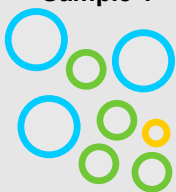


Microbiome Study Workflow Starting with Extracted Microbial Genomic DNA

Sample 1

Sample 2

Microbial Genomic DNA



Amplify 16S rRNA via PCR
with primers containing unique
barcodes for each sample

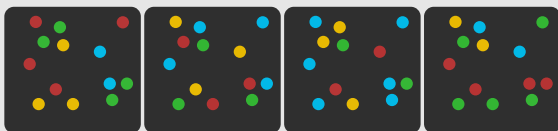
PCR Amplicons



Pool in equal concentrations

Illumina Sequencing

A
G
C
T



Demultiplex Sequences Based On
Unique Barcode Combinations

Sample 1

Sample 2

```
>Seq1
AGTCGTCGCTCGATGACTAG
>Seq2
TAGCTAGTAGCTATATAGCTA
>Seq3
ATATAGCCGCGCCCTAGCTG
```

```
>Seq1
GCGCTAGCTAGCTATATAGCAT
>Seq2
GATCGCTCTCTCTCGATCGCCC
>Seq3
TCGATAGCTTAGATCGCATCGA
```

Bin Sequences into Operational Taxonomic Units
and Assign Taxonomies

| Species | Samples → | | | | | |
|---------|-----------|---|---|---|---|-------------------------|
| | 0 | 3 | 1 | 2 | 0 | |
| 1 | 0 | 3 | 5 | 2 | | Bacteria;Proteobacteria |
| 3 | 4 | 7 | 0 | 1 | | Archaea;Euryarchaeota |
| 0 | 1 | 2 | 2 | 0 | | Bacteria;Actinobacteria |
| | | | | | | Bacteria;Chloroflexi |

Statistical Analysis

Relative Abundance

