Nanopore sequencing

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Introduction to Nanopore sequencing

It's a real-time DNA and RNA sequencing method developed by Oxford Nanopore Technologies.

Key features :

- Portable, real-time, and able to process ulta-long reads
- Suitable for analyzing complex environmental samples

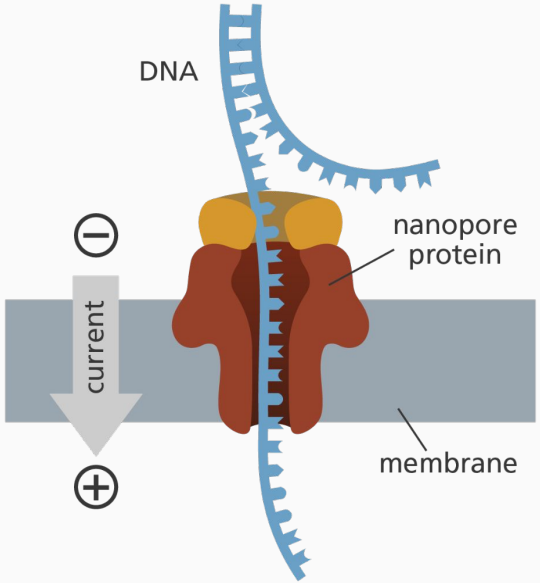
Importance in environmental microbiology :

- Identifies microbial diversity in ecosystems
- Detects low-abundance organisms and uncultivable microbes

Scientific principle

Nanopore structure :

- Proteins form pores embedded in a membrane
- A voltage gradient drives DNA/RNA strands through the pore



