Identification of body tissues/fluids in crime scene stains

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Body tissue/fluid identification for crime scene reconstruction

Crime scene stains are informative

Who? Sub-source level evaluations

Donor(s) of the stains: DNA profile

What? When? Activity level evaluations

What body fluid/tissue? When was it deposited?

For body fluid/tissue, current methods include enzymatic, immunological, microscopic, among others

Limitations in specificity and sensitivity

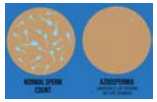
Development of novel methods based on mRNA, methylation, microbes


Luminol

Blood

Bleach

Urine, feces



Microbes as potential markers

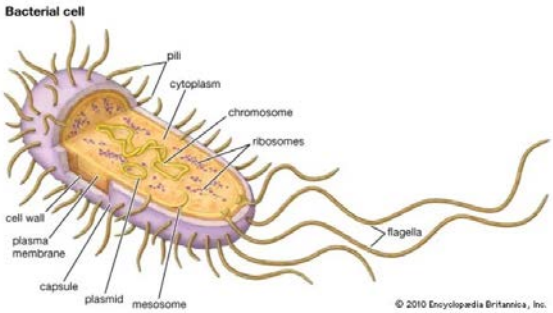
Why microbes?

- Abundant
- Robust cell walls
- Circular genomes

Survive longer than human DNA?

An estimated 30 trillion cells in your body—less than a third—are human. The other 70-90% are bacterial and fungal.

by Gaby D'Allesandro / © AMNH



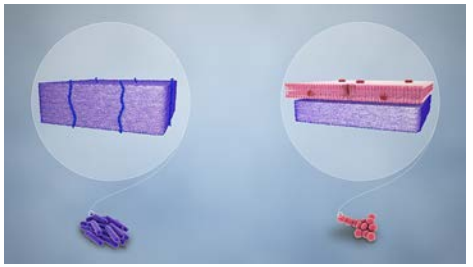
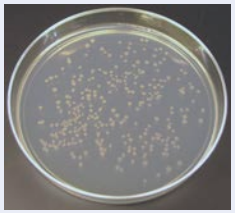


Image credits: 1 AMNH; 2 Encyclopedia Brittannica, 3 Wikipedia

The potential of microbes: insights from human microbiome projects

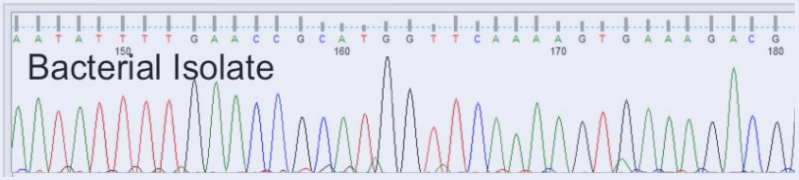
Human microbiome studies took off with NGS

Bacterial culture and Sanger sequencing



DNA extraction

PCR amplification



Next generation sequencing

DNA extraction

(PCR amplification)

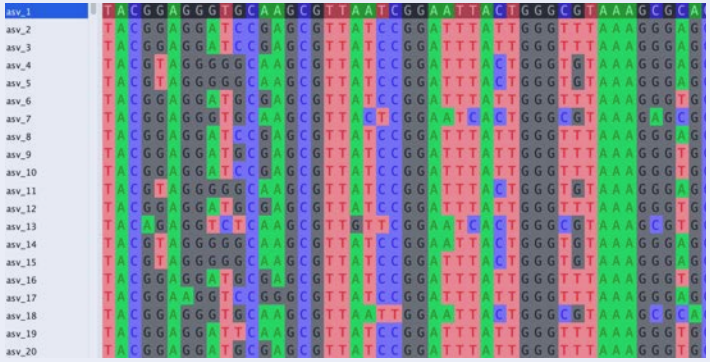
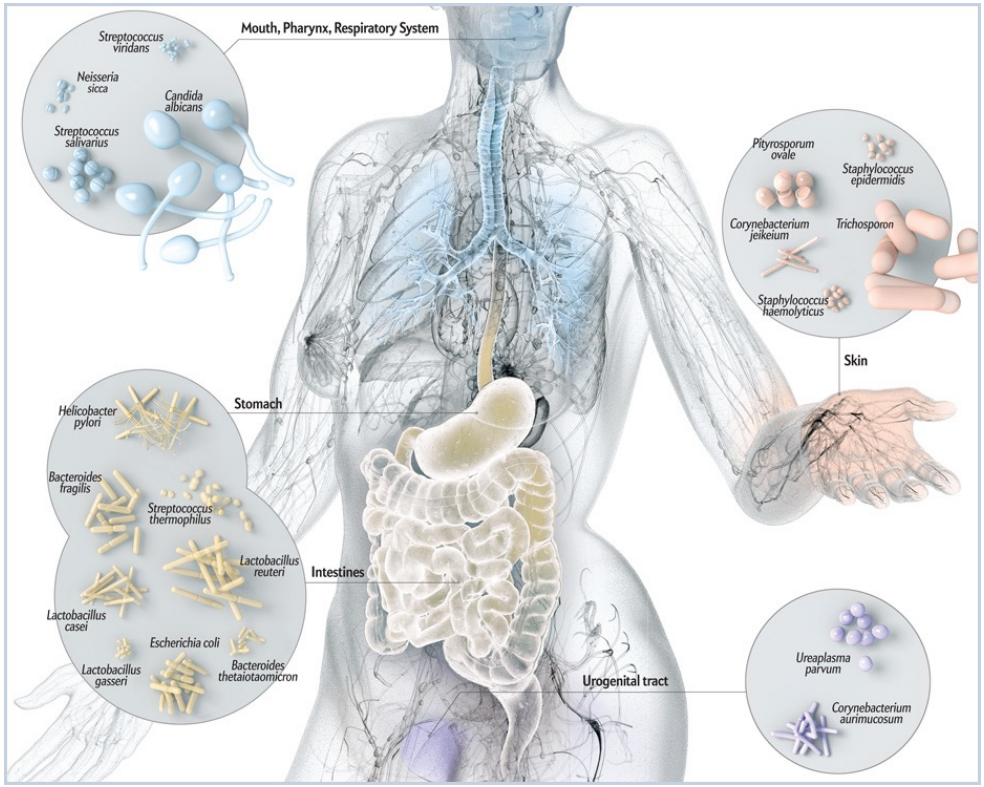


Image credits: Thermofisher, Illumina

Thousands of studies in the last decade

Main focus: healthy versus diseased states



Microbes include bacteria, fungi, archaea, and viruses

Bacteria are the most studied

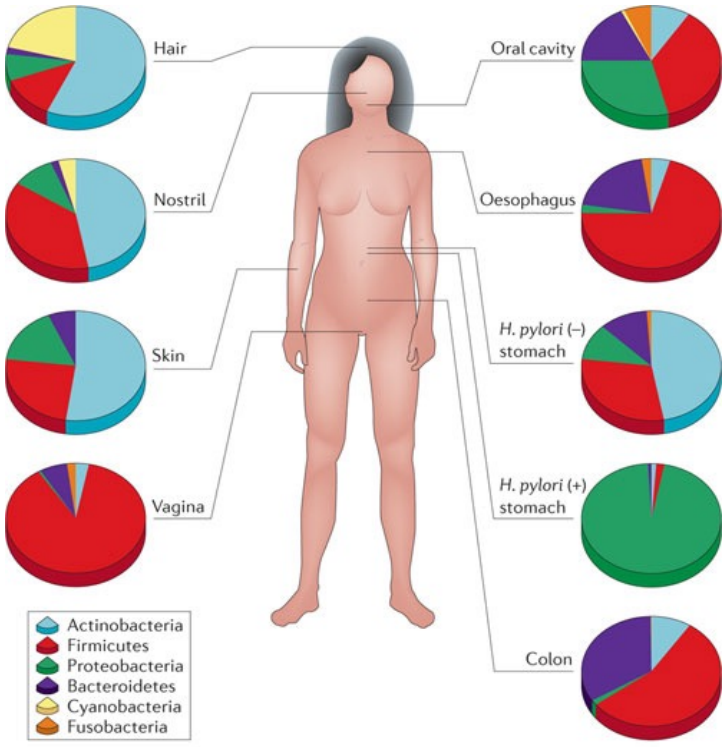
Insights:

