SPAAM

Standards, Precautions & Advances in Ancient Metagenomics

Date : 21st September 2020
Chairs: Irina Velsko

Session 1: Trash In, Trash Out

Optimizing and Standardizing Laboratory Practices in Ancient Metagenomics

Session Scope

- How to minimize the amount of contamination that becomes sequenced data?
  - Controlling for laboratory batch effects
  - Controlling for sample spill-over between high- and low-biomass samples
  - Techniques to remove potential contaminants prior to DNA extraction

- Intro to the ice breaker speakers
- Tweeting is ALLOWED

Definitions

- Contamination
  - DNA derived from sources exogenous to the sample (environmental (soil, air), worker (skin, oral/respiratory tract), laboratory (reagents, other samples))
- Blanks
  - Negative control samples included in extraction and library prep that are handled identically to real samples but do not contain sample DNA

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Reducing contamination

How to minimize the amount of trash

Introduction (ZF)

- How is contamination introduced into samples?

Excavation

Storage

Handling

Laboratory

Introduction (ZF)

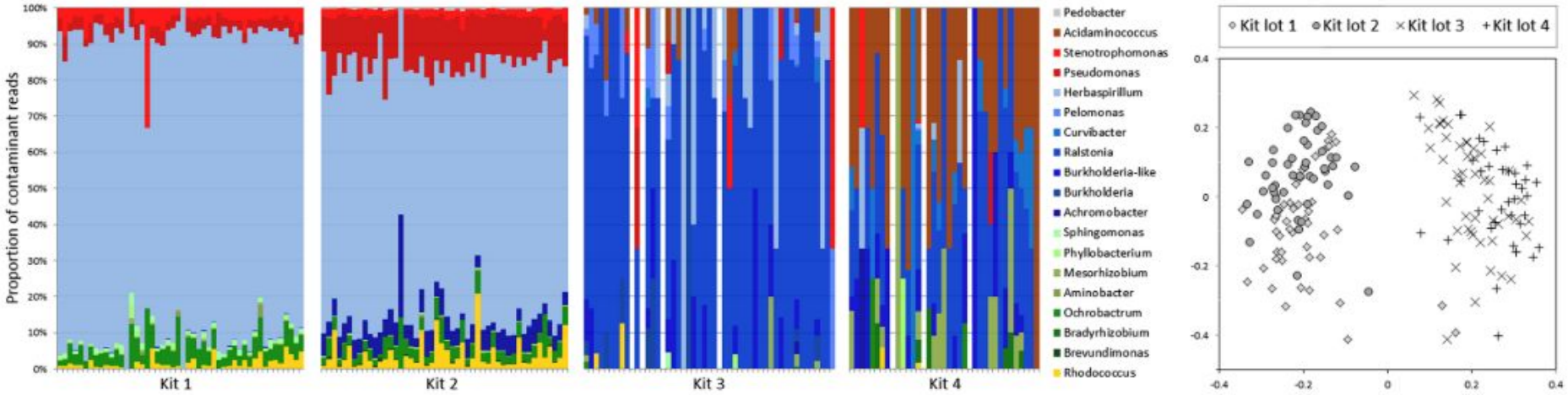
- What issues can contaminants cause downstream?
  - ‘Swamp out’ original microbial community
  - Bias results
  - Increase sequencing costs to achieve wanted results

Introduction (JB)

- What are batch effects and why should we care?
  - Samples in one batch experience the same handling
  - Differences caused by worker, reagents, minor differences in protocol etc. We are only human!
  - Can cause systematic differences to appear in downstream analyses

Batch effects (JB)

- Example: contaminants in kit lots



Contaminant OTUs and PCoA of contaminated samples - variation explained by kit lot number

Salter *et al.* 2014. *BMC Biol.*

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  - Investigate batch effects in data post sequencing

