



Netherlands Forensic Institute
Ministry of Justice

Microbial population analysis in forensic investigations

Ir. Frederike Quaak, MSc
NFI – Non Human Biological Traces

25 April 2015



Netherlands Forensic Institute



Independent national institute, residing under Ministry of Security and Justice

Three key roles:

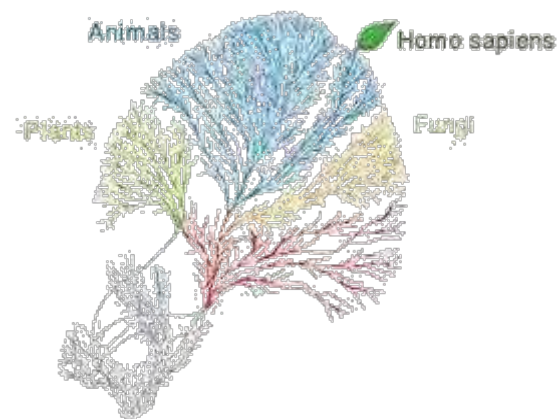
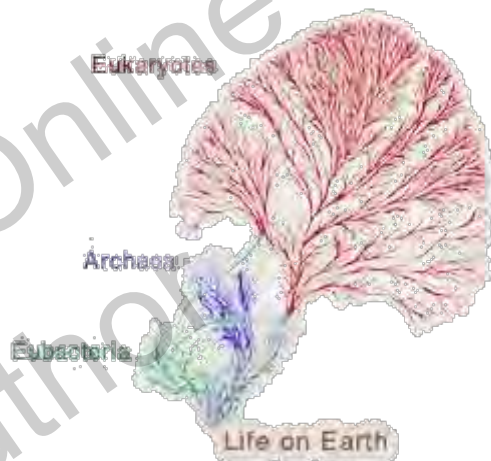
- conducting examinations in criminal cases
- conducting research & development
- being a centre of knowledge and expertise



Non Human Biological Traces department

Examination of all kinds of biological traces

- plants, animals and micro-organisms
- whole organisms or small pieces
- individual organisms or populations



10 employees

Approx. 140 cases per year



Forensic microbiology

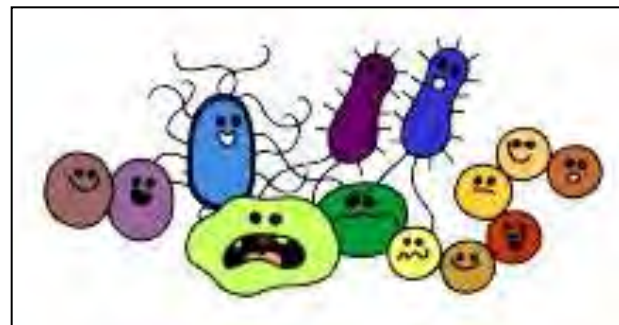
Using specific micro-organisms or microbial populations in forensic investigations

Micro-organisms are most abundant forms of life on earth

- over 10^{30} bacteria present on earth!
- large numbers present in small samples
- organisms or populations are specific to habitat or niche

More attention to forensic microbiology since 2001

Driven by technological developments





Forensic microbiology at NFI

- Deliberate infections/bioterrorism
- Linking traces to each other, to suspect(s) or to crime scene
 - Homicide, sexual assault cases, burglaries
- Determination of origin of a human trace
 - Homicide, sexual assault cases
- Post Mortem Interval estimation



Forensic microbiology challenges

Typical challenges in forensics:

- Non ideal samples
 - Degraded
 - Contaminated/mixed
 - Etc.
- Very small sample sizes
- No databases available
- Criminalistic challenges in data interpretation and evaluation of the evidential value
 - Never 100% match
 - Biological (and geological) variation
 - Differences due to sampling, degradation, random variation



Revolution in DNA techniques

Started with techniques like DGGE, tRFLP

- Bacterial marker 16S
- Fungal marker 18S
- No (direct) information on taxonomy
- tRFLP easily incorporated in forensic DNA lab (equipment is present)

