AARHUS UNIVERSITY
DEPARTMENT OF ENVIRONMENTAL SCIENCE
ENVIRONMENTAL MICROBIAL GENOMICS GROUP

MILJØ DNA

FRA EN MIKROBIOLOG'S SYNSPUNKT

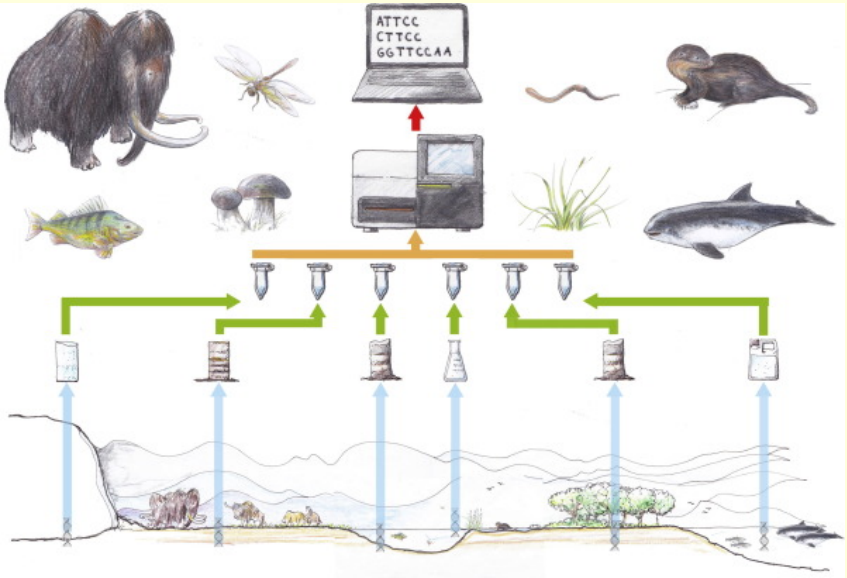
Lars Hestbjerg Hansen, Head of section EMBI @ENVS

I dag:

- Hvad er e-DNAs rolle generelt og i mikrobiologien?
- Hvad kan vi gøre?
- Hvordan gør vi?
- Spørgsmål & hvad kan man efter min mening ikke gøre?

Hvad er e-DNA?

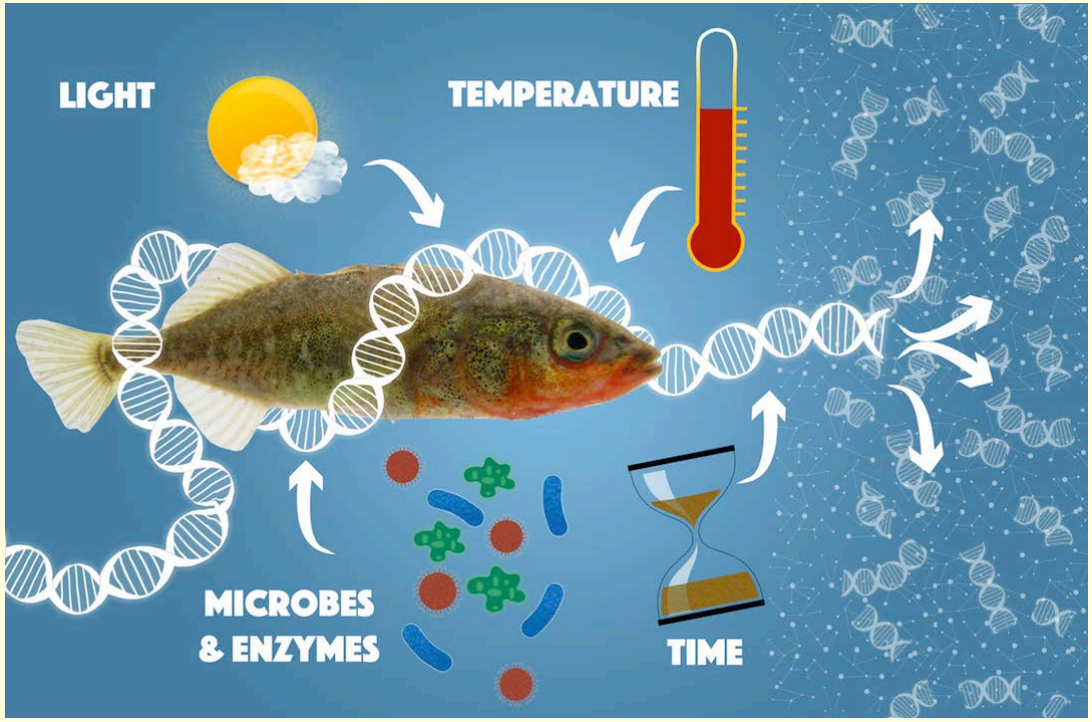
- Environmental DNA (eDNA) – defined here as: *genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material.*



Thomsen & Willerslev. 2015 **Biological Conservation**, Vol., March 2015, Pages 4-18

Hvad er e-DNA?

- DNA er ikke særligt holbart i naturen.



http://fishbio.com/field-notes/conservation/traces-left-behind

Hvad er e-DNA?

- Men alt hvad der bevæger sig gennem naturen efterlader celler.

http://fishbio.com/field-notes/conservation/traces-left-behind

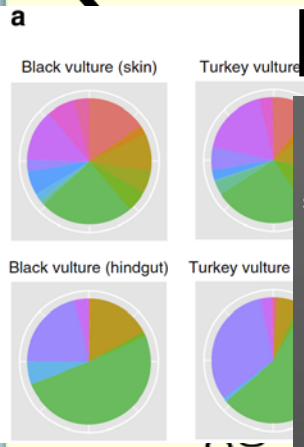
Hvad er e-DNA?