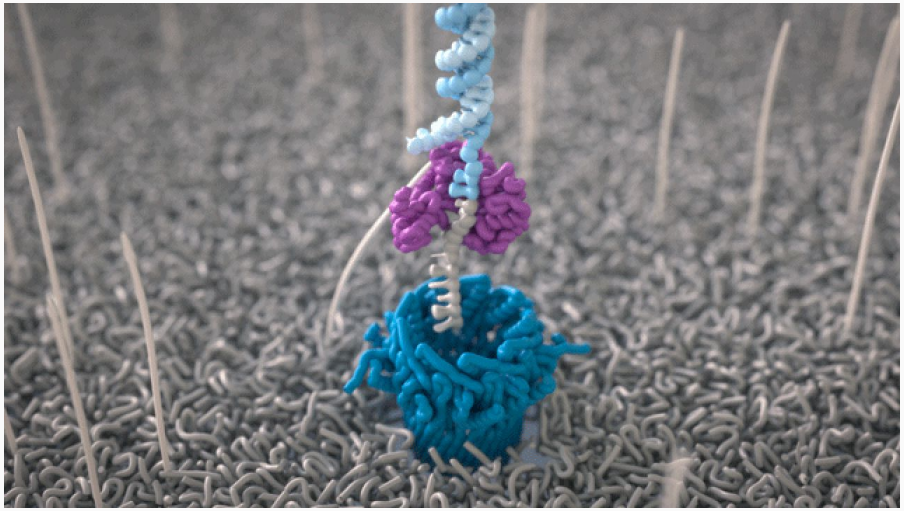
Zoom into the nanopore

Purple : helicase, or motor protein, unzipped the double strand DNA

Light blue : Double strand DNA

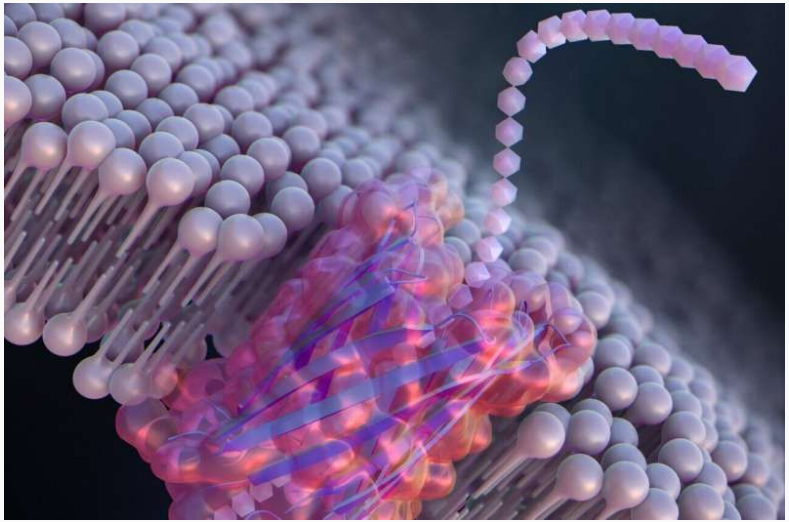
Blue : pore protein

Grey : Polymer membrane



Zoom on the membrane

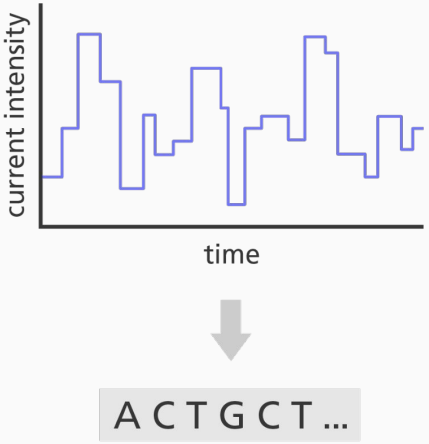
- Either biological (lipid bilayer) or solid state (ex : graphene)
- Electrically insulating
- Surrounded by electrolyte solution
- Nanopore : protein that self assembles (or is inserted) into the membrane
- Most common is α-hemolysin protein



Scientific principle

Signal measurement :

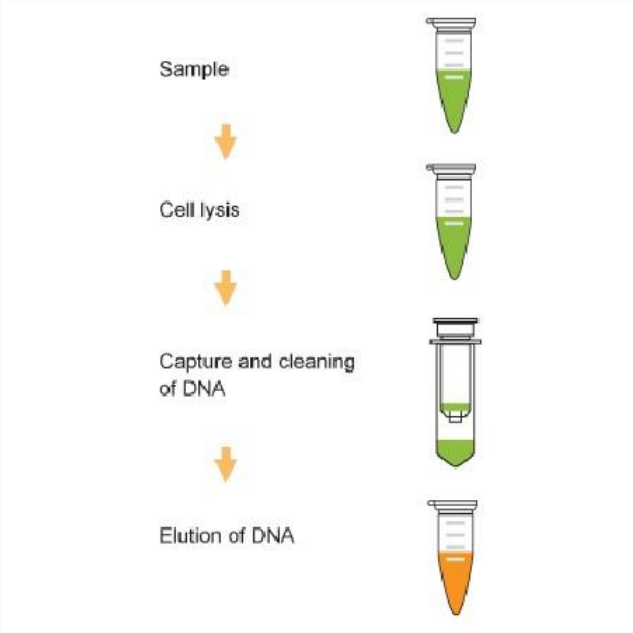
- Differents nucleotides cause unique disruption in the electric current
- These disruptions are decoded into sequences



How does Nanopore sequencing work ?

Step 1 - Sample preparation :

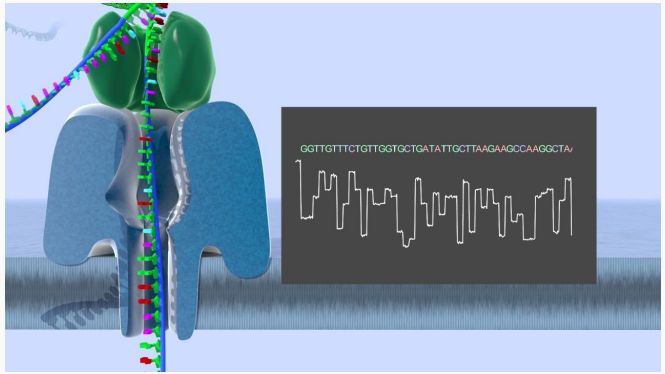
- Extract DNA/RNA from the samples
- Add adapters for nanopore compatibility



How does Nanopore sequencing work ?

