mawi
Biosampling Reinvented™

OMICs (including RNA) From the Same Sample: A Feasibility Study for Non-invasive Collection and Room Temperature Storage of Buccal Cells

Christopher Gregg[1], Hyacinth Ntchobo[2,3], Bassam El-Fahmawi[2]

1. University of Utah School of Medicine (https://gregglab.neuro.utah.edu)
2. MagBio Genomics, Inc. Gaithersburg, MD USA (http://www.magbiogenomics.com)
3. Mawi DNA Technologies, Hayward, CA USA (http://www.mawidna.com)

Introduction

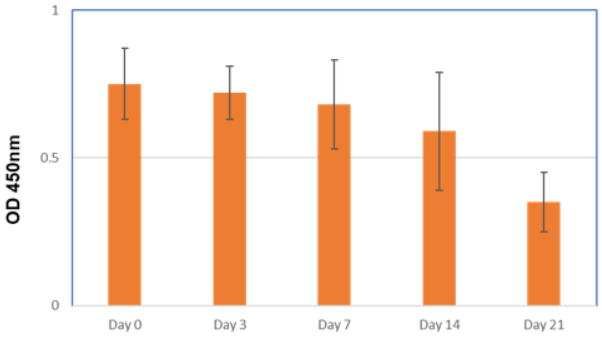
To create a more comprehensive profile of our health and wellness vs. disease, multiple analytes need to be included in the analytical mix such as DNA, RNA, proteins, and cell morphology. Unfortunately due to the limitations of current sample collection practices this means multiple samples need to be collected per individual to achieve this objective, with heavy cold chain involvement in both transport and storage. These limitations can make this type of analysis not only expensive but sometimes non-accessible which results in inconclusive research outcomes.

Usable and/or intact RNA is the most challenging macromolecule to obtain in a format suitable for analysis. Maintaining integrity of the RNA is important, but also keeping expression levels relatively stable is crucial to achieve somewhat meaningful insight into the sample status. Ideally, the RNA profile should be representative of the sample when it was collected or at the point of collection. Several oral invasive and non-invasive collection approaches have been developed specifically targeting RNA expression integrity and expression profiles (Archer et. al., 2016; Haque et. al., 2017; Attar, et. al. 2018; Hustler, et. al, 2018). Even though all samples were stored at -80°C immediately after collection, the tissue biopsy was the only sample that produced a significant RNA expression profile. However, invasive sample collection methods such as tissue biopsies are tedious, require professional expertise and heavy cold chain involvement, and limit discovery efforts in terms of accessibility and high cost (Lim and Punyadeera, 2018).

Clearly there is a need for a universal sample collection method that enables isolation and analysis of multiple analytes from the same sample to allow more precise medical discoveries. In addition, ideally this method should be simple and suitable for self or assisted collection, any population segment, and requires no specialized expertise to use (Basil et. al., 2018).

To address this need Mawi DNA Technologies has developed iSWAB-Cells, a universal non-invasive cell collection device,from which gDNA, RNA, proteins, and cell morphology can be analyzed from the same sample.

Epidermal Growth Factor Receptor Levels Remained relatively Unchanged in iSWAB-Cells



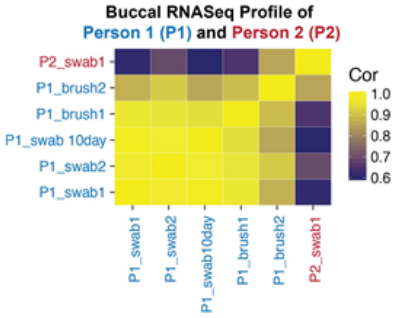
Experiment: Evaluation of the performance of iSWAB-Cells stabilization of membrane proteins at room temperature for extended periods of time utilizing Cell based colorimetric ELISA. Cell based ELISA analysis allows for protein analysis without the need for cell lysis and thus chosen accordingly. Pooled buccal cells sample collected with iSWAB-Cells from 10 subjects (Age: 21-60 Years) was tested at various time points for the stability of Epidermal Growth Factor Receptor, EGFR, Day 0, Day 3, Day 7, Day 21 post collection, while remained stored at room temperature. The stability level was measured in correlation to the intensity of the colorimetric intensity in each well at OD 450nm (10000 buccal cells/well).

Cell Based ELISA: Human EGFR In-Cell ELISA Kit from abcam (Cat No. ab126419)

EGFR Stability Assessment: Measurement of the loss of colorimetric intensity of the HRP substrate overtime at OD 450nm. All OD reading were normalized to iSWAB-Cells buffer before use.

Data Analysis: There is insignificant change in Epidermal Growth Factor Receptor levels in iSWAB-Cells after 14 days of room temperature storage.

RNAseq Profile of Buccal Cells from iSWAB-Cells: One Day vs. 10 Days Room Temperature Storage



Experiment: Comparison of Person 1 and Person 2, and repeated buccal cells collection with iSWAB-Cells of Person 1 by flocked swabs, or cytobrushes one day after 10 days post collection at room temperature storage.

RNA Extraction: Zymo Quick-RNA™ MiniPrep Plus (Cat. Nos. R1057/R1058)

Data Analysis: The figure is a correlation matrix of the FPKM read counts for every gene expressed in the RNASeq data, clustered by similarity (Pearson correlation)

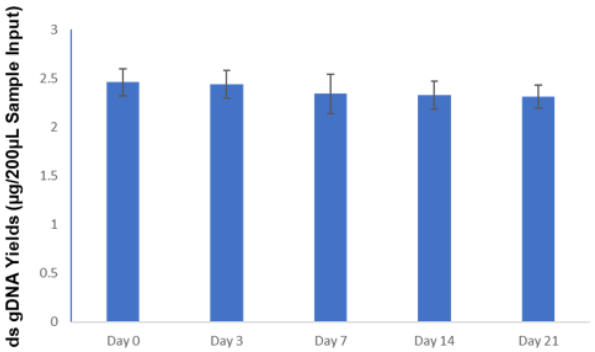
RNASeq Conditions: 60 million single end 50 base pair reads on an Illumina HiSeq using the Illumina TruSeq RiboZero stranded RNASeq kit.

