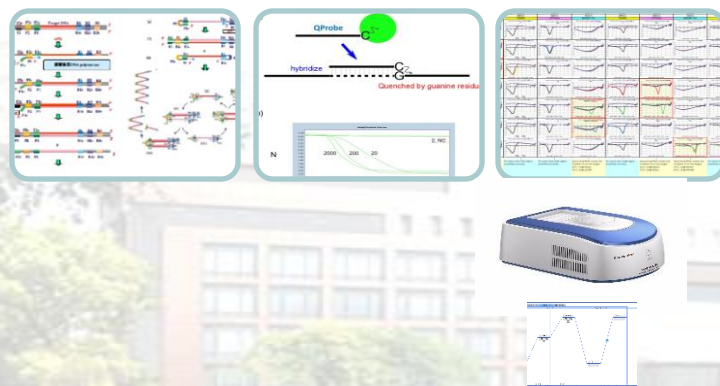
Igenesis Galaxy icassette



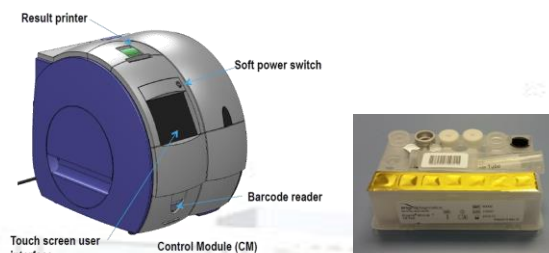
MPL-RAP



MDR-LAMP



Enigma MTB/RIF/INH



Truenat MTB/RIF





Moderate complexity automated NAATs

Innovo GenMax 16



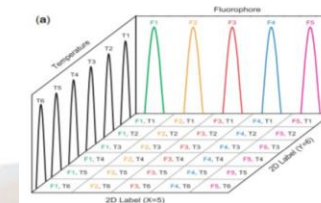
AutoSAT



GeneXpert Infinity



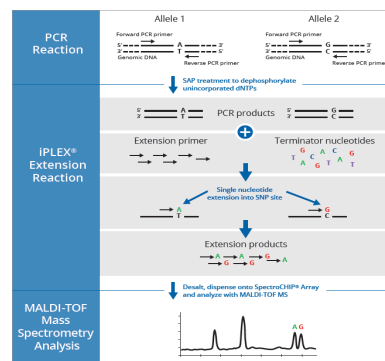
Sanity





Novel MALDI-TOF and microarray-based platforms