Step 2 - Sequencing :

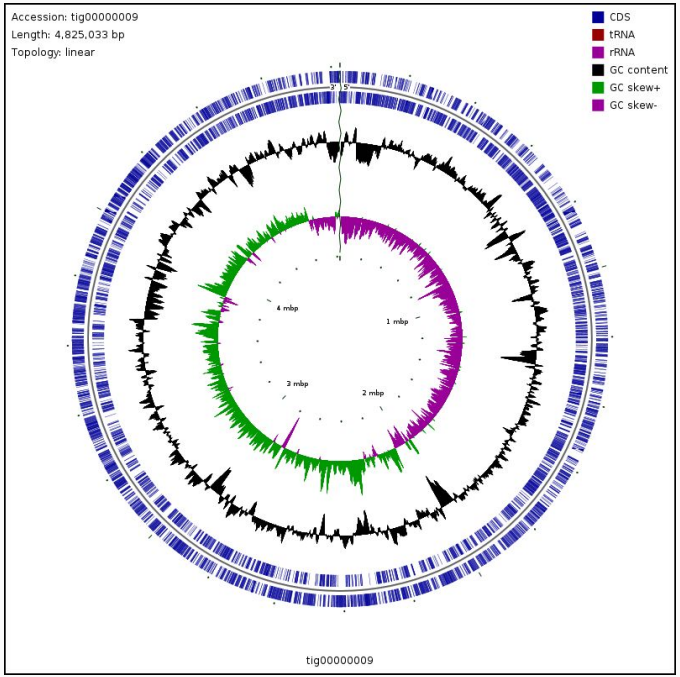
- 💻 DNA/RNA strand passes through a nanopore
- 💻 Electrical signals are measured and converted into nucleotides sequences



How does Nanopore sequencing work ?

Step 3 - Data analysis :

- 🔍 Translate signals into sequence using base-calling software
- 🔍 Perform bioinformatics analyses for organism identification and functional annotation



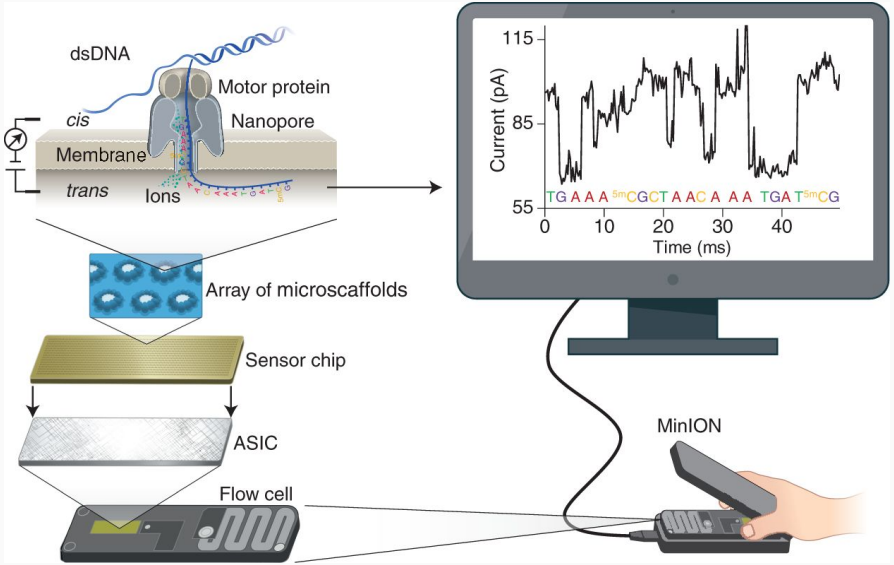
Main differences with traditional (Sanger) sequencing

	Sanger Sequencing	Nanopore sequencing
Speed	Batch (PCR needed)	Real TIme (no PCR needed)
Length	Short to intermediate reads	Very long reads possible
Accuracy	Very high (99.9%)	High (98%)
Detection method	Light detection (color)	Electric conductivity
Cost	High	Medium

Zoom into MinION fonctionnement

Flow cell design :

- 512 channels with 4 nanopores per channel = 2'048 nanopores
- Polymer membrane supported by microscaffold arrays connected to a sensor chip



Example of a scientific paper that uses Nanopore sequencing

Application in microbial ecology

Objectives : Explore microbial community structure and metabolic functions in high-altitude glacier meltwaters of Qilian Mountain, China

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SHORT RESEARCH AND DISCUSSION ARTICLE

In situ Nanopore sequencing reveals metabolic characteristics of the Qilian glacier meltwater microbiome

