OMNIgene®•DISCOVER stabilizes microbial DNA profiles in oral fluid samples, enables more precise characterization of oral flora

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Abstract

Powerful analytical methods like Microarrays and Next Generation Sequencing give researchers the ability to easily detect and quantitate molecular targets in a highly multiplexed fashion. However, to take full advantage of such analytical capabilities, care must be taken to ensure that what is captured at the time of sample collection does not change by the time samples are processed and analyzed.

Due to the complex nature of samples required for analysis of the oral and gut microbiome, bias can be easily introduced through improper sample collection, stabilization and transport. In particular, the relative abundance of microbial species or "microbial profile" can change rapidly in an non-stabilized sample. Likewise, integrity of nucleic acids can rapidly degrade due to chemical and enzymatic activity.

DNA Genotek, in collaboration with the Forsyth Institute (developer of The Human Oral Microbe Identification Microarray (HOMIM)), designed this study to demonstrate the impact of standardized sample collection and stabilization. The HOMIM array contains hybridization probes to the 16S rRNA gene sequence of around 300 of the most prevalent oral bacterial species and has been shown to provide highly correlated results with 16S rRNA pyrosequencing at the phylum level, and is suitable for high-throughput oral microbiome studies. Importantly, HOMIM is also lower cost and less labour intensive than sequencing (Ahn et al., 2011)[1]. In this study, untreated oral fluid samples were compared on the HOMIM array to oral fluid samples collected and stabilized in OMNIgene•DISCOVER. The microbial DNA profiles of the untreated samples showed significant variation over the different temperatures and time points, whereas when stabilized with OMNIgene•DISCOVER the microbial profiles remained constant regardless of storage time and temperature.

Methods

Saliva from 10 healthy donors was collected into conical tubes. Equal volumes of saliva from each donor was transferred into a 50 mL conical tube and mixed 10 times using a sterile pipette. 1 mL of the pooled saliva sample was transferred into (a) 9 Control tubes containing 1 mL of Saline and (b) 9 OMNIgene•DISCOVER OM-505 kits. For each set of samples 3 tubes DNA was extracted immediately after collection, 3 tubes were incubated at room temperature and 3 tubes were incubated at 37°C. DNA from these tubes was extracted after 5 or 21 days. Bacterial DNA from the Control tubes was extracted using the protocol recommended by Dr. Bruce Paster, Forsyth Institute (HOMIM DNA Isolation Protocol). Bacterial DNA extraction from OMNIgene•DISCOVER/saliva samples was done as follows: the whole sample was heated for 1 hour at 50°C followed by heating for 15 minutes at 85°C; a 500 µL aliquot was transferred to a new 1.5 mL tube where it was disrupted using a bead beater for 1 minute. The DNA was quantified using PicoGreen. Equivalent amounts of DNA from the control and the OMNIgene•DISCOVER/saliva samples were used for establishing the oral microbiome profiles on the HOMIM microarray. The data from the HOMIM array was analyzed with Minitab 16 using a Cluster Analysis of Variables, based on complete linkage and a final partition of 3 clusters.