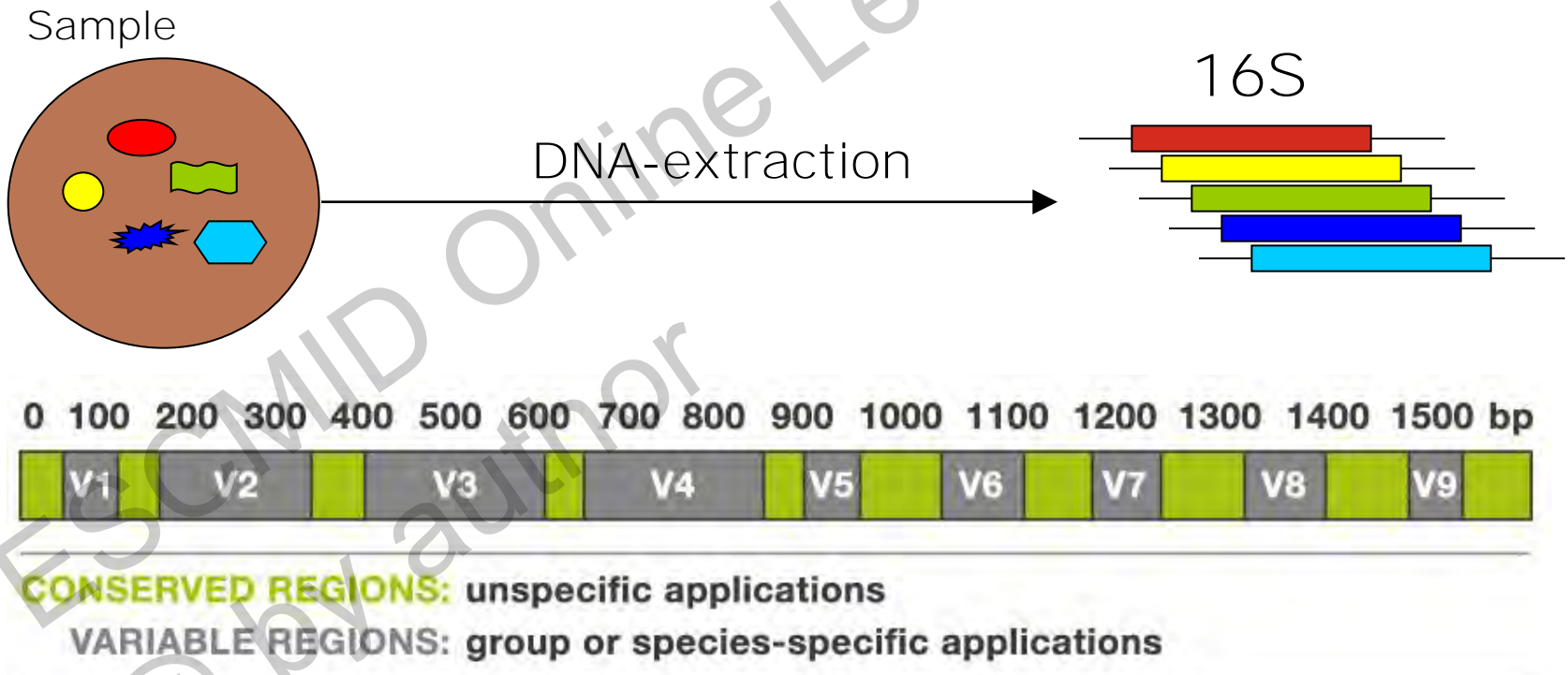
Followed by microarray analysis and NGS

- Taxonomy information
- More complex techniques
 - Interpretation!



