

# Cultivation strategies for prokaryotes from extreme Environments

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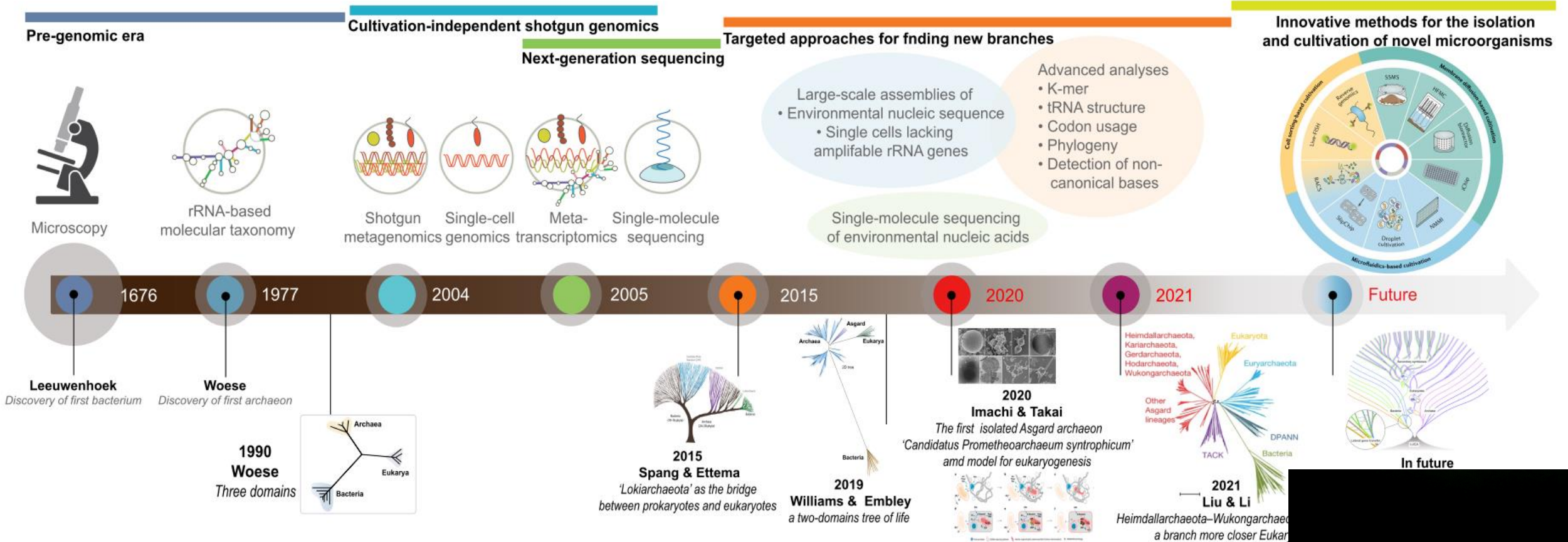
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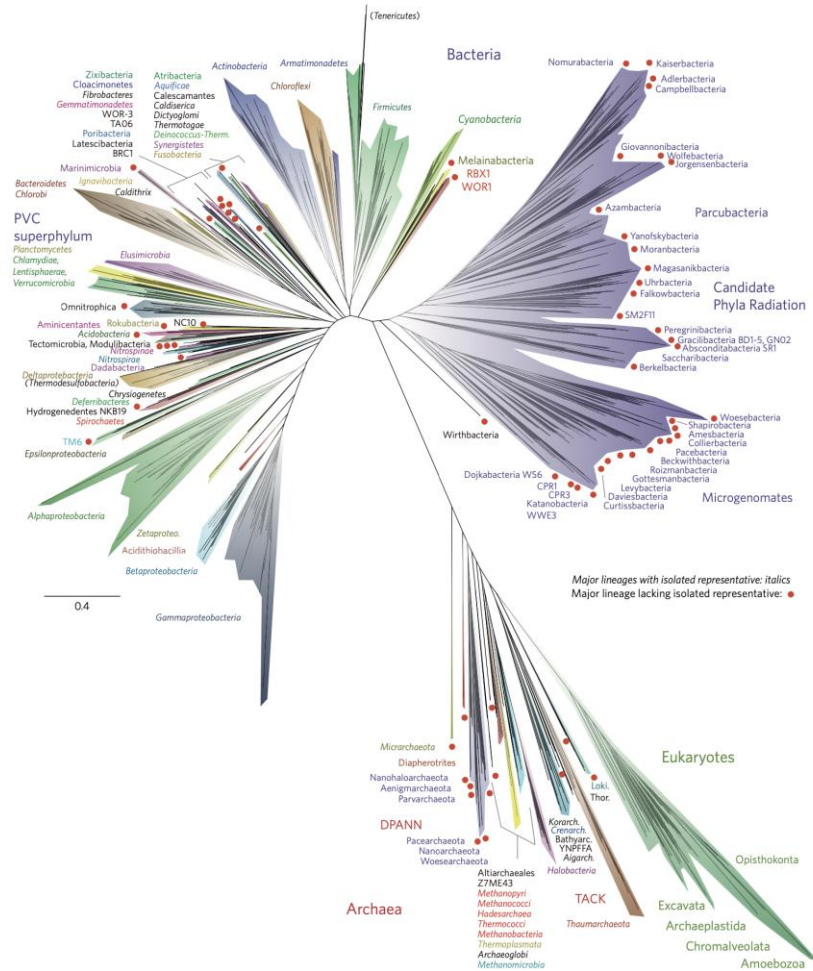
# Introduction