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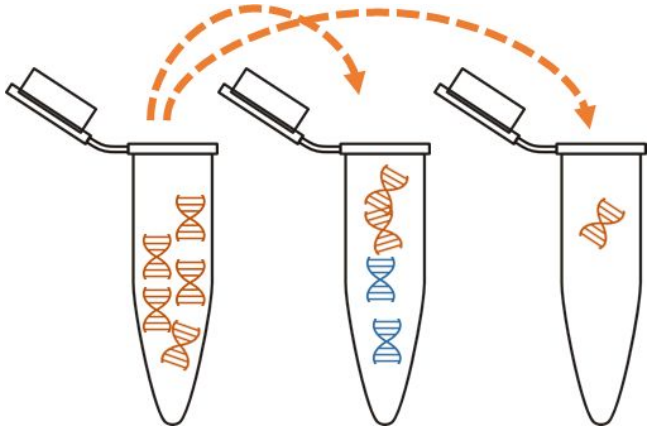
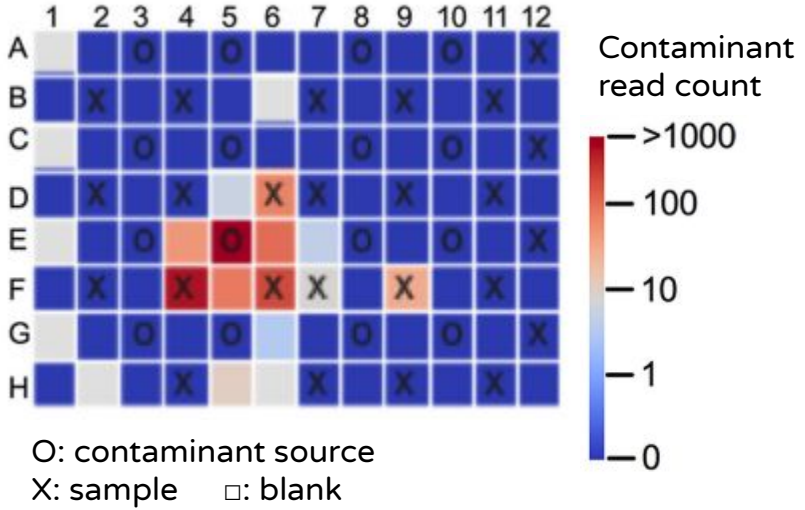
  - Randomise samples across batches
  - Record batch number, reagent and kit lot numbers, etc
  - Investigate batch effects in data post sequencing
  - Replicate samples in different batches (difficult if sample is limited)
  - Process blanks and mock sample (with known composition) in each batch

Cross-contamination (JB)

- Spillover of samples with high biomass into nearby low biomass samples





O: contaminant source
X: sample □: blank

Minich *et al.* 2019. *mSystems*

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- Possible solutions:
  - Randomise and record sample placement
  - Process samples of similar biomass together (if possible)
  - Change gloves between samples
  - Automated vs manual set-ups: test your system!

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  1. Reducing amount of contaminants already present on/in sample
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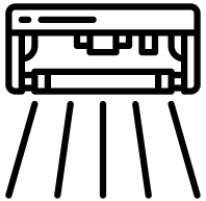
  - Only areas exposed to light source are decontaminated
  - Less efficient with low molecular weight fragments



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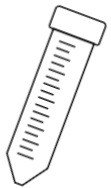
- EDTA
  - Short pre-digestion increases endogenous DNA percentage (15-30 min)
  - Longer pre-digestions cause sample loss
  - No change in damage
  - DNA can be extracted from removed fraction



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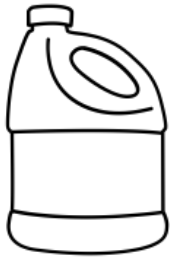
- Bleach wash (<3% solution)
  - Seems to only work occasionally
  - Sample loss (57% in Korlevic et al. 2018)
  - No indication of increased fragmentation or damage
  - Removed DNA is destroyed



Decontamination (ZF)

- Bleach wash (<3% solution)
  - Seems to only work occasionally (but when it does, it is great)
  - Sample loss (57% in Korlevic et al. 2018)
  - No indication of increased fragmentation or damage
  - Removed DNA is destroyed
- Phosphate treatment
  - Decreases DNA binding to hydroxyapatite
  - Less efficient than bleach, but lower sample loss
  - Released DNA can still be extracted

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- Randomise samples across batches
- Record everything!

Discussion Points:

- What are common best practices?
- Are there common methods that should be avoided?
- Is standardization of protocols across labs possible/desirable?

- What biases exist between different extraction/library build techniques?
- Does different background in different labs impact meta-analyses?
- What differences need to be considered for different sample types? (calculus, palaeofeces, bone, ice cores, etc?)

- Is USER enzyme treatment acceptable for ancient metagenomics?
- Is enriching endogenous DNA rather than reducing contamination a feasible technique?
- Feasibility of control spike-ins of known composition?

- All questions/topics that attendees want to discuss ...

References

- Damgaard *et al.* (2015). Improving access to endogenous DNA in ancient bones and teeth. *Scientific Reports.*
- Korlević *et al.* (2018). Reducing microbial and human contamination in DNA extractions from ancient bones and teeth. *BioTechniques.*
- Llamas *et al.* (2017) From the field to the laboratory: Controlling DNA contamination in human ancient DNA research in the high-throughput sequencing era. *STAR: Science & Technology of Archaeological Research.*
- Minch *et al.* (2019) Quantifying and understanding well-to-well contamination in microbiome research. *mSystems.*
- De Goffau *et al.* (2018) Recognizing the reagent microbiome. *Nature Microbiology.*