# Applied Metagenomics I

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Seminar “Metagenomics”

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# Introduction

## What we know already:

- **What** is metagenomics?
- **Sequencing** techniques
- Metagenome **analysis** with MEGAN

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## What I am going to explain:

- What is metagenomics **used** for?
- **Who** uses metagenomics?

# Overview

1. Applications overview
   - Bioprospecting
   - Phylogenetic Analysis
   - Functional Analysis
2. Complete Neanderthal Mitochondrial Genome
   - Introduction
   - Preparations & Procedures
3. Human Microbiome Project
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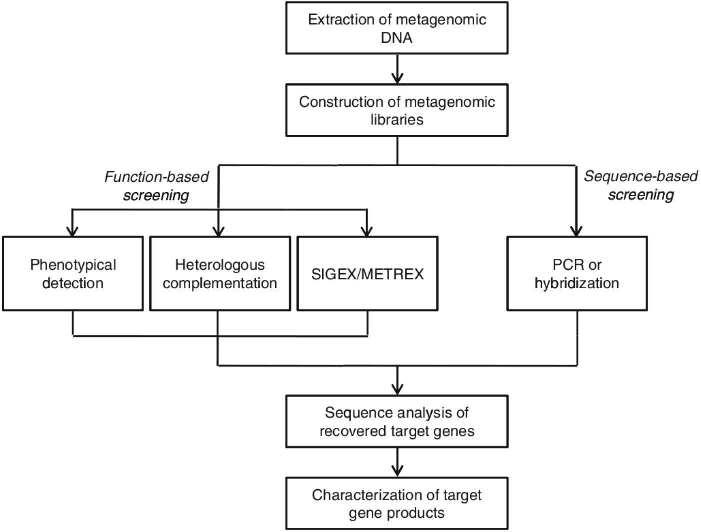
⇒ Instead of exploring single organisms let's look at **whole communities**

⇒ **Increased chances** to be successful

# Overview



Extraction of metagenomic DNA

Construction of metagenomic libraries

*Function-based screening*

*Sequence-based screening*

Phenotypical detection

Heterologous complementation

SIGEX/METREX

PCR or hybridization

Sequence analysis of recovered target genes

Characterization of target gene products

Source: [Simon and Daniel, 2009]

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- **Examples:**
  - “Subtractive hybridization magnetic bead capture”
  - “Metagenomic walking”
  - Microarrays

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- **False-negative results** possible due to host's inability to adapt

# Function-Based Screening Methods

## Direct Detection

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## Induced Gene Expression

**Green fluorescent protein** is inserted together with the target gene via operon-trap expression vector. Relevant host cells are thus **visibly marked**.

# Screenings Summary

| | **Function-based** | **Sequence-based** |
|---|---|---|
| **Advantages** | • Only complete genes are found | • No need for a foreign host to obtain gene expression data |
| **Disadvantages** | • Relies on a foreign host, which might induce false negative results | • Cannot find entirely unknown genes<br>• Might yield incomplete genes |

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⇒ **Shotgun sequencing** to avoid PCR

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- **Tools** like MG-RAST are available already

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# About the Project

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- 38,000 years old **Neandertal bone** found in Vindjia Cave (Croatia)

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- **Goal:** Finding new information about the **relationship** between modern humans and Neandertals

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- PCR using these primers allowed for **quantification** of the contained DNAs
- Contamination with unwanted modern human DNA was **below 1%**

# Considerations

- Ancient DNA is subject to **degradation processes**
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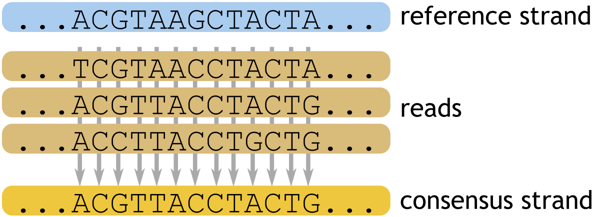
- E.g. **deamination** of cytosine results in uracil residues, which are read as thymine by the DNA polymerase
- Previous studies allowed **thorough understanding** of these disturbances
- To compensate for these expected problems **mitochondrial DNA** was chosen over nuclear DNA
- Each cell contains it in **huge abundance** and the **shorter length** works well with **454 sequencing**

# Assembly Process

- Nucleotide misincorporation is a **problem**
- Mitochondrial sequence from modern humans used as **reference strand**



...ACGTAAGCTACTA... reference strand

...TCGTAACCTACTA...
...ACGTTACCTACTG...
...ACCTTACCTGCTG...
reads

...ACGTTACCTACTG... consensus strand

- Sequencing reads **aligned** with the reference
- **Majority base** identified for each column

