



LEIDEN UNIVERSITY MEDICAL CENTER

MetaFor

Metagenomic analysis of Forensic biological traces

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Metafor

NWO Forensic Science grant.

Metagenomic analysis of Forensic biological traces.

- Analyse all present DNA in a forensic trace.
- Single molecule sequencing.

Pilot *ancient DNA* conducted at the LUMC.

<http://www.nwo.nl/forensicscience/>

Current approach



Figure 1: HiSeq 2000.

Current approach



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Figure 2: Flowcell.

Current approach



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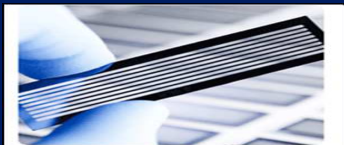


Figure 2: Flowcell.

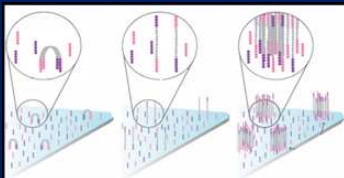


Figure 3: Amplification.

Helicos true Single Molecule Sequencing



Characteristics:

- No amplification.
- Short reads (± 32 bp).
- Relatively high error rate ($\pm 4\%$).
- Direct RNA sequencing.

Figure 4: HeliScope.

Single molecule sequencing

Pacific Biosciences Single Molecule, Real-Time



Figure 5: PacBio RS.

Pacific Biosciences Single Molecule, Real-Time

Characteristics:

- Long reads (several kilobases).
- High error rate (15-20%).
- Relatively high throughput (comparable with the Roche 454).

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Circular consensus sequencing.

- Sequence the same molecule several times.
- Extremely high accuracy.
- Acceptable read length (± 250 bp).

Ancient DNA

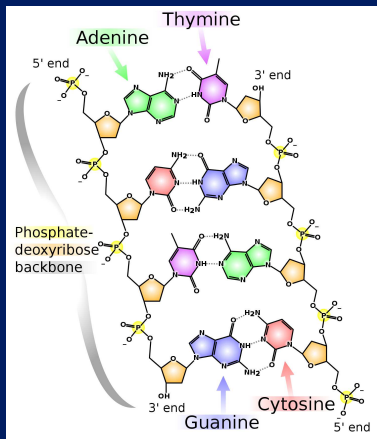


Figure 6: Structure of DNA.

DNA damage:

- Physical:
 - Fragmentation.
- Chemical:
 - Oxidation.
 - Hydrolysis (deamination, depurination, and depyrimidination).
 - ...

Mixed DNA.

- Hard to find with NGS.

Full genome analysis

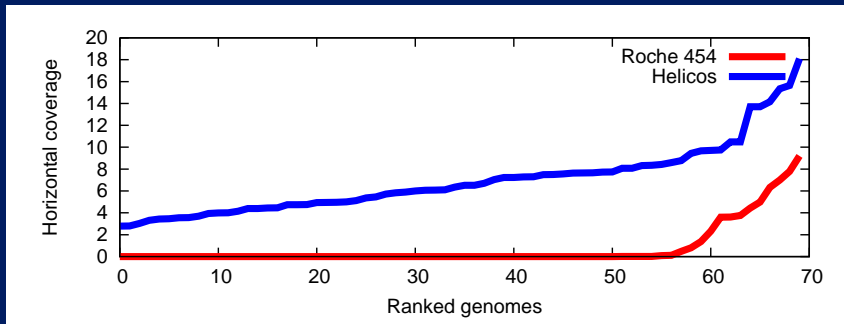


Figure 7: Horizontal coverage of ranked genomes.

Connection with metagenomic DNA

What applies for ancient DNA, also applies for mixed samples i.e., a metagenome:

- Human DNA has a GC percentage of around 40%.
- E.coli DNA has a GC percentage of around 50%.
- May vary greatly for the organisms of interest.

Apart from the biases, we also have a hard time to find under-represented species.

Therefore, single molecule sequencing.

Acknowledgements:

Lusine Khachatryan
Irina Pulyakhina
Peter-Bram 't Hoen
Johan den Dunnen
Peter de Knijff