- Diverse and complex ecosystems
- Communities are tissue specific

Illustration by Bryan Christie, Scientific American

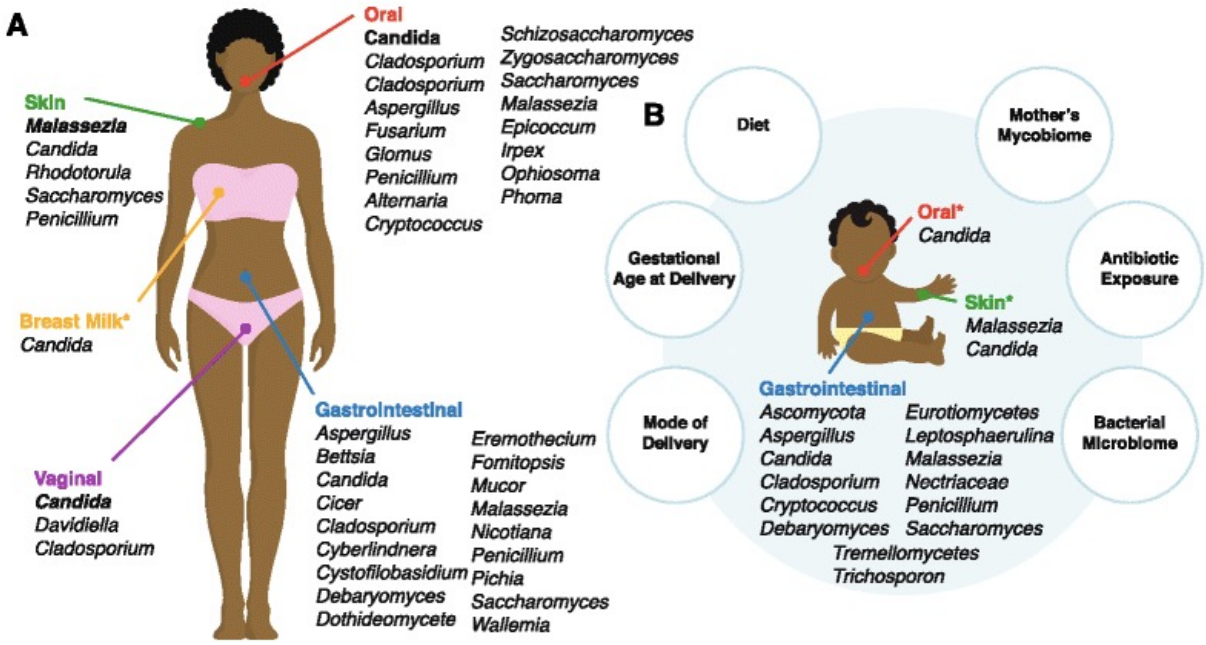
Bacterial communities vary across body sites

phyla



Nature Reviews | Genetics
Cho & Blaser 2012

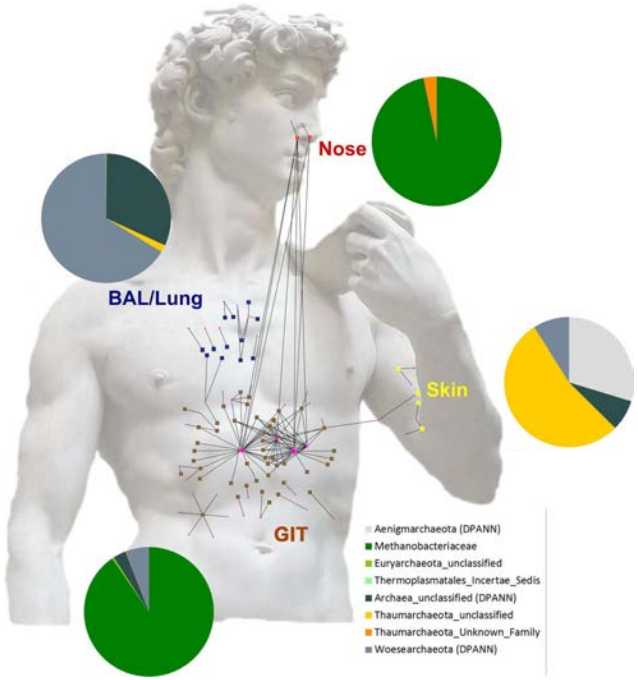
Other microbes are underexplored but also vary across body sites



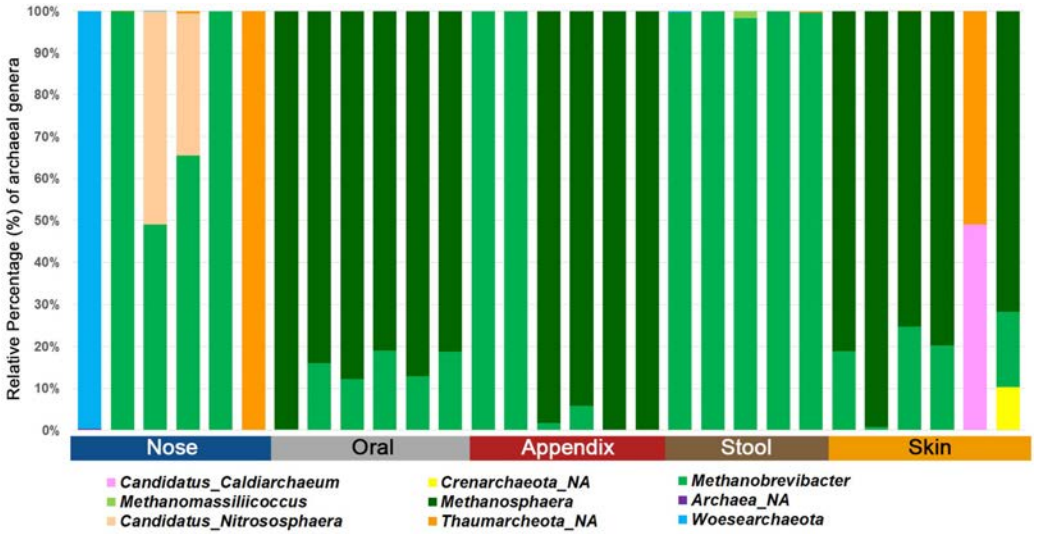
Fungal genera associated with healthy adults and infants. Body sites with an asterisk represent those that have only been characterized by culture or targeted PCR approaches.

Ward et al. 2017. Biomedicine

The archaeome



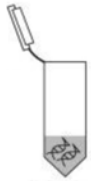
The archaeal taxonomic landscape of the human body visualized as a network



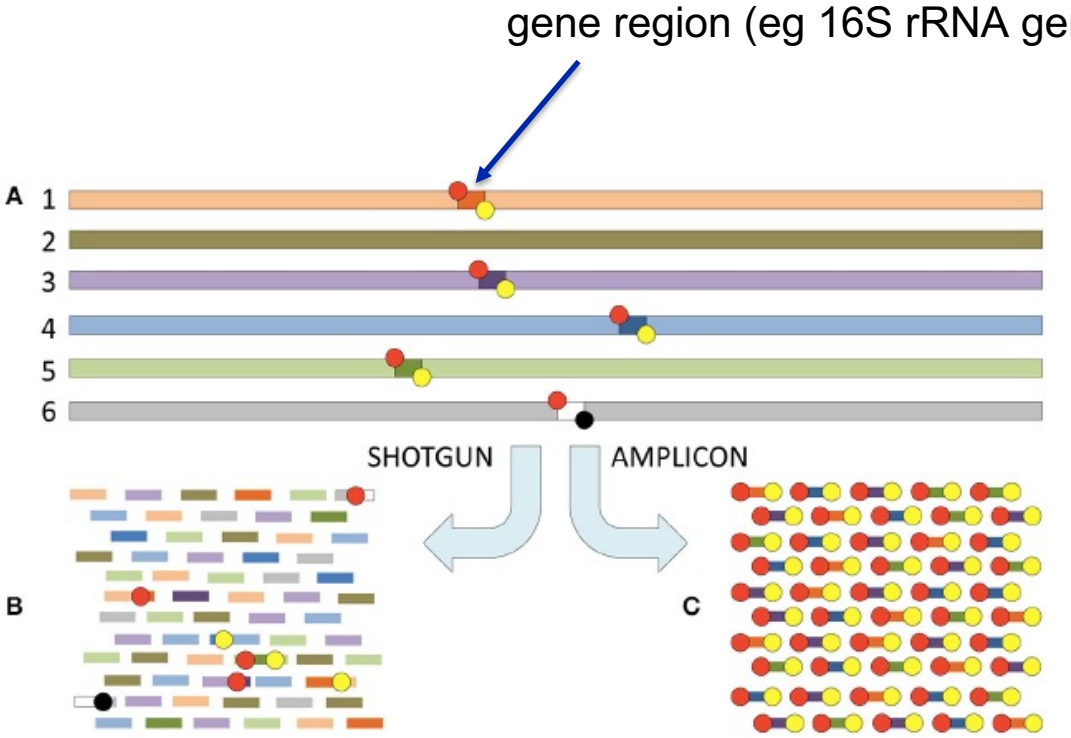
Kaisa Koskinen et al. mBio 2017; doi:10.1128/mBio.00824-17, Pausan et al. 2019 fmib