- Microorganisms form the widest distribution, the most abundant and the largest biomass on earth



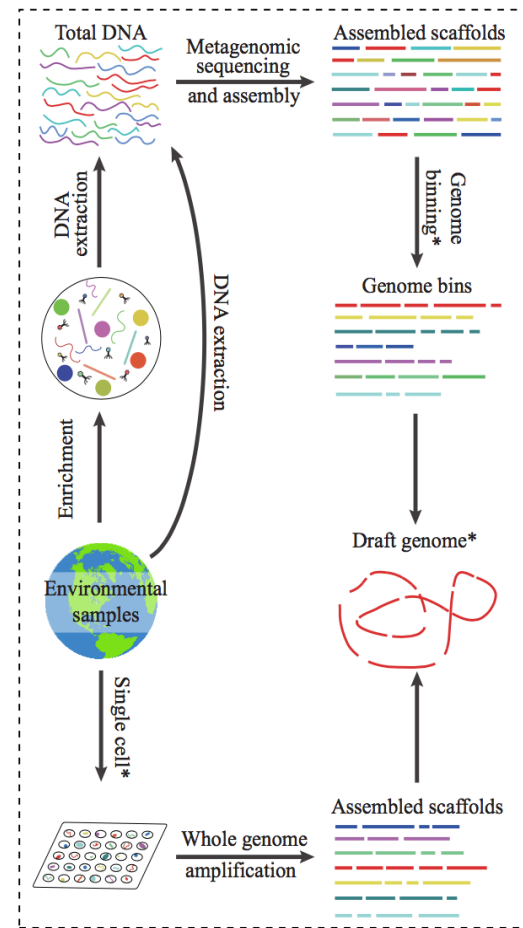
# Introduction

➤ Mining "microbial dark matter", acquiring pure cultures of uncultured microorganisms is both an opportunity and a challenge under the new situation

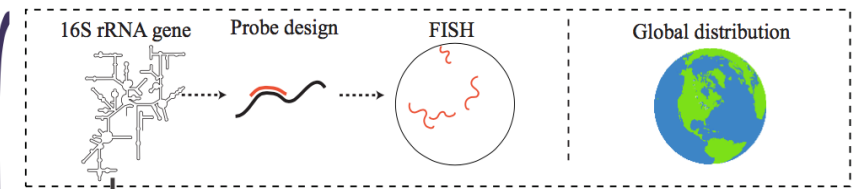


(Hug *et al.*, Nature Microbiology, 2016)