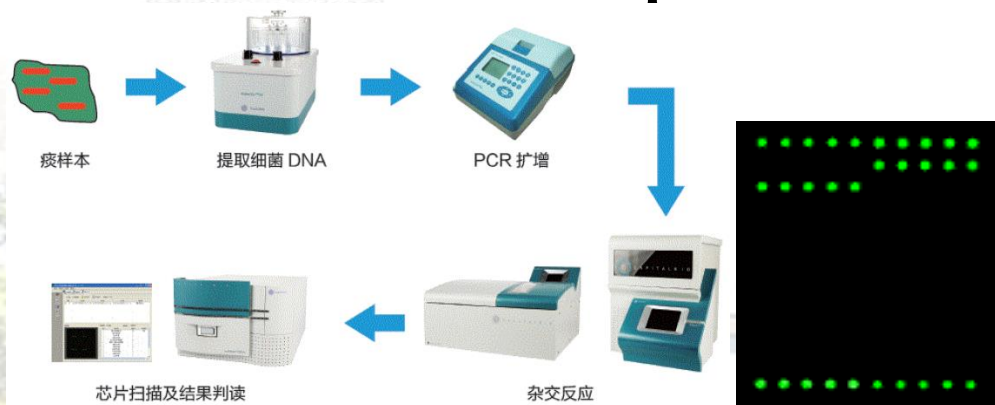
Nucleic Acid Mass spectrometry



Veredus VerePLE platform

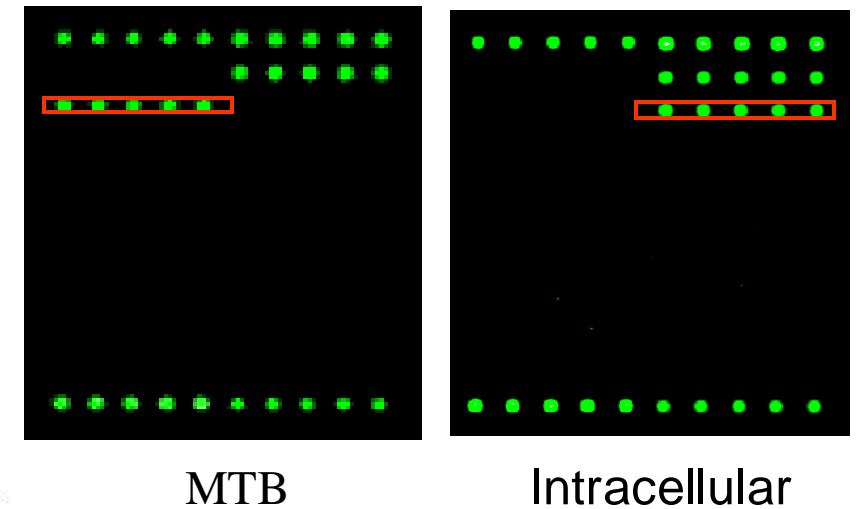


Gene Chip



Luminex xMAP

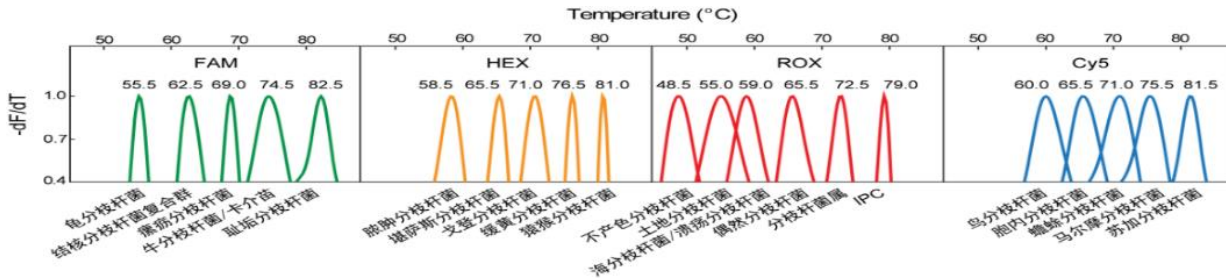




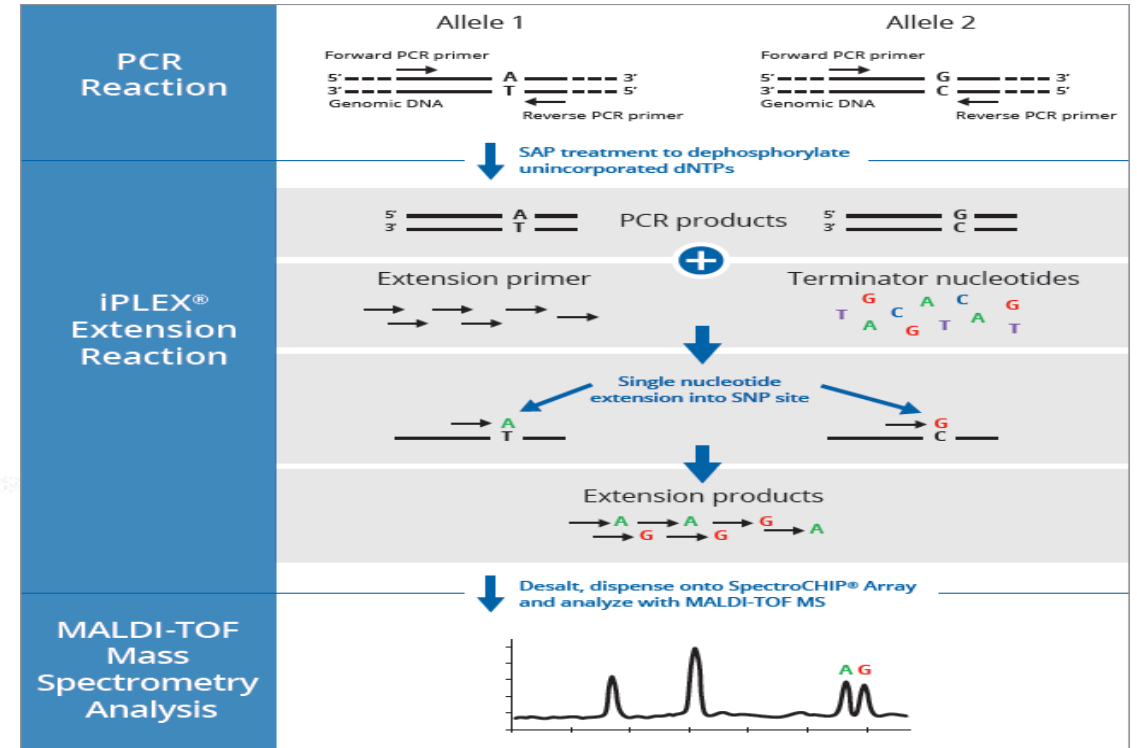


NAA Ts for species identification

■ Melting Curve



■ MALDI-TOF -MS





Digital PCR

ddPCR (Droplet digital PCR), cdPCR (Chip digital PCR)