16S rDNA

- 16S rDNA: parts are hypervariabel -> species specific sequence





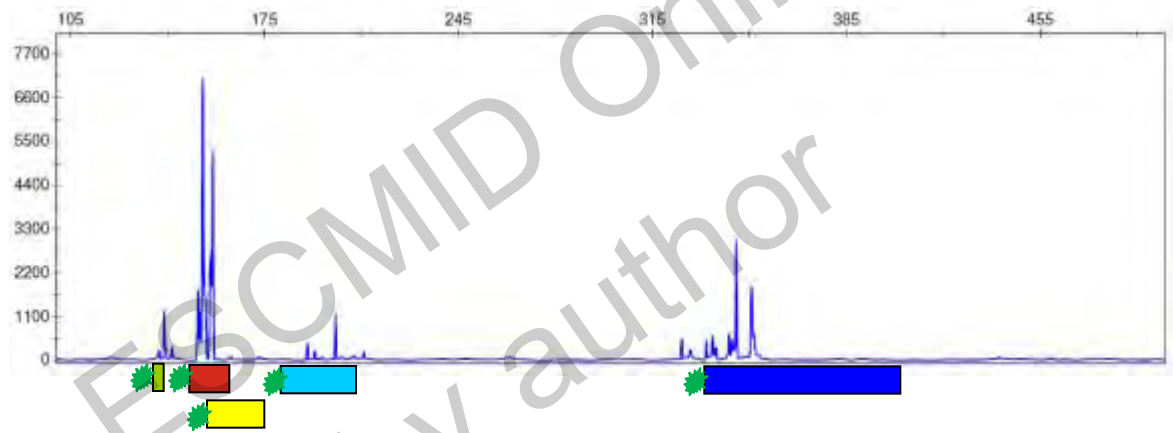
T-RFLP analysis

16S

Amplification 16S gene with PCR

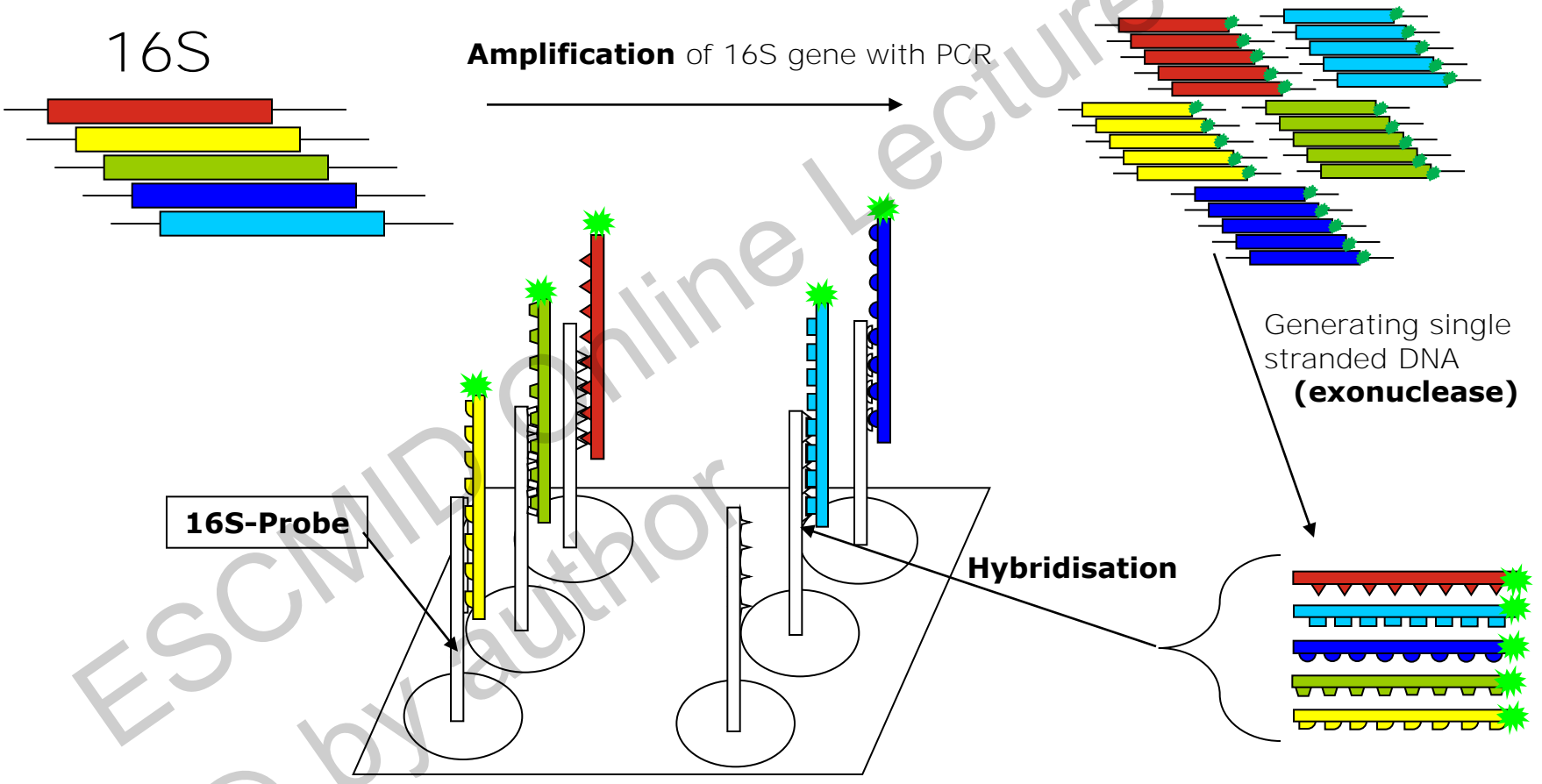
Digestion of DNA fragments with restriction enzyme

