齐碳科技
QITAN TECH

# Nanopore Sequencing Technology
# QITAN TECH

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**Director of Product (Microbiology and Infections)**

# Principles of Nanopore Gene Sequencing

## Nanopore Protein

The channel through which ions and nucleic acid molecules move under electrophoresis

## Motor Protein

To control the speed of single-stranded nucleic acid passing through the nanopore

## Flow Cell

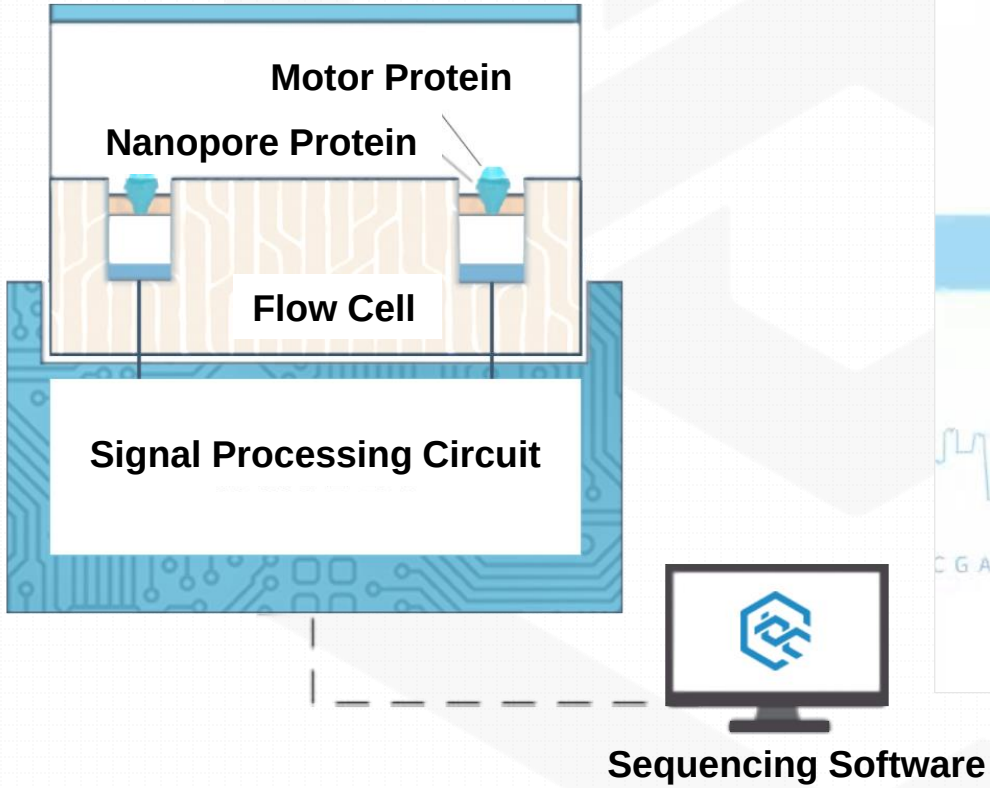
To provide structural support for nanopores and an electrochemical environment for electrophoresis

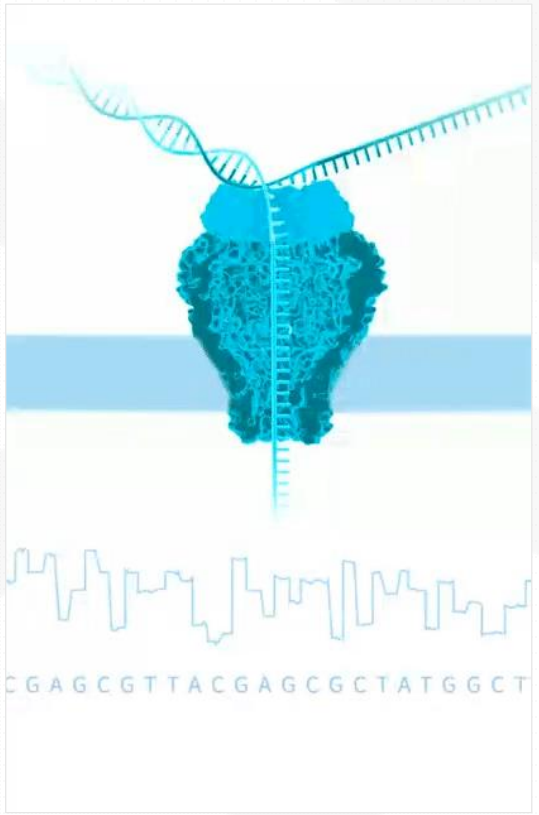
## Signal Processing Circuit

To provide biased voltage for electrophoresis and measure the current in each channel