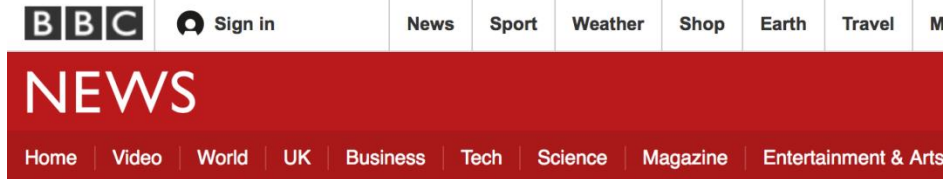
- Advantages : Higher sensitivity; Less influenced by inhibitors; Resistant strains was detected in a 1000:1 of sensitive and resistant strains.
- Disadvantages: Lower-throughput, Expensive, High quality of nucleic acid



ddPCR workstation: DW3200



Whole Genome Sequencing



Health

British scientists in world-first TB breakthrough

© 24 March 2017 | Health |

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THE LANCET Infectious Diseases

Volume 17, Issue 4, April 2017, Pages 359-361



Comment

Tuberculosis is changing

Timothy M Walker ^a✉, Ana Louisa Gibertoni Cruz ^a, Tim E Peto ^a, E Grace Smith ^c, Hanif Esmail ^b, Derrick W Crook ^{a, d}

Species identification and prediction of drug-resistance by WGS has been launched in the UK from 2017





Whole Genome Sequencing



Nanopore: DNA sequencer for identification of anti-TB drug resistance in sputum specimens

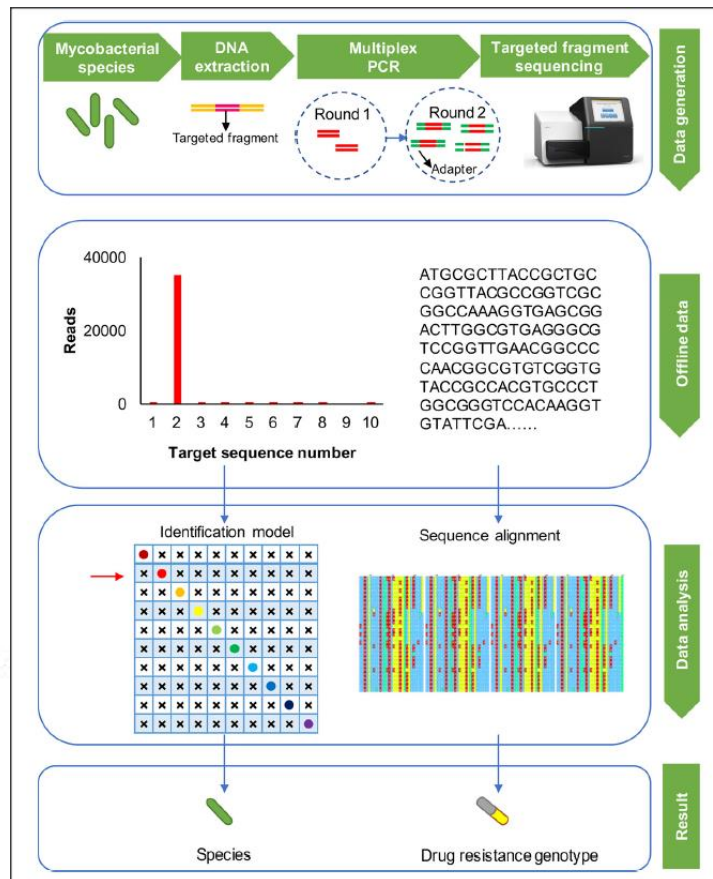
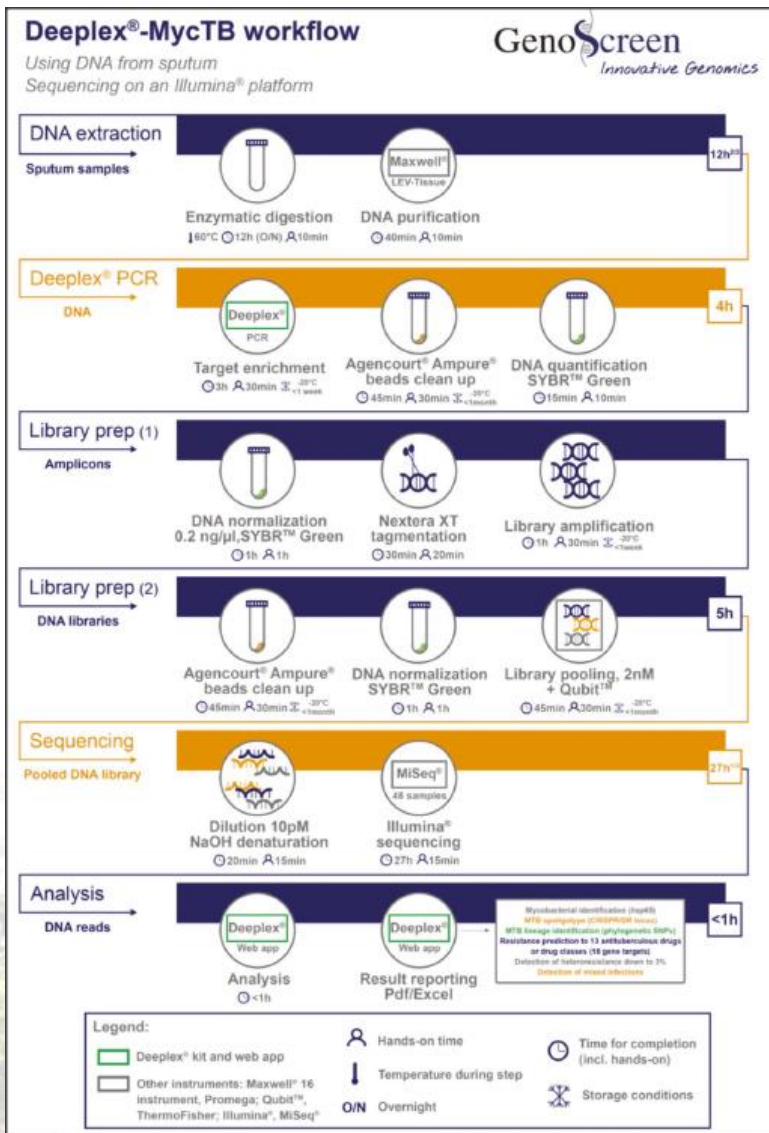
Web server: ReSeqTB, CASTB, PhyResSE, TBProfiler, GenTB, SAM