Separation based on fragment length





Microarray analysis

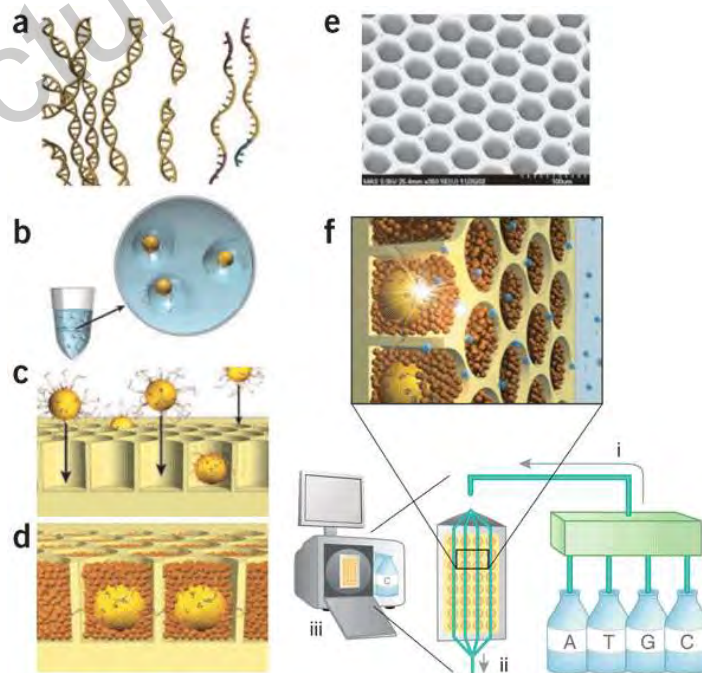




Next Generation Sequencing

Complex communities in one run
Sanger sequencing only one species at a time

De Novo sequencing of whole genomes





Linking traces to each other or suspect(s)

- Soil comparisons
 - Linking shoeprint to shoe
 - Linking spade to grave
 - etc.



Statistical data analysis of bacterial t-RFLP profiles in forensic soil comparisons

Frederike C.A. Quaak*, Irene Kuiper
Netherlands Forensic Institute, Laan Van Veenendaal 6, 3720 ZG The Hague, The Netherlands

- Linking human traces to donor based on microbial populations
 - Faecal microbial population
 - etc.



Soil comparisons at NFI

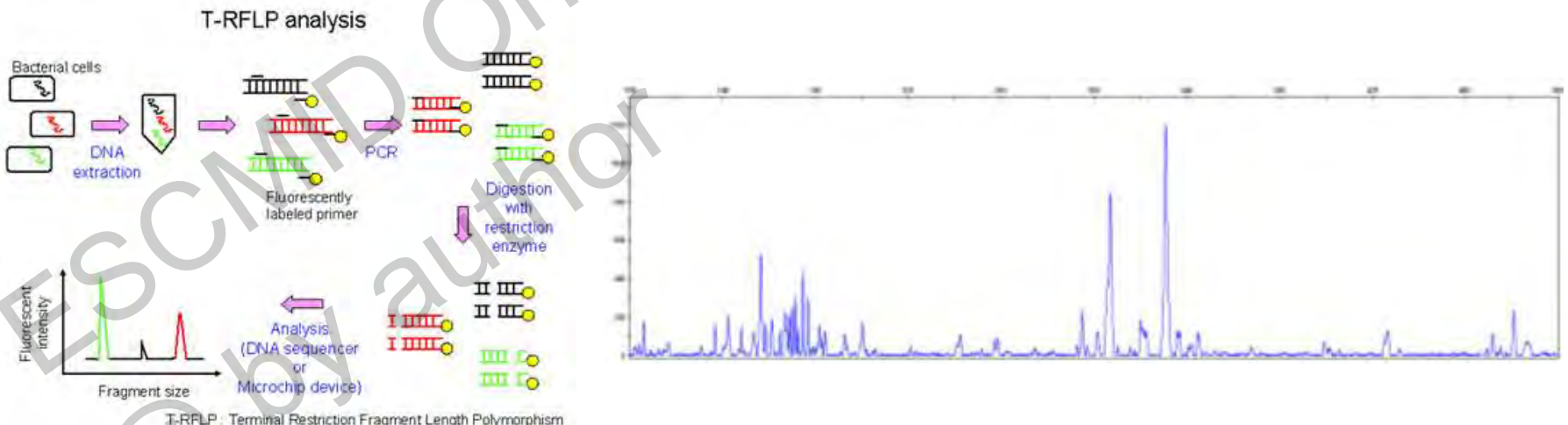
Soil is a mixture of many different types of traces in varying concentrations.





