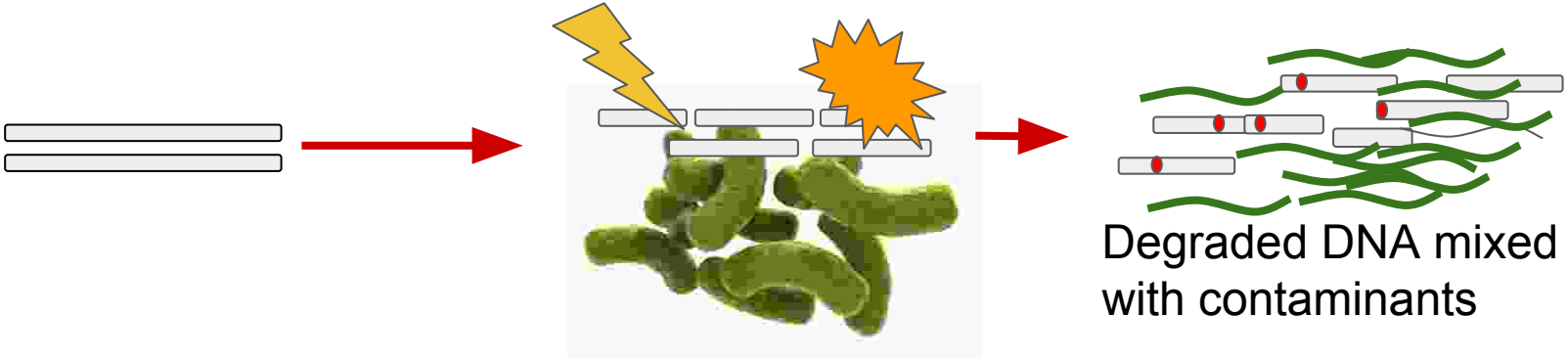
Ancient DNA In The Modern World

Alisa Vershinina
PhD Candidate, UC Santa Cruz

8th November 2017

DNA post-mortem



History

DNA sequences from the quagga, an extinct member of the horse family

Russell Higuchi*, Barbara Bowman*, Mary Freiberger*, Oliver A. Ryder† & Allan C. Wilson*

*Department of Biochemistry, University of California, Berkeley, ...lifornia 94720, USA
...search Department, San Diego Zoo, San Diego, ...rnia 92103, USA

To determine whether DNA survives and can be recovered from the remains of extinct creatures, we have examined dried muscle from a museum specimen of the quagga, a zebra-like species (*Equus quagga*) that became extinct in 1883 (ref. 1). We report that DNA was extracted from this tissue in amounts approaching 1% of that expected from fresh muscle, and that the DNA was of

Nature, 1984

120 years old

Molecular cloning of Ancient Egyptian mummy DNA

Svante Pääbo

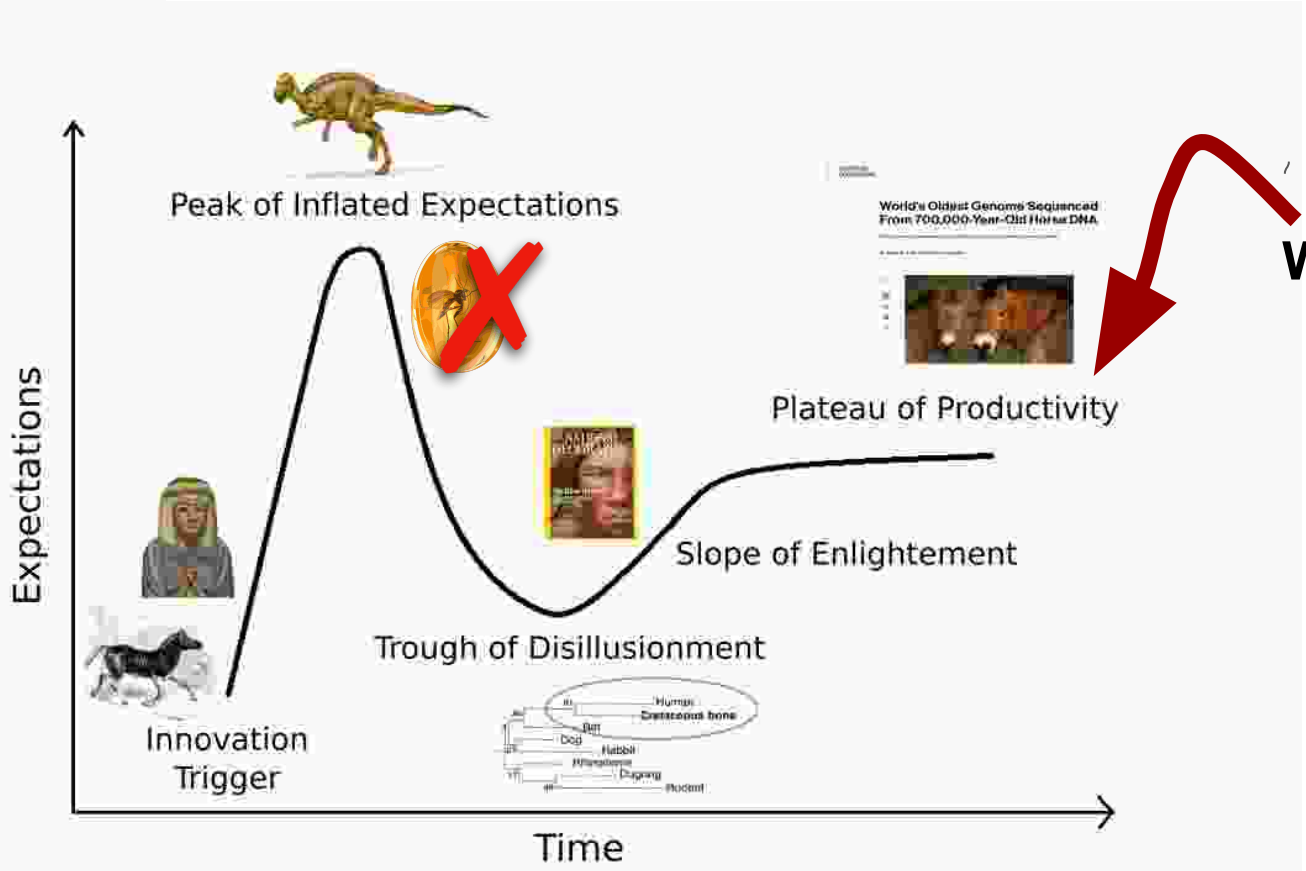
Department of Cell Research, The Wallenberg Laboratory, University of Uppsala, Box 562, S-75122 Uppsala, Sweden and Institute of Egyptology, Gustavianum, University of Uppsala, S-75120 Uppsala, Sweden