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- Some regions were **problematic** due to e.g. missing coverage
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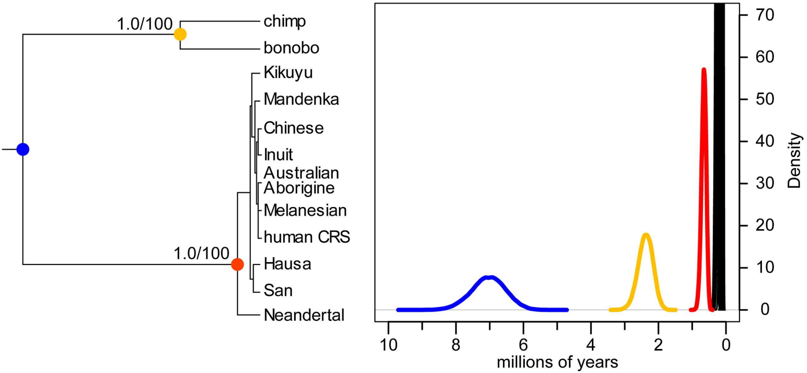
- Some regions were **problematic** due to e.g. missing coverage
- These were **extracted specifically** from another bone sample and Sanger **sequenced**
- After **repairing** the consensus strand using those results the **new** consensus strand was used as reference strand
- 721 sequences **additional** sequences were found, which the first step did not reveal

# Results

- A total of **8341 sequences** could be identified
- This leads to a **34.9-fold coverage** of the whole mitochondrial genome
- **Verification** steps showed a contamination with modern human mtDNA of 0.5%
- Trusting this to be fairly reliable the mtDNA was **analyzed** and **compared** with other data

# Results (2)

- Thus a **phylogenetic tree** could be estimated



Source: [Green et al., 2008]

# Results (3)

- The Neandertal mitochondrial genome is definitely **no mere variation** of the modern human's version
- About **660,000 years ago** both lineages diverged
- Their most recent common ancestor lived quite some time **before** the most recent common ancestor of all humans
- The results also suggest that the Neandertal **population size** was significantly **smaller** than the modern ones

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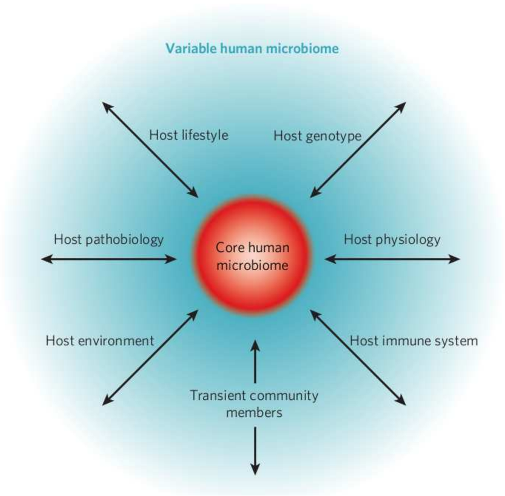
- Constant **symbiosis** with a vast number of **microorganisms** (microbiota)
- They perform tasks we therefore **never** had to do ourselves
- **Goal:** Characterize the distribution and evolution of microbiota

# Microbiome

- Entirety of **all** microbiota genomes
- HMP defines **core** and **variable microbiome**



Source: [Turnbaugh et al., 2007]

# Questions

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- How **stable** is the microbiome?
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- How do the microbiota relate to certain **diseases**?

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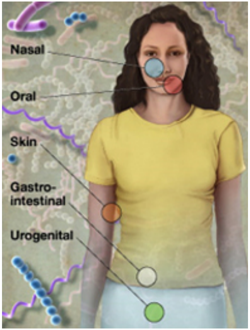
⇒ Immense **community effort**

# Fields of Interest

- **Five representative habitats** were chosen for analysis
  - Nasal
  - Oral
  - Skin
  - Gastrointestinal
  - Urogenital
- Samples from each will be analyzed once the reference data is **complete**



Source: http://www.hmpdacc-resources.org

# Metagenomics Relevance

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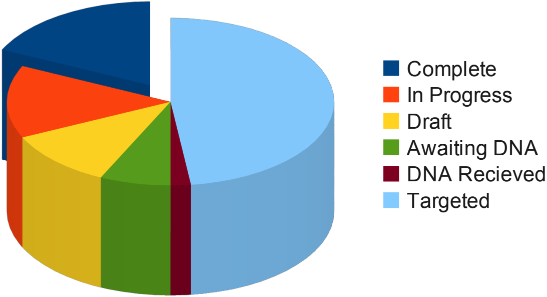
- Reads from whole-genome shotgun sequencing will be **sorted** by species or at least taxonomical groups
- **Building** and **handling** phylogenetic trees containing **millions** of sequences will have be optimized

# Current Status



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- 18% of the reference genomes **completed**
- The remaining ones in different states of **preparation** or **precessing**

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# What You Should Take Home

- The number of **possible applications** for metagenomics is immense
- The spectrum reaches from **narrowing down** on one **specific** genome to looking at a **vast number** of organisms **at once**
- Due to fast growing projects with **increasing needs** for efficient methods the field of metagenomics will keep **growing** fast
- You definitely have not heard the last of **applied metagenomics** and **metagenomics in general**

**Thank you very much for your attention.**

Questions? Remarks?

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