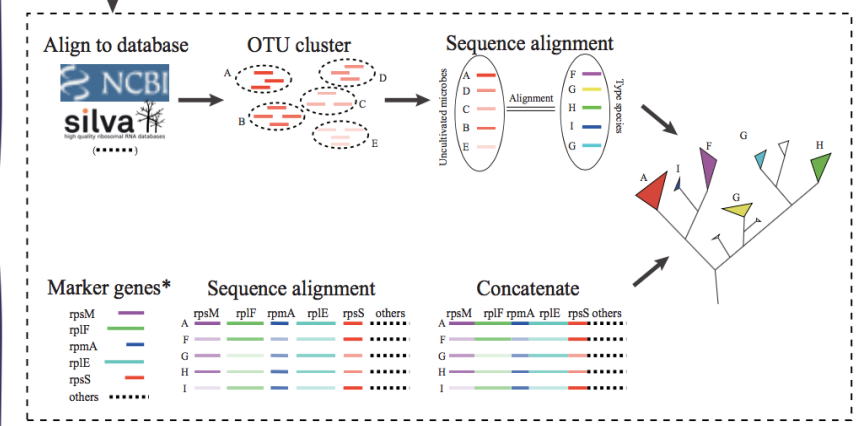
Recovery of genomes from environmental samples



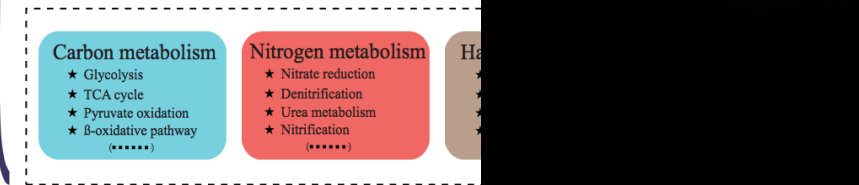
Where are they?



Who are they?