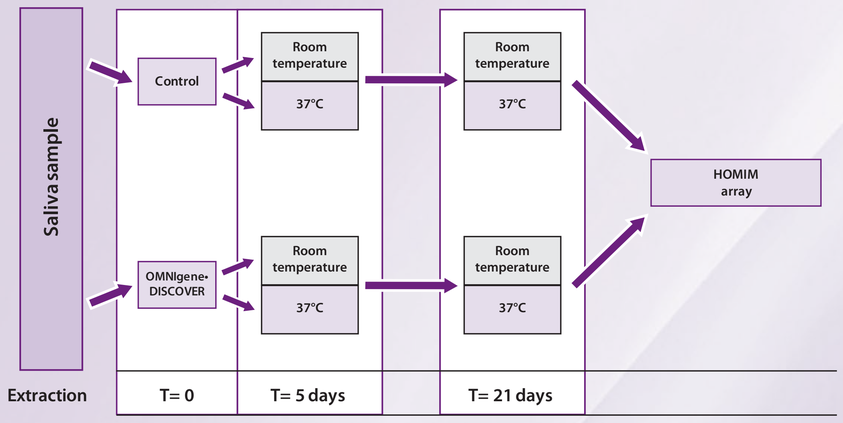
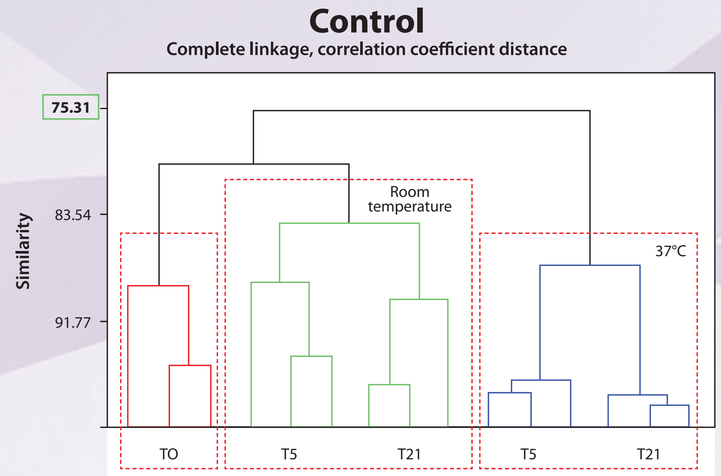


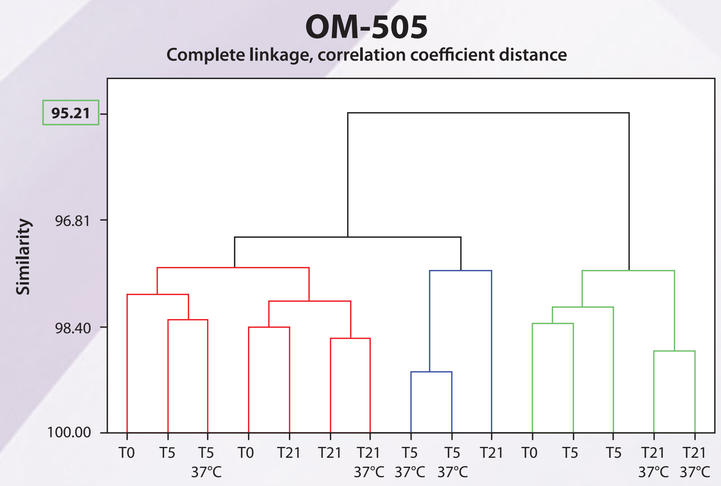
***Figure 1**: Experimental workflow.*

Results

After extraction, the integrity of the DNA was assessed by agarose gel. DNA extracted from all samples showed a single, high molecular band (data not shown). After quantification using PicoGreen® (Invitrogen), similar amounts of DNA were used for the establishment of microbial DNA profiles using HOMIM microarray. Cluster Analysis was used to analyze the data. The most relevant findings are:

- The similarity between the triplicates of the non-stabilized control samples processed immediately after collection was 89.09%. This represented the inherent variability of the HOMIM microarray. Triplicate control samples for all conditions presented a comparable or higher similarity levels than the control at time 0.
- The statistical analysis (Cluster Analysis of Variables) determined that samples incubated at room temperature or at 37°C are grouped in the same cluster, irrespective of the incubation time (similarity = 83.9% and 87.6% respectively). The similarity between the time 0 Control and these clusters were 79.4% (room temperature) and 75.3% (37°C) (Figure 2a). These results suggested that without proper stabilization the microbial DNA profile is significantly impacted by time and environmental conditions, most likely by differential bacterial growth and survival.
- In contrast to the non-stabilized control samples, clustering among samples collected and stabilized in OMNIgene•DISCOVER OM-505 is not based on storage time or temperature. Similarity among all samples was 95% (Figure 2b). Of note, one cluster contained a sample processed immediately after collection and a sample processed after 21 days at 37°C. This clearly demonstrates that collection and stabilization in OMNIgene•DISCOVER negates the effects of time and temperature on microbial profiles and DNA integrity.