Shotgun (metagenomic) sequencing versus targeted amplification

sample

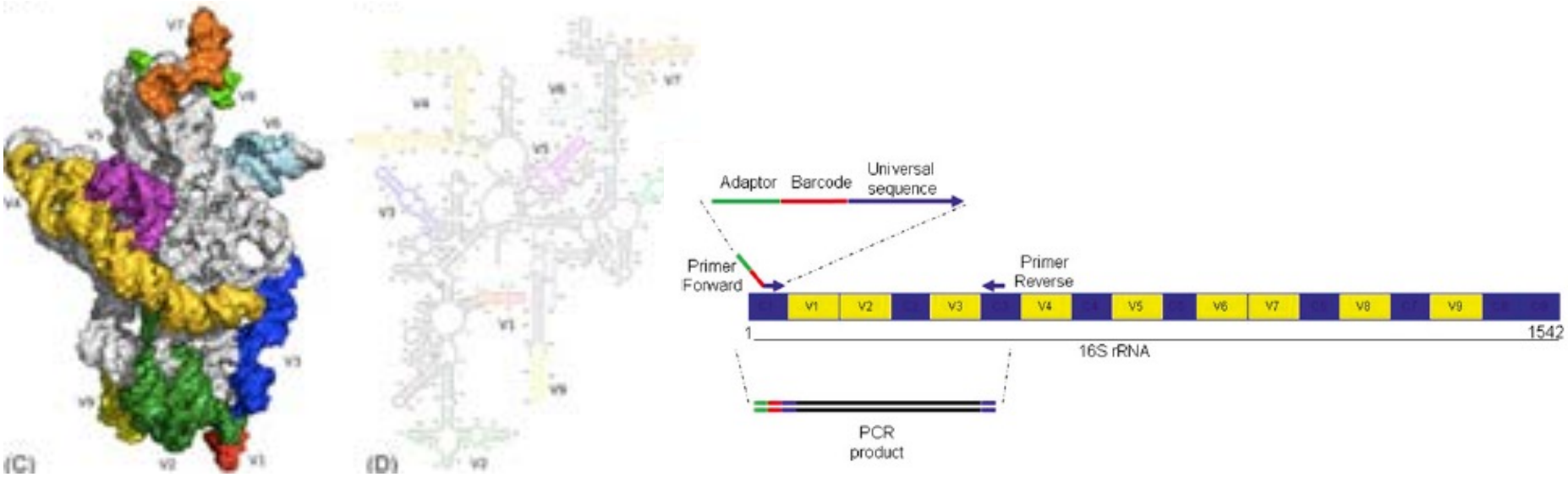


DNA extract



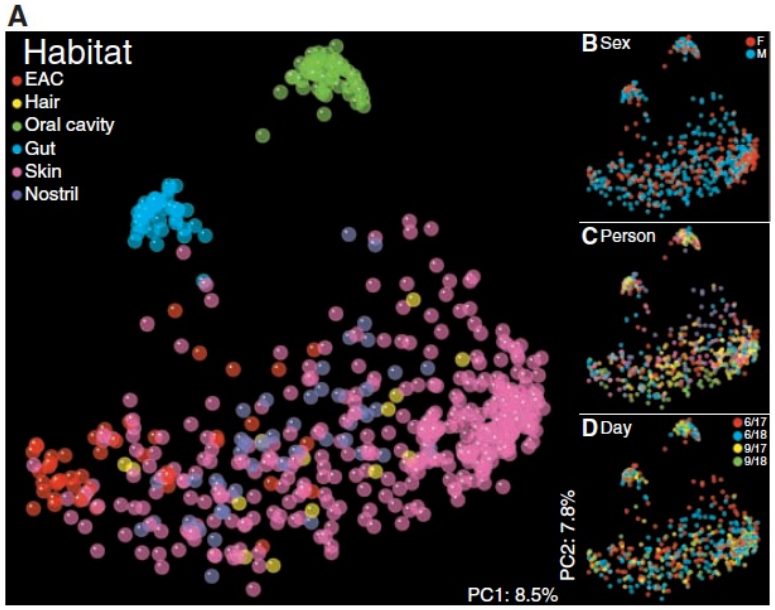
Barbosa et al. 2016, Sekse et al. 2017

Amplicon sequencing of the 16S rRNA gene regions

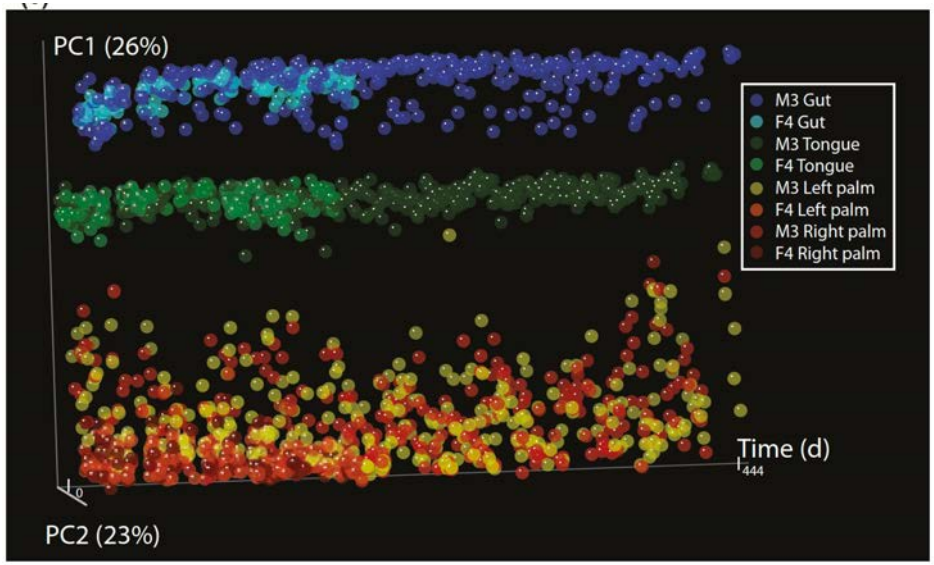


16S rRNA forms part of 30S ribosomal subunit

Early studies of the 16S rRNA gene v4: tissue specificity



6 body habitats (18 skin sites), 9 individuals, twice on 2 consecutive days

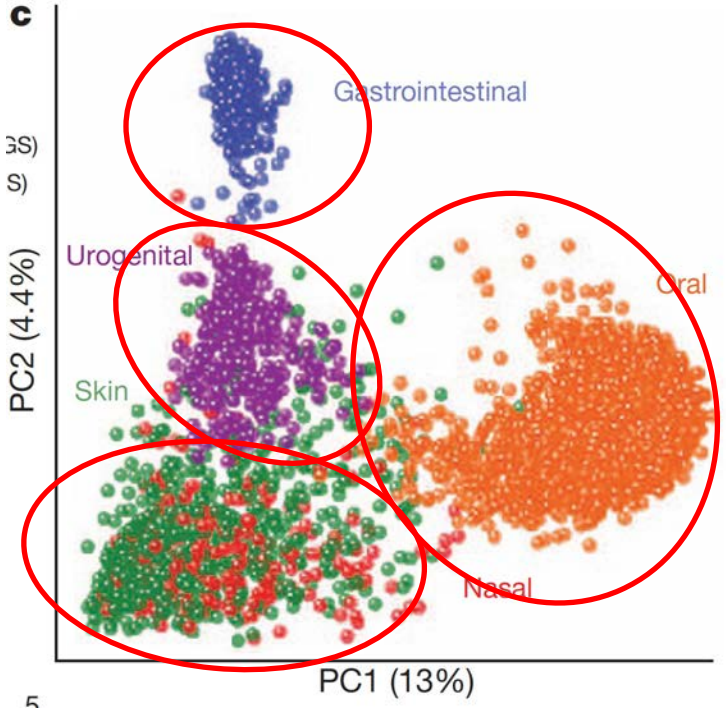


3 body habitats, 2 individuals, daily 15 months

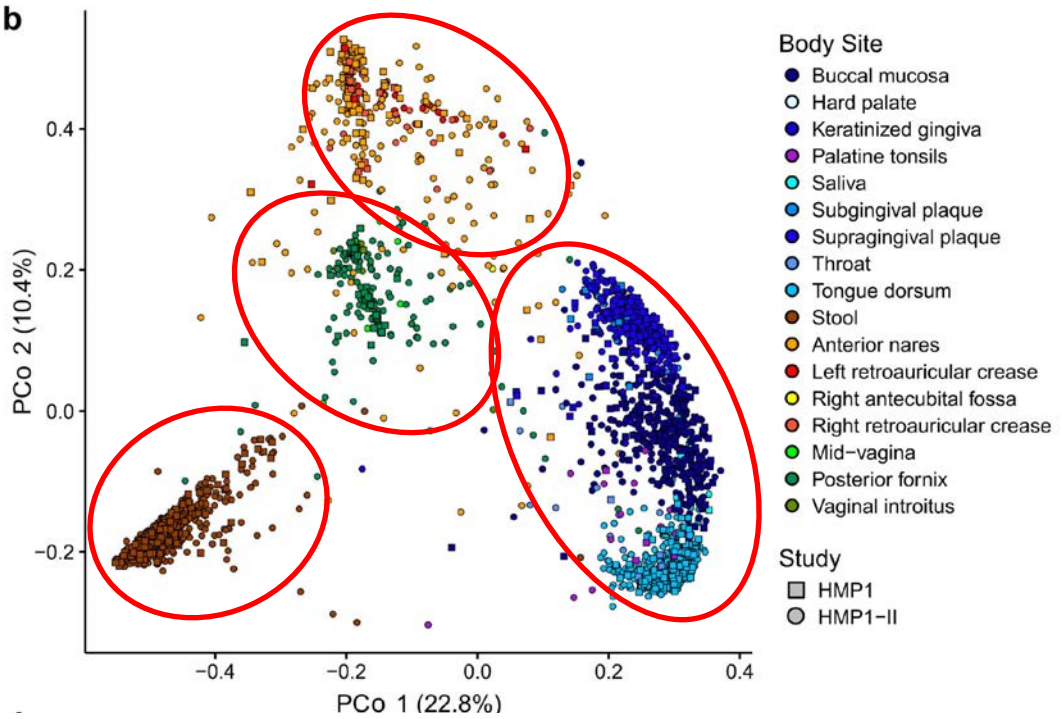