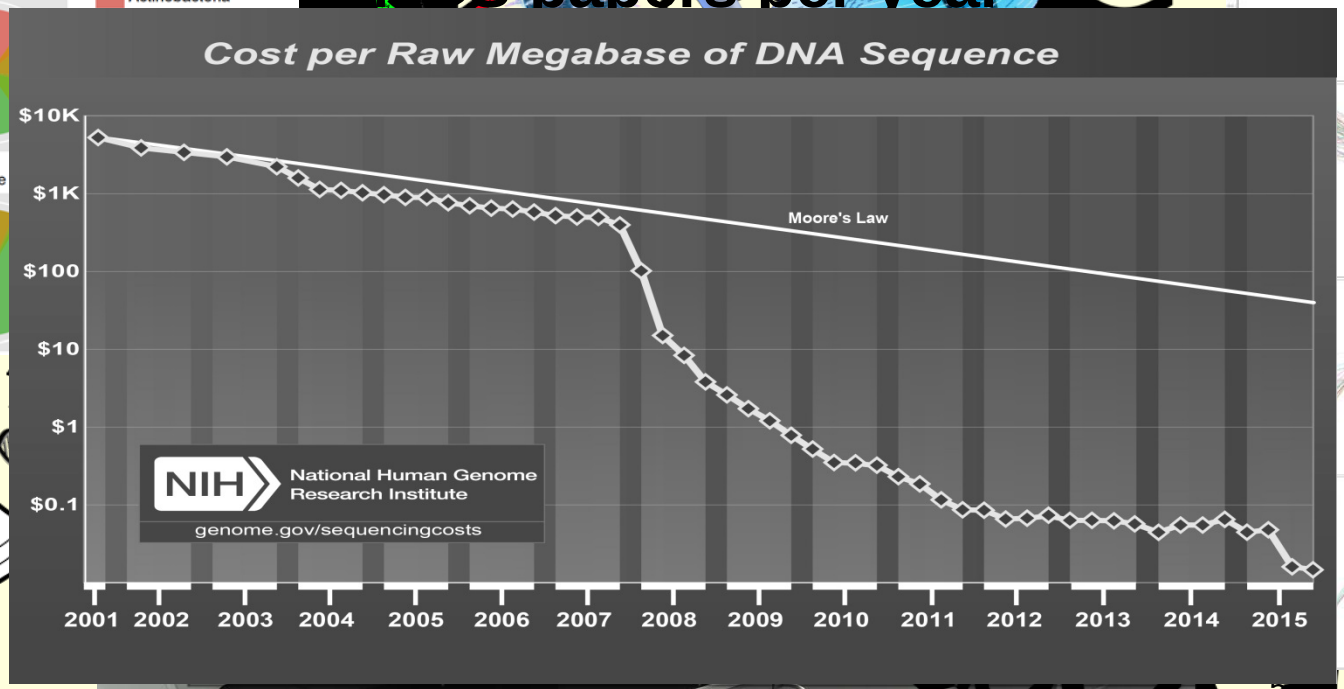
- Det gælder alt levende fra store dyr til insekter som her fra jord og så helt til bakterier, svampe og vira.

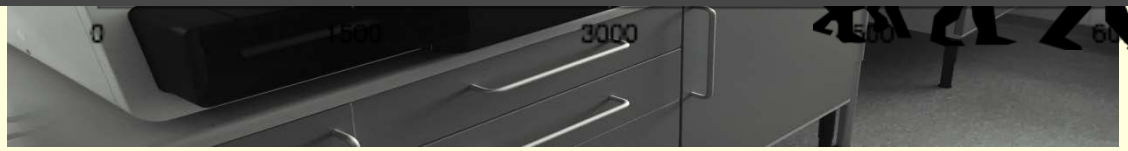
https://www.wur.nl/en/

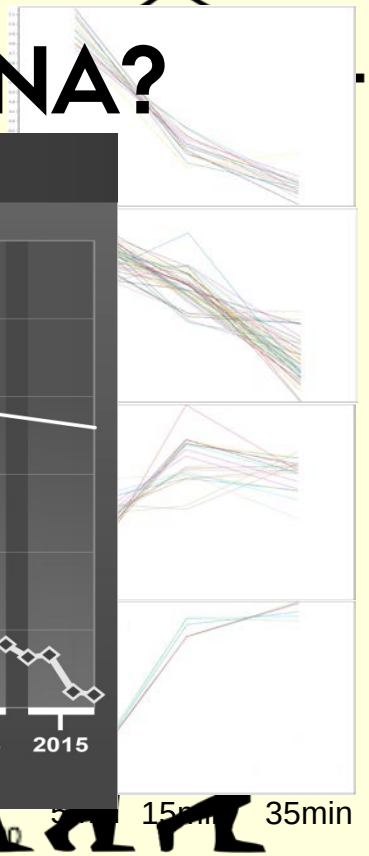
Hvad kan vi gøre?

Hvorfor er der fremtid for eDNA?









35min

Hvad kan vi gøre?

