SeeGene

Instruction Manual ShengTing Medical Technology Co., Ltd

*Mycobacterium N*ucleic Acid And *M. tuberculosis D*rug Resistance Gene Detection Kits

（Nanopore Sequencing）

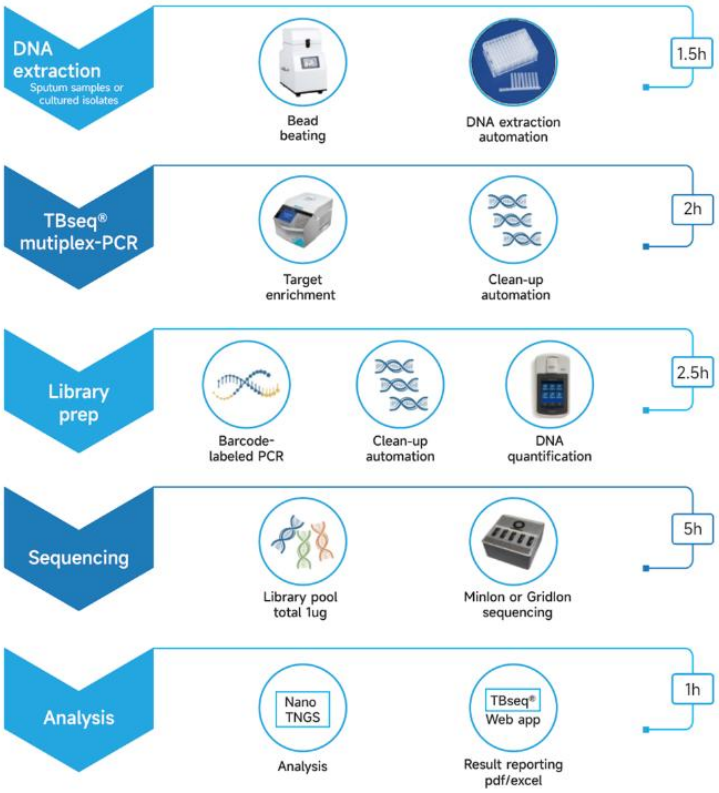
Drug-resistant Tuberculosis Diagnosis And Treatment Solutions

1. Product description

1.1. Principle

ShengTing Medical Technology Co. has a targeted Next Generation Sequencing (NGS)-based kit (TBseq®) for the simultaneous identification of *mycobacterial species* and prediction of drug resistance of *Mycobacterium tuberculosis* complex (MTBC) strains, directly applicable to clinical specimens such as sputum, bronchoalveolar lavage fluid, pleural effusion or cultured bacteria. The assay relies on deep sequencing of a paired-end barcode-labeled primer multiplex amplification mix and targets 21 main MTBC gene regions associated with resistance to first and second line anti-tuberculosis drugs (rifampicin, isoniazid, pyrazinamide, ethambutol, fluoroquinolones, amikacin, kanamycin, capreomycin, streptomycin, para-aminosalicylic acid, cycloserine, ethionamide/protionamide, bedaquiline, clofazimine, linezolid). Mycobacterial species identification is performed by targeting the 16s and *hsp65* regions.

The assay is performed using the Universal Gene Sequencing Kit (ShengTing) to generate libraries that are sequenced on either a MinIon and/or GridIon platform (Oxford Nanopore Technologies). The solution includes automated analysis software [Nano TNGS] for sequencing data processing and a secure web application (TBseq® Web App) with integrated



databases for result interpretation.

Figure 1 - TBseq Workflow（Estimated times provided for 24 tests sequenced on a GridIon）

Table 1 - TBseq specifications for the ONT platforms

Platform	Kit	Run time	Number of samples
MinIon/GridIon	Ligation Sequencing Kit V14 (SQK-LSK114)	~5h	24

The coverage of Mtb drug-resistant genes is as follows:

Table 2 - TBseq mycobacterial targets

Gene region	Target	Gene region	Target
16s, hsp65	Species ID	*gyrA, gyrB*	Fluoroquinolones
		rrs	Amikacin
		eis, rrs	Kanamycin
		tlyA, rrs*	Capreomycin
rpoB	Rifampicin	*folC, thyA**	para-aminosalicylic acid
ahpC, katG, inhA	Isoniazid	*ethA*, ahpC, inhA*	Ethionamide/protionamide
*pncA**	Pyrazinamide	*rv0678*, atpE**	Bedaquiline, clofazimine
embB, embA	Ethambutol	*rplC**	Linezolid
rrs, rpsL, gibB**	Streptomycin	*alr**	Cycloserine

(* full genes).

1.2. Context

Mycobacterium tuberculosis (TB) remains a leading cause of bacterial infectious disease worldwide. The World Health Organization reports that there were 10 million new cases of TB in 2020, resulting in 1.5 million deaths. In addition, more than half a million new cases were rifampicin-resistant (RR) or multidrug-resistant (MDR) forms, including more than 25,000 pre-extensively drug-resistant (pre-XDR: MDR also resistant to fluoroquinolones) or extensively drug-resistant (XDR: MDR also resistant to fluoroquinolones and at least one additional group A drug [bedaquiline, linezolid]) forms.

Ensuring early and accurate detection of drug resistance or susceptibility in TB is essential to rapidly determine the appropriate treatment and prevent the transmission of drug resistance.

1.3. 24-Test kit content

Component A

No.	Name	Volume (Tube count)	Major components
1	Targeted PCR primer mix	150μL (1)	Targeted primer
2	Barcode PCR primer mix	2μL (24)	Barcode primers
3	PCR Mix	375μL (2)	Taq DNA polymerize, dNTP,Mg^{2+}, buffer etc.
4	Lysozyme	125μL (1)	Lysozyme
5	Lysing enzymes	250μL（1）	Lysing enzymes

6	Positive control	1.0 mL（1）	Inactivated bacteria
7	Negative control	1.0 mL（1）	Sterile physiological saline solution

Component B

No.	Name	Volume (Tube count)	Major components
1	Magnetic beads solution	450μL (2)	Magnetic beads
2	Elution solution	500μL (2)	Purified water
3	Proteinase K	500μL (1)	Proteinase K
4	OT conditioning fluid	50 mL (1)	NaOH and other sputum liquefaction fluid

Note: Ingredients from different product batches should not be mixed or interchanged.

1.4. Storage conditions

The reagents in Component A and B are stored and shipped in dark containers at -20℃ and 2~8℃, respectively. Both have a shelf life of 12 months. Please check the production date and expiration date before use. The reagents are stable within three cycles of freezing and thawing.

1.5. Equipment and consumables to be supplied by user

Supplied	Not supplied but required
Reagents	
Component A Targeted PCR primer mix Barcode PCR primer mix PCR Mix Lysozyme Lysing Enzymes Positive control Negative control	Ultra-pure PCR-grade water
Component B Magnetic beads solution Elution solution Proteinase K OT conditioning fluid	Universal Gene Sequencing Kit (Shengting, Registeration Certificate No.: Zhejiang Device Registration Approval No. 20220004)
	Fluorometer assay reagents (e.g.Thermo Fisher™,Q32851)
	nucleic acid extraction or purification Kits (Shengting, Registeration Certificate No.: Zhejiang Device Registration Approval No. 20201178)
	Ethanol, molecular grade (e.g. Sigma Aldrich™ 32221)
Consumables	
	Personal protective equipment
	0.5mm zirconia grinding beads (e.g. Next Advance™, ZSB05)
	0.2 mL 96-well plates for PCR amplification or PCR microtubes or strips

	(e.g.Sigma Aldrich™,CLS9898)
	1.5 mL microtubes (e.g. Sigma Aldrich™,AXYMCT150CS)
	Filter tips (e.g. Biohandler)
	deep-well plate (e.g. Biohandler)
	tip combs (e.g. Biohandler)
Equipment	
	Single channel and multi-channel pipettes (p10, p100, p200 and single channel p1000, e.g. Eppendorf®)
	Fluorometer (e.g. Thermo Fisher™,Q33216)
	Beadbeater (e.g.Retsch™)
	Microcentrifuge (e.g.Thermo Fisher™)
	Vortex mixers (e.g.Thermo Fisher™)
	Thermostatic water bath pot or metal bath (e.g.Thermo Fisher™, 88880030)
	ONT sequencer (e.g.Oxford Nanopore Technologies, GridION)
	PCR amplification systems (e.g. Biorad,1861096)
	Magnetic stand (e.g. Thermo Fisher™,12321D)
	Computer
	Automatic Nucleic Acid Extraction System (e.g. Biohandler, CANTUS SCREEN）
Software Nano TNGS V1.0 TBseq®Web app	

1.6. Precautions

Due to the potential infectious risk, all steps prior to completion of heat inactivation of biological specimens must be performed according to local precautions and prescribed procedural guidelines.