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Abstract
Nanopore metagenomic sequencing enables rapid annotating microbiological ecosystems, and the previous glacier-related sequencing applications (e.g., targeted ice sheets, ice lake, and cryoconite holes) inspire us to explore high-altitude glacier meltwater at Qilian Mountain, China (3000 to 4000 m above sea level, MASL). Our findings suggest that (1) despite only several hundred meters apart, the microbial communities and functionalities are quite different among vertical alpine distributions; (2) the high-altitude Qilian meltwater microbiome serve several main metabolic functions, including sulfur oxidation, selenite decomposing, photosynthesis, energy production, enzymic, and UV tolerant activities. Meanwhile, our Nanopore metagenomic results indicate that the microbial classifications and functionalities (e.g., chaperones, cold-shock, specific tRNA species, oxidative stress, and resistance to toxic compounds) of Qilian meltwater are highly consistent with the other glacial microbiome, emphasizing that only certain microbial species can survive in the cold environment and the molecular adaptions and lifestyles remain stable all over the world. Besides, we have shown Nanopore metagenomic sequencing can provide reliable prokaryotic classifications within or among studies, which therefore can encourage more applications in the field given faster turnaround time. However, we recommend accumulating at least 400 ng nucleic acids (after extraction) and maximizing Nanopore library preparation efficiency before on-site sequencing to obtain better resolutions.

Keywords Nanopore sequencing · In-field sequenced · High-altitude glacier · Meltwater

Introduction

Globally, 25% of the land surface is defined as the cold environment, including glaciers, sub-glaciers, and ice lakes, etc. In China, Qilian glacier is a typical cold environment, and it is distributed throughout the entire Qilian Mt.,

The authors Xiang Li and Miao Zhang contribute equally to this work.

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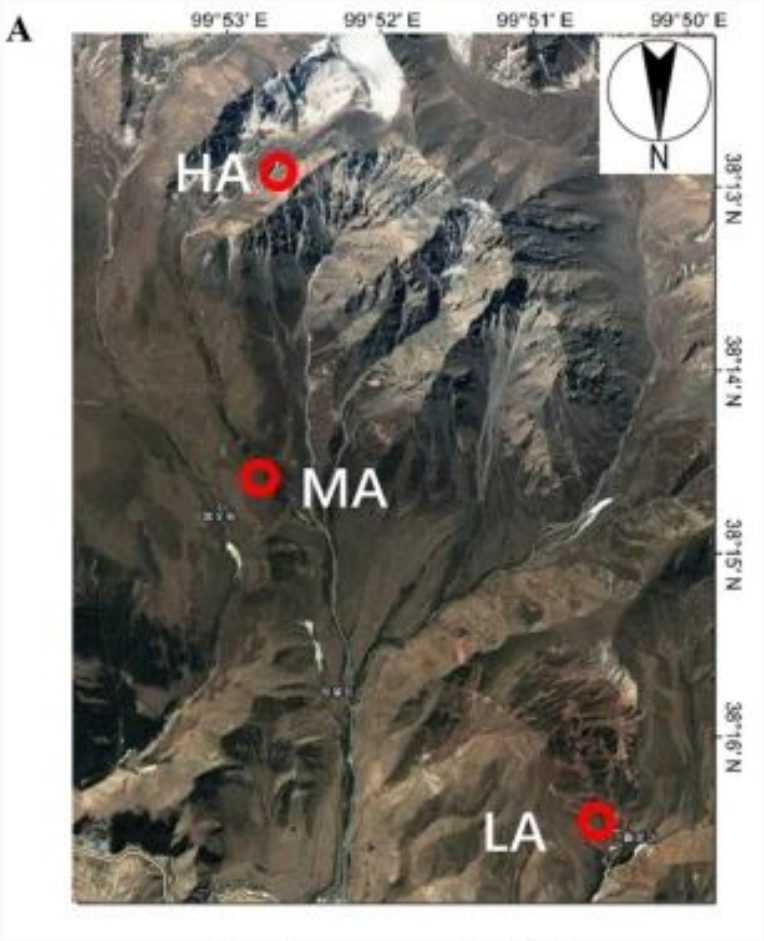
Methods

- 3 sites
  - HA : 4000m
  - MA : 3600m
  - LA : 3000m
- Glacial meltwater filtered and collected
- Mini PCR
- MinION sequencing
- Metagenomic analysis with Kaiju and eggNOG

