Diagnosis of Major Depressive Disorder in Adolescents through Analysis of Oral Microbiome Compositions

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Introduction

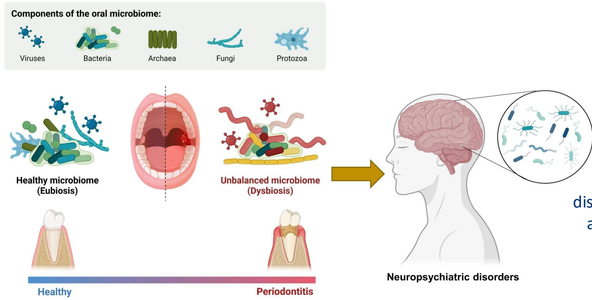


Figure 1: Studies utilizing the oral microbiome composition to diagnose neuropsychiatric disorders (Alzheimer's and Parkinson's)[1,2]

- Major Depressive Disorder (MDD) affects around 280 million people worldwide.
- Current diagnosis are subjective while physical, neurological, and laboratory tests operate on a rule-out basis.
- Varying compositions of the oral microbiome are associated with depression in adolescents.
- Bacteria biomarkers can be identified utilizing whole genome shotgun metagenomic sequencing.

Methods

- To estimate the relative composition of two bacterial biomarkers, we used CRISPR Cas 12a dsDNA detection.
- A CRISPR composition assay was used to generate a calibration curve that compared the relative composition of two bacterial types: *Haemophilus Parainfluenzae* (HP) and *Parainfluenza Nigrescens* (PN).

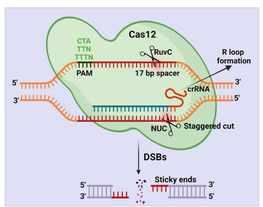
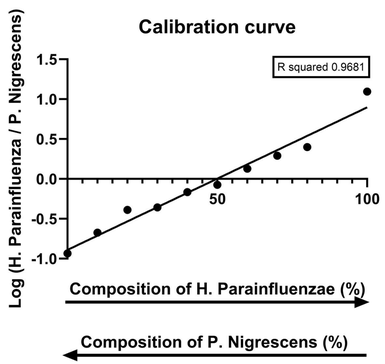


Figure 2: How the Cas 12a mechanism works to cleave the target DNA region and form a R loop.[3]

Figure 3: A calibration curve of the composition of HP and PN



Results

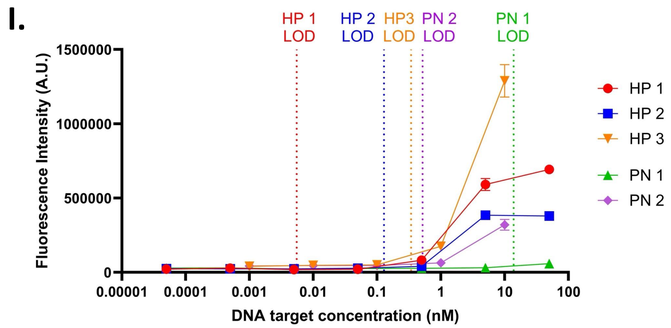


Figure 4: Determining the limit of detection (LOD) with different guide RNAs for HP and PN targets.

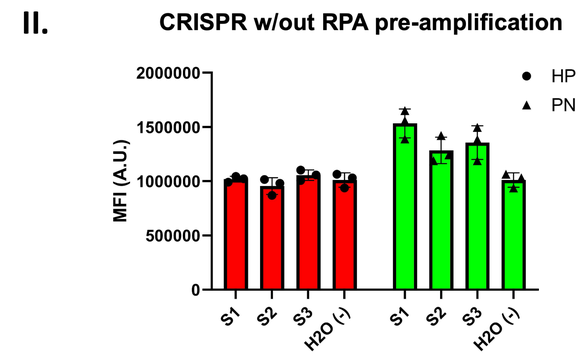


Figure 5: The measured fluorescence intensity from 3 different healthy samples to quantify the amount of HP and PN using a CRISPR assay (without RPA).