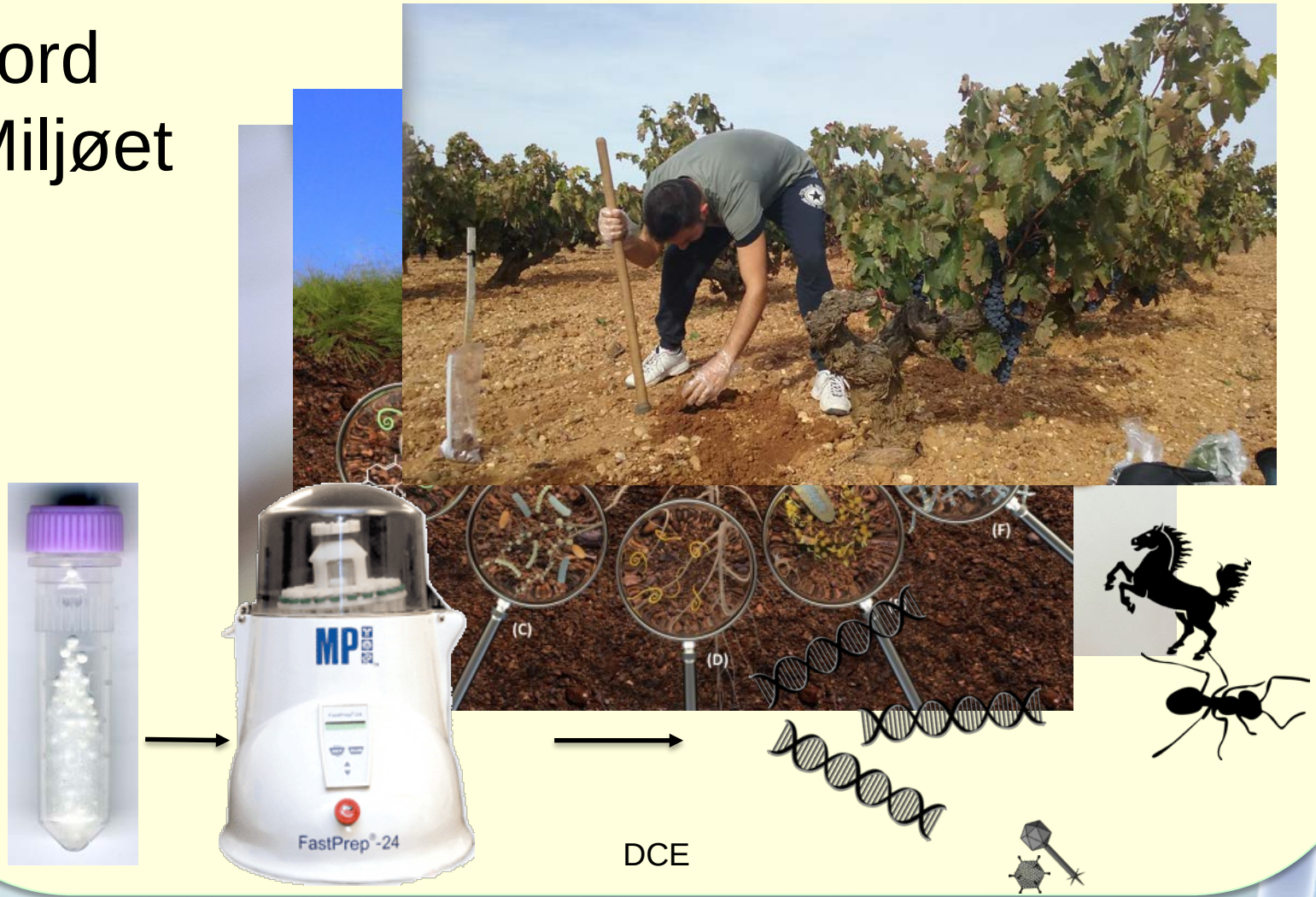
- Mekanismer bag og potentiale for bioremediering af xenobiotika på molekylært niveau (sekventering, qPCR)
- Diversitet af alle mikroorganismer, som bakterier, svampe, protister, vira ved sekventering.
- Diversitet i mikroorganismers funktion – transcriptomics ved sekventering.
- Vand, Jord, sandfilter, plante og dyre mikrobiomer.
- Detektion af specifikke mikroorganismer typisk ved qPCR
  - Human-, Plante- og Fiskepatogener (fx E.coli, men også skimmelsvampe osv).
  - Produktionsorganisme detektion i f.eks vandmiljøer
  - allergene partikler i luften og vand (fx svampesporer og alger).

Hvordan gør vi?

Vand
miljø

Hvordan gør vi?

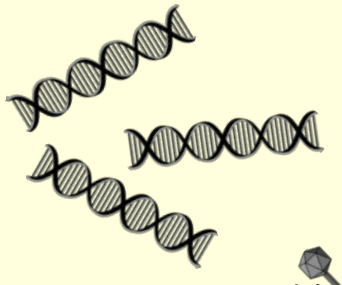
Jord
Miljøet



DCE

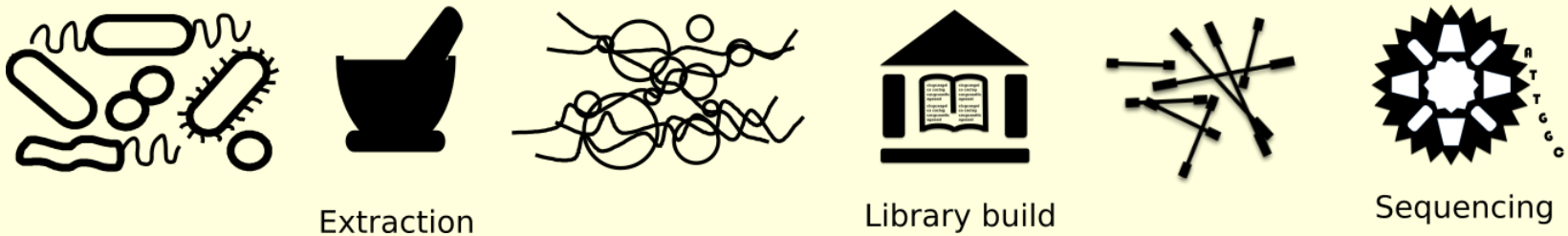
Hvordan gør vi?

Luften



DCE

Hvordan gør vi?



Shotgun sekventering

Amplicon sekventering

Metabarcoding

Hvordan gør vi?

