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MICROBIOME SCIENCE
microorganisms in lab have replication time of 20 minutes, in the real world the faster rate is about 1 hour
the total weight of all microorganisms after 1 night of replication would be higher that planet's weight
with climate change temperature increases, so microorganisms grow faster, so we need to control the temperature
microorganism in permafrost *
microbiome centrality for the ecosystem (provide health of plants, animals and environment)
earth microbiome project is a systematic attempt to characterise global microbial taxonomic and functional divesity for the benefit of the planet, you can collect data there
to study microbiome science (microbial behaviour) we need to know biology, chemistry, mathematics (informatics, statistics, modelling) in order to find solutions
application of microbiome science is microbiome biotechnology, whose applications are in different (but interconnected) aspects: the one health triad: human health (es. microbiome-based cancer treatment), climate change mitigation (es. circular economy and novel strategies for energy capture), food security (es. microbiome-based agrobiotech applications)
metagenomics is the untargeted sequencing of the genetic material present in a given sample
the first application of metagenomics science was provided by Craig Venter: whole genome sequencing of human genome (Venter's) with next generation sequencing technology
also in 2004: environmental genome shotgun sequencing of the sargasso sea
first to crate sintetic life designing the minimal set of genes necessary for an organism's life
before sequencing was possible only with the sanger approach, but really expensive
had only 5 microorganism samples avaiable for culture based approach, but then realized they were 0,1% of microbial diversity
today thousands of sample are needed for experiments
the new approach of the sequencing of the total genome was cheaper and became popular
microbiome: microbial community itself and its environment
metagenome: overall genome of a microbiome, the genome of each individual of a class
metagenomics: shotgun sequencing of the microbiome
sample: sample for a given host or environment containing a microbiome
covariate: a variable characterizing a given host or environment hosting a microbiome (es. age, weight, temperature, pH)
metagenome microbial community represented by the genomes of all species with different colors, each one having a proportional abundance, equal to the abundance of each specie in the community
marker genome: use primers targeting a specific region of a gene of in oreer to determine the microbial phylogenesis
whole metagenome: shot gun sequencing of the whole microbiome to determine phylogenesis and the functional repertoire
shotgun metagenomics is getting metagenome from random fragemntation of each genome type after extracting it, then annotation and aligment with a computer program to create a larger consensus sequence by putting together fragments looking for overlaps
tagmentation and sequencing power (number of reads provided with next gen seq) *
when squencing metagenome you have more genomes with different abundance, so you need to calculate the coverage
16S rDNA gene is the marker gene for bacterial phylogenesis because it is present in all organisms, it has important function, (ribosome is necessary for life) the same in all living organisms, and accumulates mutations very slowly since it is not involved in lateral gene transfer
within its sequence there are conserved regions (unspecific applications) and hyper variable regions (species-specific applications) -> by targetting the conserved regions I can identify the variable ones
Darwin tree of life (1830) explaines phylogenetic the origin and relationship (degree of similarity) among different species
basing on the percentage of similarity between the 16s rDNA gene (as a reference gene) we computed a new phylogenetic tree
when we have the seq of the hypervariable region we can construct the phylogenetic tree basing on the homology of the annotated sequences, and then assign the taxonomy to each branch
shotgun metagenomic: we sequence the entire gene, provides compositional structure and functions of microbic ecosystem
marker gene approach: we sequence only the marker gene, heap and fast, but provides only compositional structure of the microbic ecosystem
info provided: we look for association (is the micriobiome populating a given holobiont associated with a specific phenotypic envisonmental feature?) and causality (is the microbiome a given holobiont a determinant of a specific phenotypic and/or env feature?)
any feature can affect the microbiome disease
metagenomic studies are comparative
for case (disease) and control (health) we have metagenomics features and covariates, and specificities among them

