GeneLab: Multi-Omics Investigation of Rodent Research-1 Biobanked Tissues

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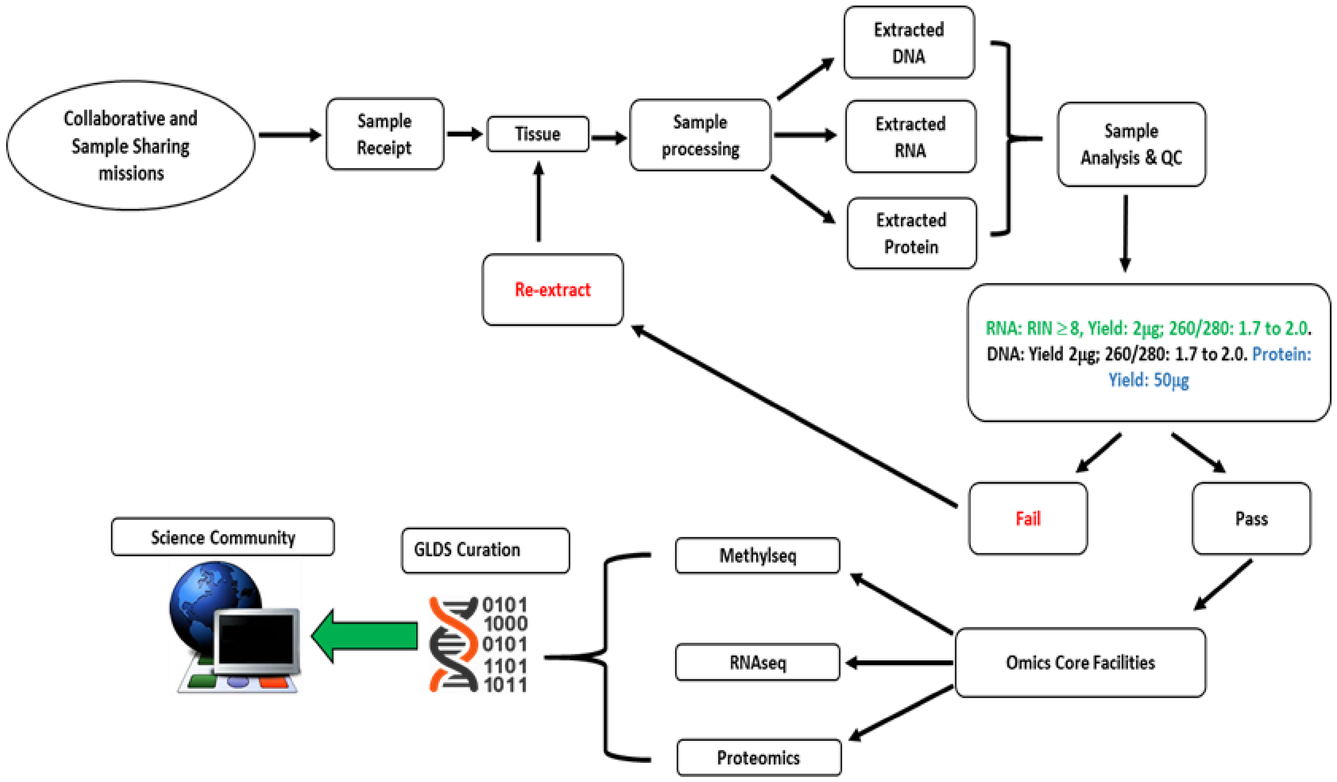
Background

Due to the limited resources for conducting biological experiments aboard the International Space Station (ISS), crew time must be used efficiently while maximizing high-quality science return. To support this endeavor, NASA initiated GeneLab a unique, open-access collaborative platform to 1) maximize the value of these experiments using a multi-omics, systems biology-based approach, and 2) disseminate these data without restrictions to the scientific community via the development of an open access data repository. The current investigation assessed viability of RNA, DNA, and protein extracted from archived RR-1 tissue samples for epigenomic (whole genome bisulfite sequencing), transcriptomic (RNAseq, miRNAseq and 5mC-RNAseq), and proteomic assays.