-sekventering

Funktioner & påvisning fra gener

- *lacZ* – koder for et enzym der spalter laktose
- *repA*- koder for et enzym der sørger for plasmid replication
- *gyrA* – koder for et enzym der sørger for for DNA struktur

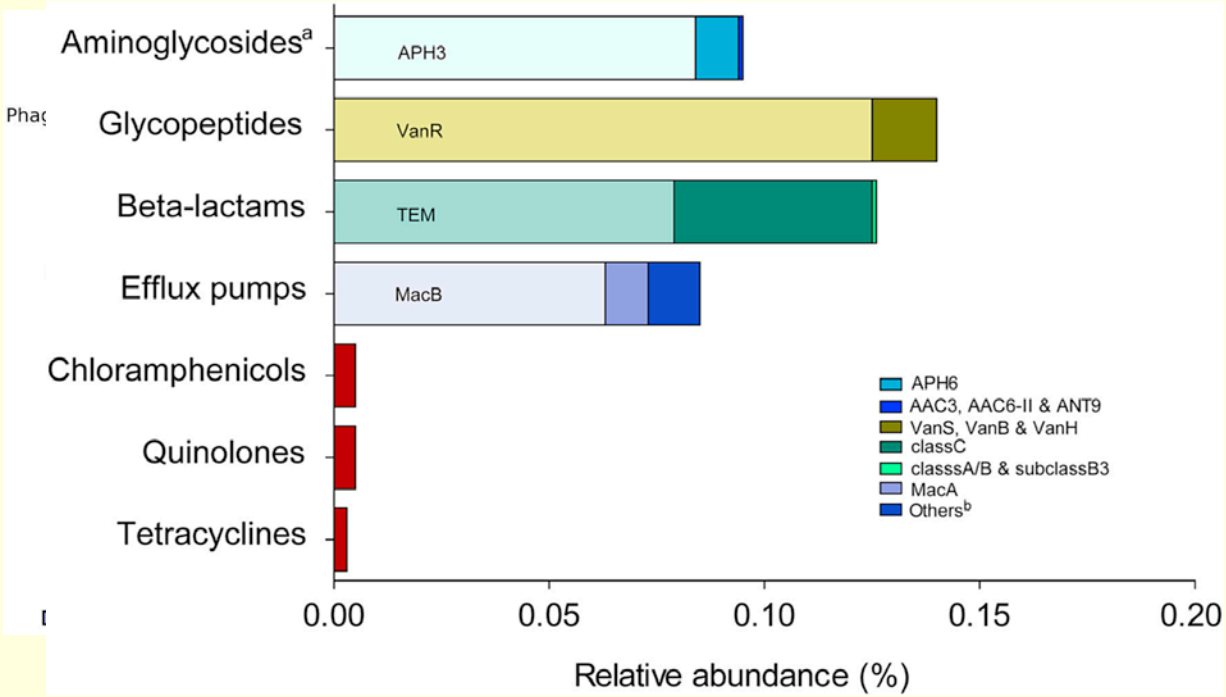
- 16S rRNA genet – et genetisk fingeraftryk til at identifisere bakterie arter

16S sequences	Fingerprint comparison	Function
AAAUUGAAGAGUUUGAUCAUGGCUCAGAUU GAACGCUGGCGGCAGGCCUAACACAUGCAA GUCGAACGGUAACAGGAAGCAGCUUGCUGCU UCGCUGACGAGUGGCGGACGGGUGAGUAAUG UCUGGGAAGCUGCCUGAUGGAGGGGGAUA...	*E. coli*	Able to eat lactose
AGAGTTTGATCCTGGCTCAGGATGAACGCTGGC GGCGTGCTTAACACATGCAAGTCGAGCGATGAA GTTTCCTTCGGGAAACGGATTAGCGGCGGACG GGTGAGTAACACGTGGGTAACCTGCCTCATAGA GTGGAATAGCCTTCCGAAAGGAAGATTAA...	*Clostridum perfringens*	Can kill humans

Bakteriers funktioner og resistens i forurenet jord?

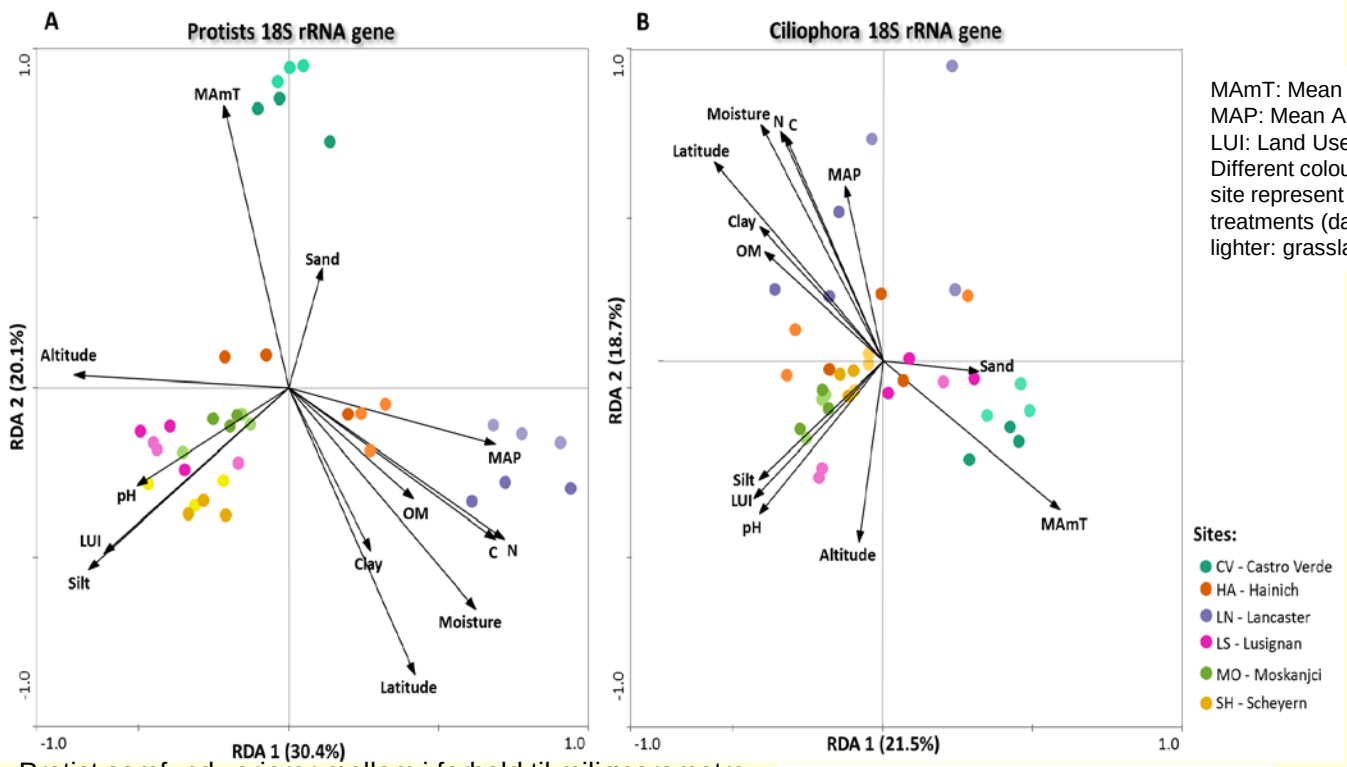
Hvilke funktioner dominerer i forurenet jord