Clinical specimens should be collected according to standard procedures and transported to the laboratory in a timely manner at a temperature between 2°C and 8°C prior to processing.

As TBseq® relies PCR amplification, appropriate procedures should be followed to avoid the risk of DNA contamination. All solutions and water used for sample processing must be PCR grade (i.e., free of DNase and contaminants). DNA extraction from samples, preparation of solutions and reaction mixtures, PCR amplification and processing of amplicons should be performed in separate rooms.

1.7.1. Applicability

The TBseq® kit is designed to predict susceptibility or resistance to 15 anti-tuberculosis drugs or drug classes, genotype MTBC strains, and identify mycobacterial species (including but not limited to MTBC).

1.7.2. Samples

The TBseq® Kit is designed to be used on DNA extracted from heat- or ethanol-inactivated clinical samples from (potential) TB patients and from heat-inactivated mycobacteria-positive

cultures. Each kit lot is validated for successful mycobacterial identification, drug susceptibility and resistance prediction, and MTBC strain genotyping at ⩾ 200 cfu/mL under the conditions described in this user manual. Performance on a user's sample will depend on the bacterial load and efficiency of DNA extraction in the sample. It is recommended that the kit be used on microscopically positive samples. Although results may be obtained from a variety of microscopically negative samples, performance on such samples cannot be guaranteed. The kit is also applicable to DNA extracted from cultured, heat-inactivated isolates. However, the kit has not been evaluated on samples from TB patients undergoing treatment.

1.7.3. Sequencing

The TBseq® kit and Web application are configured for paired-end sequencing only.

【Product Name】

Mycobacterium nucleic acid and *M. tuberculosis* drug resistance gene detection kit （Nanopore Sequencing）

【Number of Preparations】24 tests

【Intended Use】

The kit is designed for the qualitative detection of Mycobacterium nucleic acids and M. tuberculosis (Mtb) drug resistance genes in human sputum bronchoalveolar lavage fluid or Mycobacteria-positive culture. The species of mycobacteria that can be detected are listed in the table below.

Mycobacteria spp.	Chinese Name	Gram Staining
M. tuberculosis complex	结核分枝杆菌复合群	+
M. intracellular complex	鸟胞内分枝杆菌复合群	+
M. abscess complex	脓肿分枝杆菌复合群	+
M. kansasii	堪萨斯分枝杆菌	+

The coverage of Mtb drug-resistant genes is as follows:

Table 1 - TBseq mycobacterial targets

Gene region	**Target**	**Gene region**	**Target**
16s, hsp65	Species ID	*gyrA, gyrB*	Fluoroquinolones
		rrs	Amikacin
		eis, rrs	Kanamycin
		tlyA, rrs*	Capreomycin
rpoB	Rifampicin	*folC, thyA**	para-aminosalicylic acid
ahpC, katG, inhA	Isoniazid	*ethA*, ahpC, inhA*	Ethionamide/protionamide
*pncA**	Pyrazinamide	*rv0678*, atpE**	Bedaquiline, clofazimine
embB, embA	Ethambutol	*rplC**	Linezolid
rrs, rpsL, gibB**	Streptomycin	*alr**	Cycloserine

(* full genes).

Specific mutants and corresponding drug resistances are described in the Result Interpretation sections. It should be noted that the tests using this kit can detect the bacteria described above but cannot distinguish between different species of Mycobacteria. In clinical practice, the kit is suitable for ancillary diagnosis of MTB and nontuberculous mycobacteria (NTM).

For MTB-positive specimens, variants of TB drug-resistance genes can be further detected using this kit according to the instructions provided.

【Test Procedures】

The kit is based on a master mix ready for multiplex PCR amplification and then

attaches adapters to PCR products for nanopore sequencing. The detailed protocol is as follows: (1) grind the sputum and bronchoalveolar lavage fluid to break down bacterial cell walls, (2) extract nucleic acids; In the third step, (3) amplify target DNA fragments from pathogens using specific primers designed for conserved regions of the pathogen, such as the 16S region, hsp65 region, or drug resistance gene region, (4) add nanopore-specific barcodes to the above PCR products through another round of PCR amplification, (5) load the library DNA into a nanopore sequencer, (6) analyze the sequencing data through the bioinformatics pipeline (ShengTing) to identify pathogens and drug resistance gene mutations.

【Major components】

Component A

No.	Name	Volume (Tube count)	Major components
1	Targeted PCR primer mix	150μL (1)	Targeted primer
2	Barcode PCR primer mix	2μL (24)	Barcode primers
3	PCR Mix	375μL (2)	Taq DNA polymerize, dNTP,Mg^{2+}, buffer etc.
4	Lysozyme	125μL (1)	Lysozyme
5	Lysing enzymes	250μL（1）	Lysing enzymes
6	Positive control	1.0 mL（1）	Inactivated bacteria
7	Negative control	1.0 mL（1）	Sterile physiological saline solution

Component B

No.	Name	Volume (Tube count)	Major components
1	Magnetic beads solution	450μL (2)	Magnetic beads
2	Elution solution	500μL (2)	Purified water
3	Proteinase K	500μL (1)	Proteinase K
4	OT conditioning fluid	50 mL (1)	NaOH and other sputum liquefaction fluid

Reagents and Equipment Supplied by User

Reagents
Ultra-pure PCR-grade water Universal Gene Sequencing Kit (Shengting, Registeration Certificate No.: Zhejiang Device Registration Approval No. 20220004)

Fluorometer assay reagents (e.g.Thermo Fisher™,Q32851) nucleic acid extraction or purification Kits (Shengting, Registeration Certificate No.: Zhejiang Device Registration Approval No. 20201178) Ethanol, molecular grade (e.g. Sigma Aldrich™ 32221)
Consumables
Personal protective equipment 0.5mm zirconia grinding beads（e.g. Next Advance™, ZSB05） 0.2 mL 96-well plates for PCR amplification or PCR microtubes or strips (e.g.Sigma Aldrich™,CLS9898) 1.5 mL microtubes (e.g. Sigma Aldrich™,AXYMCT150CS) Filter tips (e.g. Biohandler) deep-well plate (e.g. Biohandler) tip combs (e.g. Biohandler)
Equipment
Single channel and multi-channel pipettes (p10, p100, p200 and single channel p1000, e.g. Eppendorf®) Fluorometer (e.g. Thermo Fisher™,Q33216) Beadbeater (e.g.Retsch™) Microcentrifuge (e.g.Thermo Fisher™) Vortex mixers (e.g.Thermo Fisher™) Thermostatic water bath pot or metal bath (e.g.Thermo Fisher™, 88880030) ONT sequencer (e.g.Oxford Nanopore Technologies, GridION) PCR amplification systems (e.g. Biorad,1861096) Magnetic stand (e.g. Thermo Fisher™,12321D) Computer Automatic Nucleic Acid Extraction System (e.g. Biohandler, CANTUS SCREEN）

【Storage and shipment】

The reagents in Component A and B should be stored and shipped in dark containers at -20℃ and 2~8℃, respectively. Both have a shelf life of 12 months. Please check the production and expiration dates before use. The reagents are stable within three freeze-thaw cycles.

【Sample Requirements】

1. Specimen types: sputum, bronchoalveolar lavage fluid, mycobacteria-positive culture

2. Specimen collection:

2.1 Sputum: Collect 0.5~3 ml of sputum expectorated from the deep part of the lung. The specimen should be stored in a sterile container and sealed tightly for storage and/or shipment.

2.2 Bronchoalveolar lavage fluid: Collect the specimen according to the Chinese Expert Consensus on the Detection of Bronchoalveolar Lavage Pathogens in Pulmonary Infectious Diseases (2017 Edition). Store a minimum of 5 ml of fluid in a sterile specimen collection tube and seal tightly for storage and/or shipment.