Costello et al. 2016, Caporaso et al. 2011

Human Microbiome Project data: 16S rRNA and shotgun

16S rRNA gene v3v5 (OTUs)



Shotgun (species level)

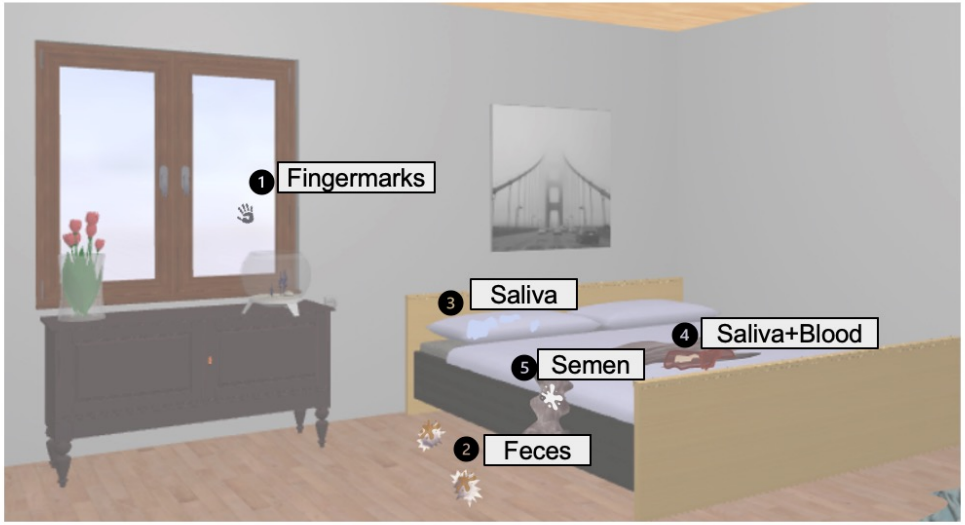


HMP 2012, Lloyd-Price et al. 2017

Applying microbiome tools in forensics

- Possibilities and limitations
- What techniques to use?
- Reporting the evidence

Assessing possibilities and limitations



Biological stains:

- Exposed over time
- On substrates (surfaces, textiles, others)
- As mixtures of various fluids/tissues

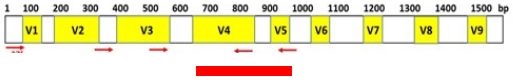
How stable are microbiome signatures over time?