Hvilke antibiotika resistenser finder vi på disse?



https://doi.org/10.1016/j.soilbio.2016.07.018

Effekten af eDNA-analyse på små jorddyr og protister:



MAmT: Mean Annual Temperature
MAP: Mean Annual Precipitation
LUI: Land Use Intensity
Different colour shades within the same site represent different soil management treatments (darker colour: intensive, lighter: grassland)

Protist samfund varierer mellem i forhold til miljøparametre (temp., pH, nedbør, LUI, soil type)

Santos, S., et al., Winding, A. in prep

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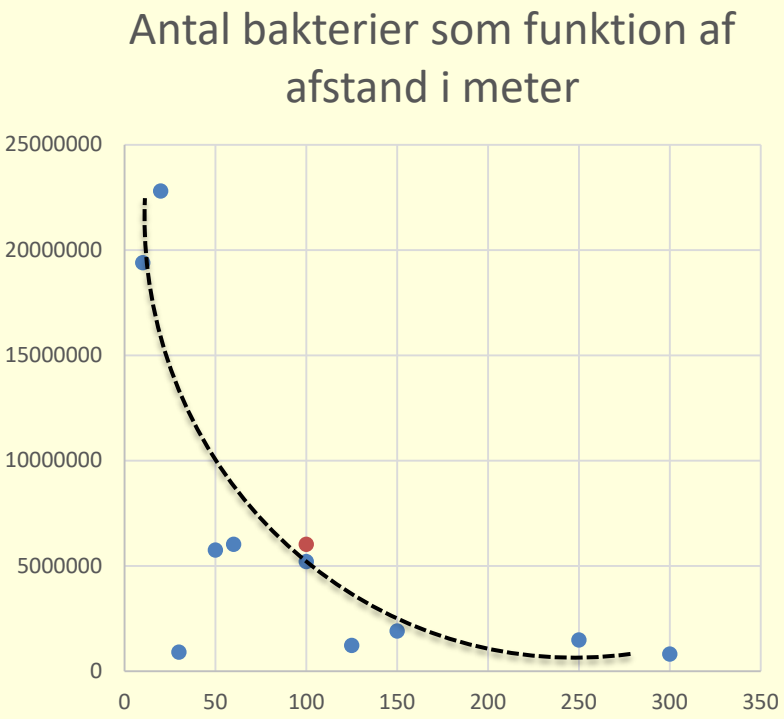
Bakterie påvirkning fra stalde



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Occurrence and co-occurrence