3.Sample and transportation: Specimens can be stored at 4℃ for 48 hours, -20℃ for

10 months, and -70℃ for 12 months. Do not freeze and thaw specimens more than four times. It is recommended that the test be performed as soon as possible after nucleic acid extraction. If necessary, purified DNA can be stored at 4°C for no longer than 24 hours and at -20°C for no longer than 32 hours.

【Test method】

1. Reagent preparation (in the reagent preparation area)

1.1 Thaw the reagents at room temperature, mix well, and place on ice for subsequent testing.

2. Sample preparation in the sample processing area (processing positive, negative, and test samples simultaneously)

2.1 Sample pretreatment

Bronchoalveolar lavage fluid:

1）Transfer 2 mL of specimen to a sterile 2 mL centrifuge tube. Shake upside down at least 5 times prior to collection. Centrifuge the tube at 12000 rpm for 4 minutes, discard the supernatant, and resuspend the sediments with 500 μL of sample in the same tube to obtain a 500 μL enriched sample. If the sample volume is less than 2.5ml, use the sample directly.

2）Add 5μL lysozyme and 10uL Lysing enzymes to the sample tube and mix thoroughly. Incubate for 15 minutes at 30°C.

3）Centrifuge the reaction for 3~5 seconds, add 20 μL Proteinase K and approximately 180 mg 0.5 mm zirconia grinding beads. Program the bead beater to grind samples for 90 seconds, pause for 15 seconds, repeat three times to break down the cell walls of pathogens.

Sputum:

1）Disperse an equal volume of OT Treatment Solution to the specimen collection tube, mix well by shaking, and incubate for 5~15 minutes. Transfer 500 μL of the liquefied sputum sample to a fresh 2 mL centrifuge tube.

2）Add 5μL lysozyme and 10uL Lysing enzymes to the tube, mix thoroughly and incubate at 30°C for 15 minutes.

3）Centrifuge the reaction for 3~5 seconds, add 20 μL Proteinase K and approximately 180 mg 0.5 mm zirconia grinding beads. Program the bead beater to grind samples for 90 seconds, pause for 15 seconds, repeat three times to break down the cell walls of pathogens.

2.2 Nucleic acid extraction

Transfer 400 μL of the ground samples, negative control, positive control into

separate 1.5 ml centrifuge tubes. Perform nucleic acid extraction using the Nucleic acid extraction or purification reagent (Registeration Certificate No.: Zhejiang Device Registration Approval No. 20201178) according to the manufacturer's instructions. Final DNA is eluted to 100 uL of TB buffer. Quantify DNA concentration by using a Qubit fluorometer per the manufacturer's recommendation.

2.3 PCR reaction setup

Pipette 9 µl of purified DNA (≤450 ng) into a 0.2 ml PCR reaction tube, then add 15 µl PCR mix, 3 µl primer mix and 3 µl ddH2O. The positive and negative are set up similarly.

3. PCR amplification

3.1. Targeted PCR amplification（Please refer to the instruction manual of each instrument for setting）

3.1.1 Each set of PCR reactions should include test samples, a positive and a negative control.

3.1.2 Cycle parameter setting:

Temperature	Time	No. of Cycles
105℃	Heated cover	1
95℃	3 min	
95℃	15 sec	6
66℃-61℃	60 sec	
72℃	15 sec	
95℃	15 sec	29
61℃	60 sec	
72℃	30 sec	
72℃	5 min	1
4℃	Hold	1

3.1.3 Purification of targeted PCR products

(1) Allow magnetic beads to equilibrate at room temperature for 30 minutes prior to use. Transfer 18 µL of beads into each centrifuge tube and determine the total number of tubes required based on the number of PCR reactions.

(2) Remove the PCR reactions from the thermocycler and centrifuge the reactions. Then transfer the PCR product to the centrifuge tubes containing magnetic

beads, mix well by pipetting at least 10 times, incubate at room temperature for 5 min.

(3) After incubation, spin the above reactions briefly at 3000 rpm for a few seconds. Place the centrifuge tubes on a magnetic rack and remove the supernatant when the magnetic beads have settled on the magnetic rack and the solution is clear.

(4) Add 200 μL of freshly prepared 80% ethanol to the beads, incubate for 30 s, pipette ethanol and discard.

(5) Repeat step (4) once.

(6) Remove the sample tubes from the magnetic rack and spin down briefly. Return the tubes to the magnetic rack and discard any remaining ethanol.

(7) Dry magnetic beads until cracks appear (approximately 5 minutes).

(8) Transfer 20 μl elution buffer to the magnetic beads, pipette up and down to mix. Incubate for 3 minutes at room temperature, then briefly spin down. Place the tubes back on the magnetic rack and incubate for 2 min before transferring the DNA eluants to fresh tubes for the second round of PCR.

3.2. Nanopore barcoding by PCR (see instrument manual for settings)

3.2.1 Set up the second round of PCR reactions as follows: Pipette 13 μL of the first round PCR products into a fresh 0.2 mL PCR tube, add 15 μL PCR mix and 2 μL mixed primers with indexed nanopore adapters. Mix well and centrifuge briefly. Each batch of PCR reactions includes a positive and negative control set up in a similar manner.

3.2.2 Cycle parameter setting:

Temperature	Time	No. of Cycles
105℃	Heated cover	1
95℃	3 min	
95℃	30 sec	14
64℃	30 sec	
72℃	1 min	
72℃	3 min	1
4℃	Hold	1

3.2.3 Purification of barcode PCR products

(1) Allow magnetic beads to equilibrate at room temperature for 30 minutes prior

to use. Transfer 18 μL of beads into each centrifuge tube and determine the total number of tubes required based on the number of PCR reactions.

(2) Remove the PCR reactions from the thermocycler and centrifuge the reactions. Then transfer the PCR product to the centrifuge tubes containing magnetic beads, mix well by pipetting at least 10 times, incubate at room temperature for 5 min.

(3) After incubation, spin the above reactions briefly at 3000 rpm for a few seconds. Place the centrifuge tubes on a magnetic rack and remove the supernatant when the magnetic beads have settled on the magnetic rack and the solution is clear.

(4) Add 200 μL of freshly prepared 80% ethanol to the beads, incubate for 30 s, pipette ethanol and discard.

(5) Repeat step (4) once.

(6) Remove the sample tubes from the magnetic rack and spin down briefly. Return the tubes to the magnetic rack and discard any remaining ethanol.

(7) Dry the magnetic beads until cracking occurs (approximately 5 minutes).

(8) Transfer 15 μL Elution Buffer to the magnetic beads using a pipette. Incubate for 3 minutes at room temperature, then spin down briefly. Place the tubes back on the magnetic rack and incubate for 2 minutes. Pipette 13 μL of the purified product into a 1.5 mL centrifuge tube and store at -20°C for the desired downstream application.

4. Sequencing

The universal gene sequencing kit (Nanopore sequencing method) (Registeration Certificate No.: Zhejiang Device Registration Approval No. 20220004) is used for library quality control, end repair, adapter ligation and sequencing.

5. Date analysis

Data analysis includes an easy-to-use web application for uploading and analyzing raw sequencing data and quickly interpreting the results. Specifically, initial basecalling relies on the Guppy software to generate pass filter reads in FASTQ format with a mean quality score > 7, followed by adapter and barcode trimming. Further data processing is performed using NanoFilt to remove reads of small size and/or low quality. After aligning each read to the human genome using Minimap2, reads derived from human DNA are removed and the unmatched reads are assumed to be from a microbe.