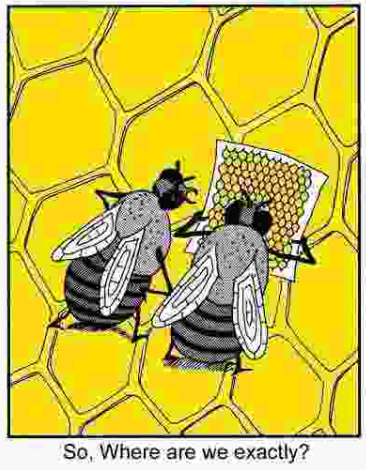
Artificial mummification was practised in Egypt from ~2600 BC until the fourth century AD. Because of the dry Egyptian climate, however, there are also many natural mummies preserved from earlier as well as later times. To elucidate whether this unique source of ancient human remains can be used for molecular genetic analyses, 23 mummies were investigated for DNA content. One 2,400-yr-old mummy of a child was found to contain DNA that could be molecularly cloned in a plasmid vector. I report here that one such clone contains two members of the *Alu* family of human repetitive DNA sequences, as detected by DNA hybridizations and nucleotide sequencing. These analyses show that substantial piec... of mummy DNA (3.4 kilobases) can be cloned and that the DN... fragments seem to contain little or no modifications introduc... postmortem.

Nature, 1985

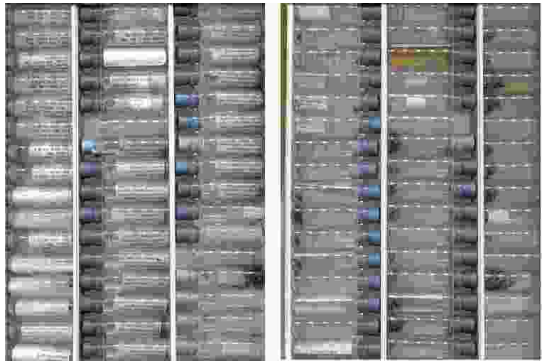
2,400 years old

The Hype Cycle





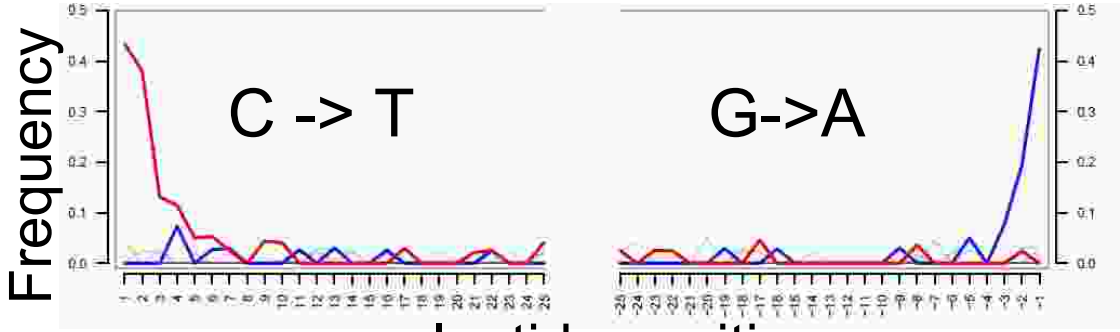
Source dictates approach

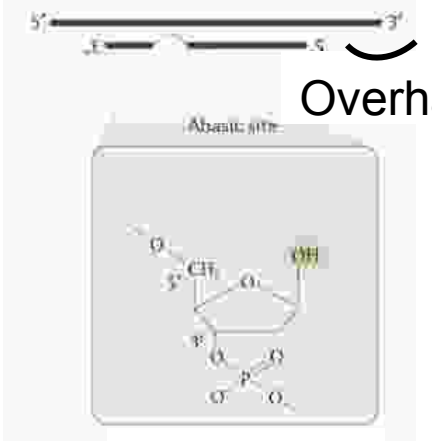


DNA damage

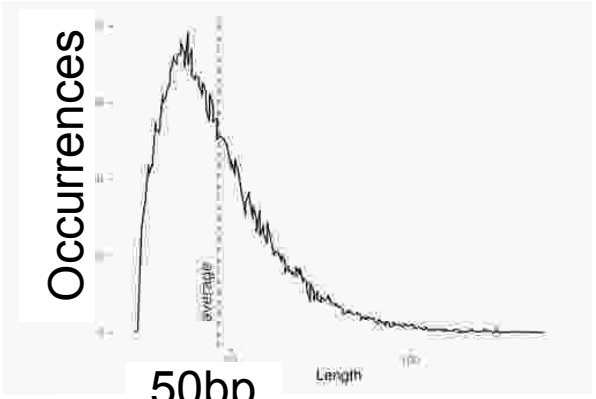
aDNA:

- Degraded
- Short
- Contaminated



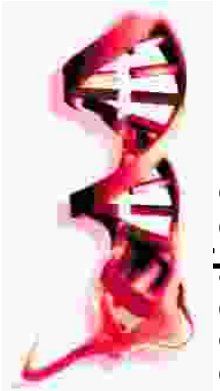


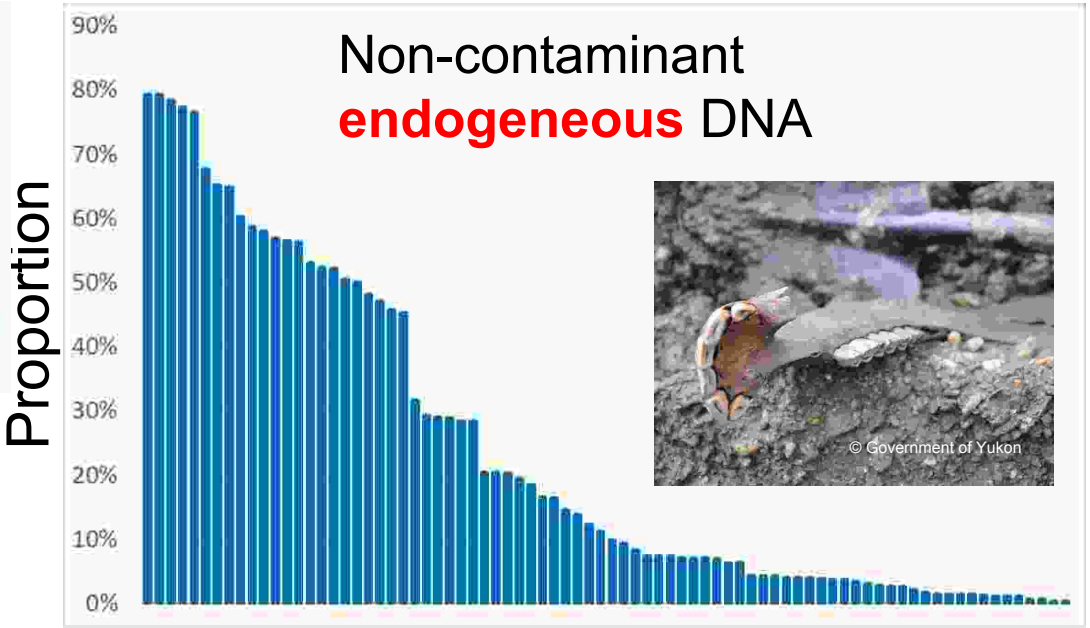
Abasic site

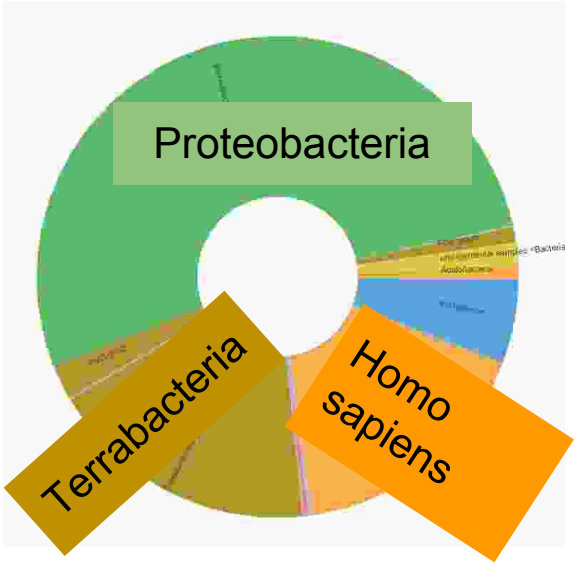


Length of aDNA fragments

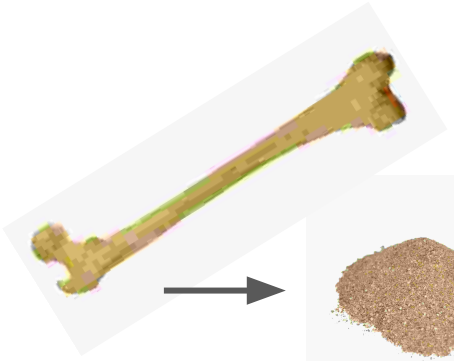
Contamination



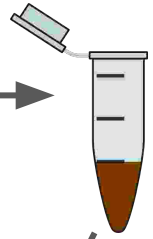


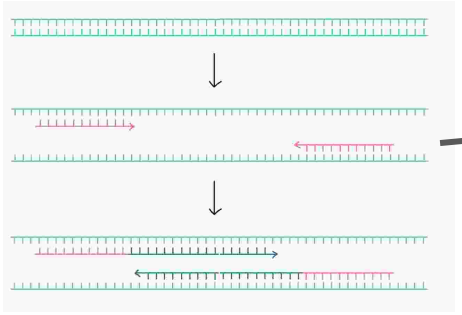


Sequencing aDNA

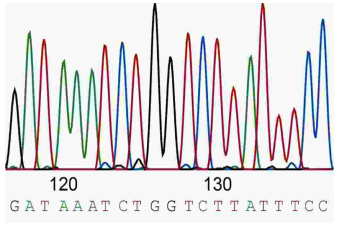


PCR



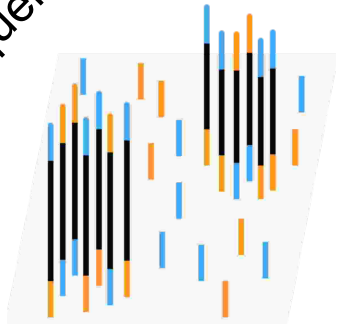


Sanger



Adapter ligation
Next-gen sequencing

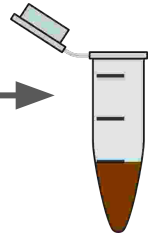
illumina®

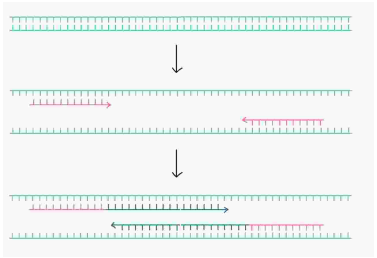


Problems:

- too short to PCR :(
- PCR inhibitors

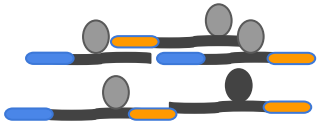
Sequencing aDNA: next-gen





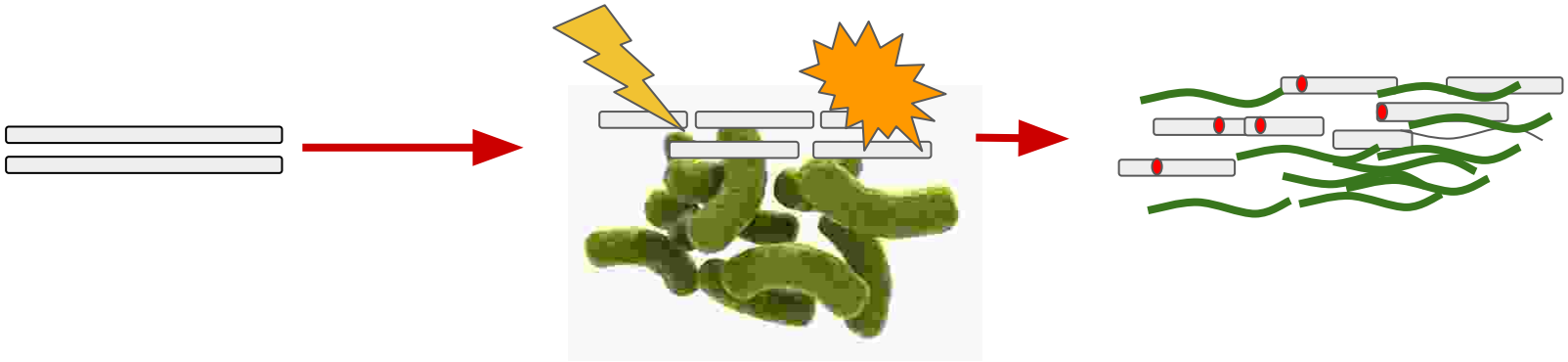
Amplicon sequencing

“shotgun” whole genome amplification



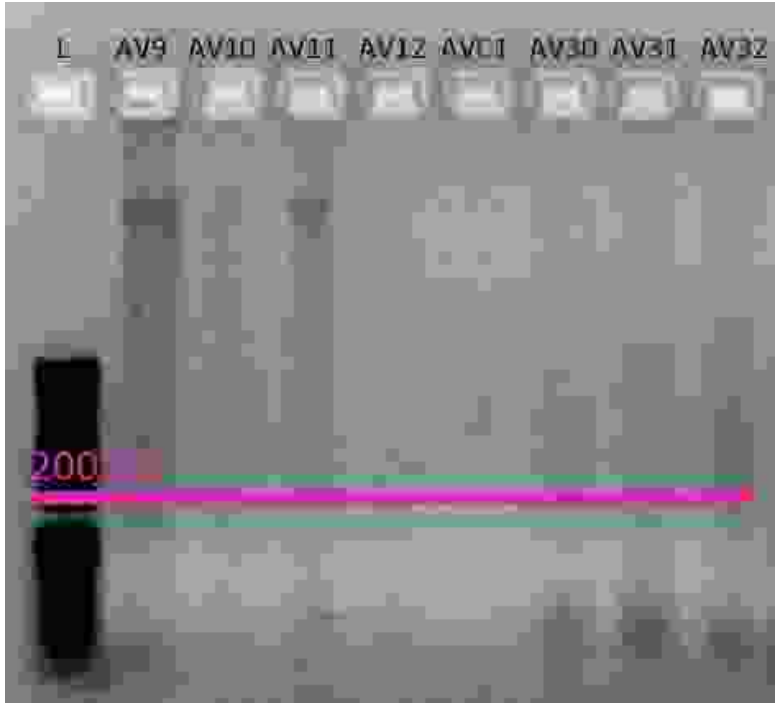
Capture with biotinylated probes

Dealing with problems

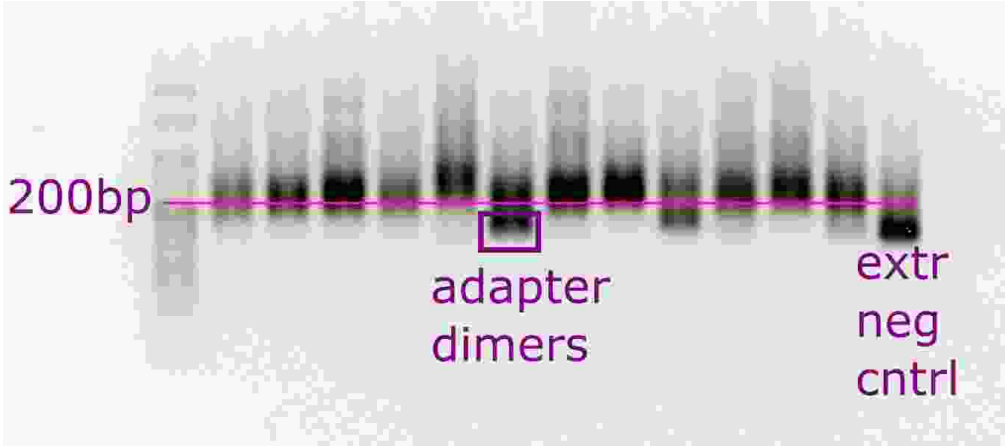


Dealing with problems:
1. decontamination

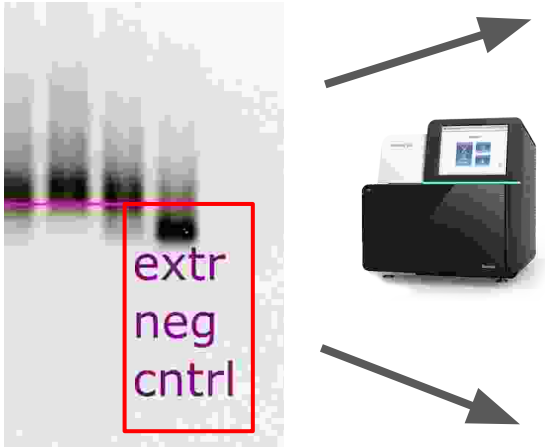
DNA extracts from
20-40k years old bones

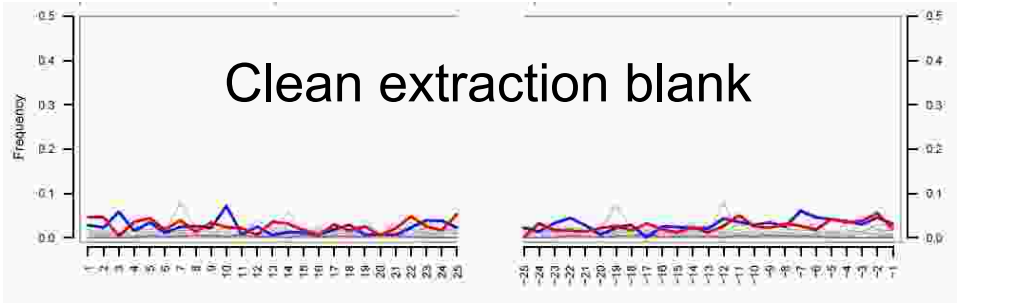


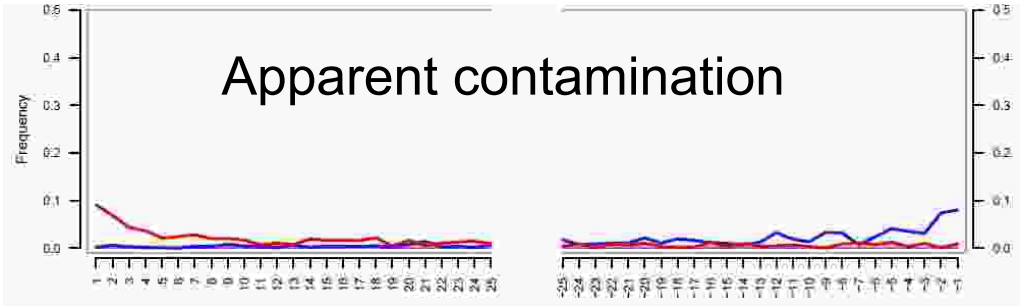
Genomic DNA library
after 25 cycles of PCR