## Sequencing Software

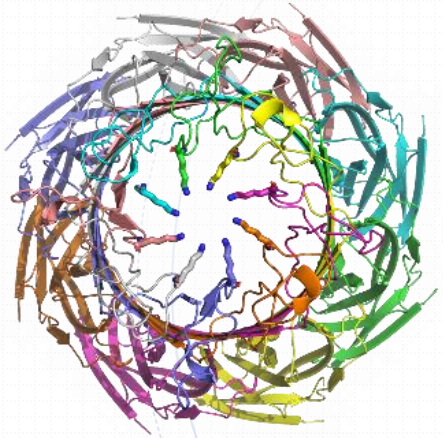
To control the sequencer, analyze the current signal generated in the sequencing process, and obtain the sequencing results.



# Core Technologies of Nanopore Sequencers

**Focus on multidisciplinary, cutting-edge technologies to form a complete nanopore gene sequencing technology layout**



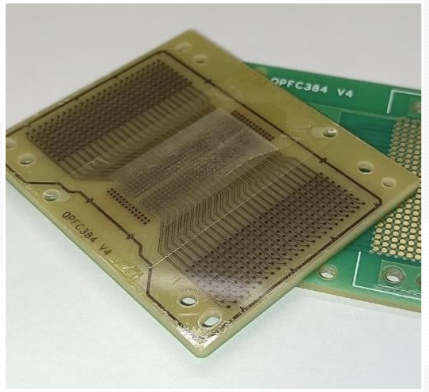
## Nanopore Protein

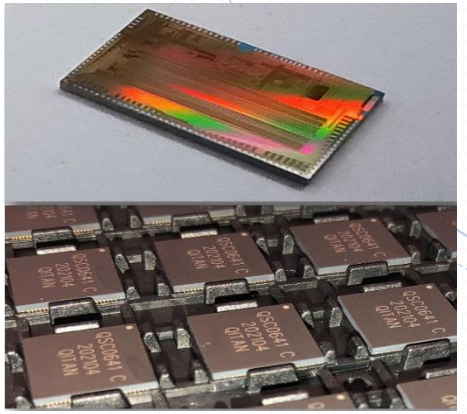
Continuous improvement of accuracy. It is expected that biochemical reagents that can approach the accuracy rate of next-generation sequencing will be launched next year

## Biochip

Detachable biochip eliminates the need for sample accumulation. Ready for use and measurements.

The consumables do not contain circuits, which can effectively reduce the cost of sequencing