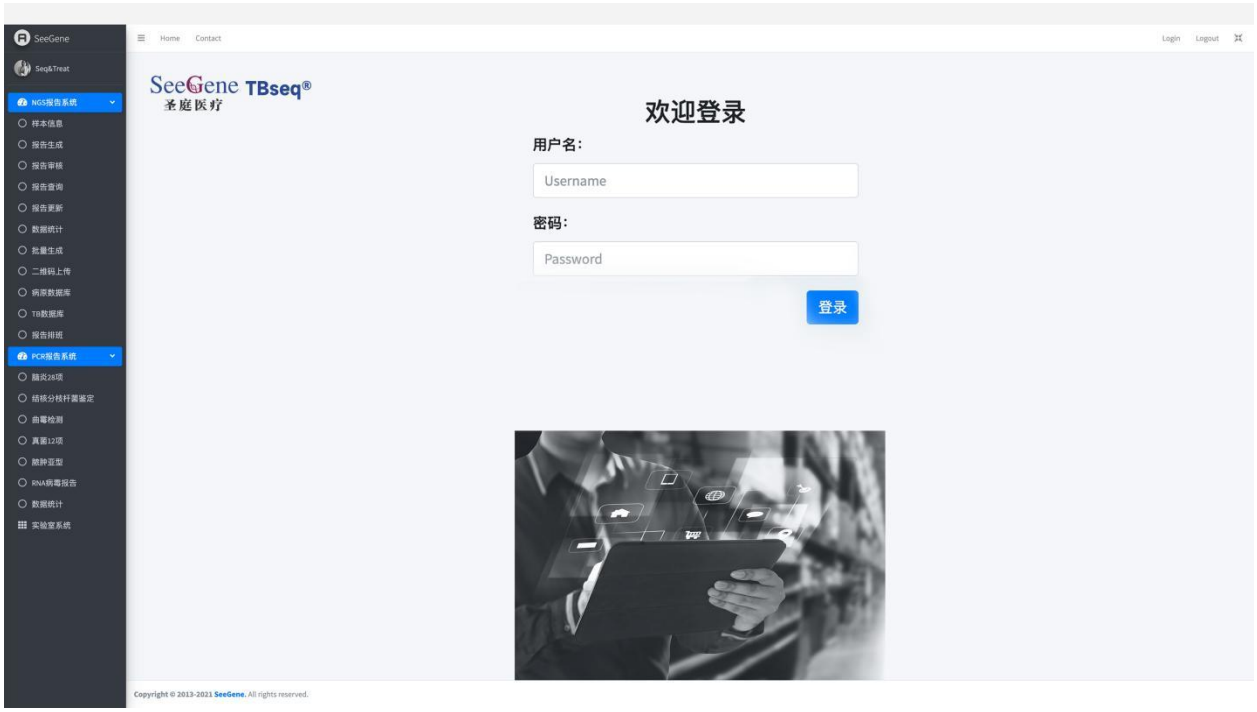
For microbial species identification, Minimap2 is used to align the unmatched reads against the microbial database, NanoTargetDB (ShengTing). Within the database, each read can be aligned to two or more species of a genus, and the one with the highest score in the comparison is considered to be the species corresponding to the query sequence. If a read perfectly aligns to two or more species from different genera, it cannot be correctly assigned and will be categorized at a higher classification level.

The process for identifying drug resistance in tuberculosis involves comparing the query sequences to the reference genome of *Mycobacterium tuberculosis*. The sequences are then subjected to variant detection using VarScan2 and annotated using SnpEff software. Finally, the variant results are compared with the Tuberculosis Drug Resistance Database (ShengTing) to obtain drug resistance information.

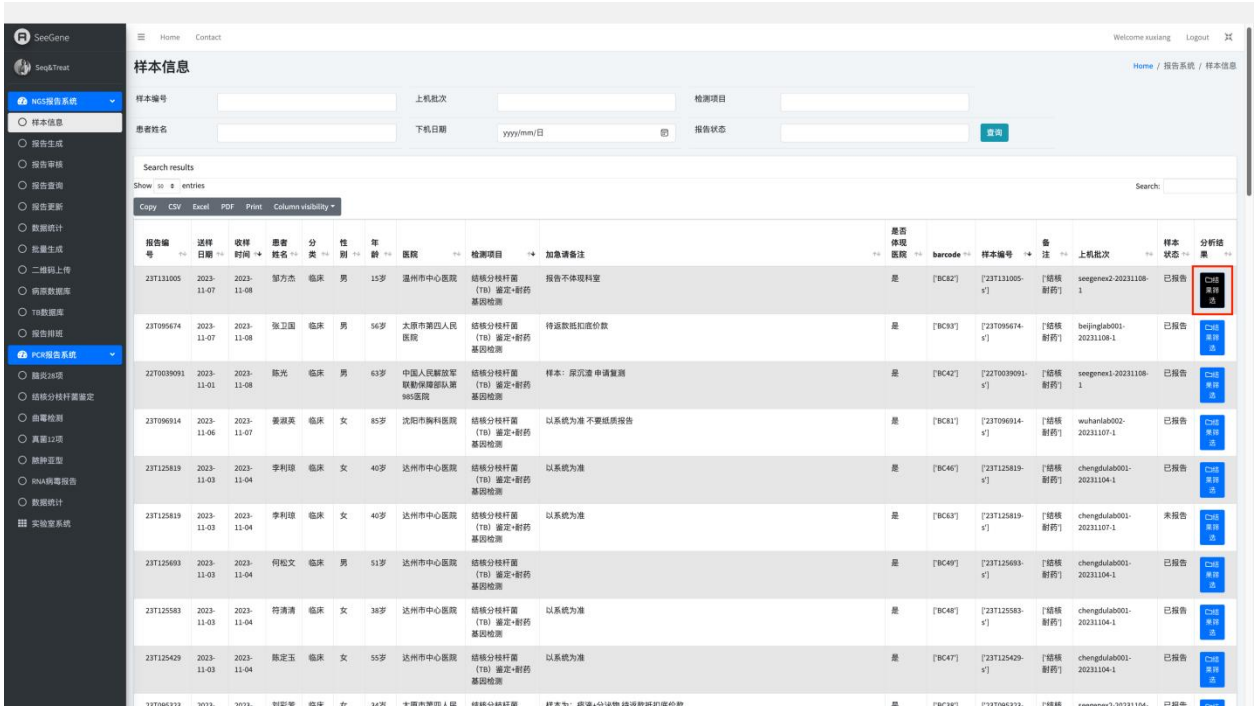
Recording and reporting

TBseq automatically generates reports that include sample information, date, analysis mode, quality summary, experiment set, and any mutation details inferred by the software. The TBseq Web App can integrate all the results of a sample and generate a report in docx or pdf format. Users can download reports directly from the TBseq Web App. Users should follow national requirements for reporting results. Please be aware that laboratory registries and reporting forms may need to be revised without notifying the user.

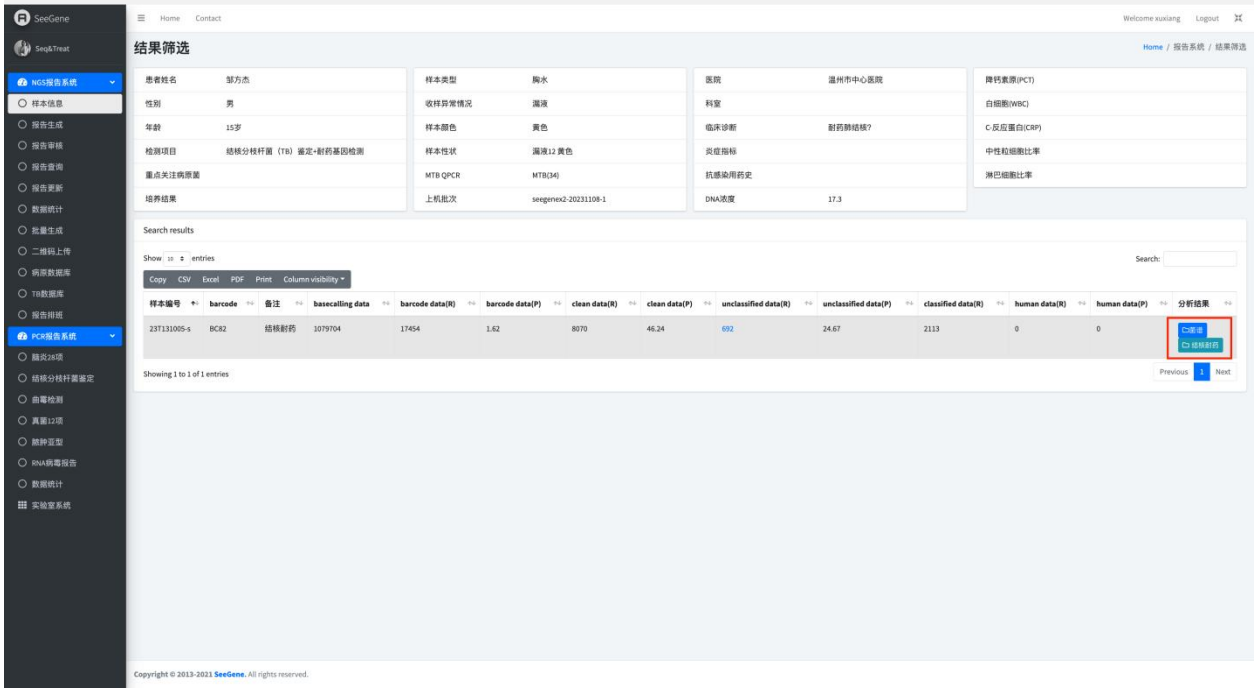
5.1 login the TBseqWeb App by typing the username and password.