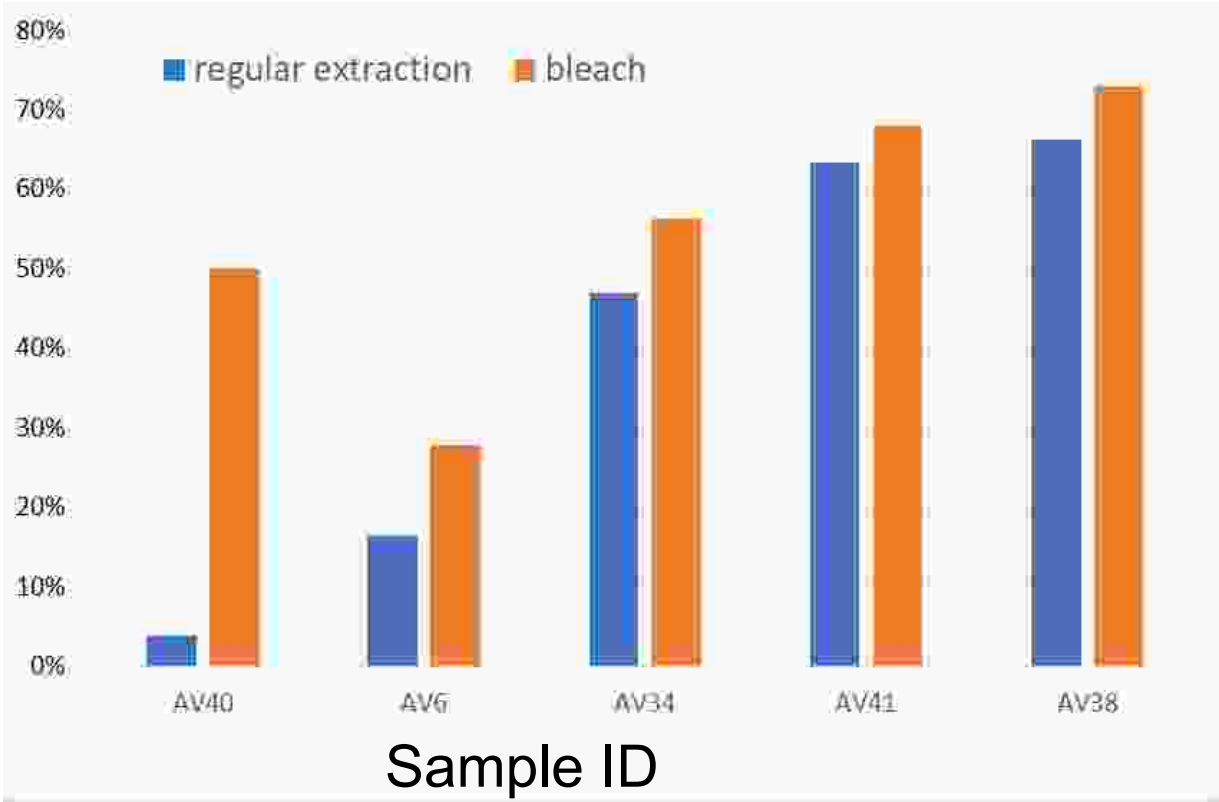
Sequence your extraction controls







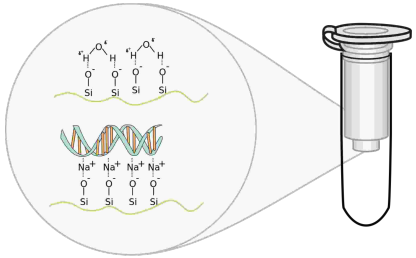
Decontaminate the object



Also see Boessenkool et al., 2017

Dealing with problems:
2. target short molecules

Silica-based extraction

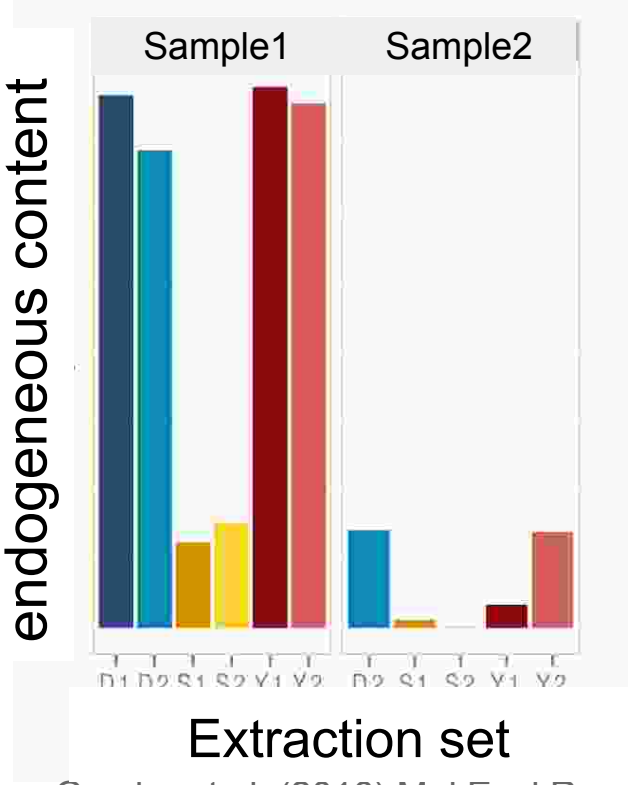


Hints:

- remove inhibitors
- increase volume of the binding buffer
- check pH of the binding buffer
- decrease volume of the elution buffer
- warm up the elution buffer