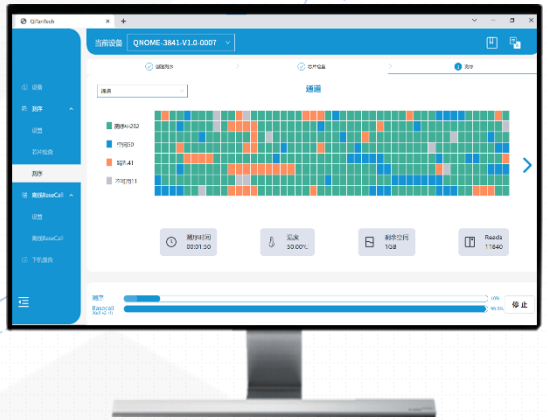



## ASIC Chip

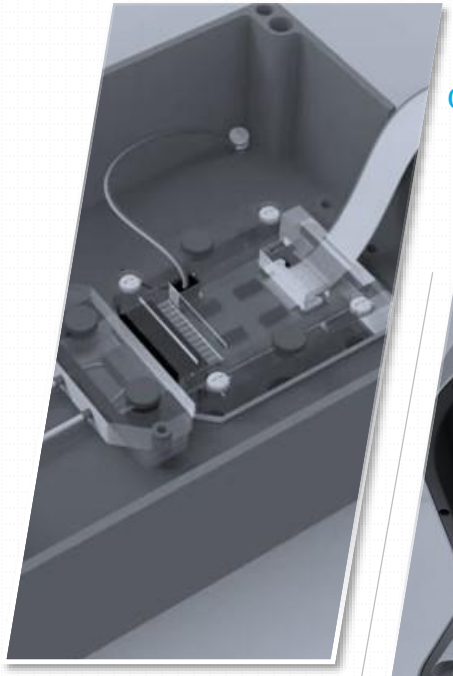
Independent intellectual property rights and localized production. The whole production process is independent and controllable. Medium-to-high throughput chips will realize simultaneous detection of thousands to tens of thousands of nanopores on a single chip

## Sequencing Algorithm

Self-developed algorithm model: high-precision + high-speed algorithm meets the different needs of accurate and efficient sequencing applications



# Road to the Research and Development of Domestically-developed Nanopore Sequencers



**2017.10**

Completion of the principle prototype Establishment of the basic platform

**2018.12**

Completion of the engineering prototype

Application of patents for the motor and flow cell

**2020.01**

Minimum viable product

Application of patents for the nanopore protein

**2020.09**

QNome-9604

The first self-developed nanopore sequencer in China

**2021.12**

QNome-3841

The first commercial nanopore sequencer in China by independent research and development

**2022.06**

QNome-3841hex

Supports up to 6 flow cells operating independently

# Nanopore strand sequencer by independent research and development

**QNome-3841**
**The first commercial nanopore sequencer in China**

**QNome-3841hex**
**A benchtop nanopore sequencer**

- Single read accuracy: **92%（V1 flow cell and kit）/ 97% （V2 flow cell and kit ）**
- Consensus accuracy (70x): **99.99%（V1 flow cell and kit ）/ 99.999% （V2 flow cell and kit ）**
- Throughput: **3Gb/flow cell**