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info from metagenomic studies: first level is finding association to explain hypothesis
covariates can impact the microbiome since diff species respond different to various; they are classified in env factors (in a context of physical or chemical variables, diet, xenobiotics), microbiome products, or microbiome activities
the general comparative design of metagenomic studies (cases and controls): es. search the diff between the micriobiomes of modern human beings (control) or hunter gatherers (case), key point is adaptation (different phenotypes) is provided by microbiome changes? have to find association and determinants
huge dimensionality of metagenome dataset, need of statistics
for 1 person, we need 1 microbiome sample, including 150 different microorganisms
now imagine we have two populations, case and control, we need to have 100 subjects per population
so 200 subjects in total
200 x 150= 30.000 microbiome variables
for 1 person we need 1 set of metadata, so 20 different metadata
so 200 x 20= 4.000 microbiome metadata
in total 34.000 variables
lot of matrices to deal with:
microbiome variables where species are the human beings :

	species n	species 1	species 3
sample 1			
sample 2			
sample 3			
sample 4			

subject metadata:

	category	time	metadata 1	metadata 2
sample 1				
sample 2				
sample 3				
sample 4				

general overview of the procedure for cross sectional metagenomic studies:
select case and control, collect sample for running the microbiome analysis, extract the DNA, library preparation for next gen seq, bioinformatics to provide a meaning to the sequence and biostatistics to search for the associaton
need to define controls and exclusion criteria, longitudinal approach repeat sampling from the same subject or habitat, calculate the power (the number of cases and controls selected is enough to detect differences?), collect metadata and covarieties, set up proof of concepts
sampling: be sure to collect sufficient microbial DNA for sequencing
after sampling and storage you need to stop the growth through preservation methods like freezing (the best one), exsiccation and DNA preservation buffers
total DNA extraction from the sample with different kits that provide small affinity columns: lysis of microbial cell, deactivation of the DNase from the cell, get a suspension including DNA and purification of the DNA using affinity column
then check for the quality, quantity, purity, dimension and integrity of the extracted and purified DNA
work under UV-light hood to avoid contamination
shotgun metagenomic sequencing: metagenome is the total of hundreds of different genome soecies esch in multiple copies, whose proportional abundance reflects the proportional abundance of the corresponding species > random fragmentation of the metagenome, then for each genome type a series of random fragments is obteined, proportional to the number of copies of each genome type
genome proportional abundance related to the proportional abundance of copies per genome region
same process for metagenome marker gene sequencing: instead of random fragmentation you consider only the 16s rDNA amplicons, again proportional to their abundance in the genome
tagmentation: dividing dna into random 300 bp reads, then sequence the short reads (300 bp) in two payrents (we sequence first the left side of the fragement (150 bp of one strand 5'to3') and then the right side (150 bp from the end of the other complementary strand 3'to5')
the cuts to create 300 bp fragments by using two oligonucleotide tags on two transposons
amplification of the fragments in index PCR: couple of primers having two barcode indexes and p5 and p7 regions > 5' primer provides the sequence of the left side, the 3' one the sequence of the right side
while for marker gene PCR fragmentation is needed, amplification occurs on v3 and v4 regions in the 16s rDNA gene

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illumina sequencing approach is a surface sequencing: surface and one copy of the fragment with the two adaptors
F strand is the 5' left part attached to the surface, R is the 5' right part attached to the surface
sequencing starts from the 3' of both sequences, one forward the other reverse
illumina sequencing technology * video on youtube
you know the combination of index 1 and 2 by making contiguous sequences throurh the two payrents
how can we figure out the total sequencing power?
scales in metagenome sequencing
i need to seq each genome fragment al least 10 times to have an accurate sequencing of the genome of a microorganism
so i need 10 copies of the e.coli DNA (2,5 Mbp), in total 10x2,5Mbp
then random fragmentation of the 10 copies of the e.coli DNA, get a total of 10K 300bp per genome copy
must be avaiable on the flow cell at least 100000 sequencing clusters
in a metagenome you have a solution with the major and minor components (two different microorganisms, major 10 times the minor
have to provide 10% coverage for the minor
good practices per sample

lesson 22 marzo *******

taxonomic assigment of asv or otu:
V search finds the 100 best matching 16

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