Rodents in the RR-1 mission were launched on Space-X4 and exposed to microgravity for 38 days. Upon culmination, a variety of tissue types were harvested either on-orbit or post-flight. The harvested organs and carcasses underwent a slow freeze in the **M**inus **E**ighty-degree **L**aboratory **F**reezer for **I**SS (MELFI) and were returned to Earth. Subsequently, mice were thawed and tissues were harvested and snap-frozen or preserved in RNAlater and were stored at least a year at -80^{O}C. Select tissues were made available to GeneLab through the Bio-specimen Sharing Program (BSP) managed by the Ames Life Science Data Archive and included: mouse adrenal glands, quadriceps, gastrocnemius, tibialis anterior, extensor digitorum longus, soleus, eye, and kidney. Protocols for and results of these tissue extractions, and the feasibility and value of these kinds of omics analyses are reported here.

GeneLab has selected a specific subset of biobanked RR-1 tissues for processing and omics analyses. Prioritization of the analyses is based on the scientific significance of spaceflight effects on these tissues. Priority is also given to the technical value of the omics analyses, which is designed to complement existing morphological, histological and gene expression data. A logistical approach enables maximum utilization of frozen carcass tissues from RR-1, and encourages a persistent collaboration between GeneLab and the PI community in order to provide, useful, complementary and novel data to the entire scientific community. In addition to providing opportunities for investigation of spaceflight effects on the mouse transcriptome and proteome in new kinds of tissues, these results may also be of value to program managers for the prioritizations of ISS crew time for rodent research investigations.

Omics Preparation Facility Workflow



Experimental Design

		Flight	Ground	Pooled	Flight + Ground Samples: ribodep-RNAseq	WGBS	Proteomics	miRNAseq (**)	RNAmethylseq
	RR1 Tissues	N	N	(L+R)	RNA	DNA	Protein	RNA	RNA
1	Adrenals	6(4)	5(4)	X	11	11	8		
2	SLS	6	6	X	12	12			
3	EDL	6	6	X	12	12			
4	TA	6	6		12	12	12	12	12
5	Kidney	6	6		12	12	12		12
5a	Kidney (Trizol)	5	6					11	
6	Gastroc *	6	6		12	12	12	12	12
7	Quad *	6	6		12	12	12	12	12
8	Eye	6	6		12	12			
9	Liver	6	6						12
9a	Liver (Trizol)	4	6					10	
				Total	95	95	56	57	60
				Final (N)	363				

RNA, DNA and protein were extracted for downstream omics analyses. Note tissue samples (*) were snap frozen in LN2, rest were preserved in RNAlater. SLS (soleus), EDL (extensor digitorum longus), TA (tibialis anterior), Gastroc (gastrocnemius) , and Quad (quadriceps). Omics analysis of miRNAseq was unsuccessful due to undetected levels of microRNA. (**).

Methods

NASA Animals & Euthanasia

- ~20 week old Female C57BL/6J mice in both flight (N=10) and ground (N=10) cohorts were injected with Euthasol (Virbac, TX) followed by cervical dislocation.

Dissection

- Mice tissues and carcasses underwent a slow freeze on-orbit in the MELFI and upon return to Earth they were thawed for harvesting. Harvested tissues samples were preserved with either LN2 or RNAlater, and were frozen at -80^{O}C and archived in the BSP
- Tissues used in RNA, DNA and protein extractions were frozen for ~1 year at -80^{O}C