**Long Read Length**
> 2Mb

**Fast Speed**

**Low Cost**

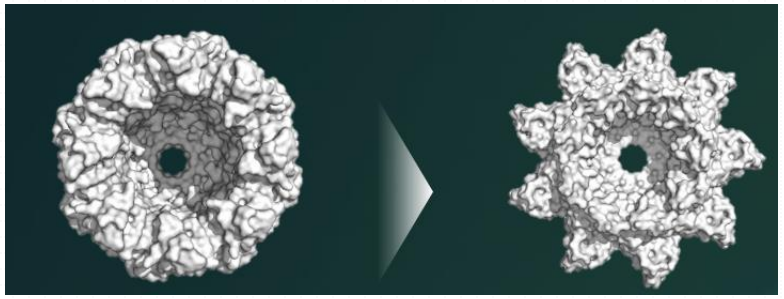
**Simple Operation**

**Direct sequencing**

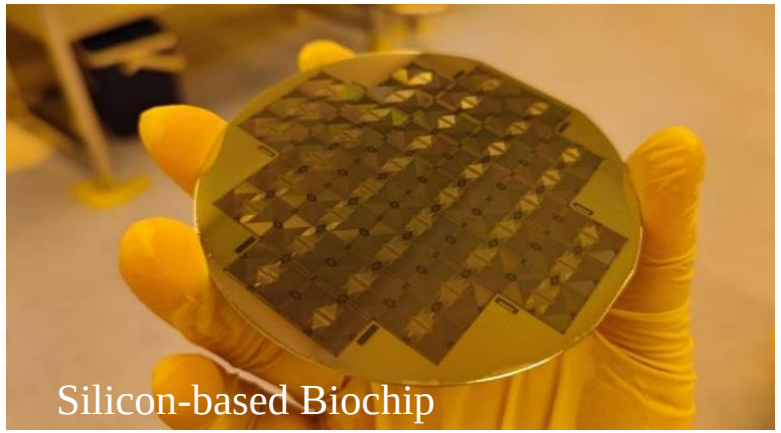
**Small & Portable**

# K2 System

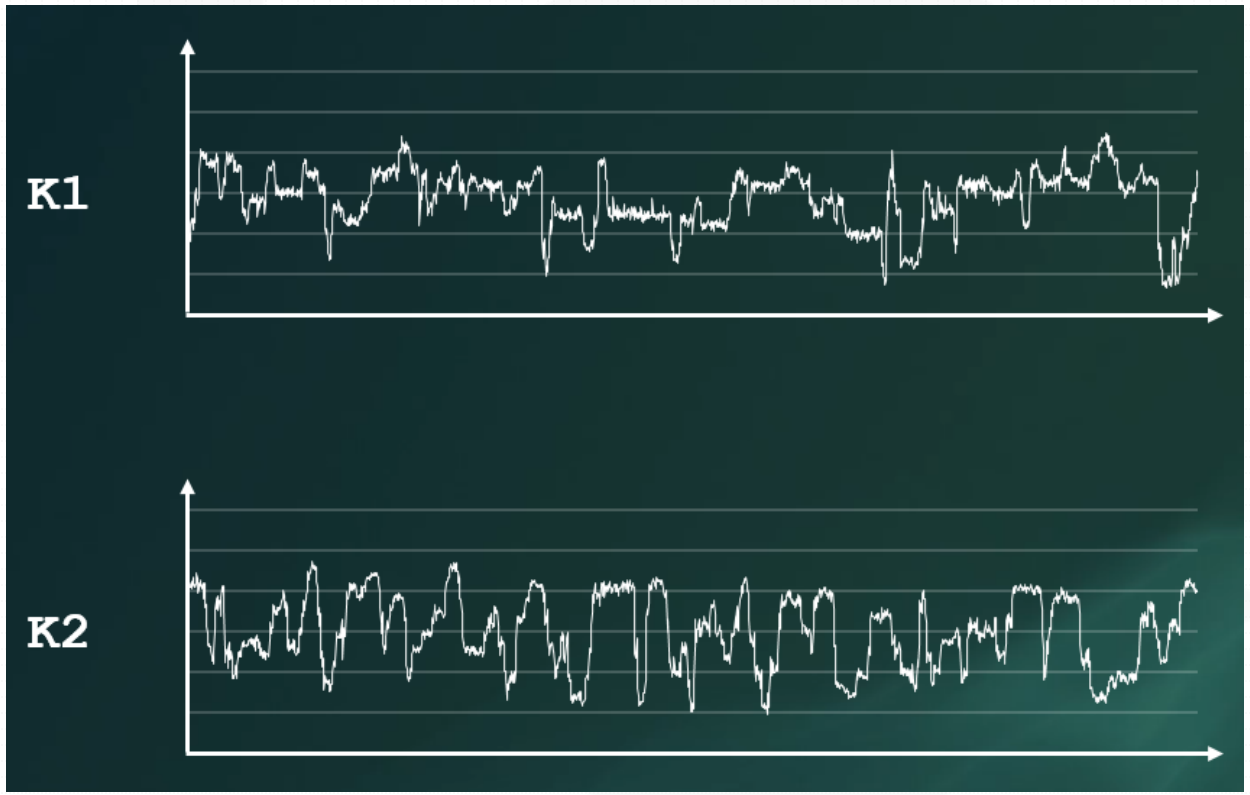
- **Nanopore Protein K2: allows for more uniform effective signal distribution, higher signal-to-noise ratio, and clearer current surges**
- **A new generation of silicon-based biochips: more stable sequencing performance and data output**