Microbial population analysis in soil comparisons

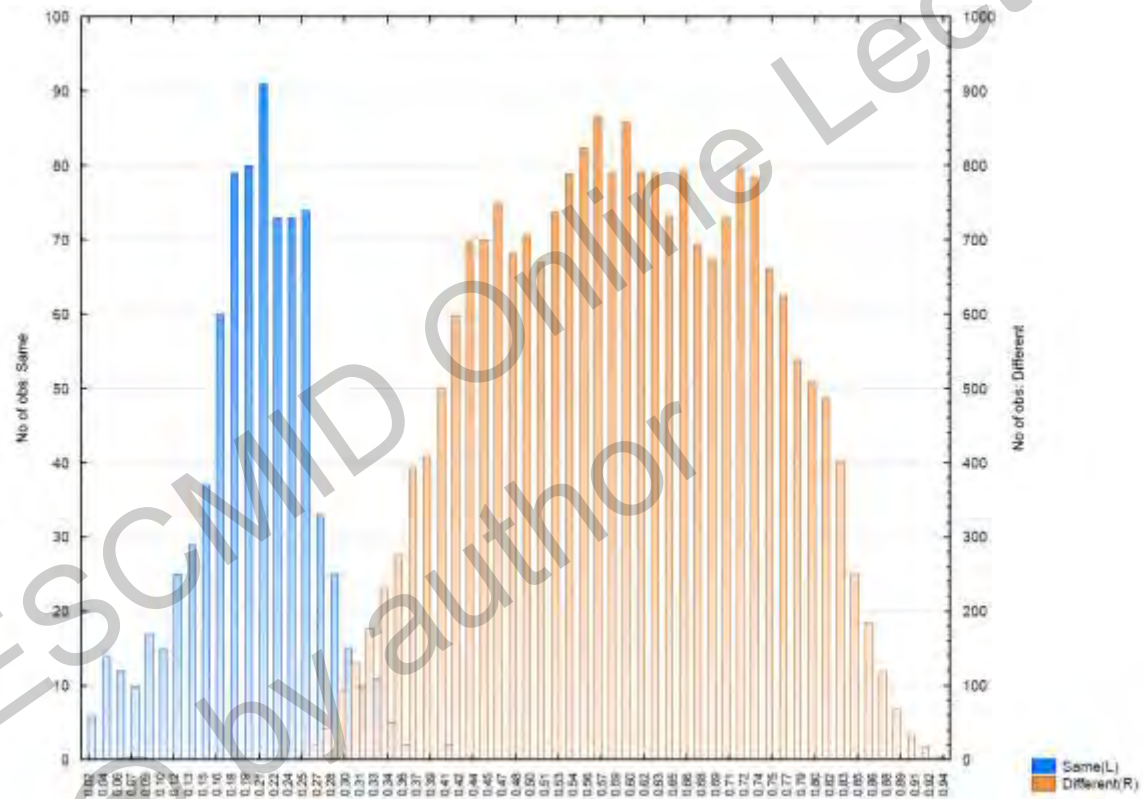
- 250 µg soil
- Direct DNA-extraction
- Amplification bacterial marker
 - > 16S rDNA, 1 labeled primer
- Restriction 16S rDNA
- Detection of labeled fragments with capillary electrophoresis





