Introduction to Metagenomics

Hadrien Gourlé – SLU Global Bioinformatics Centre, Sweden
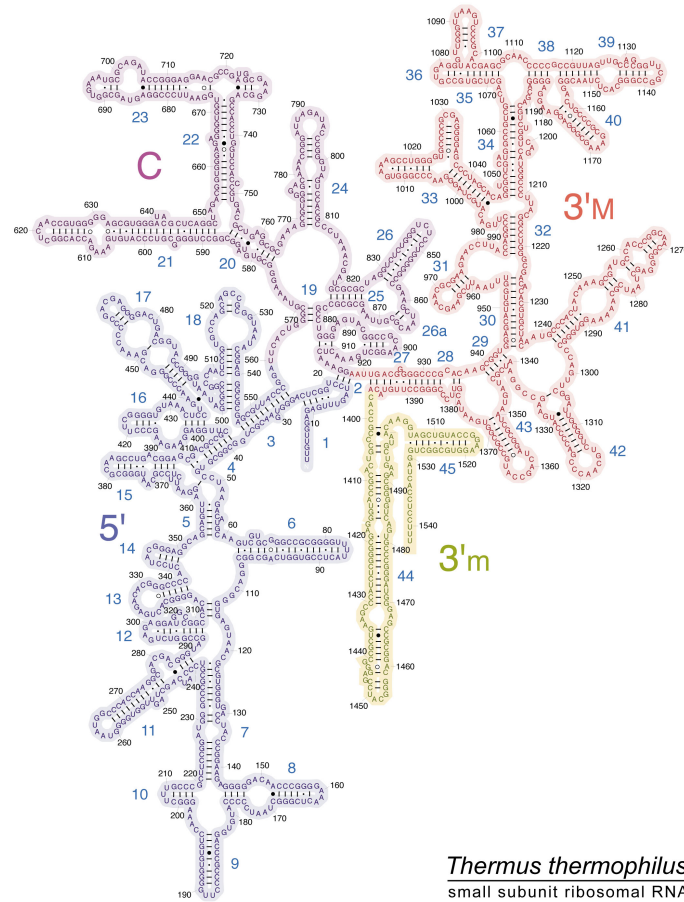
Table of Contents

- 16s Metabarcoding
  - The 16s rRNA
  - Workflow
- Whole Metagenome Sequencing (WMS)
  - General principle

16s Metabarcoding
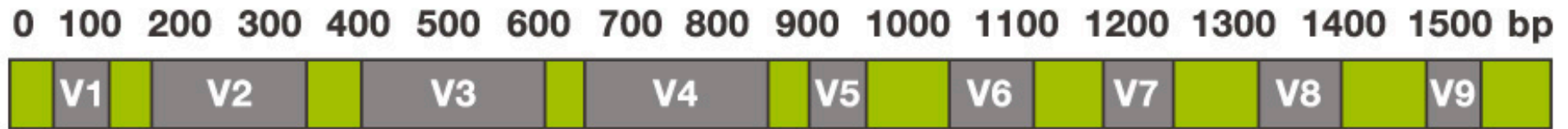
What is the 16s rRNA?

Thermus thermophilus
small subunit ribosomal RNA

What is the 16s rRNA?

- 16s rRNA gene codes for the SSU of the prokaryotic ribosome

- Ribosomes (and DNA that codes for them) have been mostly conserved over time

- Relatively short (1.5Kb), easy, fast and cheap to sequence (relative to other genes)

What is the 16s rRNA?

0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300 1400 1500 bp

| V1 | V2 | V3 | V4 | V5 | V6 | V7 | V8 | V9 |

CONSERVED REGIONS: unspecific applications

VARIABLE REGIONS: group or species-specific applications
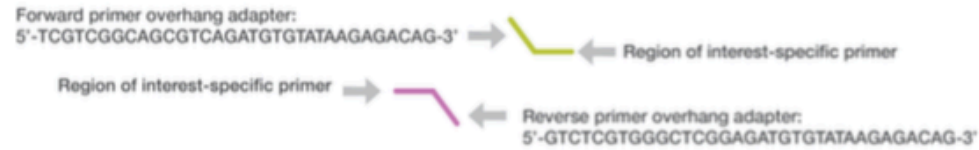
What is the 16s rRNA?

- 16s rRNA gene codes for the SSU of the prokaryotic ribosome

- Ribosomes (and DNA that codes for them) have been mostly conserved over time

- Relatively short (1.5Kb), easy, fast and cheap to sequence (relative to other genes)
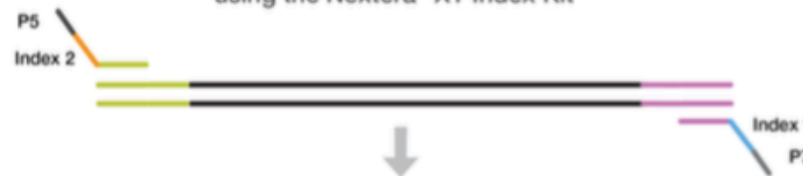
16s Metabarcoding workflow