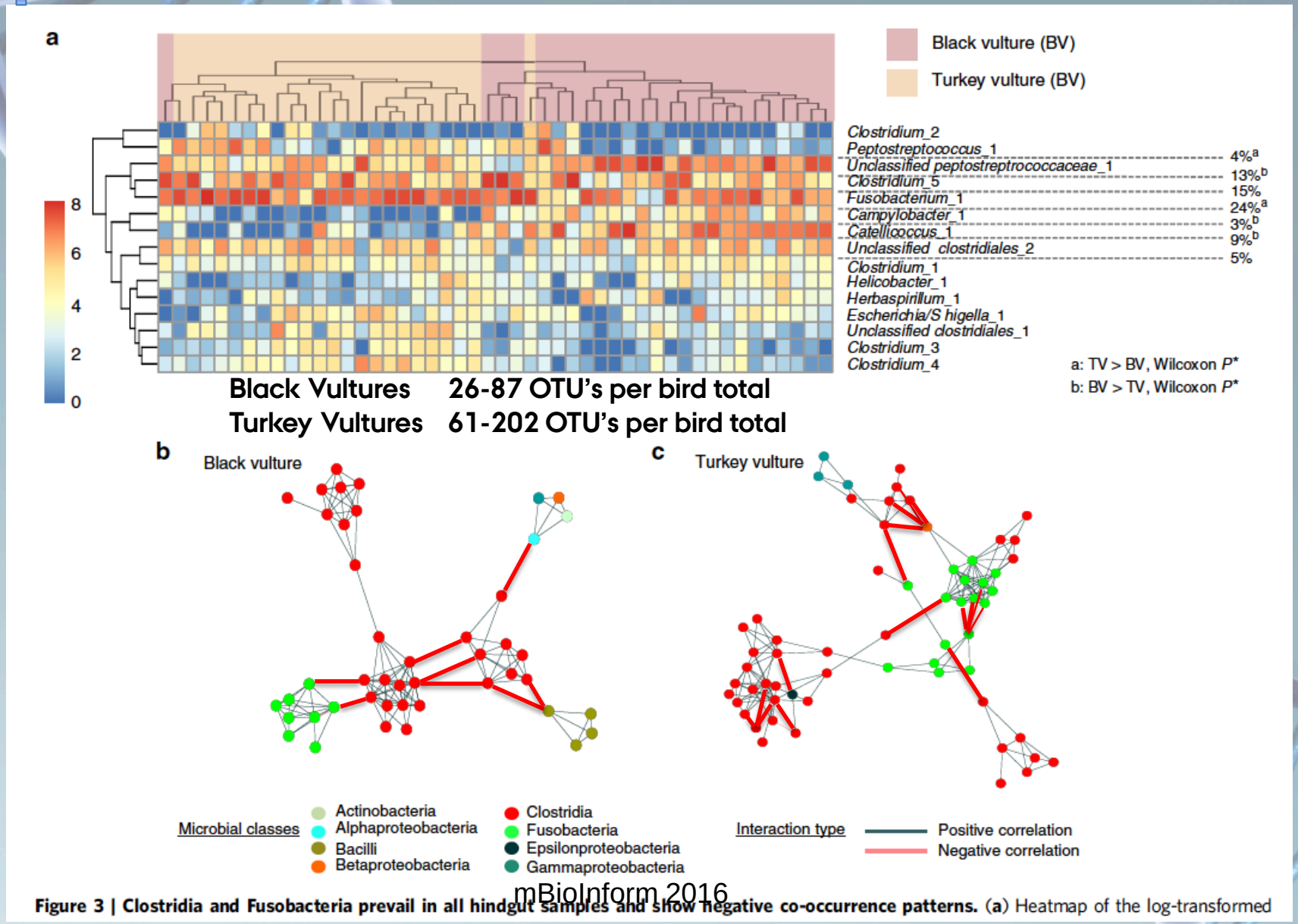

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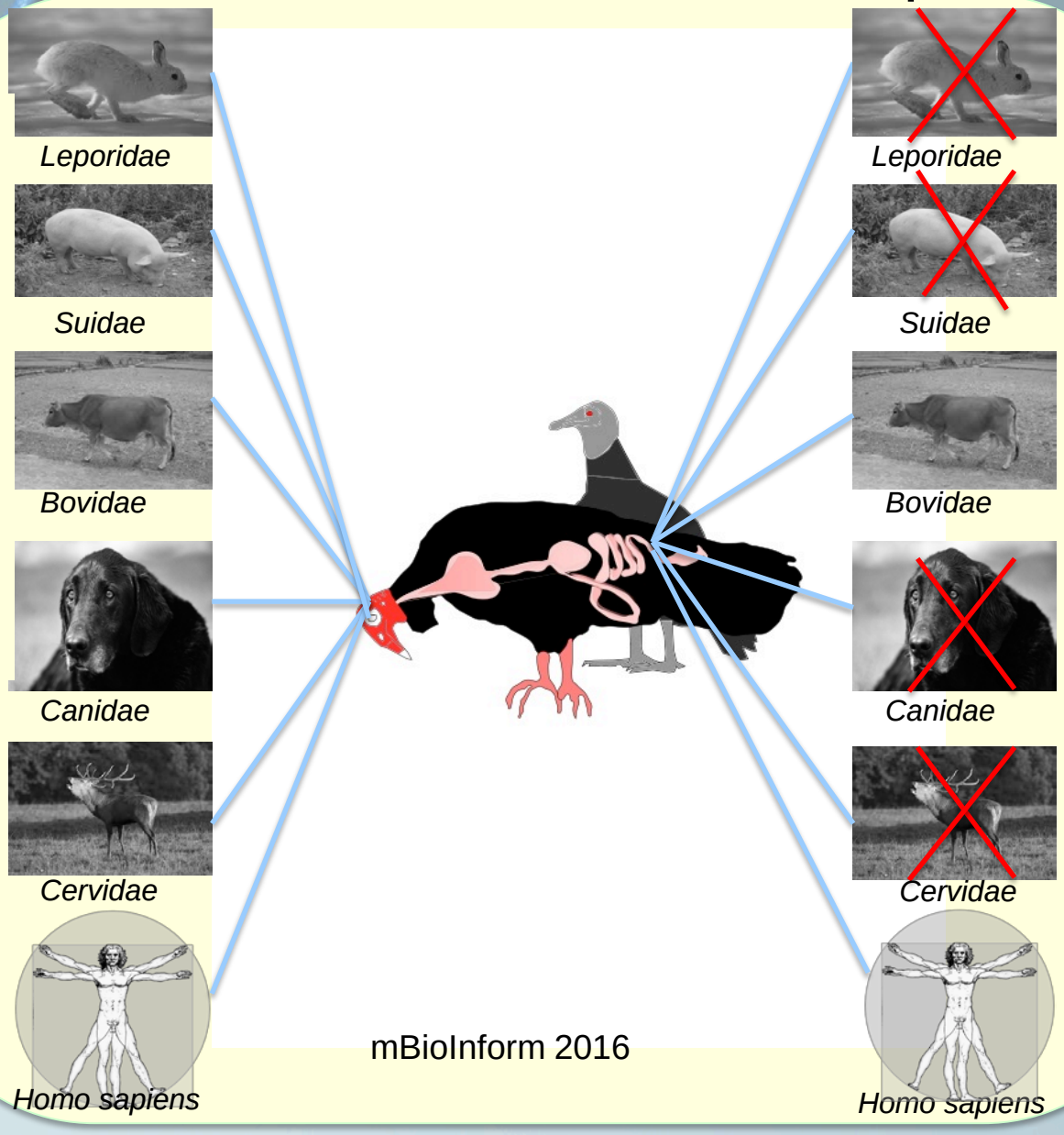
Figure 3 | Clostridia and Fusobacteria prevail in all hindgut samples and show negative co-occurrence patterns. (a) Heatmap of the log-transformed

Vulture diets

(Mammalian 16S PCR-illumina seq)

Does DNA pass through the vulture gut?

Compare face and gut prey DNA!