***Figures 2a and 2b**: Stabilization reduces variability in sample after collection.*

Conclusion

The benefits of optimized sample collection and stabilization with OMNIgene•DISCOVER:

- The ability to collect and stabilize 'microbial profiles' over time, even at elevated temperatures
- Reduces bias. Efficient recovery/detection of nucleic acids from both gram positive and gram negative bacteria
- Applicable to research on microbiome, dentistry, and infectious diseases
- Cost effective, reliable self-collection by untrained users – increase participation and ensure study protocol compliance
- Standardized sample collection ensures samples collected at different times/locations within a study can be meaningfully compared.

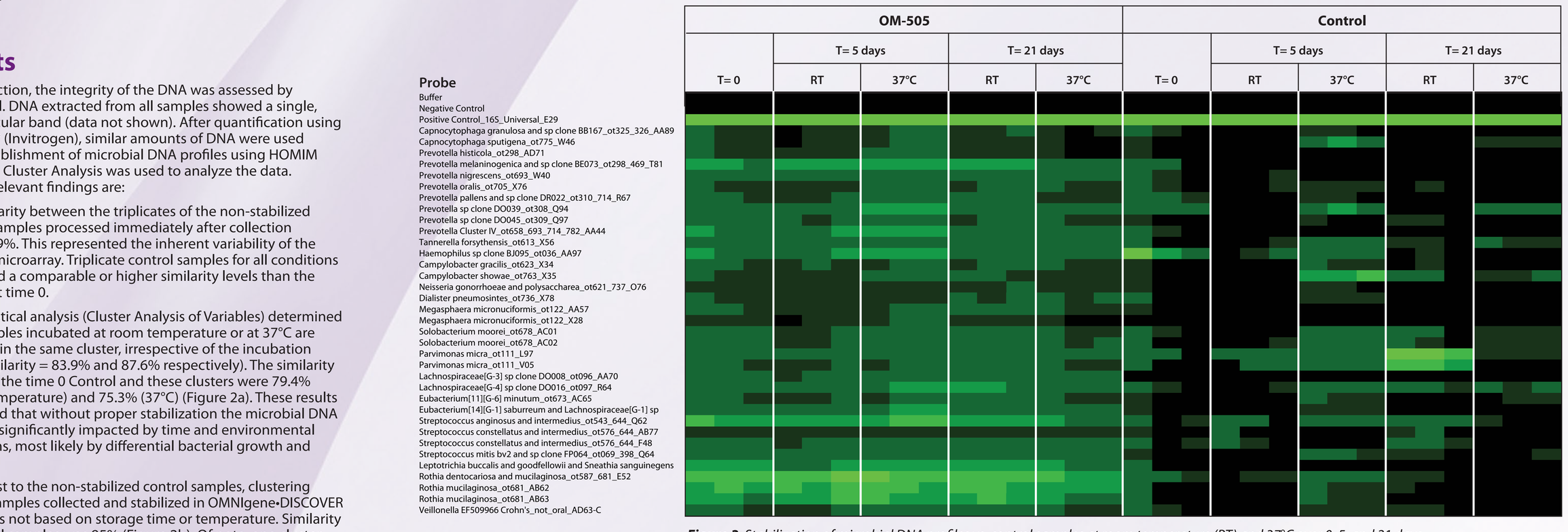


***Figure 3**: Stabilization of microbial DNA profiles vs. control samples at room temperature (RT) and 37°C over 0, 5, and 21 days.*

"By using HOMIM (Human Oral Microbe Identification Microarray), we demonstrated that saliva samples stored in OMNIgene were indeed stabilized for at least 3 weeks, even at 37 degrees. This is outstanding! Consequently, I think OMNIgene would be very useful for remote collections alleviating the need for a -80 freezer or, for that matter, any refrigeration to preserve samples. This is an incredible benefit for any investigator who is interested in preserving precious clinical samples, especially for use with molecular analyses of genomic material."

Bruce J. Paster, Ph.D., Director, Human Microbe Identification Microarray Core
Head, Department of Molecular Genetics, The Forsyth Institute
http://mim.forsyth.org

Reference

[1] Ahn JY, Yang LY, Paster BJ, Ganly I, Morris L, Pei ZH, Hayes RB. Oral microbiome profiles: 16S rRNA pyrosequencing and microarray assay comparison. PloS One2011;6(7):e22788

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