K1 Nanopore K2 Nanopore



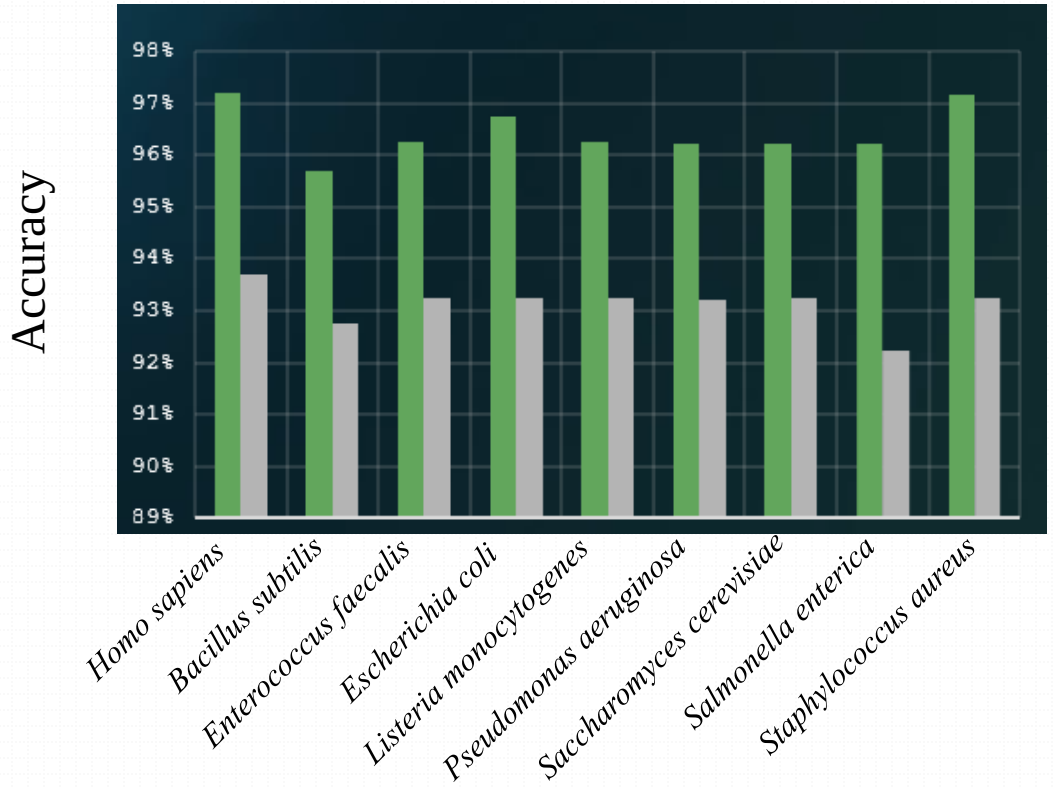
Silicon-based Biochip



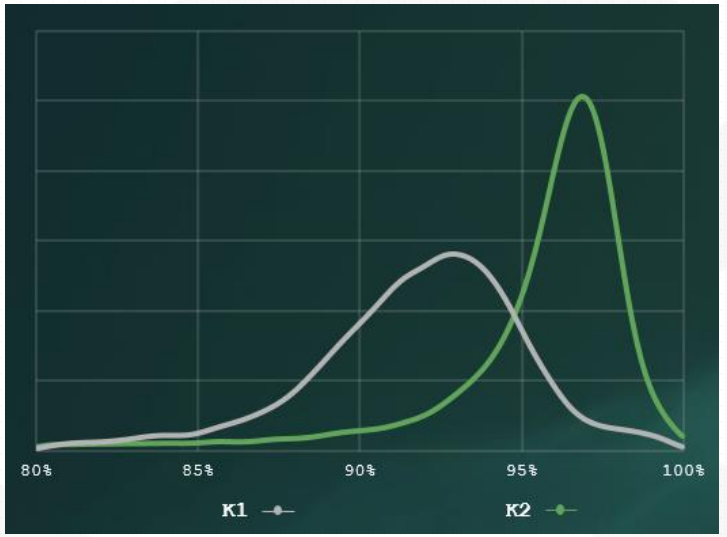
# K2 System

- **Significantly improved Homopolymer resolution**
- **The single molecule accuracy rate reached 97%, and the consensus accuracy rate (70×) reached Q50**

Single molecule accuracy




Single molecule accuracy distribution



# Complete Process of QNome Nanopore Sequencing

QPreasy

QNome-3841hex

QNome-3841

QCell-384

Ligation kit

Sequencing kit

Barcode kit

**Sample Collection**

Microbial culture, plant tissues
Animal tissue, blood, saliva
Hair, feces ····

**Nucleic Acid Extraction**

**Library Preparation**

**Sequencing**

**Real-time Data Analysis**

# Academic papers using QNome nanopore sequencing platform

**2021.11.12**


Controlled movement of ssDNA conjugated peptide through Mycobacterium smegmatis porin A (MspA) nanopore by a helicase motor for peptide sequencing application