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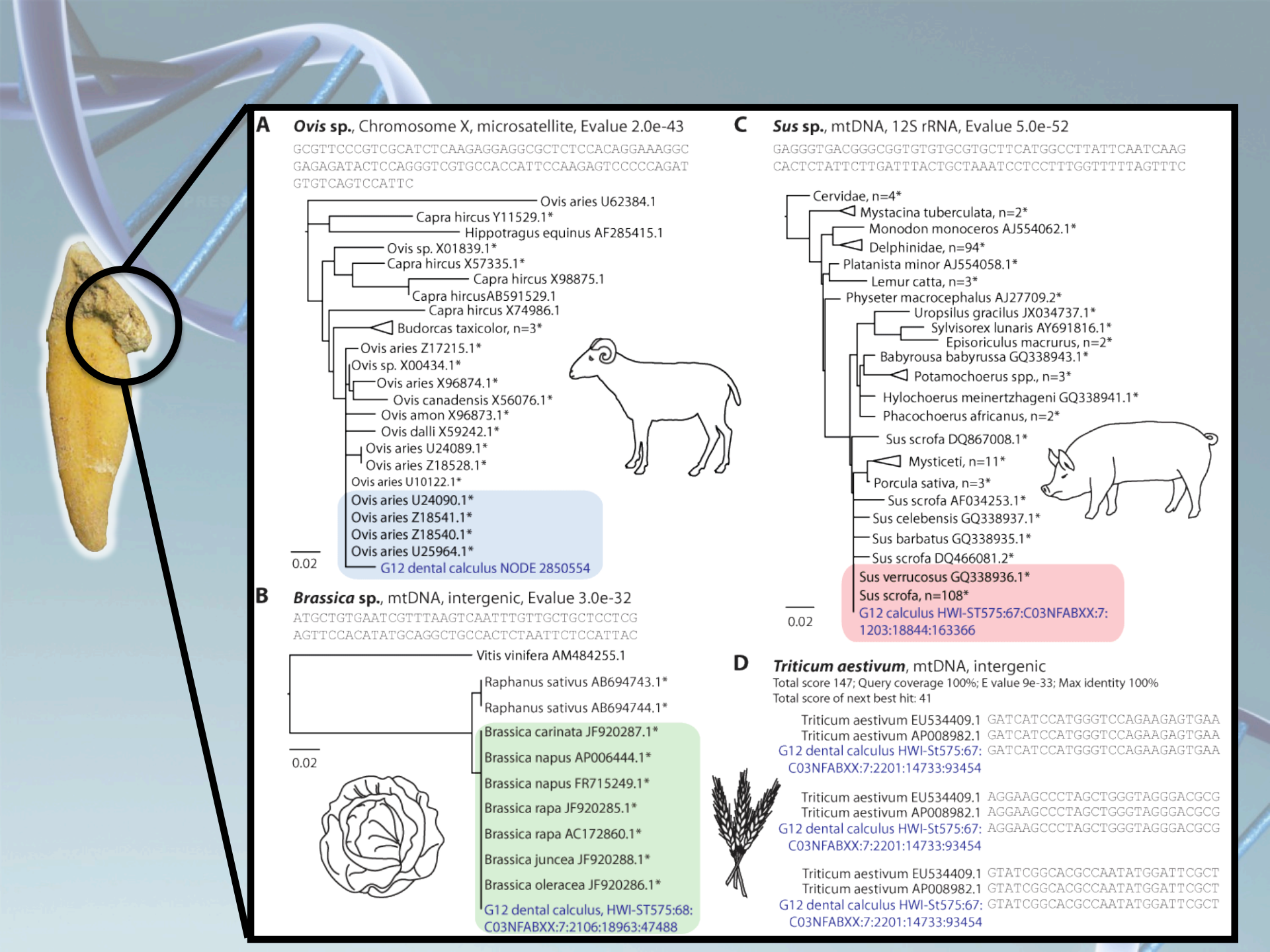
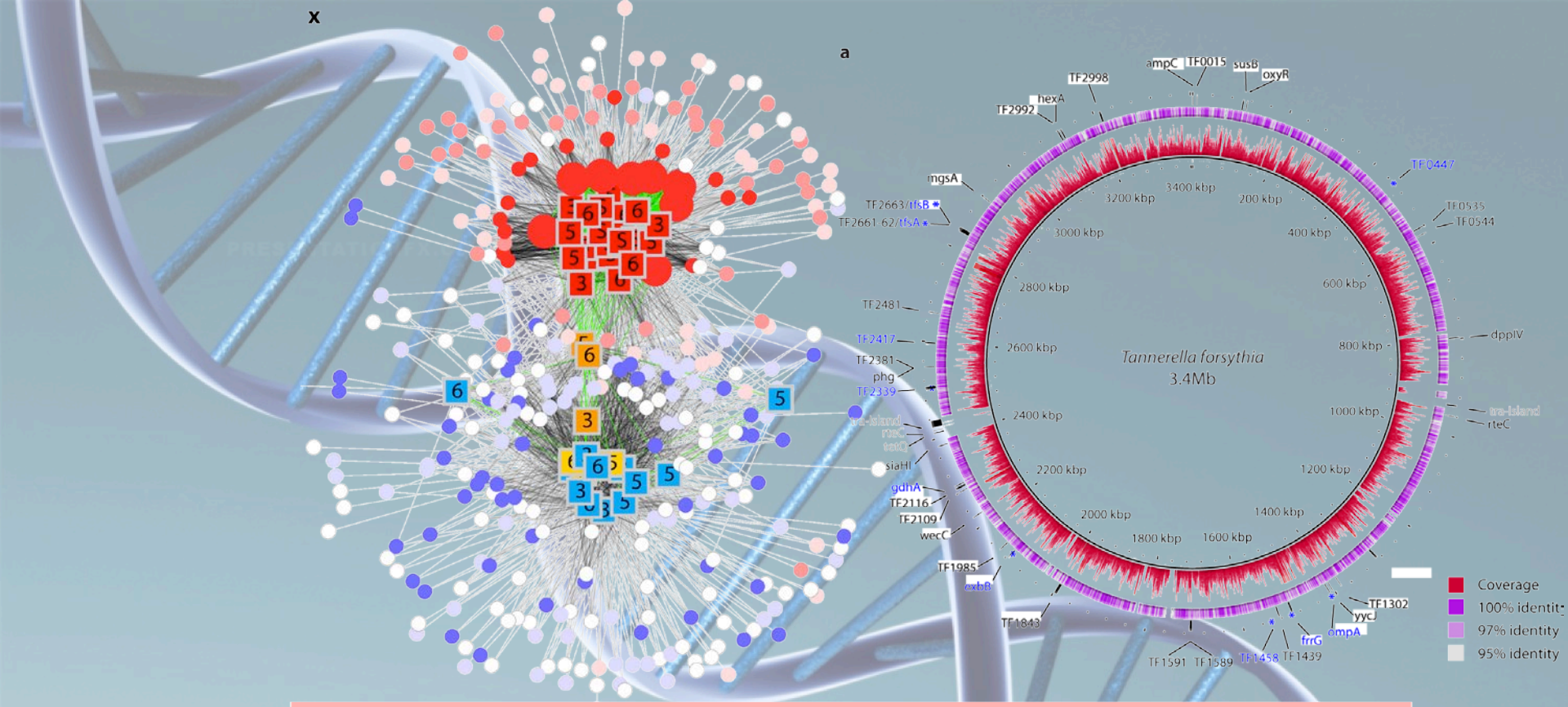