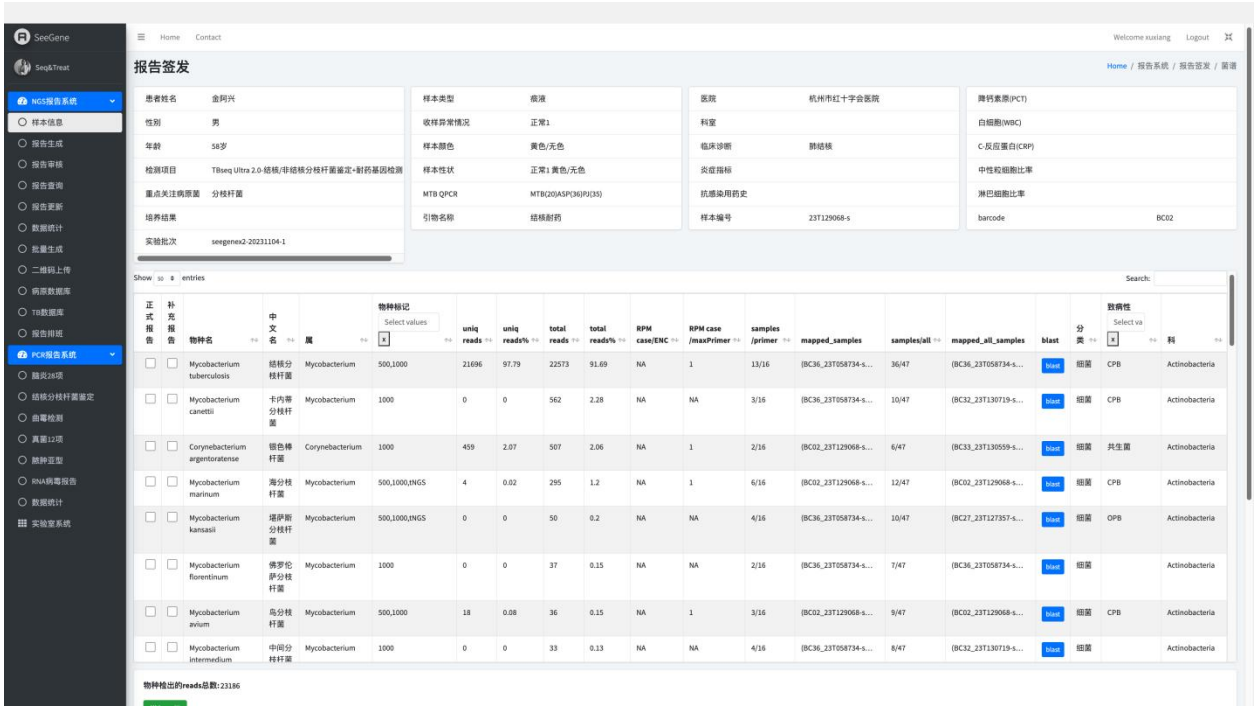
5.2 To review the analysis results of a sample, click the blue button with “结果筛选”on it in the red box.



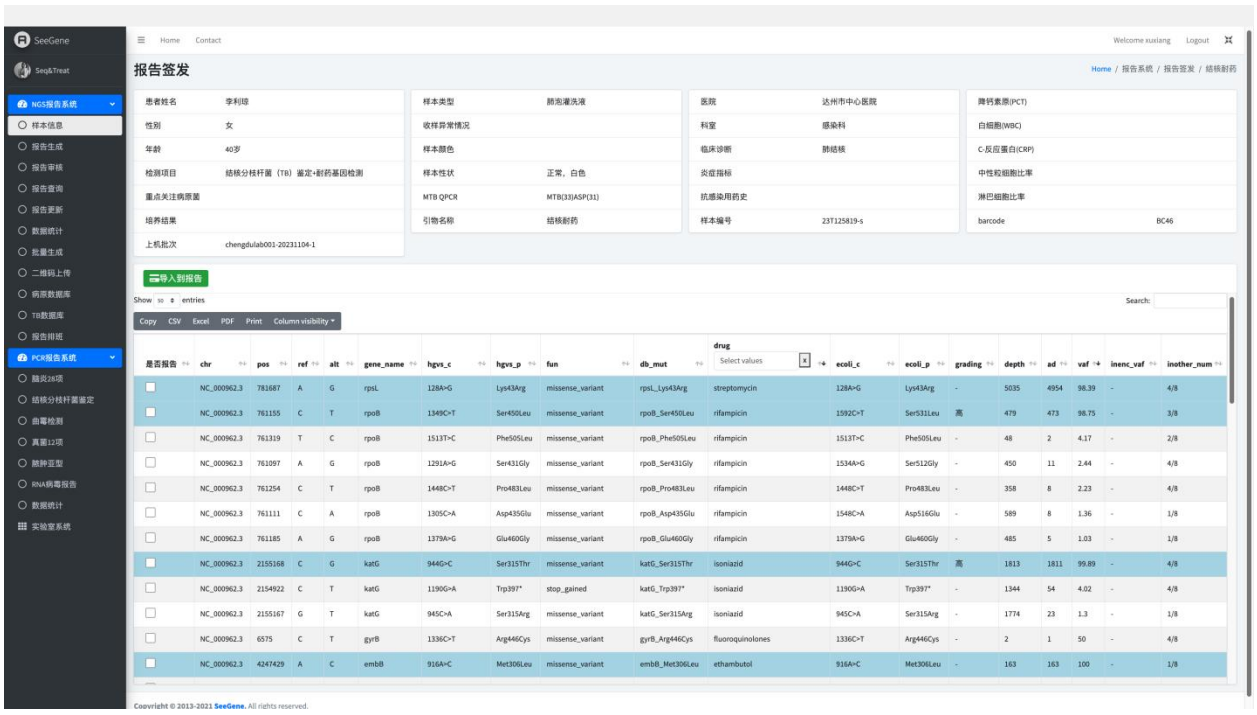
5.3 Users can check the clinical information of a specimen on the "结果筛选" page. The blue button with "菌谱" on it in the red box links to the *Mycobacterium tuberculosis* detection results. The green button with "结核耐药" on it in the red box links to the DST results of this sample.