**2021.12.27**


Forensic nanopore sequencing of microhaplotype markers using QitanTech′s QNome

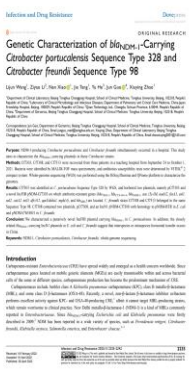
**2022.03.23**


QitanTech Nanopore Long-Read Sequencing Enables Rapid Resolution of Complete Genomes of Multi-Drug Resistant Pathogens

**2022.04.21**


Nano2NGS-Muta a framework for converting nanopore sequencing data to NGS-liked sequencing data for hotspot mutation detection

**2022.04.28**




Genetic Characterization of blaNDM-1-Carrying Citrobacter portucalensis Sequence Type 328 and Citrobacter freundii Sequence Type 98

**2022.05.20**


MAECL:A pipeline for generating consensus sequence with nanopore sequencing long-read assembly and error correction

**2022.09.15**


NanoCoV19: An analytical pipeline for rapid detection of severe acute respiratory syndrome coronavirus 2

**2022.11.24**


Distribution and spread of the mobilised RND efflux pump gene cluster tmexCD-toprJ in clinical Gram-negative bacteria: a molecular epidemiological study

**2023.01.04**


Complete Genome Sequence of a Cognatishimia activa Strain Assembled from the Phycosphere of the Marine Diatom Skeletonema tropicum

**2023.01.18**


NanoSTR: A method for detection of target short tandem repeats based on nanopore sequencing data

# Genome level surveillance of pathogenic bacteria

Recently, *Klebsiella pneumoniae* isolates harboring *tmexCD-toprJ*, which encodes a transmissible resistance-nodulation-division family efflux pump, have been widely discovered in samples from patients, animals and food in China. These isolates displayed multidrug resistance (MDR), particularly to tigecycline, one of the 'last-line' antimicrobials to defense MDR Enterobacteriaceae.

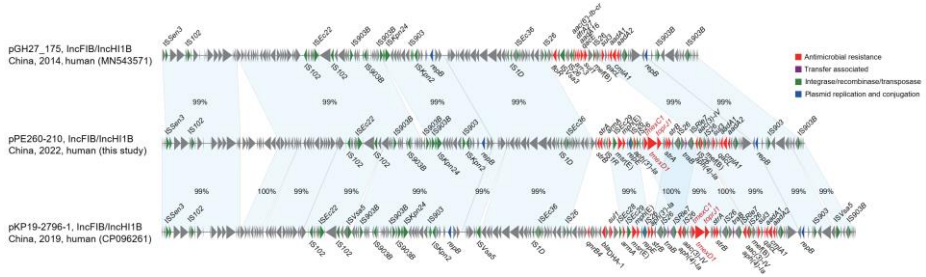
QITAN TECH has collaborated with Peking University Third Hospital and Beijing Tsinghua Changgung Hospital and identified new *tmexCD-toprJ*-positive *K. pneumoniae* subtypes using the QNome nanopore sequencing platform. The result has been published on the Lancet microbe, one of the top international journals focused on microbiology. Furthermore, the group has collaborated with Yangzhou University, to perform surveillance and research both in health care settings and food animal production industry.


## Contents

We performed comprehensive research of the newly emerging *tmexCD-toprJ*-positive *K. pneumoniae* subtypes using nanopore sequencing technology, including genome level surveillance in the hospital.

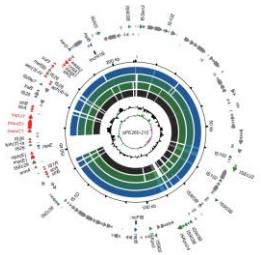
## Results

We found the first case of clonal dissemination of *tmexCD-toprJ*-positive *K. pneumoniae* between human and food animals using the QNome nanopore sequencing platform.




Annotation and alignments of *tmexCD1-toprJ1* harboring plasmid from the clinical isolate together with published plasmid sequences.