A
Ovis sp., Chromosome X, microsatellite, Evalue 2.0e-43
GCGTTCCCGTCGCATCTCAAGAGGAGGCGCTCTCCACAGGAAAGGC
GAGAGATACTCCAGGGTCGTGCCACCATTCCAAGAGTCCCCCAGAT
GTGTCAGTCCATTC
Ovis aries U62384.1
Capra hircus Y11529.1*
Hippotragus equinus AF285415.1
Ovis sp. X01839.1*
Capra hircus X57335.1*
Capra hircus X98875.1
Capra hircusAB591529.1
Capra hircus X74986.1
Budorcas taxicolor, n=3*
Ovis aries Z17215.1*
Ovis sp. X00434.1*
Ovis aries X96874.1*
Ovis canadensis X56076.1*
Ovis amon X96873.1*
Ovis dalli X59242.1*
Ovis aries U24089.1*
Ovis aries Z18528.1*
Ovis aries U10122.1*
Ovis aries U24090.1*
Ovis aries Z18541.1*
Ovis aries Z18540.1*
Ovis aries U25964.1*
G12 dental calculus NODE 2850554
0.02
B
Brassica sp., mtDNA, intergenic, Evalue 3.0e-32
ATGCTGTGAATCGTTTAAGTCAATTTGTTGCTGCTCCTCG
AGTTCCACATATGCAGGCTGCCACTCTAATTCTCCATTAC
Vitis vinifera AM484255.1
Raphanus sativus AB694743.1*
Raphanus sativus AB694744.1*
Brassica carinata JF920287.1*
Brassica napus AP006444.1*
Brassica napus FR715249.1*
Brassica rapa JF920285.1*
Brassica rapa AC172860.1*
Brassica juncea JF920288.1*
Brassica oleracea JF920286.1*
G12 dental calculus, HWI-ST575:68:
C03NFABXX:7:2106:18963:47488
0.02
C
Sus sp., mtDNA, 12S rRNA, Evalue 5.0e-52
GAGGGTGACGGGCGGTGTGTGCGTGCTTCATGGCCTTATTCAATCAAG
CACTCTATTCTTGATTTACTGCTAAATCCTCCTTTGGTTTTTAGTTTC
Cervidae, n=4*
Mystacina tuberculata, n=2*
Monodon monoceros AJ554062.1*
Delphinidae, n=94*
Platanista minor AJ554058.1*
Lemur catta, n=3*
Physeter macrocephalus AJ27709.2*
Uropsilus gracilus JX034737.1*
Sylvisorex lunaris AY691816.1*
Episoriculus macrurus, n=2*
Babyrousa babyrussa GQ338943.1*
Potamochoerus spp., n=3*
Hylochoerus meinertzhageni GQ338941.1*
Phacochoerus africanus, n=2*
Sus scrofa DQ867008.1*
Mysticeti, n=11*
Porcula sativa, n=3*
Sus scrofa AF034253.1*
Sus celebensis GQ338937.1*
Sus barbatus GQ338935.1*
Sus scrofa DQ466081.2*
Sus verrucosus GQ338936.1*
Sus scrofa, n=108*
G12 calculus HWI-ST575:67:C03NFABXX:7:
1203:18844:163366
0.02
D
Triticum aestivum, mtDNA, intergenic
Total score 147; Query coverage 100%; E value 9e-33; Max identity 100%
Total score of next best hit: 41
Triticum aestivum EU534409.1 GATCATCCATGGGTCCAGAAGAGTGAA
Triticum aestivum AP008982.1 GATCATCCATGGGTCCAGAAGAGTGAA
G12 dental calculus HWI-St575:67: GATCATCCATGGGTCCAGAAGAGTGAA
C03NFABXX:7:2201:14733:93454
Triticum aestivum EU534409.1 AGGAAGCCCTAGCTGGGTAGGGACGCG
Triticum aestivum AP008982.1 AGGAAGCCCTAGCTGGGTAGGGACGCG
G12 dental calculus HWI-St575:67: AGGAAGCCCTAGCTGGGTAGGGACGCG
C03NFABXX:7:2201:14733:93454
Triticum aestivum EU534409.1 GTATCGGCACGCCAATATGGATTCGCT
Triticum aestivum AP008982.1 GTATCGGCACGCCAATATGGATTCGCT
G12 dental calculus HWI-St575:67: GTATCGGCACGCCAATATGGATTCGCT
C03NFABXX:7:2201:14733:93454



Pathogen[a,b]	Genes (contigs)	Proteins (peptides)	Virulence	Resistance[c]	Plasmid	CTn/Phage
Streptococcus pneumoniae	144 (339)	1 (8)	+	+		+
Streptococcus pyogenes	14 (32)	1 (8)		+		+
Haemophilus influenzae	19 (39)	1 (4)				+
Neisseria meningitidis	336 (821)	1 (2)	+	+		+
Porphyromonas gingivalis	802 (2588)	7 (72)	++	+		+
Tannerella forsythia	1099 (11279)	10 (137)	++	+		+
Treponema denticola	917 (6106)	3 (15)	++	+	+	+

Hvad kan man ikke?

- Analysere for alle arter
- Kvantificere noget som helst andet end bakterier
- Adskille visse arter
- Se om noget stadig lever i miljøet