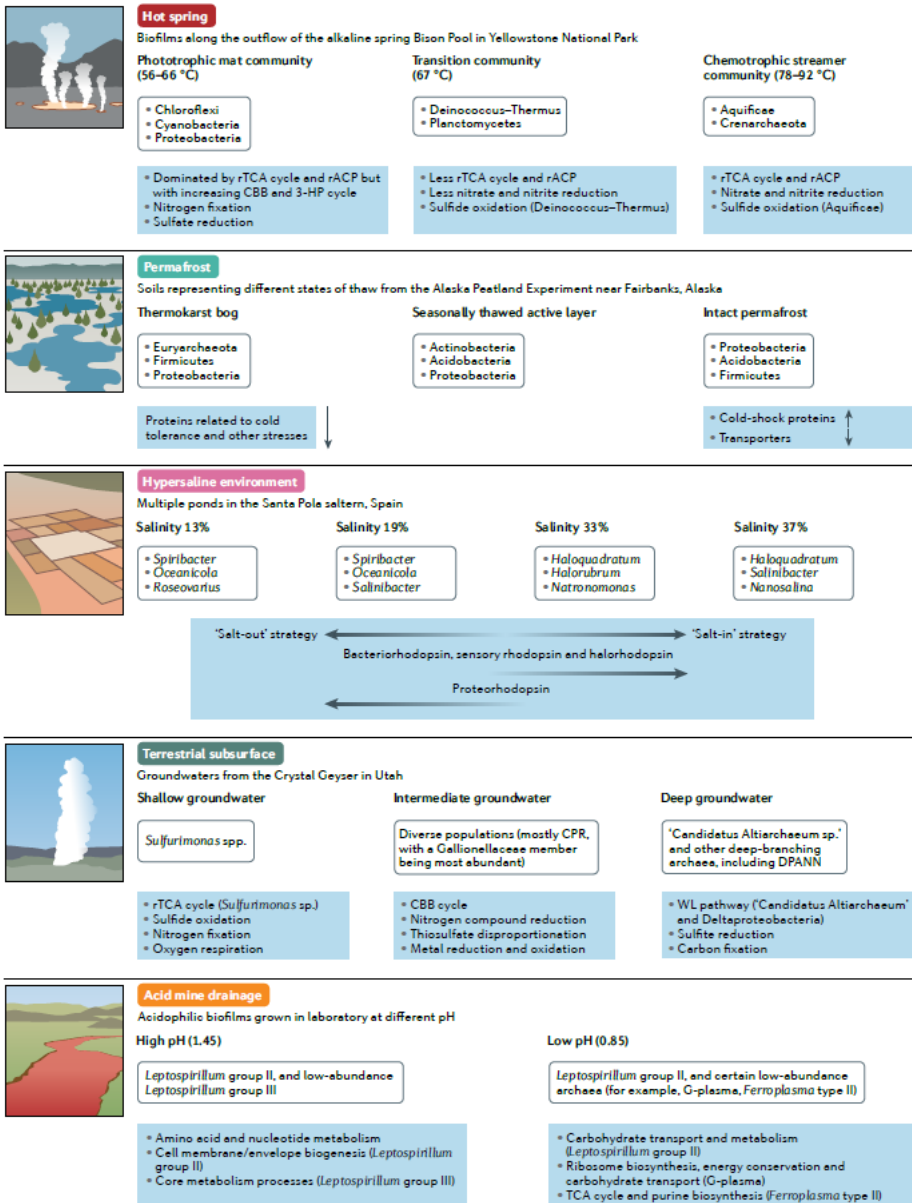
What they can do?\*



(Jiao *et al.*, 2021, Nation

Design targeted cultivation approach

# Introduction



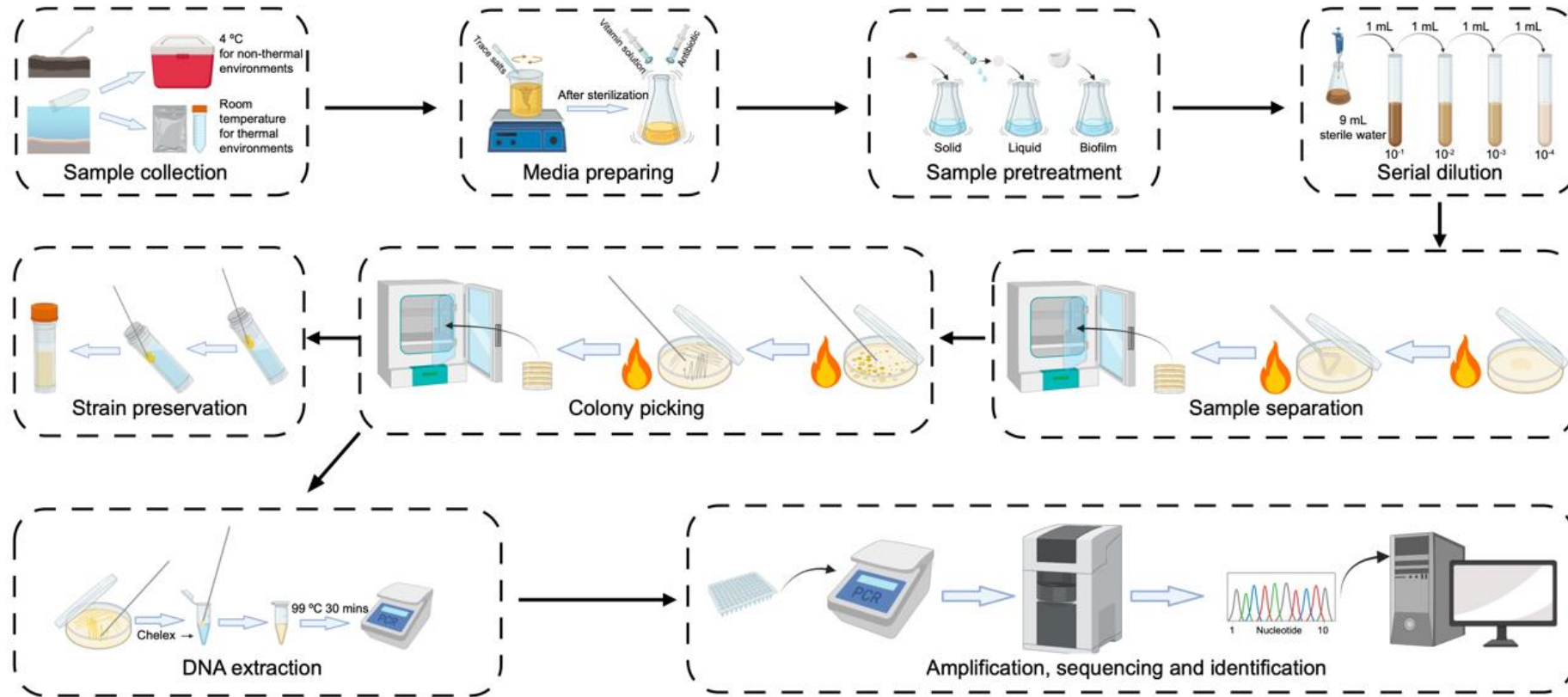
➤ Extreme environments are significant geological forms on Earth, which harbor unexpectedly diverse extremophiles from all three domains of life;

