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Identification of Contaminant Using Hypothesis Testing in Marker Gene and Metagenomics Data

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Abstract

Background: The measurement of microbial community suffers from contaminant DNA sequences that are not truly present in the sample (Figure 1). *Decontam* has been introduced to identify contaminant sequences using a classification procedure based on a pattern that contaminant appears high frequencies in low-concentration samples (Figure 2). However, it has no false discovery rate control, and clear guidance is missing to help users choose an interpretable threshold.

Results: We propose a hypothesis testing procedure, *Tcontam*, to detect contaminants using statistical p-value and control the false discovery rate using multiple testing correction procedure. We confirmed validity of *Tcontam* using simulation. In a human oral dataset, *Tcontam* reports the contaminants with false discovery rate under control and has low chance to classify the sequences with small sample size as contaminants.

Contaminants

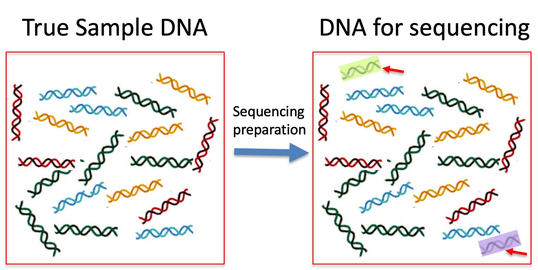


Figure 1: Schematic of contaminant DNA sequences that are not truly present been introduced in marker-gene and metagenomic sequencing (MGS) procedure.

Modeling of Contaminants

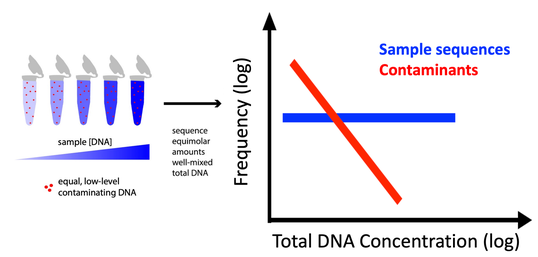


Figure 2: Mixture model of contaminants and true sample sequence in MGS experiments.

(Davis, et al. Microbiome, 2018)

Methodology of Hypothesis Testing

Step 1: Fit two models

Contaminant model (C)
$\log(freq) \sim (-1) * \log(conc)$

True Sample model (S)
$\log(freq) \sim (0) * \log(conc)$

Step 2: Compute R

$$R = \frac{RSS_c}{RSS_s}$$

***Decontam* Step3: classification procedure**

- Transform R to value P between 0 and 1 with adjusting sample size.
- Set a threshold P^* and ASVs with $P < P^*$ are classified as contaminants

***Tcontam* Step3: hypothesis testing procedure**

1. Define the null and alternative hypothesis:
   H_0: *True sample model fits better or equally better*
   H_a: *Contaminant model fits better*
2. Obtain the null distribution based on ratio of dependent Chi-square distribution
3. Compute p-values for each ASV based on null distribution giving R values
4. Perform FDR correction using q-value procedure. Reject null, i.e., ASVs classified as contaminants for q-values less than a threshold, e.g., 0.05.

Figure 3: Overview of hypothesis testing procedure for contaminant identification

Comparison between *Tcontam* and *Decontam*: Human Oral Microbiome Dataset

A

Decontam \ *Tcontam*	True Sample	Contaminant
True Sample	789	0
Contaminant	43	15

B

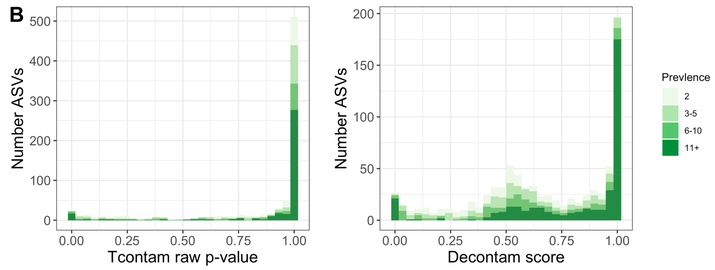
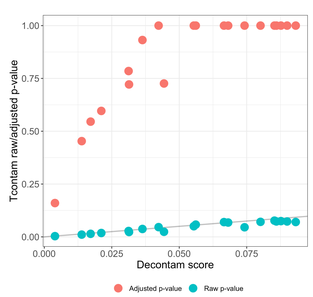


Figure 5: Difference of overall findings and scores/p-values between *Tcontam* and *Decontam* from an oral 16S rRNA gene dataset **A**: Contingency table of findings between *Decontam* and *Tcontam*; **B**: Histogram of *Tcontam* raw p-values (left panel) and *Decontam* scores (right panel) for each ASVs colored for different sample size.

A



B

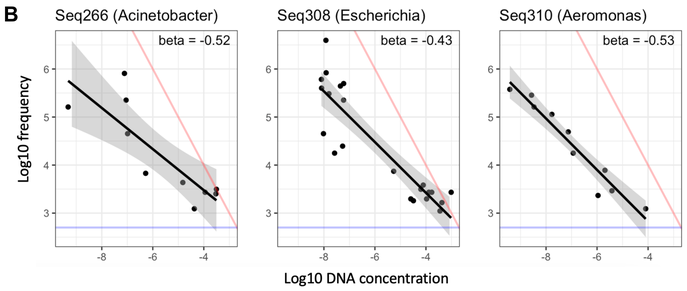


Figure 6:ASVs reported as contaminants only by *Decontam*. **A**: Scatter plot of *Tcontam* p-values vs. *Decontam* scores for ASVs with sample size < 5. **B**: Scatter plot of DNA concentration and frequency in log10 scale for 3 ASVs with sample size ≥ 10.

Validity of *Tcontam*

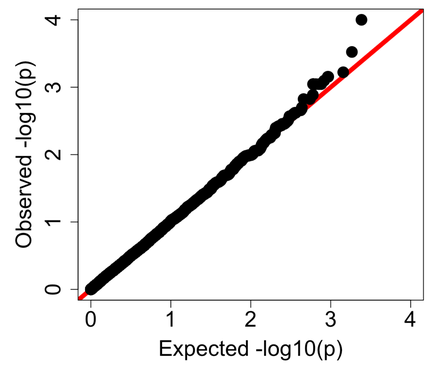


Figure 7: QQ plot of *Tcontam* p-value. It follows a uniform distribution under the null hypothesis.

Discussion and Conclusion

Tcontam is a hypothesis testing-based procedure, which assumes most of the DNA used to do marker gene or metagenomics sequencing are from true sample. *Tcontam* will call a ASV from true sample unless we have enough evidence from DNA concentration and frequency data. Compared with *Decontam*, which is based on a classification procedure, *Tcontam* reports a valid p-value, which can be (1) better interpreted with a significant level (2) connected to FDR correction procedure to control the overall FDR.

Specifically, *Tcontam* prefers not to call a ASV as a contaminant for the following two cases: (1) a strong correlation between DNA concentration and frequency with small sample size (e.g., < 5); (2) a weak to mild correlation with large sample size (e.g., > 10).

Next Steps

1. Conduct power analysis using simulation for different sample size.
2. Perform genus-level contaminant analysis using the oral dataset and validate findings use known contaminants or oral taxa reference database.
3. Conduct other real data analyses comparison between Tcontam and Decontam.

Acknowledgement

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