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Performance Comparison of MagBio Genomics RNA Extraction Chemistry with other Commercial kits for Buccal Cells Collected and Transported at Room Temperature With iSWAB-RNA-v2

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Introduction

Usable and/or intact RNA is the most challenging macromolecule to obtain in a format suitable for downstream 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, moreover they limit discovery efforts in terms of accessibility and high cost (Lim and Punyadeera, 2018).

The iSWAB-RNA-v2 from Mawi DNA Technologies allows for the non-invasive collection, concentration, and stabilization of intact buccal cells and/or any mammalian cells collected with a swab or cytobrush allowing for real time ambient stabilization of total RNA from the point of collection to processing. Maintaining RNA stability and protecting it from degradation is a significant challenge, current RNA stabilization methods such as PAXgene, Tempus, RNAgard or dry blood spots require invasive blood collection resulting in low compliance. The need to collect blood results in a very limited shelf life, forces labs to invest in cold storage infrastructure or commit resources to immediate sample processing. Other options for non-invasive collection also suffer from short shelf life storage as well as low quality and unusable RNA.

Comparing different RNA extraction chemistries is essential to optimize the yield and quality of RNA from any sample. Therefore in this study, column and para-magnetic bead based commercially available RNA extraction chemistries were evaluated with iSWAB-RNA-v2 samples stored at room temperature for 5-17 days. The objective is to identify reliable manual and automation friendly RNA extraction methods.

Summary and Conclusions

-There was insignificant change in RNA yields from MagBio HighPrep RNA Kit (no ProK & β-ME treatments) between day 5 and day 17 within evaluated extraction chemistries. This is a strong indication of the capability of iSWAB-RNA-v2 in stabilizing RNA samples stored at room temperature for a long period of time.

- Performing re-elution from both column-based chemistries improved the total RNA yield significantly. However, the RNA resulting from Zymo had better quality compared to Qiagen RNeasy kit.

-Two RNA extraction methods have been identified with high quality RNA outcome; column and para-magnetic bead based for both manual and automation friendly sample processing. However, the RNA produced by MagBio Genomics' extraction chemistry provided the highest RNA yield, quality and least number of steps as compared to column-based RNA extraction

-MagBio's Extraction kit can efficiently co-purify DNA and RNA from the iSWAB-RNA-v2

References

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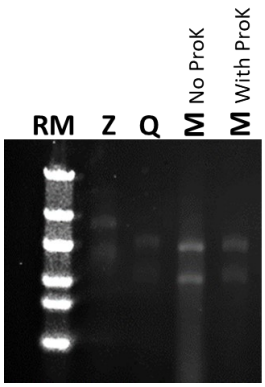
RNA Yields from Buccal Cells Collected and transported at Room Temperature with iSWAB-RNA-v2 Using Different Commercially Available Chemistries 5 Days Post Collection*



RNA Extraction Chemistry	RNA Conc (ng/µl)	Yield (ng)
Z	5.64	169.2
Q	3.27	98.1
M without ProK	**22.8**	**684**
M with ProK	5.15	154.5

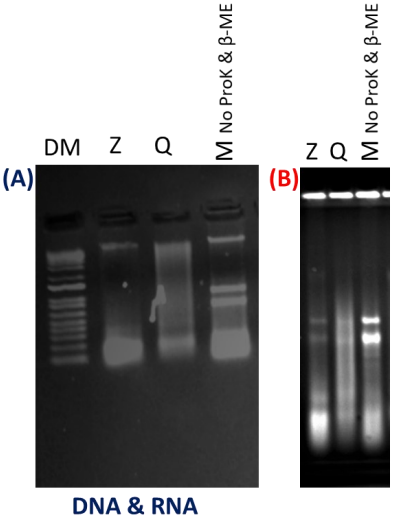
*Yields are from 200µL sample input from iSWAB-RNA-v2. Total sample volume is 1mL.

Experiment: Comparison of two different RNA extraction chemistries (column and magnetic bead based) from 200 µL input of buccal cells collection with iSWAB-RNA-v2 (A total of 7 samples where collected and pooled). To simulate standard shipping conditions, samples were shipped at room temperature from Hayward, CA to Gaithersburg, MD via FEDEX 3 days shipping.

RNA Extraction: RNA isolation was performed from iSWAB-RNA-v2 5 days post collection. Lane (Z) Zymo Quick-RNA™ MiniPrep Plus (column-based chemistry), **single elution** (Cat. Nos. R1054, R1055, R1057 and R1058), Lane (Q) RNeasy (column-based chemistry), **single elution** (Cat. Nos. 74104/74106), Lanes (M) MagBio's bead based HighPrep Total RNA Plus kit without and with proteinase K treatment (Cat. Nos., HPTOR-R50, HPTOR-R100, HPTOR-R400). Lane (RM) RNA marker.

Data Analysis: The figure shows RNA integrity of pooled iSWAB-RNA-v2 collected samples that were incubated for **5 days** at room temperature. A 1.2 % RNA denaturing gel (Lonza) was used and each well was loaded with 2.5 µL from 50 µL elution volume from perspective extraction chemistry. RNA yield was measured using Qubit RNA HS Kit.

DNA & RNA Yields from Buccal Cells Collected at and transported at Room Temperature with iSWAB-RNA-v2 Using Different Commercially Available Chemistries 17 Days Post Collection*



Experiment: Comparison of different DNA/RNA copurification chemistries from 200 µL input of buccal cells collection with iSWAB-RNA-v2 (A total of 7 samples where collected and pooled). To simulate standard shipping conditions, samples were shipped at room temperature from Hayward, CA to Gaithersburg, MD via FEDEX 3 days.

Nucleic Acid Extraction: Buccal cells (A) DNA & RNA co-purification and (B) RNA extraction protocol from iSWAB-RNA-v2 5 days post collection. Lane (Z) Zymo Quick-RNA™ MiniPrep Plus, **double elution** (Cat. Nos. R1054, R1055, R1057 and R1058), Lane (Q) RNeasy, **double elution** (Cat. Nos. 74104/74106), Lanes (M) MagBio Tissue DNA/RNA Co-isolation kit without proteinase K treatment & β-mercaptoethanol (Cat. Nos. HPTOR-R5, HPTOR-R50, HPTOR-R100, HPTOR-R400).

Data Analysis: Figure (A) shows DNA and RNA co-purification extracts in 1% non denaturing gel showing total nucleic acids (8µl/well) and Figure (B) shows 1.2% RNA denaturing gel (Lonza) with 2.5 µL/well of RNA extract from pooled iSWAB-RNA v2 sample stored for **17 days** at room temperature. DNA & RNA yield was measured using Qubit ds DNA and RNA HS quantification kits.

Nucleic Acid Extraction Chemistry	RNA conc (ng/µL)	RNA yield (ng)	DNA conc (ng/µL)	DNA yield (ng)
Z	8.41	**210.25**	42.9	**1072.5**
Q	20.4	**510**	28.7	**717.5**
M without ProK	**26**	**780**	**35.2**	**880**

*Yields are from 200µL sample input from iSWAB-RNA-v2. Total sample volume is 1mL.