Magnetic bead-based clean-ups

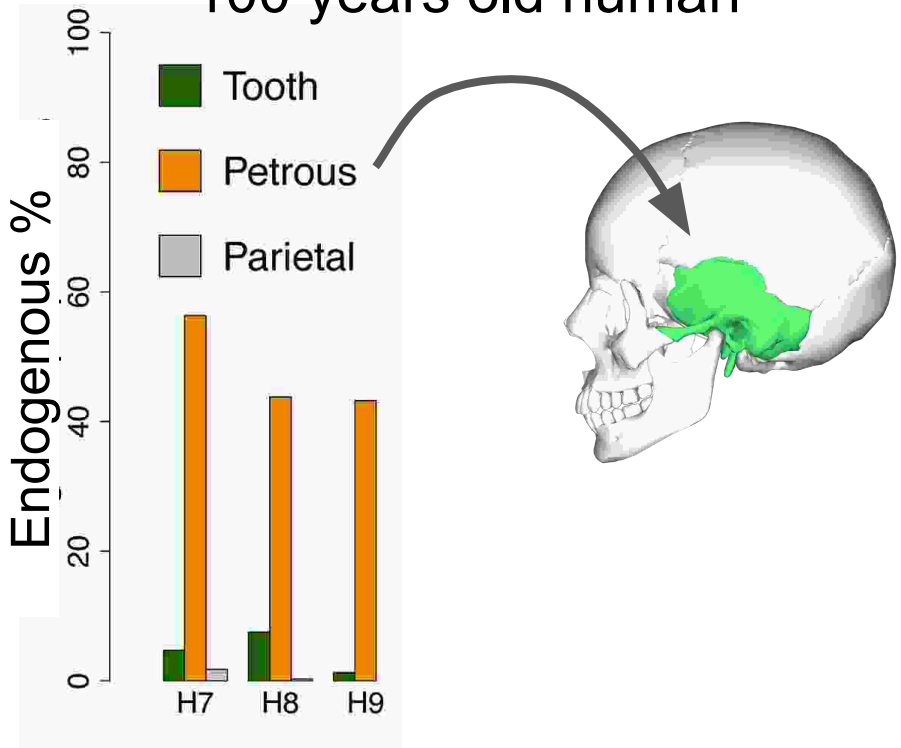
- increase bead\product ratio



Gamba et al. (2016) Mol Ecol Res

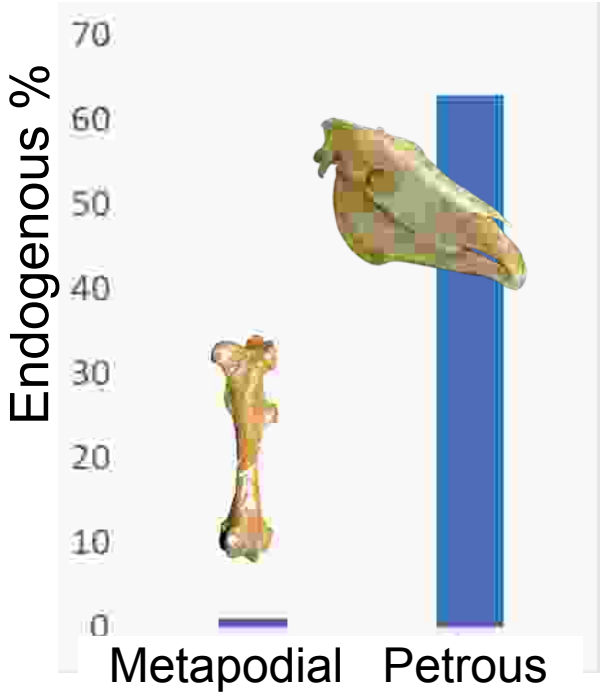
Not all tissues are made equal

~ 100 years old human



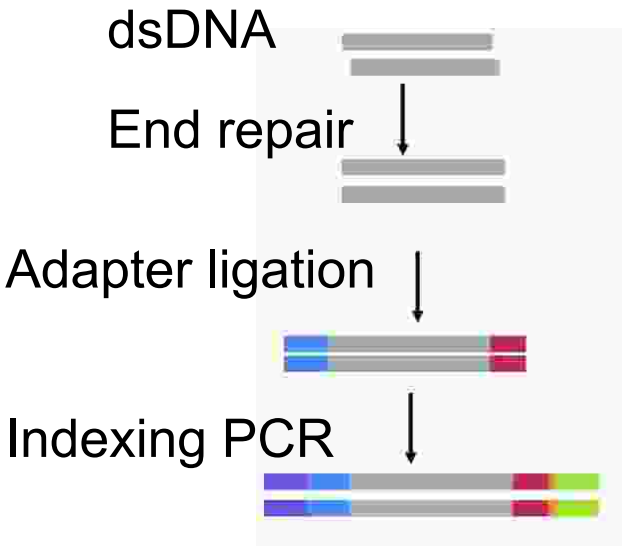
Hansen et al. (2017) PLOS One

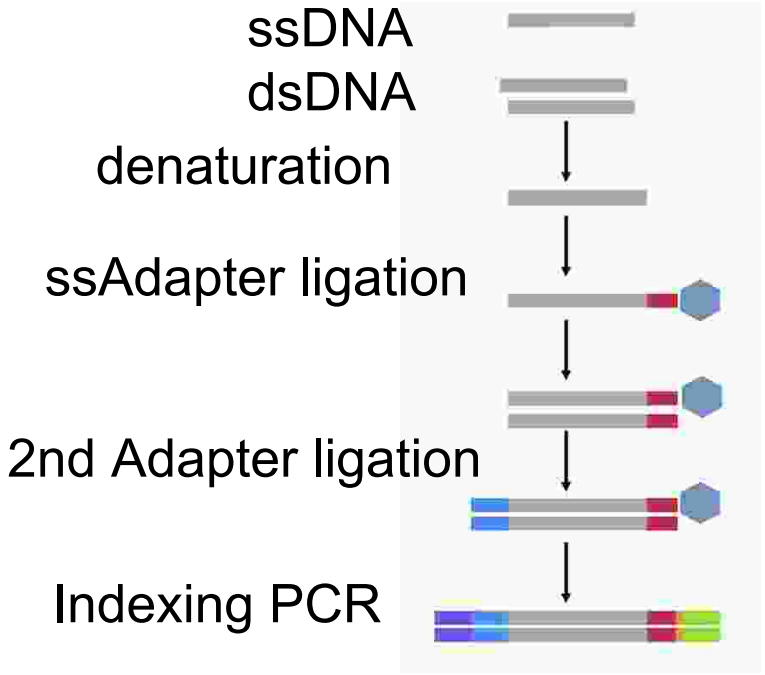
700k years old horse



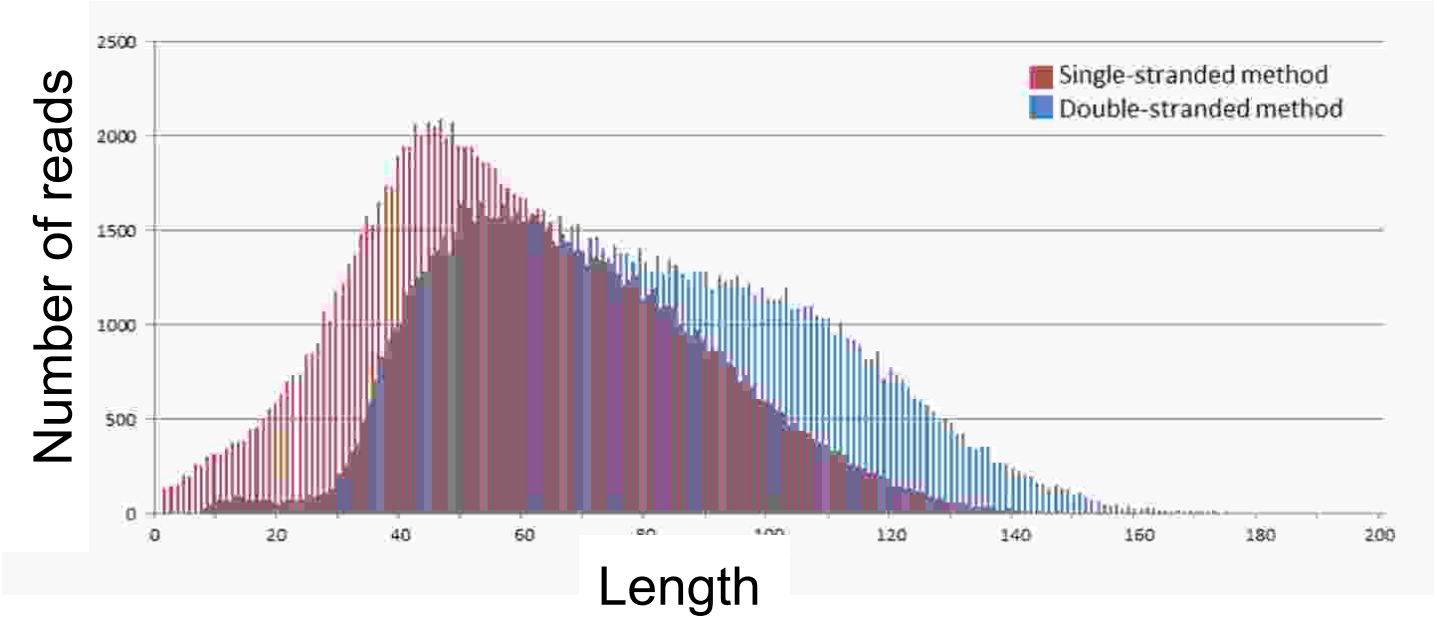
Author's data

Dealing with problems: 3. next-gen sequencing



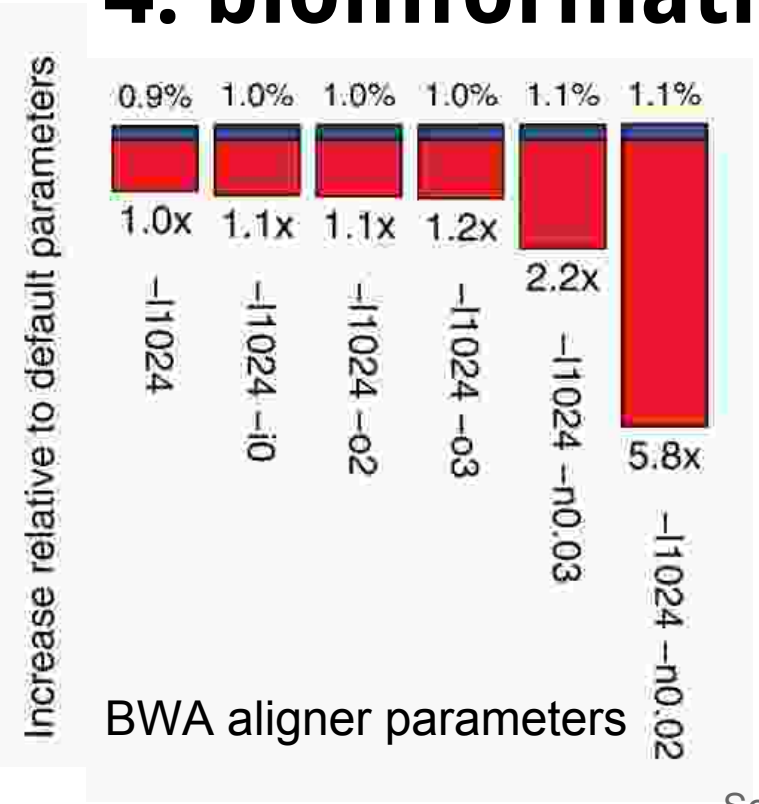


Dealing with problems: 3. next-gen sequencing



Bennett et al. (2014) BioTechniques

Dealing with problems: 4. bioinformatics



Schubert et al. (2012) BMC Genomics

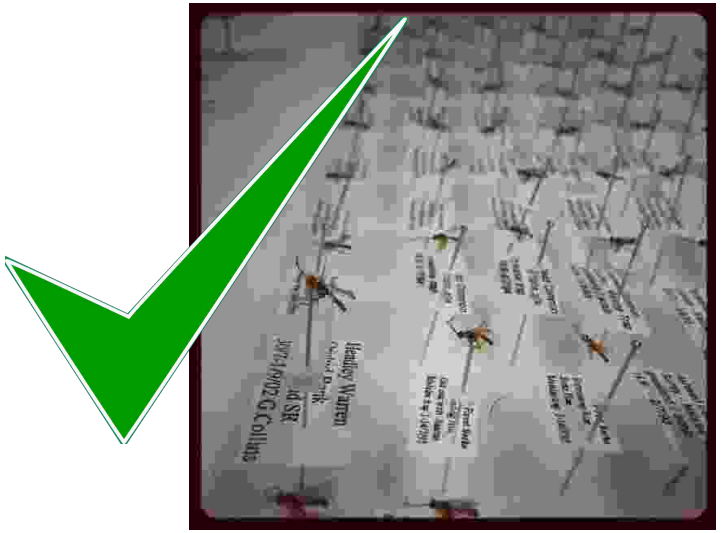
Insects?
Think twice about preservation and collection technique

Killed in ethyl acetate, stored in weak alcohol, all samples mixed together

Freeze-killed, stored in 100% ethanol in a cold dark place, individual vial