➤ Microorganisms from extreme environments are more difficult to isolate, due to their specific adaptations and metabolisms;

➤ Extremophiles can not only provide unique products but are also ideal targets for the study of microbial ecology, evolution, and environmental adaptation;

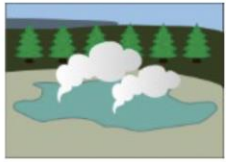
➤ Extremophiles are also regarded as optimal models for the origin and evolution of life and even the potential

# Common processes for extremophile isolation



The common processes for the isolation of extremophiles can be divided into four parts and nine steps

# Specific strategies for different extremophiles isolation



Hot spring



Hydrothermal vent

## Thermal environments

- Gelrite replace agar
- Keep Petri dish moist when incubation
- Isolation from fresh sample
- Sodium thiosulfate addition
- Both aerobic and anaerobic strains isolation

Extreme environments have distinctive limiting factors, so diverse isolation strategies and matters need attention when exploring different environments:

➤ Isolating microorganisms from thermal environments mainly requires to maintain the media state;

➤ The sample pretreatment strategies for xeric environment samples need to be more drastic;

➤ Different kinds of salts need to be added to the isolation media according to the physicochemical properties of the samples when isolating microorganisms in saline and alkaline environments;

➤ Sample enrichment and suitable medium coagulants are important for acidic environment isolation;

➤ The isolation temperature and physicochemical differences of different samples need to be attent



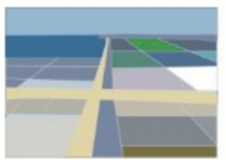
Desert



Yardang landform

## Xeric environments

- Short-time ultrasonication pretreatment
- Appropriate UV-light irradiation pretreatment
- Right amount salt addition
- Suitable antibiotic addition



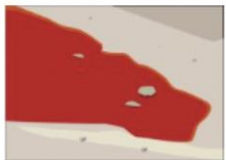
Saltern



Salinealkali land

## Saline and alkaline environments

- Drying treatment before isolation
- Salts addition based on physicochemical properties
- Chloramphenicol for archaea isolation
- Salt addition when isolation and preservation



Acid mine drainage



Acidic water

## Acidic environments

- Biomass enriched by filter membrane
- Nutrient-rich medium utilization
- Overlay technique utilization
- Sterilized media and coagulant separately
- Gelrite replace agar