C. Haas


A. Dobay

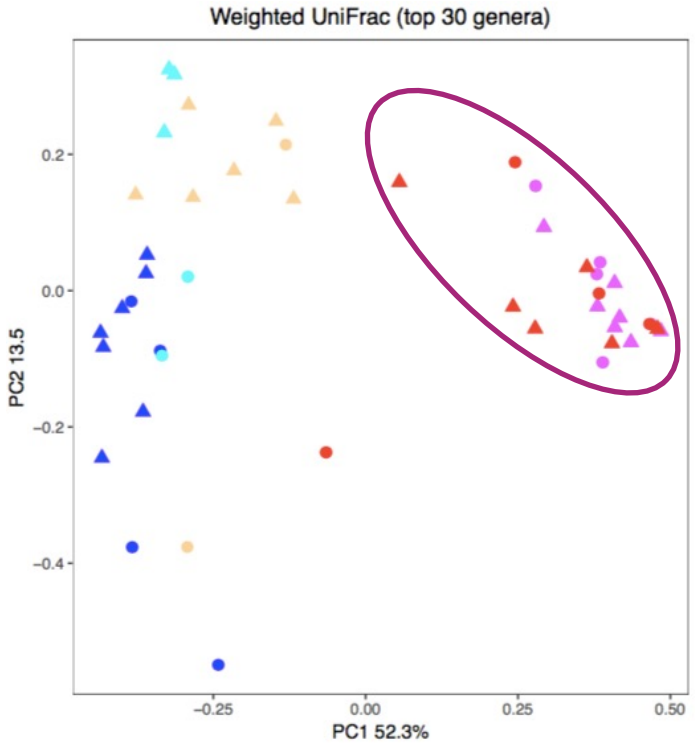
exposed
(1 month)
vs. control

16S rRNA gene sequencing



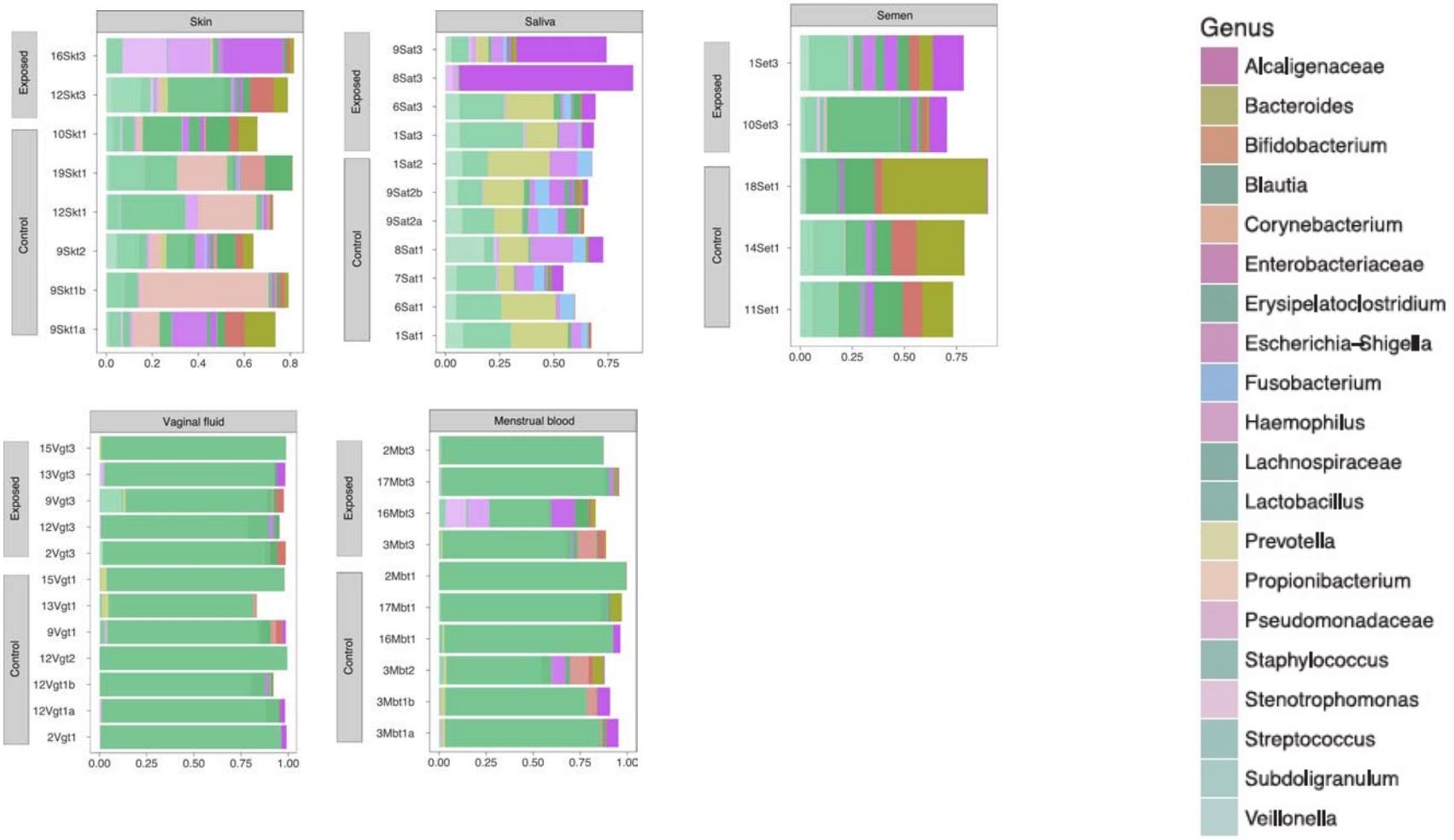
Body site
- menstrualblood
- saliva
- semen
- skin
- vaginalswab

State
- exposed
- control



Dobay et al. 2019 FSI Genetics

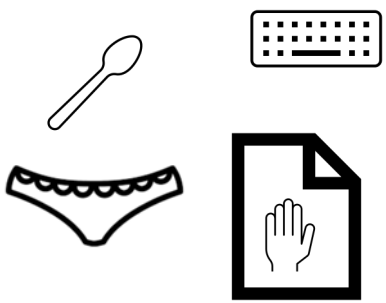
What microbes are found at what body sites?



Aged samples stored dry

Up to 7.5 years

- Vaginal, oral, skin samples
- Collected in tube or swabbed directly
- Swabbed from substrates



vagina

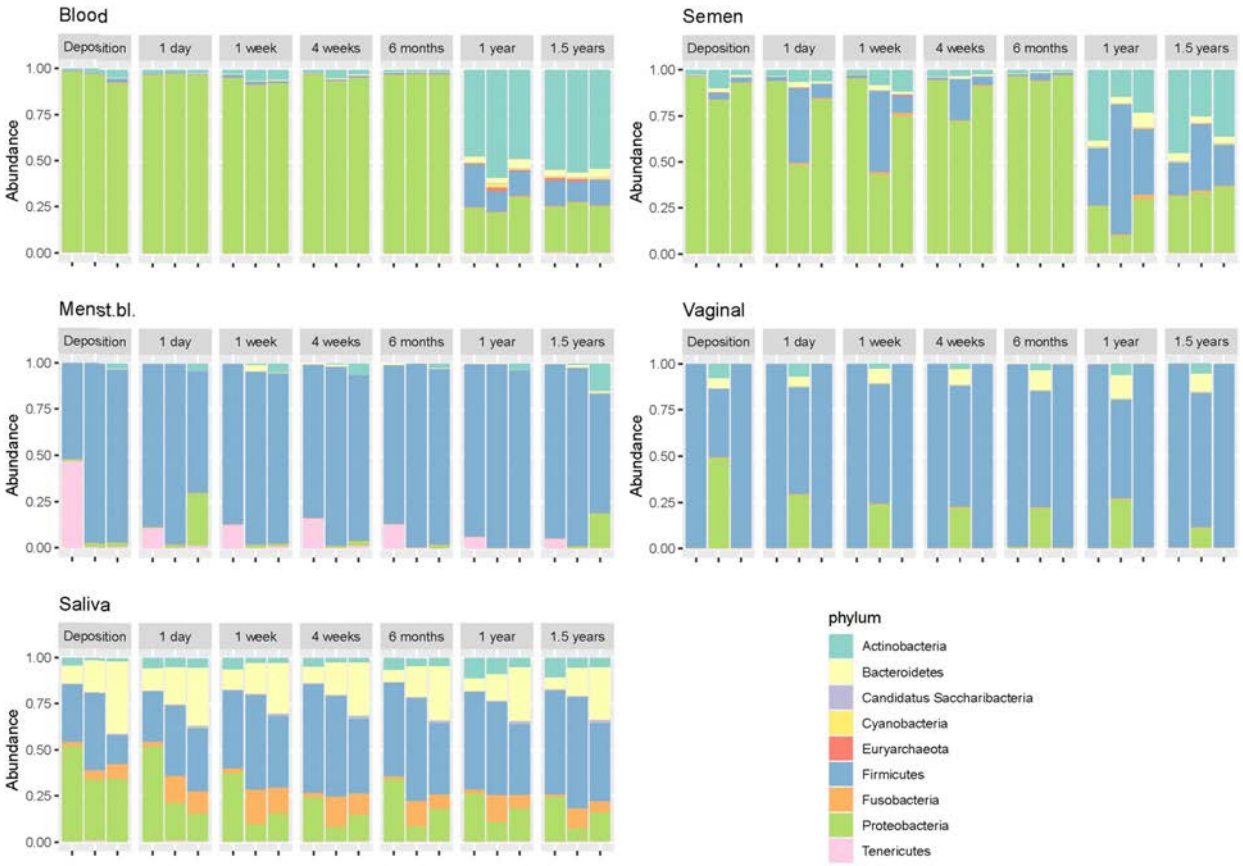
saliva

skin

Predictions with neural networks

Sample ID	Trace storage time (years)	Predicted site probabilities		
		Skin	Oral	Vagina
ZHF11	1	1.87E-05	1.12E-05	0.99997
ZHF7	2	1.64E-01	7.60E-02	0.76002
ZHF3	5.9	0.44223	4.73E-04	0.55730
ZHF20	6	2.00E-05	1.05E-04	0.99987
ZHF19	6.6	4.95E-03	1.51E-05	0.99504
ZHF16	7	6.44E-03	1.77E-04	0.99339
ZHF10	1	3.64E-06	0.99999	2.87E-06
ZHF5	2	3.87E-06	0.99999	2.71E-06
ZHF6	2	3.72E-06	0.99999	2.76E-06
ZHF24	5	3.61E-06	0.99999	2.96E-06
ZHF2	7	3.66E-06	0.99999	2.96E-06
ZHF17	7	3.18E-05	0.99997	2.96E-06
ZHF18	7	4.11E-06	0.99999	2.69E-06
ZHF14	7.6	3.61E-06	0.99999	2.93E-06
ZHF15	7.6	0.99977	2.21E-04	1.19E-05
ZHF12	1	0.99999	7.76E-06	6.45E-06
ZHF8	2	0.99999	7.38E-06	6.29E-06
ZHF4	4.9	0.99999	7.66E-06	6.44E-06
ZHF21	5	0.99999	7.36E-06	6.38E-06
ZHF22	5	0.99998	1.66E-05	7.11E-06
ZHF23	5	0.99998	8.06E-06	7.07E-06
ZHF25	5	0.99999	7.47E-06	6.27E-06

Body fluids exposed for up to 1.5 years



C. Haas

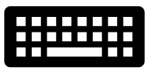
A. Salzmann

3 donors per body site
Samples exposed indoors and outdoors

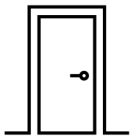
What about substrates?