Phylogenetic relationship of the *tmexCD1-toprJ1*-positive ST726 *K. pneumoniae* isolates from the patient and food animals.



Genetic composition of *tmexCD1-toprJ1* harboring plasmid from the clinical isolate and the alignments with those from the four animal isolates.

# Metagenomic Testing for Bloodstream Infections

**Bloodstream infections are serious and life-threatening, and nanopore sequencing technology provides a faster and more accurate detection method for bloodstream infections**

## Test Content

We took the peripheral blood sample of a clinical febrile patient whose traditional blood culture test was negative. By developing an efficient method to deplete the host nucleic acid, the QNome Nanopore Sequencing Platform was used for metagenomic sequencing to detect pathogens in the sample.

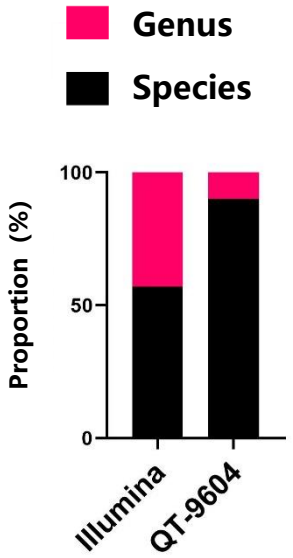
## Test Results

By using the metagenomic sequencing solution developed by Qitan Technology, the entire process comprising of host depletion, DNA extraction, and transposase-based DNA library construction can be completed within 4 hours. In the test results, the proportion of human (host) nucleic acid was reduced to 69%, and *Klebsiella aerogenes*, which accounted for 21%, were successfully detected.

**Comparison of metagenomic sequencing results (proportion of the top 5 species)**

| QNome Nanopore Sequencing Platform | | |
|---|---|---|
| Data (Mbp) | Number of Reads | Human Nucleic Acid (%) |
| 710 | 397,019 | 68.75 |
| **Species** | **Proportion（%）** | **Number of Reads** |
| *Homo sapiens* | 68.75 | 216684 |
| *Klebsiella aerogenes (G-)* | 21.42 | 67517 |
| *Klebsiella pneumoniae (G-)* | 2.84 | 8975 |
| *Corynebacterium striata (G+)* | 1.84 | 5798 |
| *Propionibacterium acnes (G+)* | 1.44 | 4553 |

| Nextseq550 (SE50) Sequencing Platform | | |
|---|---|---|
| Data (Mbp) | Number of Reads | Human Nucleic Acid (%) |
| 1231 | 24,633,277 | 62.6 |
| **Species** | **Proportion（%）** | **Number of Reads** |
| *Homo sapiens* | 62.60 | 4296785 |
| *Klebsiella aerogenes (G-)* | 10.76 | 738851 |
| *Klebsiella pneumoniae (G-)* | 3.18 | 218629 |
| *Corynebacterium striata (G+)* | 1.78 | 122623 |
| *Propionibacterium acnes (G+)* | 1.72 | 118639 |



# Metagenomic Testing for Lower Respiratory Tract Infections

**Lower respiratory tract infection is currently the main cause of death from infectious diseases in the world. Nanopore sequencing technology can provide very important etiological evidence for the diagnosis of clinical suspected infection cases**

## Test Content

We obtained the deep sputum samples of 5 patients with highly suspected lower respiratory tract infection. By developing and using an efficient method to deplete the host nucleic acid, the QNome nanopore sequencing platform was then used for metagenomic sequencing, and the sequencing results were compared with clinical culture results and next-generation sequencing results.