Permafrost

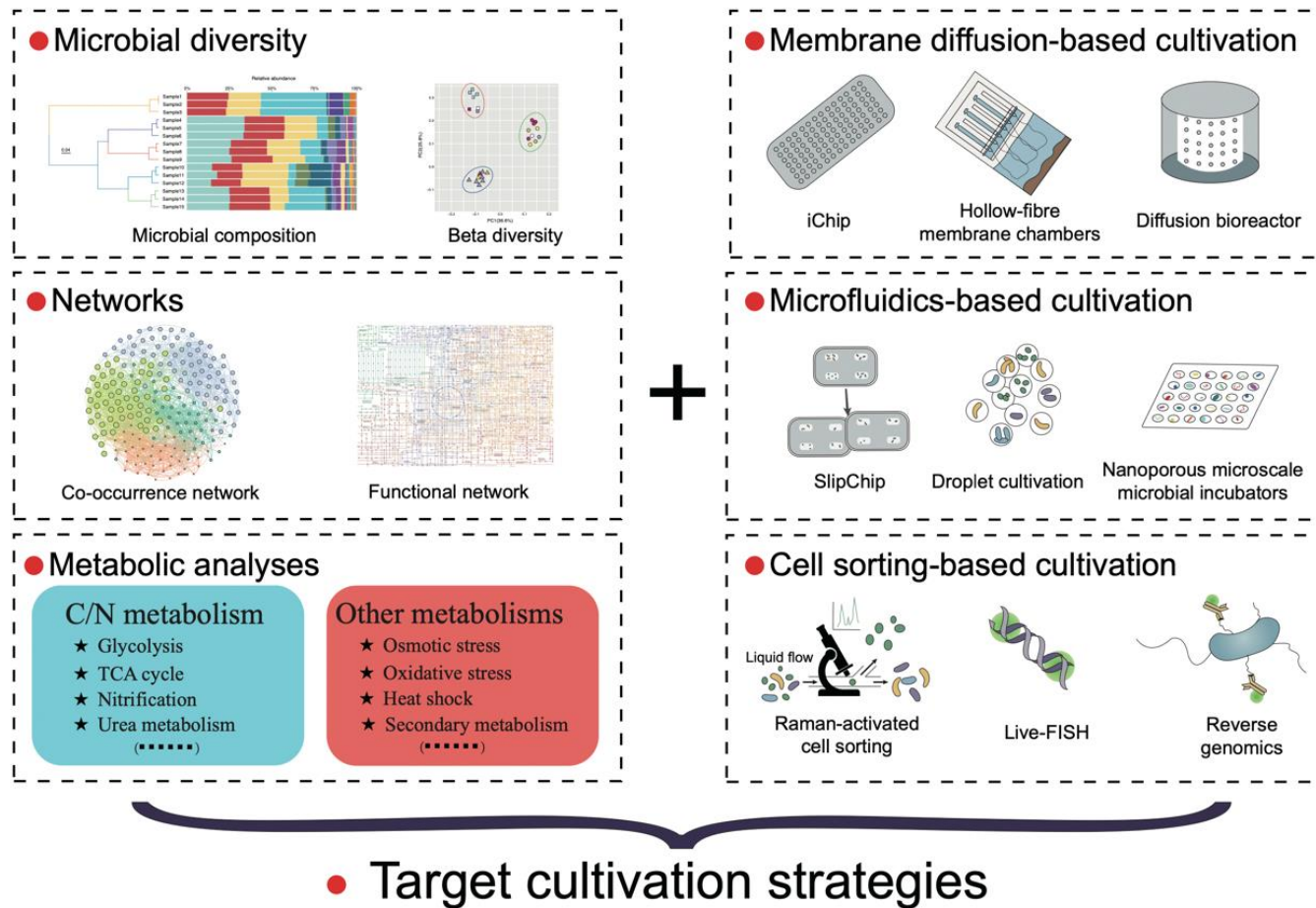


Glacier

## Cryogenic environments

- Biomass enriched by filter membrane
- Medium dilution
- Moderate temperature isolation
- Extension incubation time when isolation
- Right amount salt addition

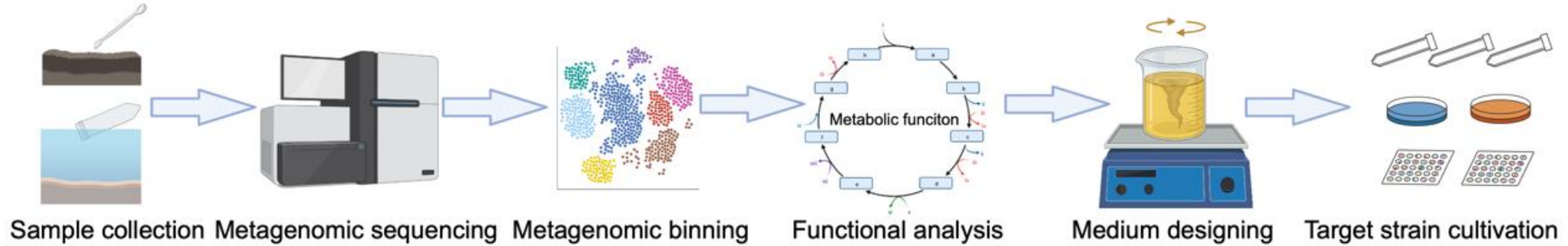
# Multi-omics-guided microbial cultivation