Software: KvarQ, Mykrobe Predictor TB



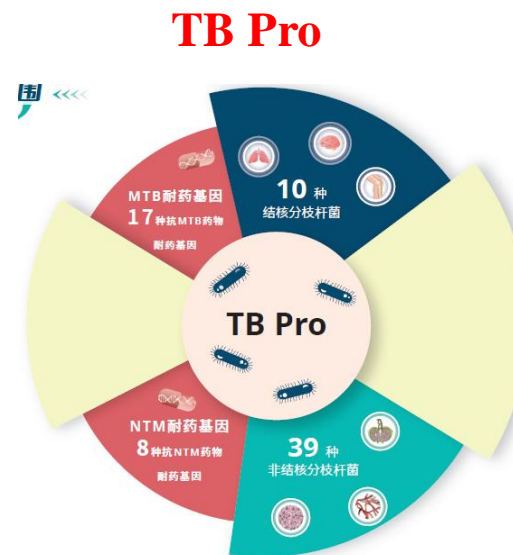


Targeted next generation sequencing



GenSeizer: a Multiplex PCR-Based Targeted Gene Sequencing Platform for Rapid and Accurate Identification of Major *Mycobacterium* Species

Bing Li,^a Liyun Xu,^a Qi Guo,^{a,b} Jianhui Chen,^{a,b} Yanan Zhang,^c Wenpan Huang,^c Zheming Zhang,^a Lihong Han,^d Xiaogang Xu,^a Haiping Chu^{a,f}



Tbseq Ultra

结核耐药检测结果					
序号	突变基因	潜在耐药药物	核酸突变结果	密码子突变结果	突变率(%)
1	<i>rpoB</i>	RIF	1592C>T	Ser531Leu	95.09
2	<i>rpoB</i>	RIF	1532T>C	Leu511Pro	99.57
3	<i>katG</i>	INH	944G>C	Ser315Thr	99.74
4	<i>katG</i>	INH	943A>G	Ser315Gly	99.12
5	<i>gyrA</i>	FQs	280G>T	Asp94Tyr	94.57
6	<i>embB</i>	EMB	916A>G	Met306Val	85.34

耐药置信度
High
low
High
-
High
High

Thanks for your attention!