Challenges in soil comparisons

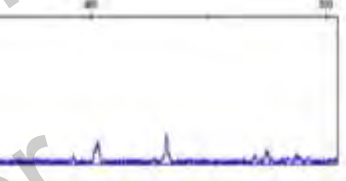
- Comparison of profiles by calculating BC-distance
- Model for evidential value determination, score based LR





Case example – Attempted rape

Woman dragged into bush, started screaming. Suspect ran away, but was apprehended by the police





Human individualisation by comparison of microbial populations

For some body sites it is known that populations are unique to individuals

Forensic identification using skin bacterial communities

Noah Fierer^{a,b,1}, Christian L. Lauber^b, Nick Zhou^b, Daniel McDonald^c, Elizabeth K. Costello^c, and Rob Knight^{a,b}

^aDepartment of Ecology and Evolutionary Biology, ^bCooperative Institute for Research in Environmental Sciences, and ^cDepartment of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309; and ¹Howard Hughes Medical Institute

→ samples from computer keys or mouse could be linked to reference samples

Development of method for faecal comparison

- Very few human cells in faeces
- 20-50% dry weight bacteria
- Comparison of stain on item with reference sample

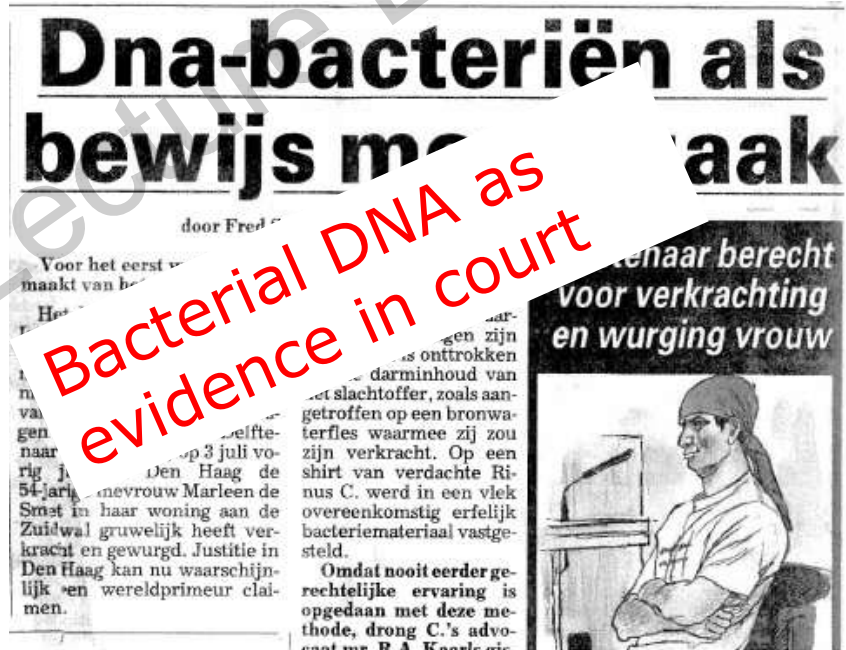


Case example - Homicide

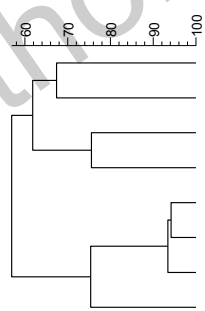
Female body discovered, possibly raped

Suspect apprehended with brown stains on his shirt

Could material on shirt have come from the faecal material found on the victim?



