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Study design

Sampling → **Biosensors**
- Replication
- Pooling issue

Handling samples
- Preventing external contamination
- Preventing cross-contamination

Storing samples
- Preserving DNA/RNA

-omics methods

Sample preparation

Meta-barcoding

·Rapid air-drying
·Freeze-drying
·Deep-freezing
·Preservation buffer

WGS
MetaG
MetaT

DNA/RNA extraction
- Method selection
- Negative, positive controls

Marker selection
- Taxonomic resolution
- Reference database

· ITS
·16S/18S
·23S/28S
·COI

Arrays qPCR ddPCR

Primer selection
- Target taxa
- Amplicon length
- Identifier tagging

PCR
- Polymerase selection
- Number of PCR cycles
- Technical PCR replicates

Fragmen-tation

RT-PCR

· one-ended
· both-ended

PCR product control
- Purification
- Normalizing

Adapter ligation

HTS

Choice of platform
- Read quality, quantity
- Read length
- Cost

Sequencing
- Library preparation
- Generation of reads

·Illumina
·Ion Torrent
· PacBio
· Oxford Nanopore

Bioinformatics

Quality control
- Quality filtering
- Chimera filtering
- Demultiplexing

· QIIME
·mothur
·PipeCraft

Demulti-plexing

Contigs

· ITSx
·Metaxa2
·V-Xtractor

Extraction of variable region(s)
- *Optional*

Clustering
- Error rate
- Barcode gap

Genome assembly

·CD-HIT
·UCLUST
·UPARSE
·DADA2

Taxonomy + functional assignment
- Similarity search
- Reference databases

·MEGAN
· INSDc
·RefSeq
·KEGG

· BLAST

· SILVA
· UNITE
· INSDc

Data management

Contaminant + tag-switch removal
- Manual data check

·LuLu

Data analysis
- Computer programs

Data formatting
- FAIR format
- Standards

· MIxS
·MIMARKS
·MIMS

Publication
- Recording options
- Link to data

Data storage
- Raw data
- Metadata

·Dryad
·DataOne

· SRA

Wet lab In situ

DNA extr. lab

PCR lab

HTS prep. lab Post-PCR lab

Office

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