Insects?
Think twice about preservation and collection technique

Killed in ethyl acetate, stored in weak alcohol, all samples mixed together



Storage in a cold, dry, dark place

Thank you!

Special thanks to Eric Gordon,
Michael Forthman,
and ESA enhancement funds!

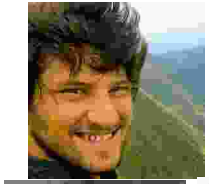
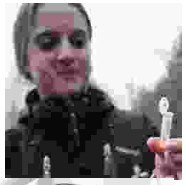
PIs:

Dr. Richard E. Green,
Dr. Beth Shapiro,
Dr. Lars Fehren-Schmitz



UCSC PALEOGENOMICS LAB

PostDocs:
Dr. Darko Cotoras, Dr. Ruth Nichols, Dr. Gemma Murray, Dr. Megan Supple, Dr. Jannine Forst

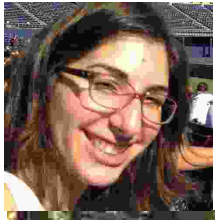
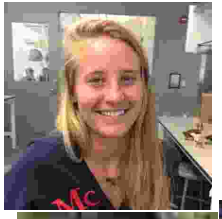




Technicians:
Miguel Onate, Heather Milne, Beth Nelson

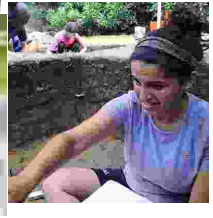
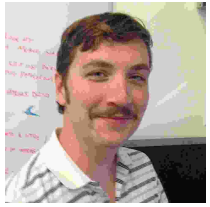
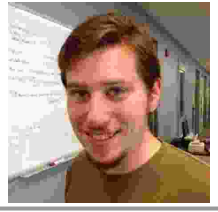


Job openings

- PostDoc on wet methods (Shapiro)
- PostDoc on genome assembly (Green)