Pearson correlation (Opt:1.50%) [0.0%-100.0%]
DGGE DGGE

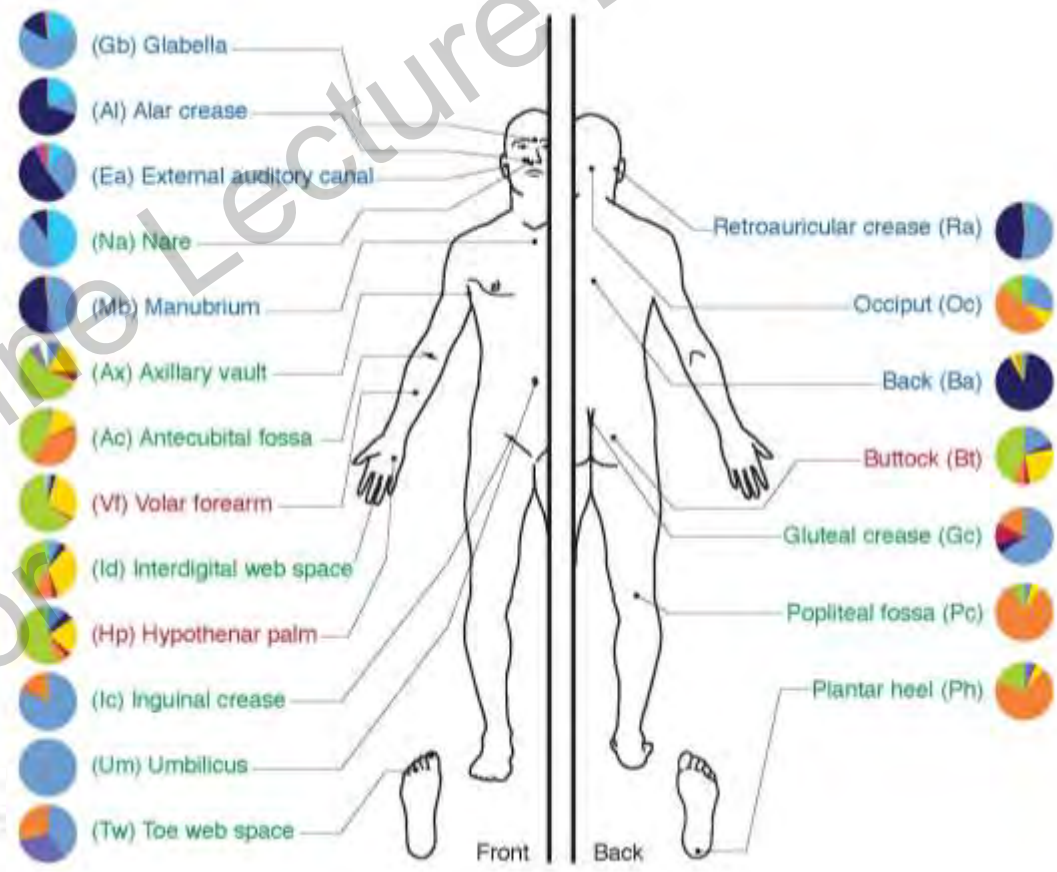


- 1903 Referentiemateriaal
- 200 Feces broek
- 0909 Referentiemateriaal
- 2512 Referentiemateriaal
- 202 Feces op stukjes shirt
- 603 Feces van fles
- 2606 Referentiemateriaal
- 2005-309 Uitstijpke



Determining origin of a trace

- Bacterial cells outnumber human cells 10 to 1
- Communities fairly unique to body site
- At some body parts larger and more diverse communities



Vaginal microbial flora analysis by next generation sequencing and microarrays; can microbes indicate vaginal origin in a forensic context?

Corina C. G. Benschop • Frederike C. A. Quak • Mathilde E. Bonn • Tina Sijpe • Irene Kuper



Case example – What could this stain be?

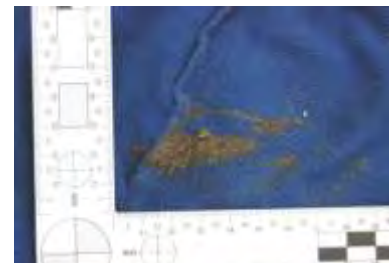
Shirt with brown stains in it found in trashcan at victim's house.
Could the stains have originated from faecal matter?

Morphological examination:

- Lots of brown fibers
- Seed-like fragments

Bacterial examination:

- Direct DNA extraction
 - Bacterial profiling using microarrays
- Faecal microbial population pattern





Further research at NFI

- Development of a forensic microbial population array
 - Determining the origin of a sample using micro-organisms (by excluding other sources for the population)
 - Ideally also to be used for comparison of (faecal) samples
- Development of a statistical method to determine the (human) origin of a sample in a probabilistic manner
- Development of a statistical method to compare faecal samples, and maybe even samples from other body sites?
- Exploring of the use of microbial populations in PMI determinations
 - on and inside corps
 - in the ground surrounding/underneath corps



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