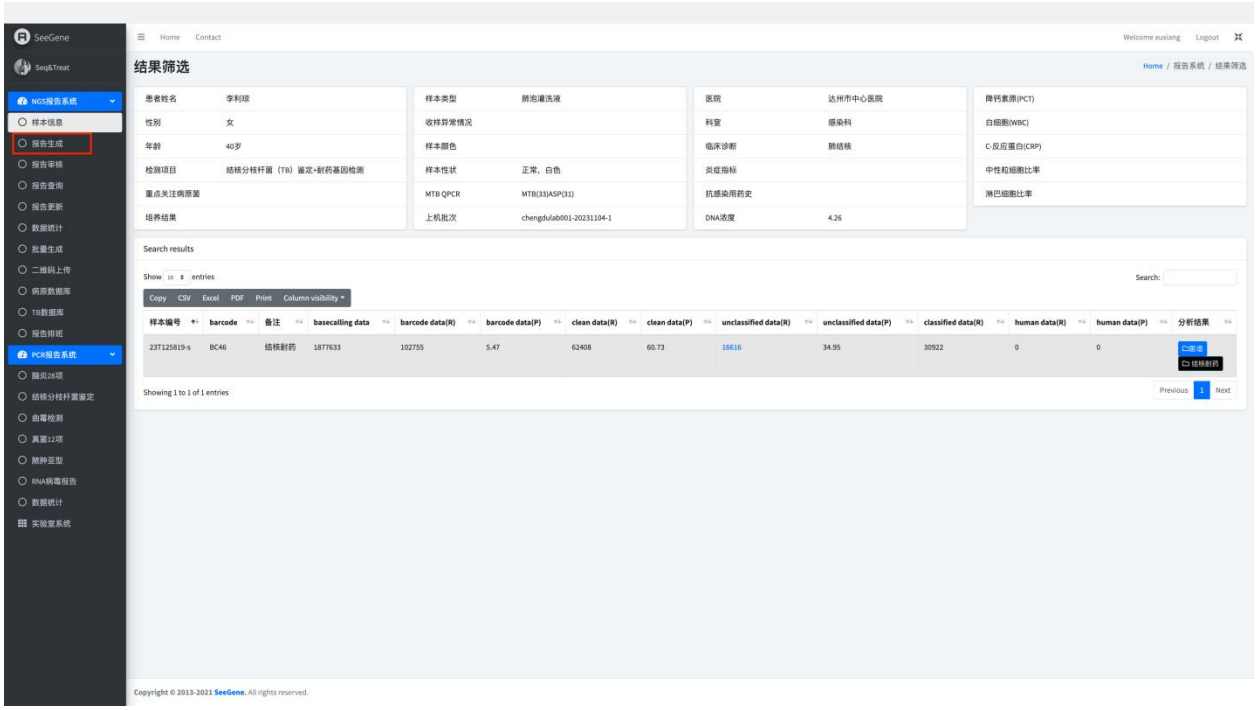
5.4 In “菌谱” page, users can see the reads of *Mycobacterium tuberculosis*, and click the box in “正式报告” column to report MTB positive. Then click the “确定” button at the bottom of this page.



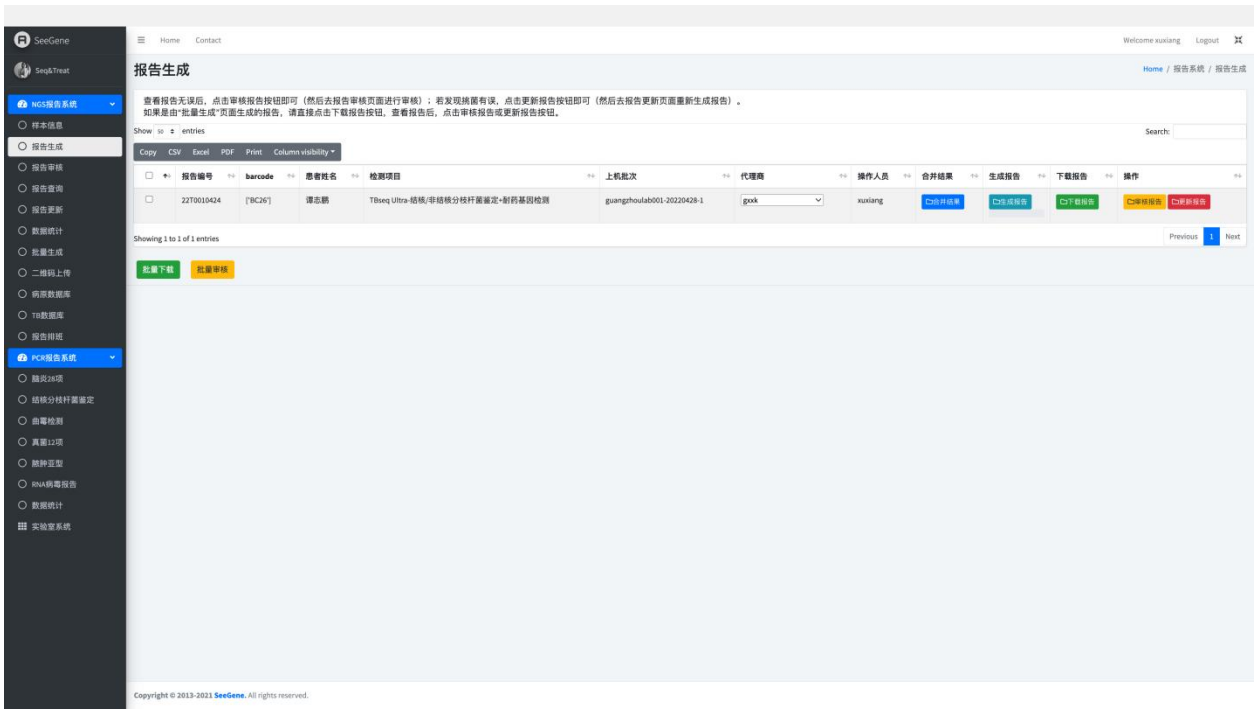
5.5 In the "结核耐药" page, the reliable mutations of Mycobacterium tuberculosis in this sample are highlighted in blue. Users can click the box in the "是否报告" column to report the reliable mutations related to MTB drug resistance.



5.6 After doing the above, click "报告生成" on the left side of the "结果筛选" page.



5.7 On the "报告生成" page, click "合并结果" to check the report results, and click "确认" after the results are confirmed. Then click "生成报告" to automatically generate the report of this sample. And users can download the report by clicking the "下载报告" button.



6. Quality control

6.1 Negative Control: Sequencing of the negative control should end with no reads. However，some commercial reagents, such as enzymes purified from bacteria, may still contain bacterial DNA and may appear in the sequencing results.

6.2 Sequencing of the positive control should show a sufficient number of reads from the relevant pathogens. Also, some commercial reagents, such as enzymes purified from bacteria, may still contain bacterial DNA and may appear in the sequencing results.

6.3 Both positive and negative results should appear as expected; if not, repeat the test.

【Positive cut-off value】

Mycobacterium: If there are more than one target pathogen sequencing reads, the sample is considered positive for the target pathogen.

Drug-resistant genes: The mutation frequency of a drug-resistant gene should be ≥ 10% in MTB-positive specimens.

【Interpretation of detection results】

1. Criteria for positive or negative results: If the sequencing reads from a *Mycobacterium* species, such as *Mycobacterium tuberculosis complex, Mycobacterium intracellular complex, Mycobacterium abscess complex*, and *Mycobacterium kansasii*, are greater than one, the specimen is considered positive. Negative results are defined as no relevant sequence reads from the above pathogens.

2. Drug resistance gene: For MTB-positive specimens, if the mutation rate of a TB drug resistance gene is greater than 10%, the specimen is considered positive. The list does not include all detectable mutations.

Gene	Mutation	Confidence grade	Drug
inhA	c-15t	Middle	INH (Isoniazid)
katG	S315I	High	INH (Isoniazid)
katG	S315N	High	INH (Isoniazid)
katG	S315T	High	INH (Isoniazid)
katG	Mixed frameshift and premature stop codons	High	INH (Isoniazid)
katG +*inhA*	S315 (T/G) and c-15t	High	INH (Isoniazid)
mabA	g609a	–	INH (Isoniazid)
rpoB	Q513K	High	RIF (Rifampicin)
rpoB	Q513L	High	RIF (Rifampicin)
rpoB	Q513P	High	RIF (Rifampicin)
rpoB	F514dupl	High	RIF (Rifampicin)
rpoB	D516A	High	RIF (Rifampicin)
rpoB	D516F	High	RIF (Rifampicin)
rpoB	D516G + L533P	High	RIF (Rifampicin)
rpoB	D516V	High	RIF (Rifampicin)
rpoB	delN518	High	RIF (Rifampicin)
rpoB	H526C	High	RIF (Rifampicin)
rpoB	H526D	High	RIF (Rifampicin)
rpoB	H526G	High	RIF (Rifampicin)
rpoB	H526L	High	RIF (Rifampicin)
rpoB	H526R	High	RIF (Rifampicin)
rpoB	H526Y	High	RIF (Rifampicin)
rpoB	S531F	High	RIF (Rifampicin)
rpoB	S531L	High	RIF (Rifampicin)
rpoB	S531W	High	RIF (Rifampicin)
rpoB	D516Y	Moderate	RIF (Rifampicin)
rpoB	S522L	Moderate	RIF (Rifampicin)
rpoB	H526P	Moderate	RIF (Rifampicin)
rpoB	L533P	Moderate	RIF (Rifampicin)
rpoB	L511P	Low	RIF (Rifampicin)
rpoB	H526N	Low	RIF (Rifampicin)
rpoB	I572F	Low	RIF (Rifampicin)
gyrA	G88C	High	MFX (Moxifloxacin)
gyrA	D89N	–	MFX (Moxifloxacin)
gyrA	A90V	High	MFX (Moxifloxacin)
gyrA	S91P	High	MFX (Moxifloxacin)
gyrA	D94A	High	MFX (Moxifloxacin)
gyrA	D94G	High	MFX (Moxifloxacin)
gyrA	D94H	–	MFX (Moxifloxacin)
gyrA	D94N	High	MFX (Moxifloxacin)
gyrA	D94Y	High	MFX (Moxifloxacin)
gyrB	D461H	–	MFX (Moxifloxacin)

