Analysis of samples with low abundance of microbial DNA

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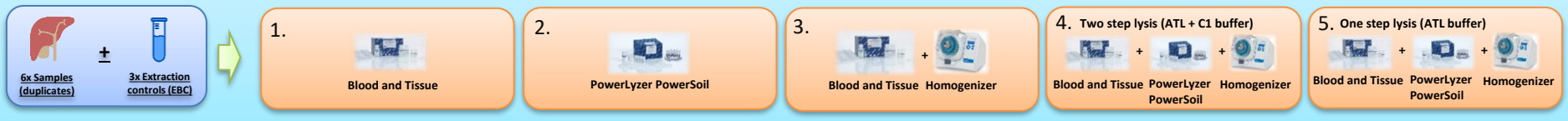
Introduction

Recent studies confirmed bacterial presence even in internal tissues or body fluids such as liver, breast tissue, or brain, blood, initially presumed as sterile in healthy individuals (1, 2, 3, 4). However, the amount of bacterial components (including DNA) could be extremely low and represents several issues during laboratory preparation: Introduction of **contaminant bacterial DNA** from the environment, isolation and PCR kit itself in the sample during laboratory preparation poses the problematic riddle in a sense of correct interpretation of the bacterial profile (5,6). Regarding the previous point, it is often difficult to decide whether is sample negative (contain no bacterial DNA at all) because of variable contamination manifestation. If a **low level of bacterial DNA** is presented, the sample is prone to manifestations of contamination on the sequencing profile because of the lower ability to compete for reagents in the PCR reaction during the library preparation (6). In the case of tissue and some body fluid samples, during library preparation a non-specific products can be amplified and sequenced because of **high proportion of present eukaryotic DNA** or reaction could be attenuated by other byproducts of tissue and body fluid isolation such as PCR inhibitors (7).

Our **goal is to identify an isolation protocol with best attributes** (highest yield of whole DNA, highest yield of bacterial DNA, lowest percentage of non-specific eukaryotic products, lowest percentage of bacterial conaminant taxa) that allows us to achieve accurate and reproducible results in the field of analysis of tissues with .

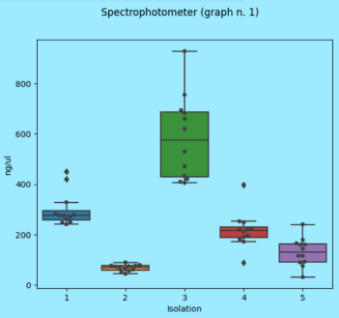
Materials and methods

Five isolation protocols were designed (combining different customs and proprietary approaches of enzymatic and mechanical lysis) was tested to obtain the highest yield of bacterial DNA from tissue and six different samples of chicken liver in duplicates have been isolated in duplicates. Each isolation has been carried out with three extraction controls to capture contamination. Quality and quantity of whole DNA has been checked spectrophotometricaly and fluorometrically (Synergy HTX). Quantitative PCR (qPCR) (LightCycler480) of controls and samples has been performed to confirm (or disprove) the presence of bacterial DNA in the isolates and determine difference of bacterial DNA amount between samples and extraction controls. Finally, a 16S library has been prepared from all samples and controls for further sequencing (Illumina MiSeq) for comparation of bacterial and eukaryotic contamination influence for each isolation.

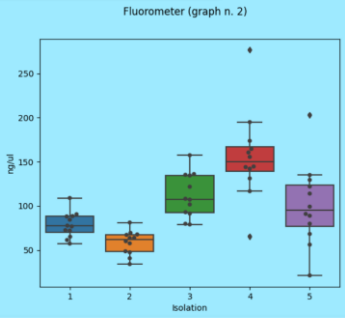


Results

Spectrophotometer (Synergy HTX)