- Sample collection / DNA extraction

- Library preparation

- Sequencing

- Downstream Analysis

Workflow: library preparation

PCR amplify template out of genomic DNA using region of interest-specific primers with overhang adapters

Forward primer overhang adapter:
5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3' → Region of interest-specific primer

Region of interest-specific primer → Reverse primer overhang adapter:
5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3'

Attach indices and Illumina sequencing adapters using the Nextera® XT Index Kit

P5
Index 2

Index 1
P7

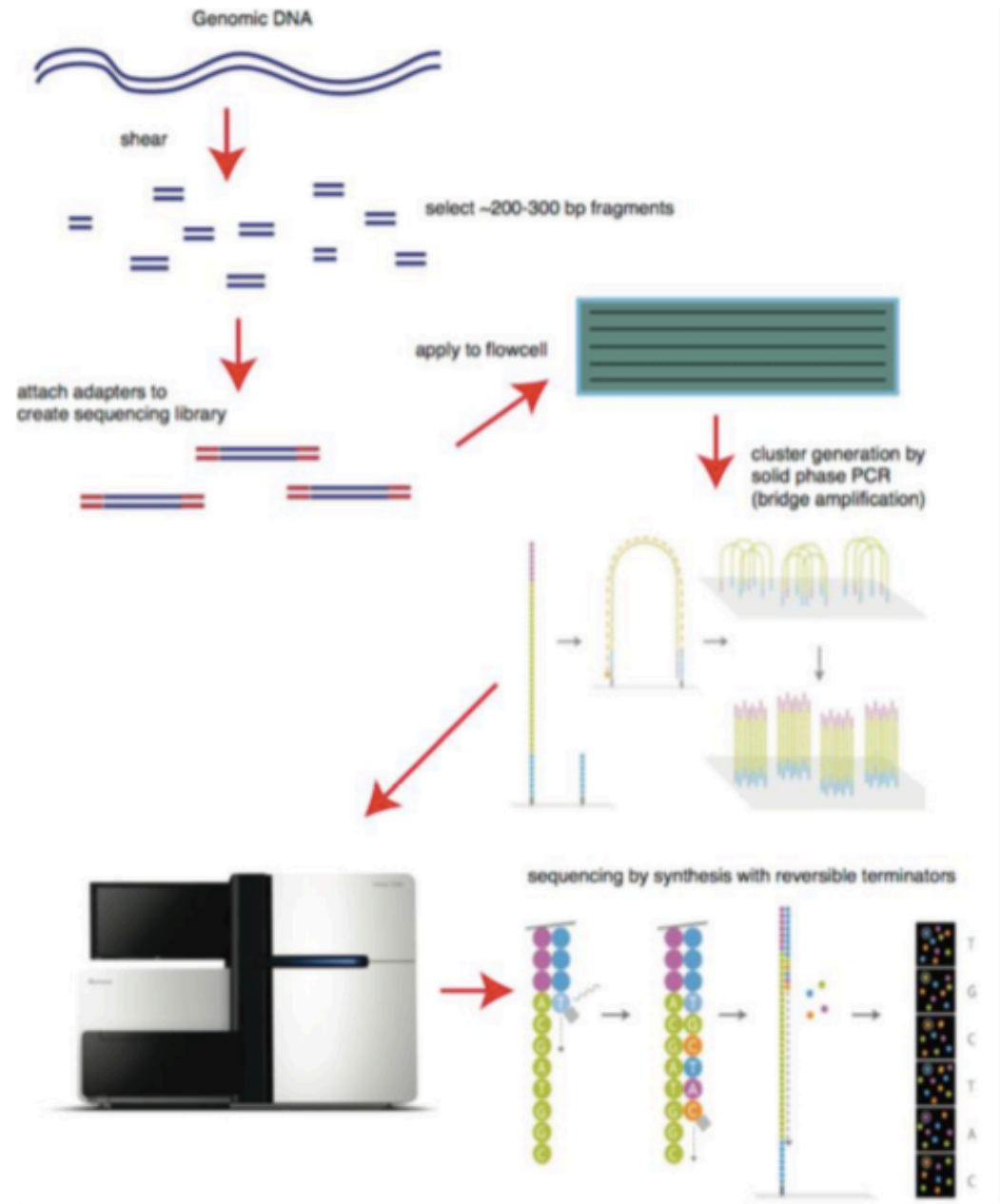Normalize and pool libraries

Sequence

Workflow: sequencing

Sequencing by synthesis

Reversible dye terminator chemis
Solid phase PCR to create cluster
of copies of one DNA fragment on
surface in a flow cell

Addition of reversible dye termina
bases Read base - then cleave of
terminator + dye

Currently most popular method

Workflow: Downstream analysis

- https://github.com/HadrienG/tutorials

- Select "16s Metabarcoding Analysis" and follow the tutorial!

Whole Metagenome Sequencing

General principle of WMS

- Relatively new and powerful technique

- Sequences all the genomic material present in the environment

- Increases the resolution, and allows the discovery of archaea and viruses

- BUT is more expensive than 16s, produces a lot more data