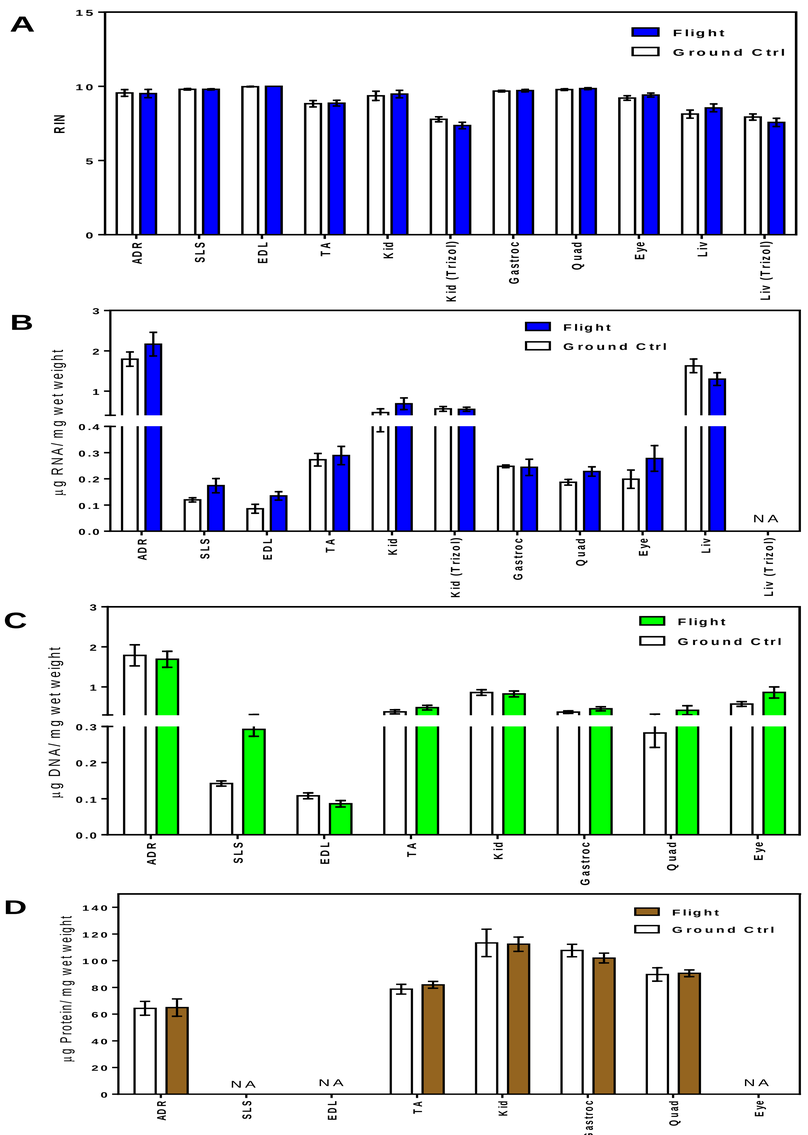
DNA and RNA

- Trizol based extractions, Allprep and PrepEase
- Buffer: 1:100 beta-mercaptoethanol to Buffer RLT (Qiagen, Valenica, CA)
- 20-30 mg of sample homogenized using handheld rotor-stator homogenizer
- DNA and RNA purified from homogenate via Qiagen's AllPrep DNA/RNA Mini Kit (Qiagen, Valencia, CA)
- Concentration and absorbance ratios measured using the NanoDrop 2000 and spectrophotometer (Thermo Fisher Scientific, Waltham, MA)
- RNA quality measured using the Agilent 2100 Bioanalyzer with the Agilent RNA 6000 Nano Kit (Agilent Technologies, Santa Clara, CA)

Protein

- Buffer: 1:100 0.1 M phenylmethanesulfonyl fluoride solution to urea-based buffer provided by Stanford University, with protease inhibitor added
- 20-30 mg of samples homogenized using handheld rotor-stator homogenizer
- Concentrations measured using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA)

Results



Results

- **A. Tissue RNA Integrity Number (RIN):** All tissues show high RIN (>7) across tissues and within cohorts and meet omics requirements.
- **B. RNA yield/mg tissue:** the yield varied across tissues but not within cohorts. SLS, Adr and EDL were pooled before extraction, all tissue yield met omics requirements
- **C. DNA yield/mg tissue:** : the yield varied across tissues but not within cohorts. SLS, Adr and EDL were pooled before extraction, all tissue yield met omics requirements
- **D. Protein yield/mg tissue**: the yield for all selected tissues met omics requirements. There was not enough tissue to perform both RNA and protein extractions for SLS, EDL and eye.

Conclusions

1. **Selected RR-1 biobanked tissues were successfully processed and analyzed for the following omics: transcriptomics, epigenomics, and proteomics.**

2. **All extracted products from 1+ year-old frozen carcass tissue preserved in LN2 or RNAlater met or exceeded omics core facility sample quality requirements.**

3. **Overall, on-orbit dissection and organ isolation have a tremendous impact on ISS crew time and limited resources. GeneLab has demonstrated the capability to perform high-quality extractions of RNA, DNA and protein from tissue isolated from mice carcasses. Although ideal, on-orbit dissections may not be necessary, since downstream processing or quality of the extractions are unaffected for these tissues**

GeneLab Data System

- All datasets obtained from omics core facilities were curated and published through the GeneLab portal **(http://genelab.nasa.gov)**. GeneLab anticipates the release of the RR-1 Biobanked Study dataset in early December 2016.

Glossary

- **Transcriptomics**: performed by analyzing the RNA or microRNA transcripts using deep sequencing methodology known as RNAseq or miRNAseq
- **Epitrancriptomics**: defined as functionally relevant changes to the transcriptome that do not involve a change in the ribonucleotide sequence
- **Epigenomics**: the study of the complete set of epigenetic modifications derived by performing deep sequencing on genomic DNA using the methodology known as DNA methyl-seq
- **Proteomics**: the global identification of proteomic depth reads and their modifications from isolated proteins

Acknowledgements

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