gyrB	D461N	–	MFX (Moxifloxacin)
gyrB	N499D	–	MFX (Moxifloxacin)
gyrB	N499S	–	MFX (Moxifloxacin)
gyrB	N499K	–	MFX (Moxifloxacin)
gyrB	A504V	High	MFX (Moxifloxacin)
pncA	a-11g	High	PZA (Pyrazinamide)
pncA	t-7c	High	PZA (Pyrazinamide)
pncA	A3E	High	PZA (Pyrazinamide)
pncA	A3P	—	PZA (Pyrazinamide)
pncA	L4S	High	PZA (Pyrazinamide)
pncA	I6T	High	PZA (Pyrazinamide)
pncA	V7A	—	PZA (Pyrazinamide)
pncA	D8G	High	PZA (Pyrazinamide)
pncA	D8N	High	PZA (Pyrazinamide)
pncA	D8A	—	PZA (Pyrazinamide)
pncA	V9G	—	PZA (Pyrazinamide)
pncA	Q10P	High	PZA (Pyrazinamide)
pncA	D12A	High	PZA (Pyrazinamide)
pncA	D12N	High	PZA (Pyrazinamide)
pncA	F13L	—	PZA (Pyrazinamide)
pncA	F13S	—	PZA (Pyrazinamide)
pncA	C14R	High	PZA (Pyrazinamide)
pncA	G16S	—	PZA (Pyrazinamide)
pncA	G17D	High	PZA (Pyrazinamide)
pncA	L19P	High	PZA (Pyrazinamide)
pncA	G24D	High	PZA (Pyrazinamide)
pncA	A28T	—	PZA (Pyrazinamide)
pncA	D33A	—	PZA (Pyrazinamide)
pncA	Y34D	High	PZA (Pyrazinamide)
pncA	Y34S	—	PZA (Pyrazinamide)
pncA	Y41H	—	PZA (Pyrazinamide)
pncA	H43P	—	PZA (Pyrazinamide)
pncA	A46V	High	PZA (Pyrazinamide)
pncA	T47P	—	PZA (Pyrazinamide)
pncA	H51Q	High	PZA (Pyrazinamide)
pncA	H51R	High	PZA (Pyrazinamide)
pncA	H51P	—	PZA (Pyrazinamide)
pncA	H51Y	—	PZA (Pyrazinamide)
pncA	P54S	High	PZA (Pyrazinamide)
pncA	P54Q	—	PZA (Pyrazinamide)
pncA	P54T	—	PZA (Pyrazinamide)
pncA	H57D**	High	PZA (Pyrazinamide)
pncA	H57P	High	PZA (Pyrazinamide)
pncA	H57R	High	PZA (Pyrazinamide)
pncA	H57Y	High	PZA (Pyrazinamide)

pncA	H57Q	—	PZA (Pyrazinamide)
pncA	H57N	—	PZA (Pyrazinamide)
pncA	F58S	—	PZA (Pyrazinamide)
pncA	S59P	High	PZA (Pyrazinamide)
pncA	P62Q	High	PZA (Pyrazinamide)
pncA	P62T	—	PZA (Pyrazinamide)
pncA	P62R	—	PZA (Pyrazinamide)
pncA	D63G	High	PZA (Pyrazinamide)
pncA	D63A	—	PZA (Pyrazinamide)
pncA	S65P	—	PZA (Pyrazinamide)
pncA	S67P	High	PZA (Pyrazinamide)
pncA	W68C	High	PZA (Pyrazinamide)
pncA	W68R	High	PZA (Pyrazinamide)
pncA	W68L	—	PZA (Pyrazinamide)
pncA	P69R	—	PZA (Pyrazinamide)
pncA	H71Y	High	PZA (Pyrazinamide)
pncA	C72R	High	PZA (Pyrazinamide)
pncA	C72P	—	PZA (Pyrazinamide)
pncA	T76P	High	PZA (Pyrazinamide)
pncA	H82R	High	PZA (Pyrazinamide)
pncA	H82Y	—	PZA (Pyrazinamide)
pncA	L85P	High	PZA (Pyrazinamide)
pncA	F94L	High	PZA (Pyrazinamide)
pncA	F94C	—	PZA (Pyrazinamide)
pncA	F94S	High	PZA (Pyrazinamide)
pncA	K96N	High	PZA (Pyrazinamide)
pncA	K96R	High	PZA (Pyrazinamide)
pncA	K96Q	–	PZA (Pyrazinamide)
pncA	K96T	–	PZA (Pyrazinamide)
pncA	G97D	High	PZA (Pyrazinamide)
pncA	G97S	High	PZA (Pyrazinamide)
pncA	G97A	–	PZA (Pyrazinamide)
pncA	T100P	–	PZA (Pyrazinamide)
pncA	S104R	High	PZA (Pyrazinamide)
pncA	G105D	–	PZA (Pyrazinamide)
pncA	G108R	High	PZA (Pyrazinamide)
pncA	T114P	–	PZA (Pyrazinamide)
pncA	N188T	–	PZA (Pyrazinamide)
pncA	L120P	High	PZA (Pyrazinamide)
pncA	L120R	–	PZA (Pyrazinamide)
pncA	R121P	–	PZA (Pyrazinamide)
pncA	R123P	High	PZA (Pyrazinamide)
pncA	V125F	High	PZA (Pyrazinamide)
pncA	V128G	High	PZA (Pyrazinamide)
pncA	V130G	–	PZA (Pyrazinamide)

pncA	V130A	–	PZA (Pyrazinamide)
pncA	G132A	High	PZA (Pyrazinamide)
pncA	G132D	High	PZA (Pyrazinamide)
pncA	G132S	High	PZA (Pyrazinamide)
pncA	G132C	–	PZA (Pyrazinamide)
pncA	I133S	–	PZA (Pyrazinamide)
pncA	A134V	High	PZA (Pyrazinamide)
pncA	T135P	High	PZA (Pyrazinamide)
pncA	H137P	High	PZA (Pyrazinamide)
pncA	H137R	–	PZA (Pyrazinamide)
pncA	H137D	–	PZA (Pyrazinamide)
pncA	C138Y	High	PZA (Pyrazinamide)
pncA	C138R	–	PZA (Pyrazinamide)
pncA	C138W	–	PZA (Pyrazinamide)
pncA	V139G	High	PZA (Pyrazinamide)
pncA	V139L	High	PZA (Pyrazinamide)
pncA	V139A	–	PZA (Pyrazinamide)
pncA	Q141P	High	PZA (Pyrazinamide)
pncA	T142A	High	PZA (Pyrazinamide)
pncA	T142K	High	PZA (Pyrazinamide)
pncA	T142M	High	PZA (Pyrazinamide)
pncA	T142P	–	PZA (Pyrazinamide)
pncA	A143G	–	PZA (Pyrazinamide)
pncA	A146V	–	PZA (Pyrazinamide)
pncA	T153I	–	PZA (Pyrazinamide)
pncA	T153N	–	PZA (Pyrazinamide)
pncA	V155G	High	PZA (Pyrazinamide)
pncA	V155E	–	PZA (Pyrazinamide)
pncA	V163G	–	PZA (Pyrazinamide)
pncA	V163A	–	PZA (Pyrazinamide)
pncA	S164P	–	PZA (Pyrazinamide)
pncA	L172P	High	PZA (Pyrazinamide)
pncA	M175T	High	PZA (Pyrazinamide)
pncA	M175V	High	PZA (Pyrazinamide)
pncA	V180F	High	PZA (Pyrazinamide)
pncA	Pooled frameshifts and premature stop codons	High	PZA (Pyrazinamide)
pncA	V7G	Moderate	PZA (Pyrazinamide)
pncA	Q10R	Moderate	PZA (Pyrazinamide)
pncA	P54L	Moderate	PZA (Pyrazinamide)
pncA	W68G	Moderate	PZA (Pyrazinamide)
pncA	K96E	Moderate	PZA (Pyrazinamide)
pncA	K96T	Moderate	PZA (Pyrazinamide)
pncA	A171T	–	PZA (Pyrazinamide)