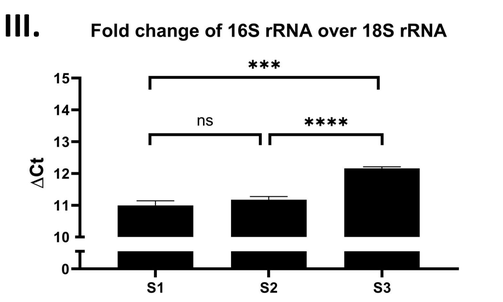


Figure 6: The fold change of 16S rRNA and 18S rRNA for three healthy saliva samples.

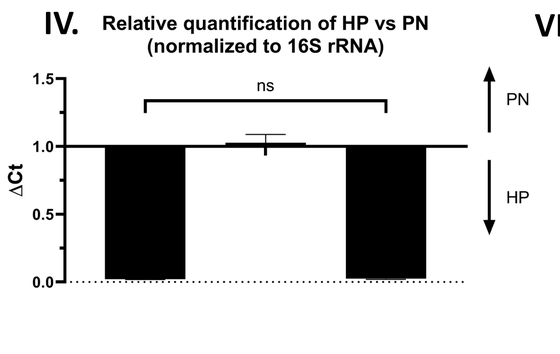


Figure 7: The relative quantification of HP and PN normalized to 16S rRNA in three different healthy saliva samples.

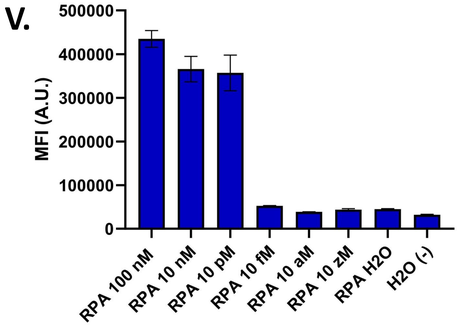


Figure 8: Results from a CRISPR assay utilizing RPA isothermal reamplified targets.

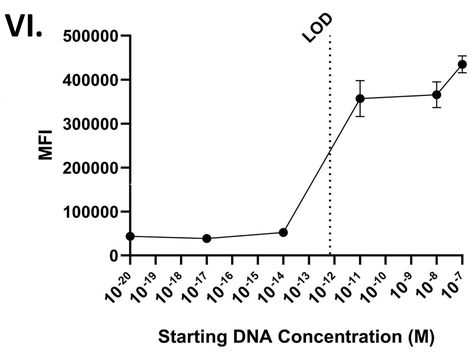


Figure 9: The determined limit of detection using CRISPR RPA targets for HP (~ 1 pM).

Conclusion & Future Work

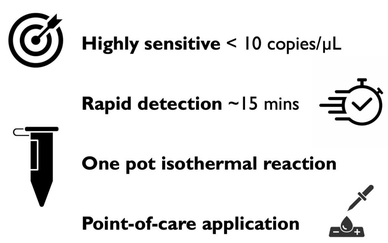


Figure 10: The advantages to using CRISPR Cas12a in the point of care setting

- Recombinase Polymerase Amplification (RPA) is required to detect lower concentrations of the synthetic DNA targets (HP and PN) and to establish an accurate lower limit of detection.
- Future work includes conducting an RPA primer screening assay to identify the most effective RPA primer, performing CRISPR-RPA assays on various saliva samples, and determining the limit of detection for the composition calibration curve of HP and PN targets.
- To incorporate this project into diagnostics, we need to use the CRISPR-RPA assay to determine the limit of detection (LOD) for a lateral flow assay test strip.

References

[1]Hashimoto, K. Emerging role of the host microbiome in neuropsychiatric disorders: overview and future directions. *Mol Psychiatry* (2023).
[2] Wingfield, B., *et al.* Variations in the oral microbiome are associated with depression in young adults. *Sci Rep* (2021).
[3]Hillary, V. E., & Ceasar, S. A., A Review on the Mechanism and Applications of CRISPR/Cas9/Cas12/Cas13/Cas14 Proteins Utilized for Genome Engineering. *Molecular Biotechnology*, (2023).

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