F1 vagina

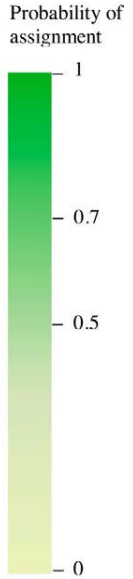

F2 saliva




F3 skin



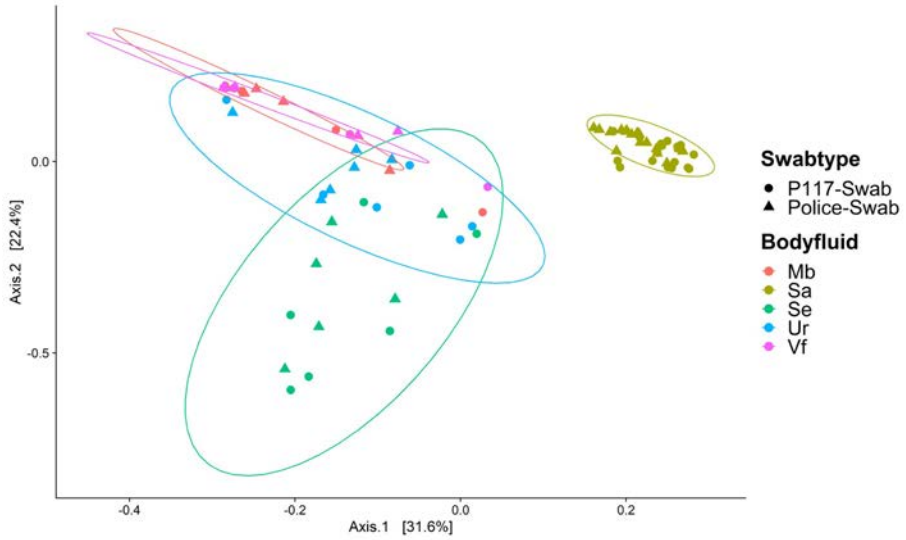

Sample ID	Trace storage time (years)	Predicted site probabilities		
		Skin	Oral	Vagina
F1A	0	1.92E-05	1.11E-05	0.99997
F1B	0	1.87E-05	1.12E-05	0.99997
F1C	0	1.67E-05	1.24E-05	0.99997
F1D	0	1.79E-05	1.15E-05	0.99997
F1E	0	2.57E-05	1.03E-05	0.99996
F2A	0	3.64E-06	0.99999	2.92E-06
F2B	0	3.59E-06	0.99999	2.96E-06
F2C	0	3.69E-06	0.99999	2.82E-06
F2D	0	3.62E-06	0.99999	2.91E-06
F2E	0	3.58E-06	0.99999	2.98E-06
F2F	0	3.61E-06	0.99999	2.94E-06
F2G	0	3.58E-06	0.99999	2.98E-06
F2H	0	3.62E-06	0.99999	2.91E-06
F2I	0	3.70E-06	0.99999	2.83E-06
F2J	0	3.60E-06	0.99999	2.94E-06
F3A	0	0.99999	7.44E-06	6.29E-06
F3B	0	0.99999	7.44E-06	6.26E-06
F3C	0	0.99999	7.53E-06	6.32E-06
F3D	0	0.99998	8.65E-06	6.46E-06

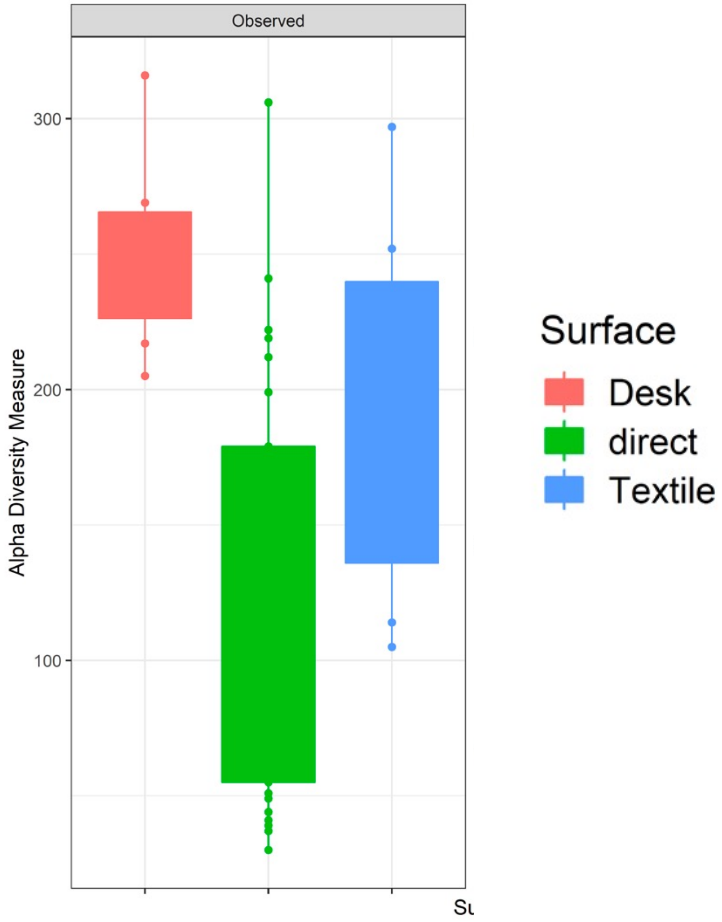


Diez-Lopez et al. 2019 FSI Genetics

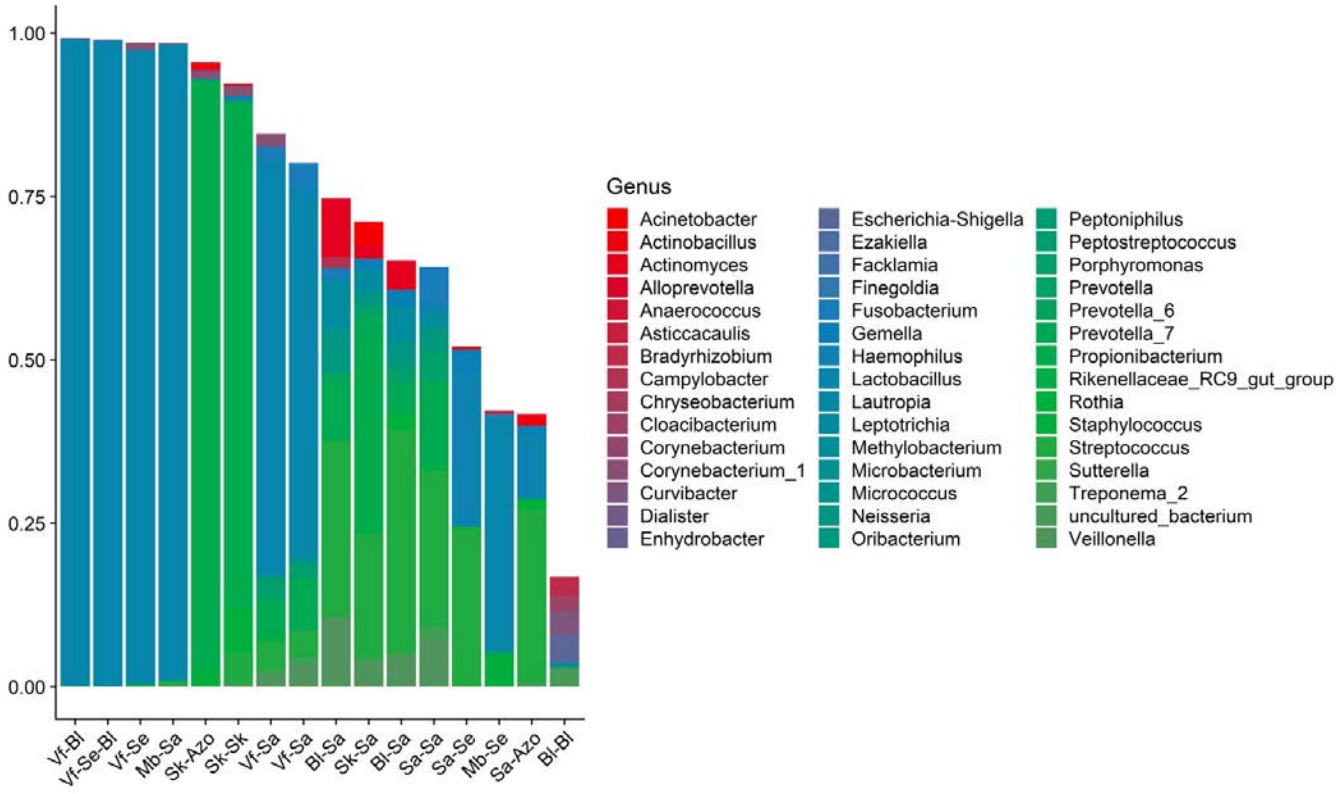
Saliva on surfaces

Saliva samples
- collected directly
- swabbed from desks
- swbbed from textiles





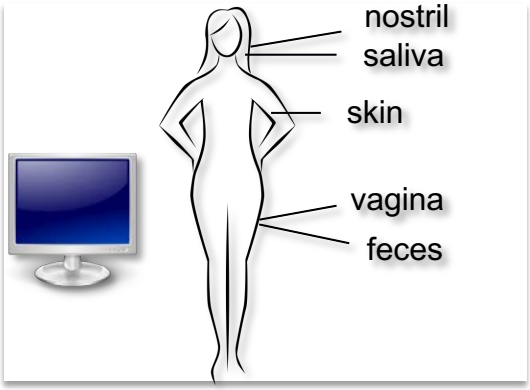
What about mixtures of more than one body fluid/tissue?