pncA	M175I	Moderate	PZA (Pyrazinamide)
pncA	D12G	Low	PZA (Pyrazinamide)
pncA	E37V	–	PZA (Pyrazinamide)
pncA	F58L	Low	PZA (Pyrazinamide)
pncA	S65A	–	PZA (Pyrazinamide)
pncA	H71R	Low	PZA (Pyrazinamide)
pncA	D110G	–	PZA (Pyrazinamide)
pncA	I133T	Low	PZA (Pyrazinamide)
pncA	I133N	–	PZA (Pyrazinamide)
pncA	A170V	–	PZA (Pyrazinamide)
pncA	V180I	–	PZA (Pyrazinamide)
rrs	a1401g	High	AMK (Amikacin)
rrs	c1402t	—	AMK (Amikacin)
rrs	g1484t	High	AMK (Amikacin)
eis	c-14t	—	AMK (Amikacin)
rrs	a1401g	High	KAN (Kanamycin)
rrs	c1402t	High	KAN (Kanamycin)
rrs	g1484t	High	KAN (Kanamycin)
eis	g-10a	Moderate	KAN (Kanamycin)
eis	c-12t	Low	KAN (Kanamycin)
eis	c-14t	High	KAN (Kanamycin)
eis	g-37t	Low	KAN (Kanamycin)
rrs	a1401g	High	CAP (Capreomycin)
rrs	c1402t	High	CAP (Capreomycin)
rrs	g1484t	High	CAP (Capreomycin)
tlyA	(Combined)	Generally High	CAP (Capreomycin)
embB	Met306Val	High	EMB (Ethambutol)
embB	Gly406Asp	High	EMB (Ethambutol)

【Limitations】

Test results are affected by how the specimen is collected, processed, transported, and stored; any errors in the process may result in inaccurate results. False positive results can occur due to cross contamination. This kit can detect rare mutations, but false negatives may occur with mutation rates below the detection limi

【Performance indicators of products】

The detection of enterprise reference products: Ten positive reference products (P1-P10) are tested, the diagnostic concordance rate is 100%. Four negative reference samples (N1~N4) are tested, and the negative concordance rate is also 100%. The precision reference materials (R0, R1-R2) have been repeatedly tested 10 times, and the detection results of the corresponding pathogens are correct. Ten enterprise reference materials (SQ1 ~ SQ10) for detection limit have been tested, and all of them meet the requirements of minimum detectability.

Limit of detection of pathogens in this kit:

Pathogen	Limit of Detection
Mycobacterium tuberculosis complex	200cfu/mL
Mycobacterium intracellular complex	500cfu/mL
Mycobacterium abscess complex	500cfu/mL
Mycobacterium kansasii	500cfu/mL

The detection limit for drug resistance genes is 10%.

Interfering substances at the concentrations listed will not affect the performance of this kit:

Interfering substances	Final concentration	Interfering substances	Final concentration
Oxymetazoline hydrochloride	100μg/mL	SDS	100μg/mL
Dexamethasone	50μg/mL	EDTA	10μg/mL
Cefmenoxime hydrochloride	50μg/mL	Urea	100μg/mL
Menthol	50μg/mL	Protoheme	10μg/mL
Zanamivir	100μg/mL	Purified mucin	20μg/mL
Ribavirin	100μg/mL	FeCl3	100μg/mL
Azithromycin	100μg/mL	Absolute ethyl alcohol	20%(v/v)
Sodium chloride	60μg/mL	Whole Blood	20%(v/v)
Beclomethasone	50μg/mL	Flunisolide	10μg/mL
Histaminum Hydrochloricum	50μg/mL	Triamcinolone acetonide	100μg/mL
Free-dried, nasal sprayed influenza attenuated vaccine	50μg/mL	Budesonide	50μg/mL
Benzocaine	50μg/mL	Mometasone	50μg/mL
Mupirocin	50μg/mL	Fluticasone propionate	50μg/mL
Tobramycin	50μg/mL	Oseltamivir	100μg/mL
Phenylephrine	100μg/mL	Peramivir	100μg/mL

Pathogens at the indicated concentrations will not cross-react with tests using this kit:

Pathogen	Concentration of pathogen (copies/mL)	Pathogen	Concentration of pathogen (copies/mL)
Bordetella pertussis	1.21×10^7	*varicella-zoster virus*	1.72×10^6
Streptococcus pyogenes	8.5×10^6	*Corynebacterium diphtheriae*	1.2×10^7
Cytomegalovirus	2.74×10^8	*Lactobacillus bulgaricus*	8.2×10^6
Streptococcus saliva	1.54×10^6	*Moraxella catarrhalis*	1.77×10^7
Nocardia farcinica	3.81×10^7	*Staphylococcus epidermidis*	3.2×10^7
Bocavirus	2.22×10^6	*Neisseria meningitidis*	5.1×10^6
Human herpesvirus 1	3.3×10^6	*Rothia mucilaginosus*	1.2×10^7
EB virus	6.97×10^6	*Aspergillus nidulans*	2.58×10^6
Penicillium citrinum	3.56×10^6	*Histoplasmosis capsulati*	1.28×10^6
Human parainfluenza virus 1	5.98×10^6	*Human parainfluenza virus* 2	4.35×10^8
Chlamydia pneumoniae	7.51×10^7	*Human parainfluenza virus* 3	6.52×10^8
Influenza A virus	8.59×10^6	*Influenza* B *virus*	7.81×10^6
Coronavirus 229E	5.36×10^6	*Mycobacterium smegmatis*	2.3×10^7
Mycobacterium chelonei	3.1×10^6	*Mycoplasma hominis*	4.2×10^7

【Precautions】

1. This kit is for *in vitro* use only. Please read this manual carefully before use.

2. Before performing the test, the user should read the instructions carefully and know how to use all relevant instruments. Also, perform quality control on each batch of tests.

3. Strict PCR laboratory management practices should be followed when performing the test. All laboratory personnel should be professionally trained. Ensure strict adherence to applicable safety protocols and guidelines throughout the experimental process. Sterilize all consumables after each use. Use specialized instruments and equipment for each step of the experimental procedure, avoiding cross-contamination between supplies throughout the process.

4. All specimens should be handled as infectious agents. Wear protective clothing, disposable face masks, and gloves throughout the assay. Change gloves frequently to avoid cross-contamination between specimens; handle specimens and dispose of waste in accordance with applicable regulations:*General Guidelines for Biosafety in Microbiology and Biomedical Laboratories and Medical Waste Management Regulations* of the Ministry of Health.

5. All frozen reagents should be thawed at room temperature and mixed thoroughly before use.

【Label Legend】

/

【Reference】

1. Global tuberculosis report 2020. Geneva: World Health Organization; 2020 (https://apps.who.int/iris/bitstream/handle/10665/336069/9789240013131-eng.pdf, accessed 10 January 2021).

2. Mohamed S, Köser CU, Salfinger M, Sougakoff W, Heysell SK. Targeted next-generation sequencing:a Swiss army knife for mycobacterial diagnostics? Eur Respir J. 2021;57:2004077.

3. Global tuberculosis report 2013. Geneva: World Health Organization; 2013 (https://apps.who.int/iris/bitstream/handle/10665/91355/9789241564656_eng.pdf, accessed 12 April 2021).

【Basic information】

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Production address:

Production license number or production record certificate number:

【 Medical device Registration certificate No. / Product technical requirements No.】

Medical device Registration certificate No. ：

Product technical requirements No. :

【Date of manual approval and modification】2023.07.31