## Test Results

By using the metagenomic sequencing solution developed by Qitan Technology, the entire process comprising of host depletion, DNA extraction, and transposase-based DNA library construction can be completed within 4 hours. Compared with clinical culture, the detection results are highly consistent and more pathogenic bacteria can be found (qPCR verification positive); compared with next-generation sequencing, the results are consistent, but the QNome nanopore sequencing platform is easier to operate and faster.

| NO. | Clinical Culture Results | Nanopore Sequencing Results | | | Next Generation Sequencing Results | | |
|---|---|---|---|---|---|---|---|
| | Species | Data/Number of Reads/Average Length | Species | Proportion (%) | Data/Number of Reads/Average Length | Species | Proportion (%) |
| 1 | *Escherichia coli (G-)*<br>*Klebsiella pneumoniae (G-)* | 485Mbp<br>0.47Mreads<br>1577 | *Homo sapiens*<br>*Escherichia coli (G-)*<br>*Klebsiella pneumoniae (G-)* | 48.90<br>28.41<br>11.89 | 1275Mbp<br>17Mreads<br>50 | *Homo sapiens*<br>*Escherichia coli (G-)*<br>*Klebsiella pneumoniae (G-)* | 92.75<br>1.56<br>0.82 |
| 2 | *Acinetobacter baumannii (G-)* | 701Mbp<br>0.58Mreads<br>1198 | *Pseudomonas aeruginosa (G-, positive by qPCR)*<br>*Acinetobacter baumannii (G-)*<br>*Homo sapiens* | 48.00<br>6.69<br>4.64 | 1653Mbp<br>22Mreads<br>50 | *Pseudomonas aeruginosa (G-, positive by qPCR)*<br>*Homo sapiens*<br>*Acinetobacter baumannii (G-)* | 56<br>29.15<br>3.64 |
| 3 | *Klebsiella pneumoniae (G-)* | 485Mbp<br>0.47Mreads<br>900 | *Klebsiella pneumoniae (G-)*<br>*Homo sapiens* | 43.90<br>5.62 | 1658Mbp<br>22Mreads<br>50 | *Klebsiella pneumoniae (G-)*<br>*Homo sapiens* | 44.78<br>19.09 |
| 4 | *Pseudomonas aeruginosa (G-)* | 554Mbp<br>0.46Mreads<br>1026 | *Homo sapiens*<br>*Pseudomonas aeruginosa (G-)* | 37.08<br>17.34 | 1840Mbp<br>24Mreads<br>50 | *Homo sapiens*<br>*Pseudomonas aeruginosa (G-)* | 76.52<br>10.83 |
| 5 | *Klebsiella aerogenes (G-)* | 533Mbp<br>0.43Mreads<br>969 | *Klebsiella aerogenes (G-)*<br>*Homo sapiens* | 81.28<br>3.03 | 1784Mbp<br>23Mreads<br>50 | *Klebsiella aerogenes (G-)*<br>*Homo sapiens* | 86.76<br>7.81 |


Medical Field
Nanopore sequencing has long reads, and more complete and detailed pathogen genome information can be obtained by spanning complex regions that are difficult to be achieved by short-read data.
Pathogen Study
Tumor Detection
Nanopore sequencing will comprehensively and accurately detect gene defects related to the occurrence and development of tumor in cancer patients, which is conducive to identifying the etiology and developing clinical management strategies.
Nanopore sequencing is more conducive to detecting long segment structural variations and obtaining more accurate locations of breakpoint, assisting to locate pathogenic genes and develop clinical management strategies.
Genetic Diseases Detection
Public Health and Epidemic Prevention
Nanopore sequencers can output sequencing results in real time and long reads sequencing is more conducive to obtaining complete and accurate pathogen detection results, which may go to the front line in major public health emergencies.
Reproductive Health, Basic Medicine Study, Pharmacogenomics, Research and Development of New Drugs
.....
Biodiversity Protection, Agricultural Breeding, Species Identification
.....
Environmental Monitoring and Protection
Nanopore sequencers is compact, portable, easy to operate and has long reads, and can be more conducive to go into different conditions on site and conduct real-time sequencing and identification of different biological samples, and facilitate the assessment of environment quality.
Disease Control of Animals and Plants
Nanopore sequencing is fast and has long reads, which can rapidly and accurately obtain pathogen genome information and facilitate timely adoption of appropriate and necessary prevention and control actions to prevent extensive economic losses caused by pathogen transmission.
Judicial Identification
Nanopore sequencing has long reads and flexible throughput, which may be simultaneously used to detect a variety of genetic markers commonly in forensic fields. The device is compact and easy to operate, which is more conducive to installation and use in primary laboratories.
Non-medical Field

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# Thank you!

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