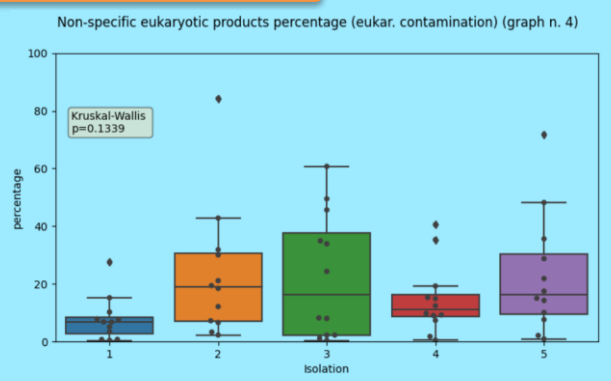


Fluorometer (Synergy HTX)



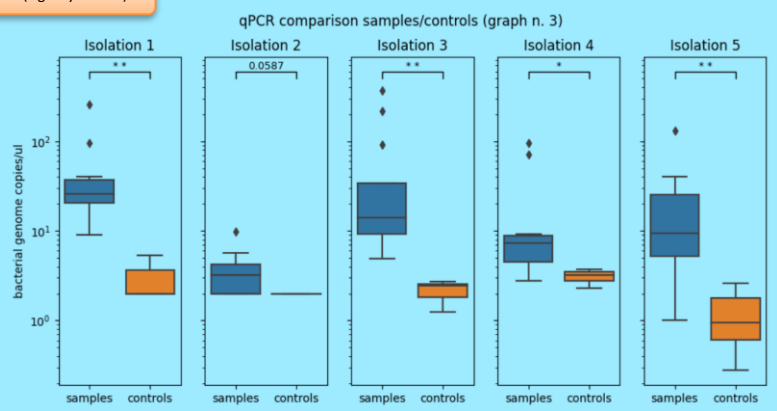
The highest yields of whole DNA (eukaryotic and prokaryotic) from tissue were obtained by isolation 3 measured spectrophotometricaly and isolation 4 measured fluorometrically.

16S rRNA library preparation (Illumina MiSeq)



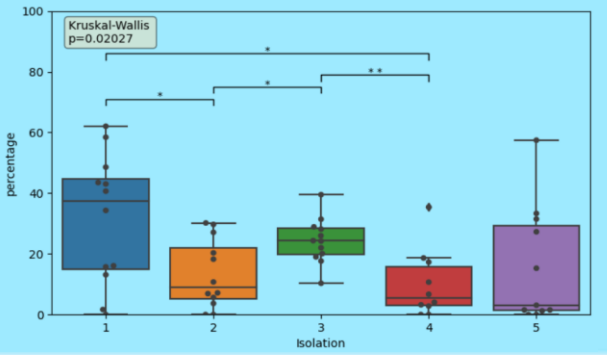
There was no significant difference between isolations with respect to the ratio bacterial/chicken ASV.

qPCR (LightCycler480)



qPCR analysis revealed a highest significant difference of bacterial DNA copy amount between samples and extraction controls in isolations 1, 3, and 5. Lowest difference between the number of copies of samples and was observed in isolation 2.

Bacterial contamination percentage (graph n. 5)



Bacterial contaminant taxa have been considered if presented in at least 1% abundance in extraction controls and at least in at least 1% abundance in samples (excluding less abundant taxa with respect of small influence on sample composition). Lowest level of bacterial contamination taxa has been observed in isolations 2 and 4. The most frequent contaminant taxa contained in controls and samples are *Moraxella*, *Streptococcus*, *Staphylococcus*, and *Micrococcus*

Conclusion

- **High yield of whole DNA** may implicate also proportionally high bacterial DNA yield. Our measurements reveals that **isolation 3** (spectrophotometer) and **4** (fluorometer) produce highest amounts of whole DNA.

- Bacterial genome copies amount difference between samples and extraction controls measured by qPCR describes **prokaryotic DNA extraction effectivity** of particular kit. Best isolations with highest ratio of extracted prokaryotic DNA in samples and contaminant DNA in controls and are **isolation 1, 3** and **5**.

- According to our data we can not conclude which isolation produce less **non-specific eukaryotic products** during library preparation.

- **Bacterial contamination** has been estimated from sequencing data. Lowest contamination levels reveals **isolation 2** and **4**. More accurate bacterial contamination would be calculated by additional statistical software.

Based on aforementioned data suitable isolation protocols are **isolation 3** and **4**. Single best isolation will be chosen after validating results on higher number of smaples.

References

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