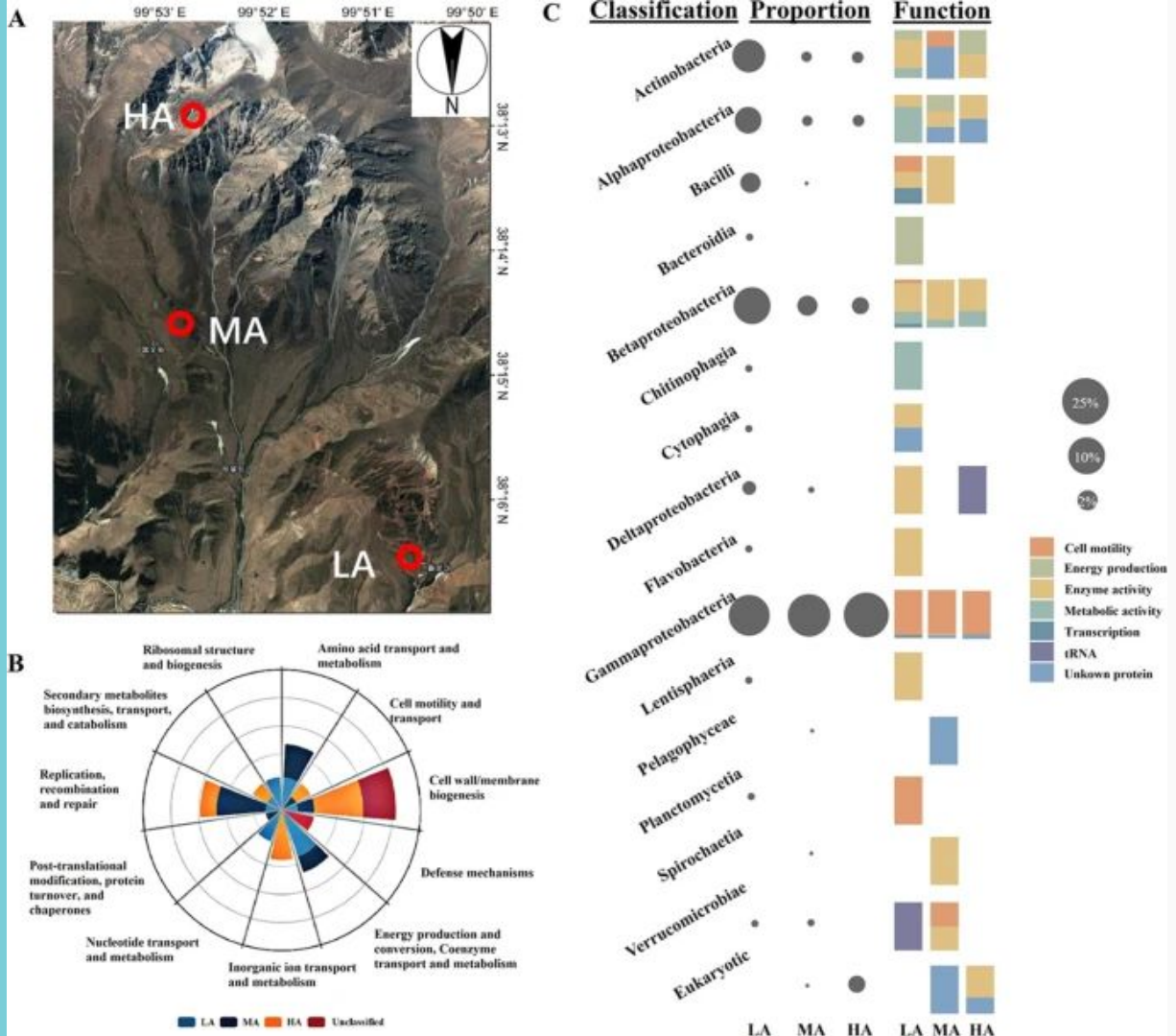


Results :

High differences in microbial communities at different altitudes

Metabolic functions :

- motility (critical trait for cold-adapted bacteria)
- sulfur oxidation detected



Take home messages

- Nanopore sequencing is portable, real-time and can process long-reads
- Can be used in very remote environment

Limitations :

- Accuracy lower than sanger sequencing
- Does not entirely replace the usual sequencing method