Module 3:
Sample collection, extraction and library preparation for 16S NGS analyses
Learning Outcomes

Describing different aspects of planning for 16S experiments; for example study design, DNA extraction methods and laboratory workflows.

• Understand what is meant by the term “16S rRNA gene” and why we are interested in this gene.

• Understand the concepts behind Sanger sequencing and high-throughput sequencing

• Know why it is important to clearly plan an experiment and how different components to the study and experimental design may influence data generated and downstream analyses.

• Know that techniques other than 16S rRNA sequencing are also available to study microbial profiles.
Module 3:
Sample collection, extraction and library prep for 16S NGS analyses

Part 3.1
16S rRNA high throughput sequencing: how it works
16S rRNA high throughput sequencing: how it works

**Advantages:** Study viable organisms

**Disadvantages:** Thought that less than 1% of all bacterial species are cultivatable
16S rRNA high throughput sequencing: how it works

Culture-independent molecular methods:

Marker gene-dependent

→

Fingerprinting techniques

Sanger sequencing

High-throughput sequencing

→

16S rRNA gene

Marker gene-independent

→

High-throughput sequencing (shotgun sequencing)

All organisms need ribosomes to make protein

Any gene that makes up ribosome may be a good marker gene

Ribosomal RNA (rRNA) never gets transcribed to protein

Gene focussed on: **16SrRNA gene** (~1500bp)

https://www.youtube.com/watch?v=8Aa_mnyXm70

**16SrRNA Intermediate Bioinformatics Online Course:**

Shantelle Claassen-Weitz
Some parts of the gene are structurally very important for the ribosome to work = **conserved regions**

Some parts of the gene have *mutations* but the ribosome still works = **variable regions**

The entire gene is too long to sequence without errors for most DNA sequencers.

Find a portions with enough variability to distinguish species

https://www.youtube.com/watch?v=8Aa_mnyXm70
16S rRNA high throughput sequencing: how it works

16S ribosomal RNA gene

V1  V2  V3  V4  V5  V6  V7  V8  V9

DNA amplicons

16SrRNA Intermediate Bioinformatics Online Course:
Int_BT_2019
Shantelle Claassen-Weitz
Sanger method only sequences a single DNA fragment at a time
16S rRNA high throughput sequencing: how it works

Sanger method sequences a single DNA fragment at a time
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- ddCTP
- ddTTP
- ddGTP
- ddATP
16S rRNA high throughput sequencing: how it works

Sanger method sequences a single DNA fragment at a time

- ddCTP
- ddTTP
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- ddATP
16S rRNA high throughput sequencing: how it works

Sanger method sequences a single DNA fragment at a time

ddCTP  ddTTP  ddGTP  ddATP

A  G  G  T  C  T  C
16S rRNA high throughput sequencing: how it works
DNA from a number of samples

Undergo amplification using a multiplex approach
16S rRNA high throughput sequencing: how it works
16S rRNA high throughput sequencing: how it works

In summary:

• 16S rRNA gene is by far the most common housekeeping gene targeted to study bacterial phylogeny and classification.

• High throughput sequencing targeting the 16S rRNA gene allows for large quantities of DNA to be sequenced much more quickly and cheaply compared to Sanger sequencing.

• High throughput sequencing of the 16S rRNA gene allows for identification as well as relative abundance quantification of all bacteria present in a sample.

• 16S rRNA high-throughput sequencing allows for processing multiple samples together via the use of barcoded (indexed) primers.