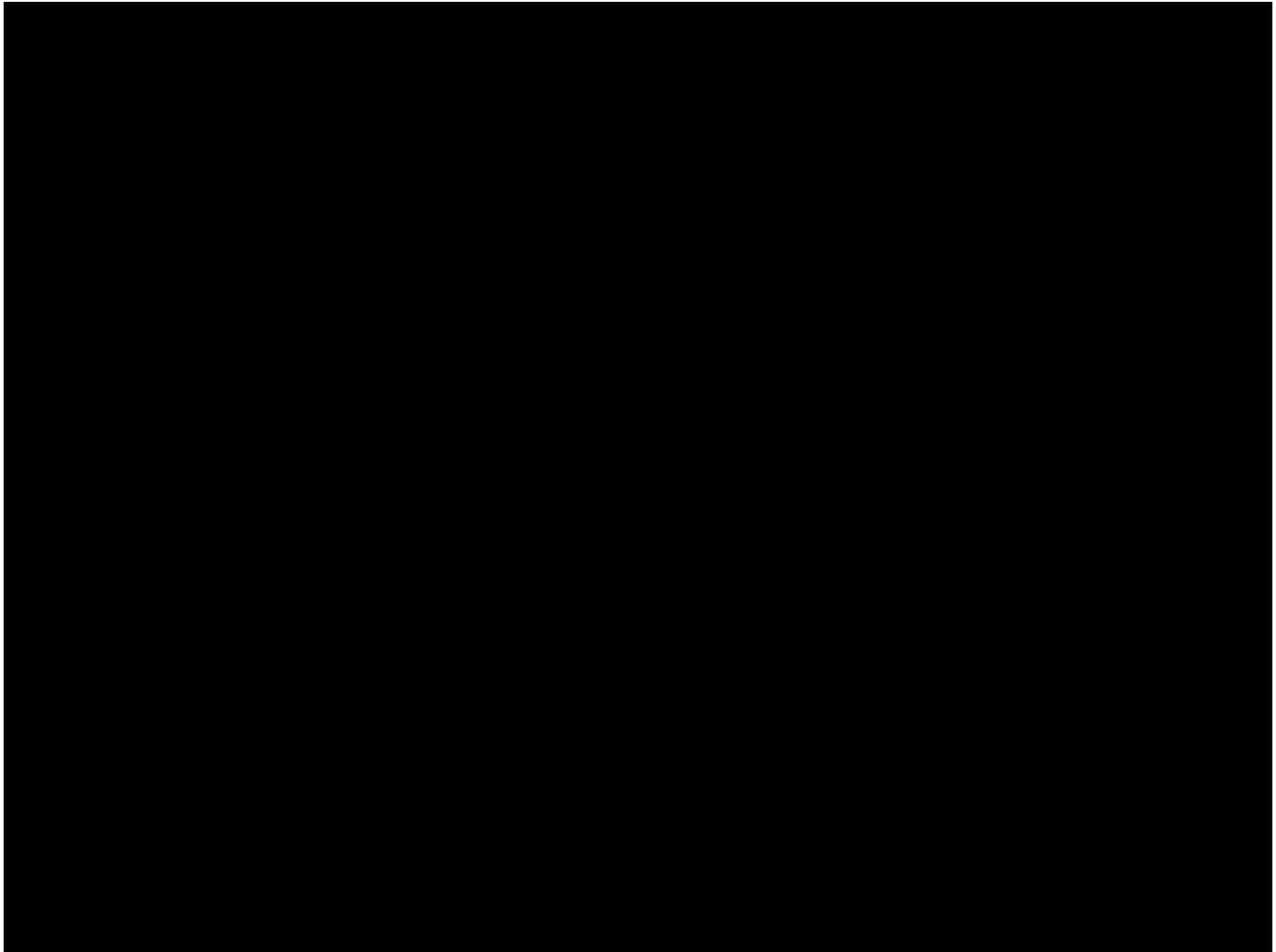





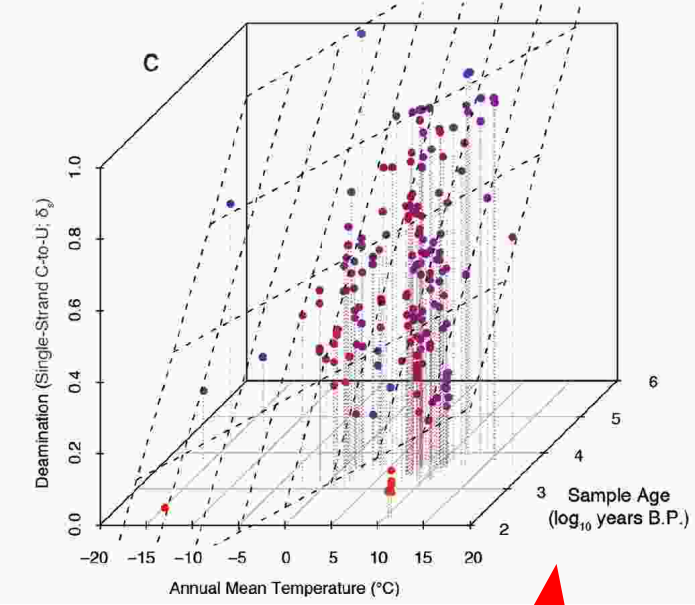

Grad students:
Nedda Saremi
Natasha Dudek
Josh Kapp
Ed Rice

Nathan Schaefer
Sidra Hussain
Sabrina Shirazi
Brendan O'Connell
Nevé Baker

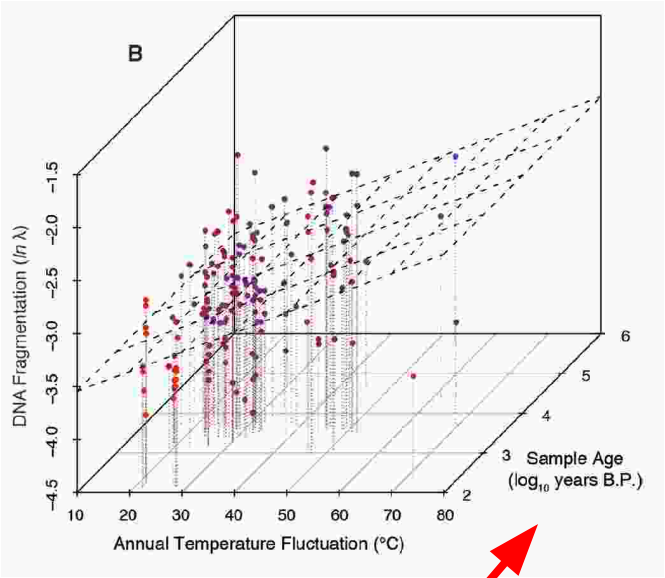


© video by illumina

200 Bones From Across The Globe



deamination increases with age



fragmentation does not

Kistler et al. (2017) Nucl Acid Res

DNA Sequences from a Fossil Termite in Oligo-Miocene Amber and Their Phylogenetic Implications

Rob DeSalle, John Gatesy, Ward Wheeler, David Grimaldi

DNA was extracted from the fossil termite *Mastotermes electrodominicus* preserved in Oligo-Miocene amber (25 million to 30 [illegible] ribosomal DNA (rDNA)] and nuclear chain reaction. Phylogenetic analysis logical cladistic analyses of living dicty fossil termite shares several sequenc of this fossil to living-species phyloge phyly and affects molecular phylogene characterized.

Science

Revival and Identification of Bacterial Spores in 25- to 40-Million-Year-Old Dominican Amber

Raúl J. Cano* and Monica K. Borucki

A bacterial spore was revived, cultured, and identified from the abdominal contents of extinct bees preserved for 25 to 40 million years in buried Dominican amber. Rigorous surface decontamination of the amber and aseptic procedures were used during the recovery of the bacterium. Several lines of evidence indicated that the isolated bacterium was bioc clos

Science

Opinion TRENDS in Microbiology Vol.13 No.5 May 2005

Geologically ancient DNA: fact or artefact?

Martin B. Hebsgaard[1,2], Matthew J. Phillips[1] and Eske Willerslev[1,2]

Trends in Microbiol