Information from omics can be combined with that from other and innovative cultivation methods to provide the framework necessary for rationally designing targeted cultivation strategies

# Multi-omics-guided microbial cultivation

## A. Function guiding microorganisms cultivation



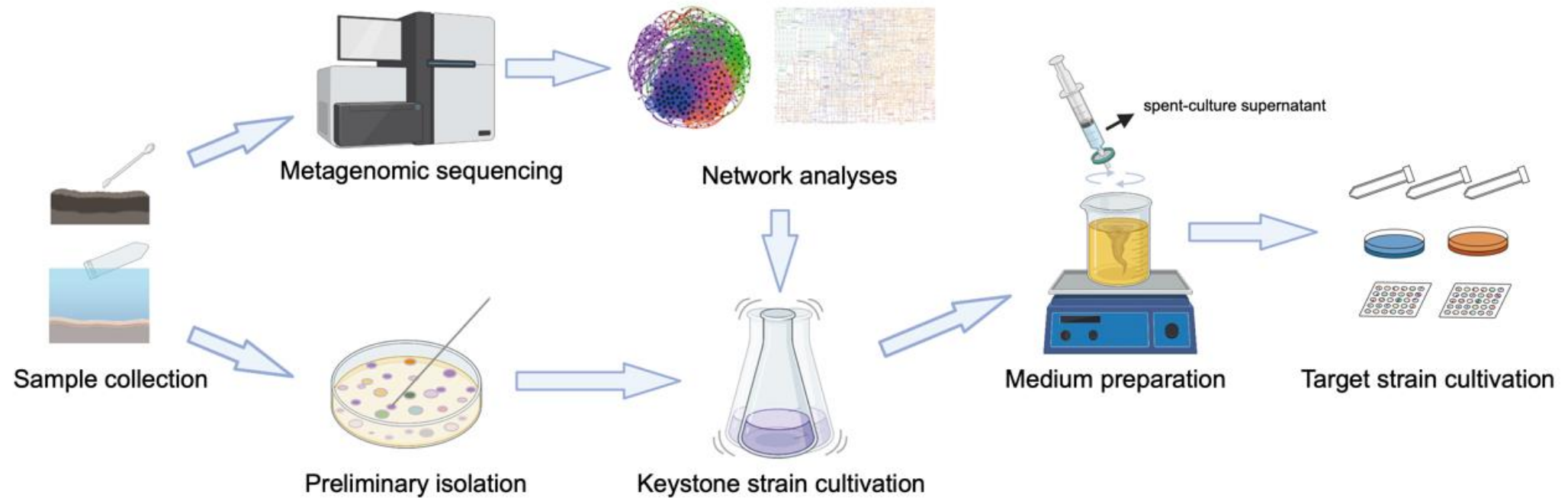
Through analyzing metagenomic data, the *Ca. Geothermincolia* were found to be putative homoacetogens

$N_2$ ,  $H_2$ , and  $CO_2$  were added to the inorganic medium to enrich this group

After 120-days enrichment, the proportion of the target *Actinomycetota* had increased drastically

# Multi-omics-guided microbial cultivation

## B. Network guiding microorganisms cultivation



the genus *Tepidimonas* had a high-degree centrality, the genus *Chloroflexus* formed the peripheral nodes

R2A containing 10% spent-culture supernatant from *Tepidimonas* sp. to target isolation of the dominant *Chloroflexota*

36 potential novel species and one novel class of *Chloroflexota* were isolated

# Summary

- A straightforward protocol for efficiently isolating microorganisms from different extreme habitats are provided;
- Specific strategies and notes for different extremophiles isolation are provided;
- Propose the use of multi-omics-guided microbial cultivation approaches for culturing these as-yet-uncultivated microorganisms and provided two cases to introduce how it works

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