From characterisation to prediction

J. Tackman

J. F. Matias



Machine Learning Methods

Check Prediction Accuracy

ca. 15,000 samples
ca. 50 studies
Different types of data:
- 16S rRNA regions

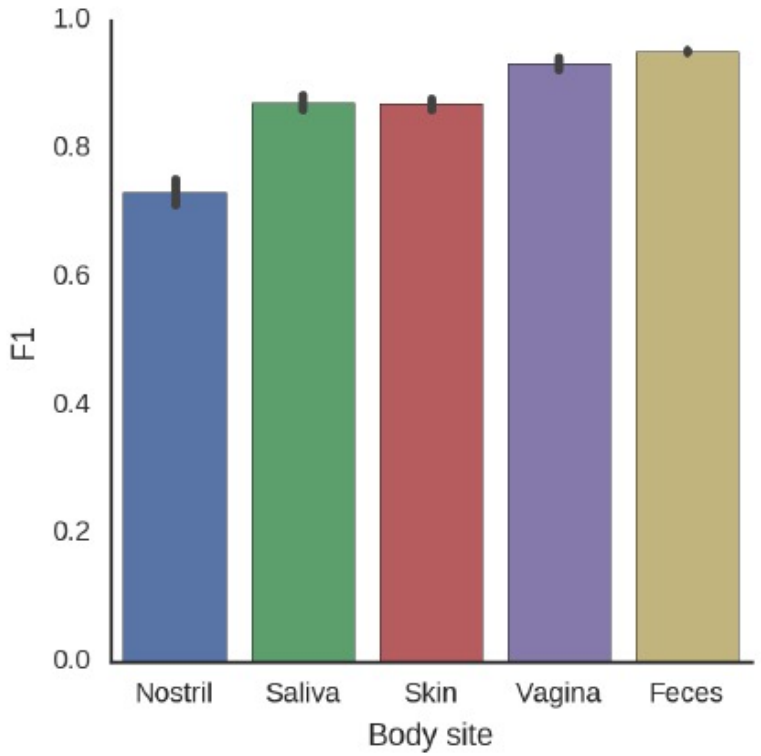
Random Forest Classifier

Single source

Mixture

Tackmann et al. 2018

Random Forest Classifier trained on global data has high prediction accuracy

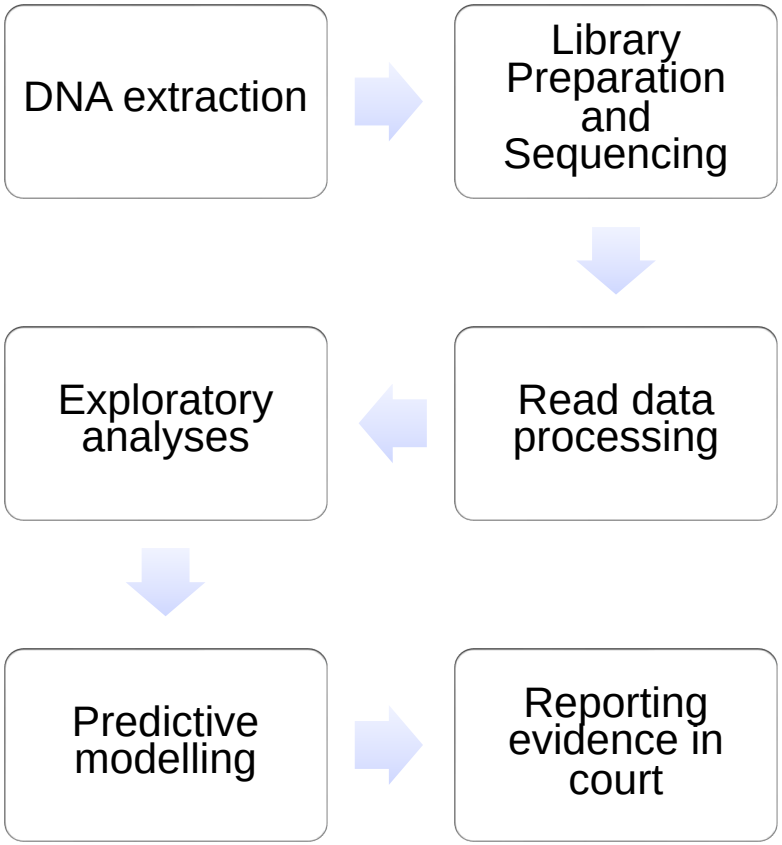


F1

Precision = $\frac{\text{Correct positive}}{\text{Total positive}}$

Recall = $\frac{\text{Correct positive}}{\text{Total body site}}$

What techniques to use? Establish workflows



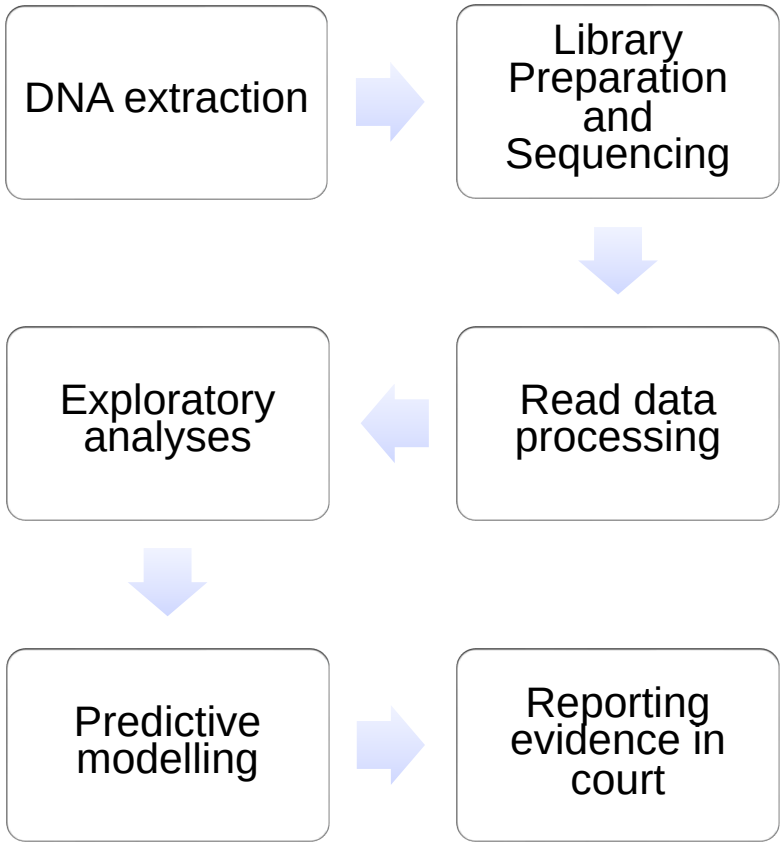
Establish a standard operating procedure (SOP)

Laboratory workflow
Bioinformatics workflow

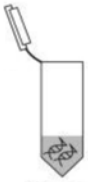
Issues:

- Validate a method
- Fast development of new methods and software, new recommendations

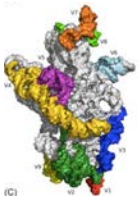
What techniques to use? Establish workflows



DNA extraction: which protocol enables streamlining human DNA profiling and microbiome profiling?



Library preparation: which region of the 16S rRNA gene to use?



Read data processing: cluster into operational taxonomic units (OTUs) or use amplicon sequence variants (ASVs)?

OTUs vs ASVs

Predictive modelling with machine learning: which classification method to use eg random forests, neural networks?