Summary and Conclusions

Enabling scientists to analyze multiple OMICs parameters within the same sample will not only provide better assessment of health and disease, but will also reduce cost and the number of samples required thus improving sample collection compliance. Also, having the ability to transport and store at room temperature allows global access. Currently our understanding of health and disease is often limited to certain regions. Having worldwide access to biological samples will enrich our knowledge of humans and animals across the globe.

iSWAB-Cells represents a true universal, non-invasive sample collection tool that allows for the analysis of genomics, transcriptions and proteomics from the same sample while eliminating expensive cold chain involvement and multi-sampling steps.

iSWAB-Cells Stabilizes Double Stranded Human gDNA at Room Temperature for Weeks



Experiment: Evaluation of the ability of iSWAB-Cells to stabilize Human gDNA at room temperature for extended period of time. Pooled buccal cells sample collected with iSWAB-Cells from 10 subjects (Age: 21-60 Years) was tested at various time points, Day 0, Day 3, Day 7, Day 21 post collection, while remaining stored at room temperature.

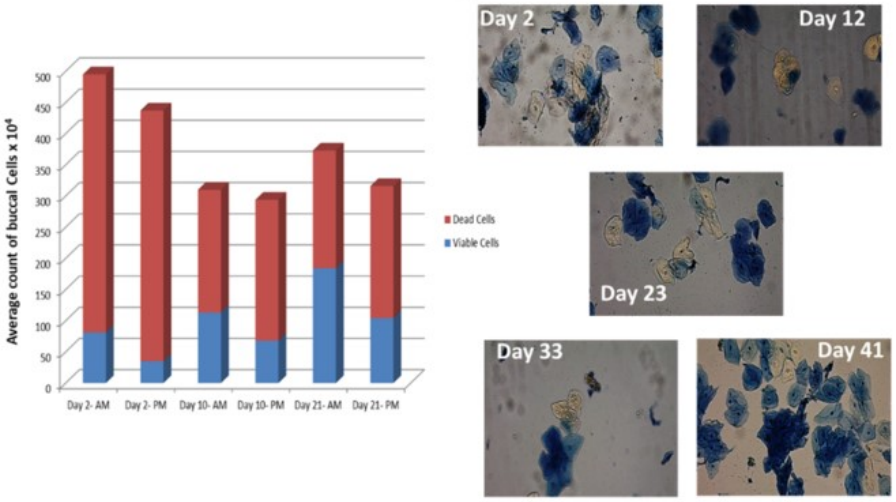
Human gDNA extraction: QIAamp DNA Blood Mini Kit (Cat. Nos. 51104/51106) from 200uL input as starting material from iSWAB-Cells

DNA Qualification: Qubit dsDNA HS Assay Kit (Cat. Nos. Q32851/Q32854)

Data Analysis: There is insignificant change in the ds gDNA output from iSWAB-Cells after 21 days of room temperature storage.

iSWAB-Cells maintains cells viable and intact for up to 4 weeks at room temperature

Time Course of Collected Buccal Cells with iSWAB-Cells: Viable vs. Dead Cells at Room Temperature Storage



Buccal Cells were collected from 6 volunteers (age: 19-50) following iSWAB-Cells collection procedure. Each volunteer provided a sample early in the morning before brushing their teeth (AM) and a second set of samples before dinner (PM). The cells were counted by light microscopy using a hemocytometer and Trypan Blue. The numbers of cells are the average of cells of the AM and PM collection samples. Buccal cells that did not take Trypan Blue appear colorless and counted to be viable, where the blue colored cells are the ones that took the Trypan Blue dye and counted as dead cells.

References

1. Archer NS, Liu D, Shaw J, Hannan G, Duesing K, Keast R (2016) A Comparison of Collection Techniques for Gene Expression Analysis of Human Oral Taste Tissue. PLoS, ONE, 2016, 11 (3)
2. Ashraful Haque, Jessica Engel, Sarah A. Teichmann, and Tapio Lönnberg. A practical guide to single-cell RNA sequencing for biomedical research and clinical applications. . Genome Medicine, 2017, 9:75
3. Moustafa Attar, Eshita Sharma, Shuqiang Li2, Claire Bryer, Laura Cubitt, John Broxholme,Helen Lockstone, James Kinchen, Alison Simmons, Paolo Piazza, David Buck, Kenneth J. Livak, and Rory Bowden. A practical solution for preserving single cells for RNA sequencing. Nature, Scientific Reports, 2018, 8:2151
4. Arianna Hustler, Ian Eardley, Jennifer Hinley, Joanna Pearson, Felix Wezel, Francois Radvanyi, Simon C Baker, and Jennifer Southgate. Differential transcription factor expression by human epithelial cells of buccal and urothelial derivation. Experimental Cell Research 2018, 369: (2) Pages 284-294
5. Yenkai Lim and Chamindie Punyadeera. A pilot study to investigate the feasibility of transporting saliva samples at room temperature with MAWI Cell Stabilization buffer. Cogent Biology, 2018, 4
6. Marcel Bassil, Aya Al Atat, and Mariam Ibragim. Detection of Oxidative Stress in Buccal Cells using iSWAB Tubes. J. Mol. Biomark. Diagn., 2018, 9:2