Reporting the evidence in court: DNA profile matches

Evidence = DNA profile

STR profile	Crime scene stain	Suspect
Locus 1	14,16	14,16
Locus 2	16,17	16,17
Locus 3	XY	XY
Locus 4	12,16	12,16

Hp = DNA from suspect
Hd = DNA from another person

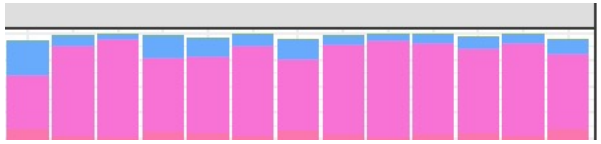
Likelihood ratio $\frac{P(E|H_p)}{P(E|H_d)}$ $\frac{1}{x}$

Probability of evidence given Hp =1

Probability of evidence given Hd
= 1 in 100
= 1 in 10,000
= 1 in 100,000

Reporting the evidence in court: microbiome analyses

Evidence = microbiome composition



Probability stool 0.9
Probability blood 0.01

Hp = defecated after burglary
Hd = other bodily source (appeared randomly)

Likelihood ratio $\frac{P(E|H_p)}{P(E|H_d)}$ $\frac{0.9}{x}$

Probability of evidence given Hp =0.9

Probability of evidence given Hd

How to take uncertainties in the predictive models into account?
What if we have mixtures?

Contents

Outlook for the not-so-distant future

Only a few steps away from doing microbiome analyses in forensic labs: what is missing?

Test the reliability of microbiome based methods (especially limitations)

- Larger sample sizes
- Different conditions
- Different donors

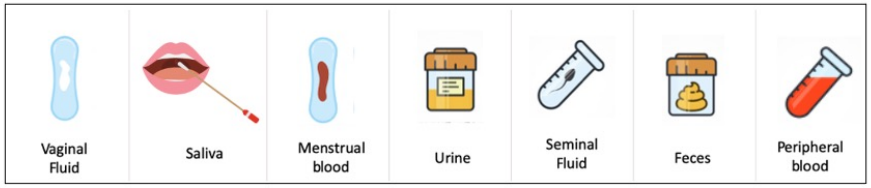
Establish an SOP including laboratory and bioinformatic workflows

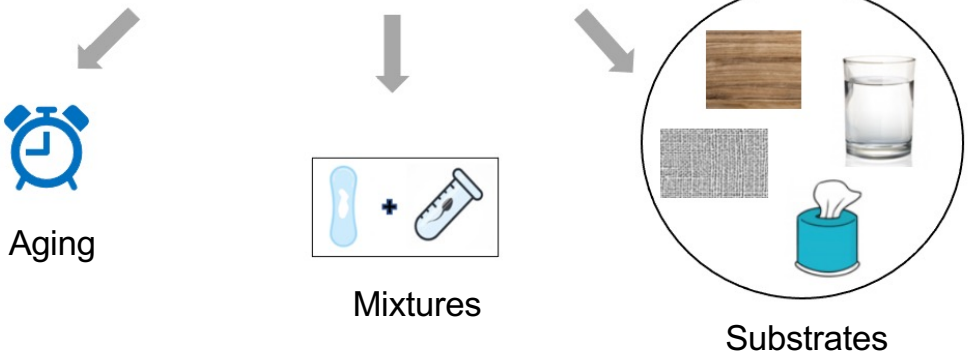
Predictive modelling: establish a reference database to train machine learning algorithms

Collaborative network of forensic scientists to conduct systematic studies

Integrate microbiome with metabolome data

Larger sample size: 20 donors per fluid/tissue





Aging

Mixtures

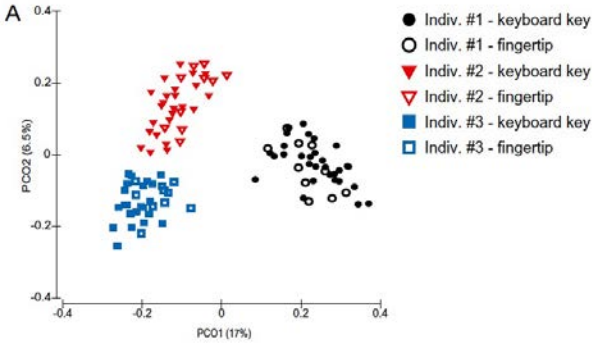
Substrates

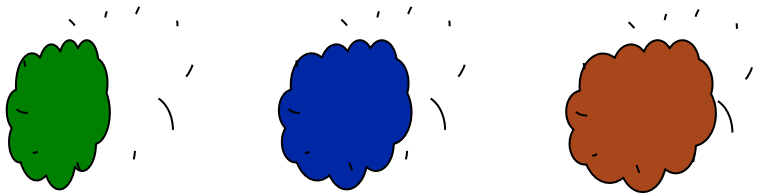
PhD student
Meghna
Swayambhu

Prof. Rolf
Kümmerli

Expand to other applications for stain analyses?

Individual identification





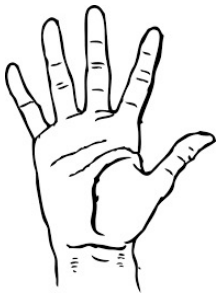
Dolosigranulum; Streptococcus: Lactobacillus

Image Credits: Steve Miller (CIRES); Viputheshwar Sitaraman (phys.org); Fierer et al. PNAS; Meadow et al. PeerJ.

Forensic panel for individual identification from skin

hidSkinPlex (Schmedes et al. 2017)
286 bacterial and phage markers
>65% markers are from Propiniobacterium acnes - cause of acne

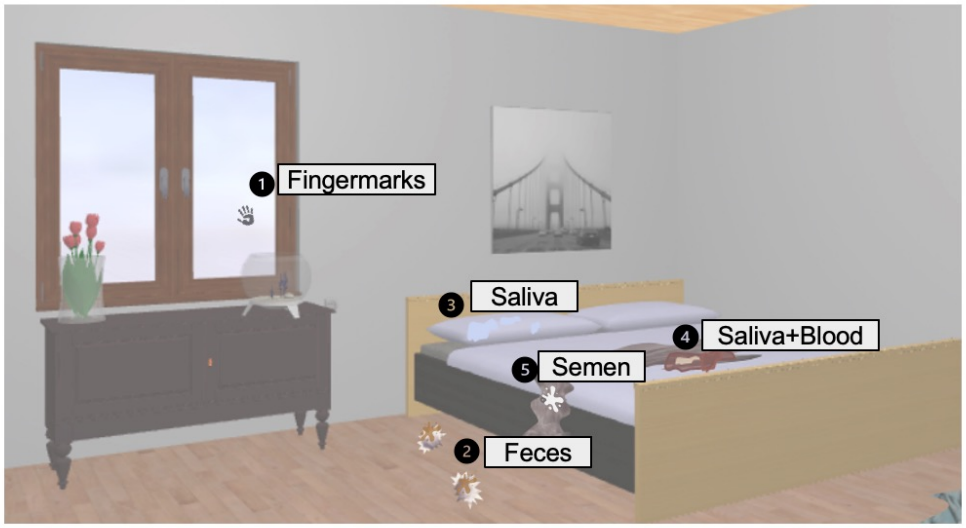
Tested: 8 individuals, 3 samples from Hand, Collar, Foot



Classification accuracy	96-100%	71-96%	54-83%

Expand to other applications for stain analyses?

Time since deposition (TsD)



C. Haas

L. Walser

16S rRNA amplicon sequencing:

Simultaneous identification of body fluid and TsD?

Caveat: Mixtures

Investigative leads on stain donors?

Microbiome analyses contain information on health

- Example: donor of skin stain → microbes associated with skin conditions?
- Ethical and legal issues

Clinical diagnostics:

- Stool microbiome
  - Inflammatory bowel disease
  - Colorectal cancer
- Urine microbiome
  - Prostate cancer high risk patients and metastatic

Summary

- Almost ready to use microbiome analyses for body fluid identification in forensic laboratories: valuable complement or alternative
- Feasibility of other applications related to stain analyses
  Eg individual identification and TsD
- Potential for other applications: geo-location, post-mortem interval, others
- Clinical and forensic applications face similar challenges to incorporating new method
  - Understanding how well it works in the applied setting (forensic setting)
  - A (simple) validated SOP

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Images

Links to images

1. Microscope: https://easydrawingguides.com/how-to-draw-a-microscope/
2. https://www.amnh.org/explore/science-topics/microbiome-health/meet-your-microbiome
3. https://kids.britannica.com/students/assembly/view/204048
4. http://www.scientificanimations.com/wiki-images/
5. https://depts.washington.edu/molmicdx/mdx/tests/NGS16S.shtml
6. https://medium.com/analytics-vidhya/create-a-deep-neural-network-from-scratch-15c9b07c1388