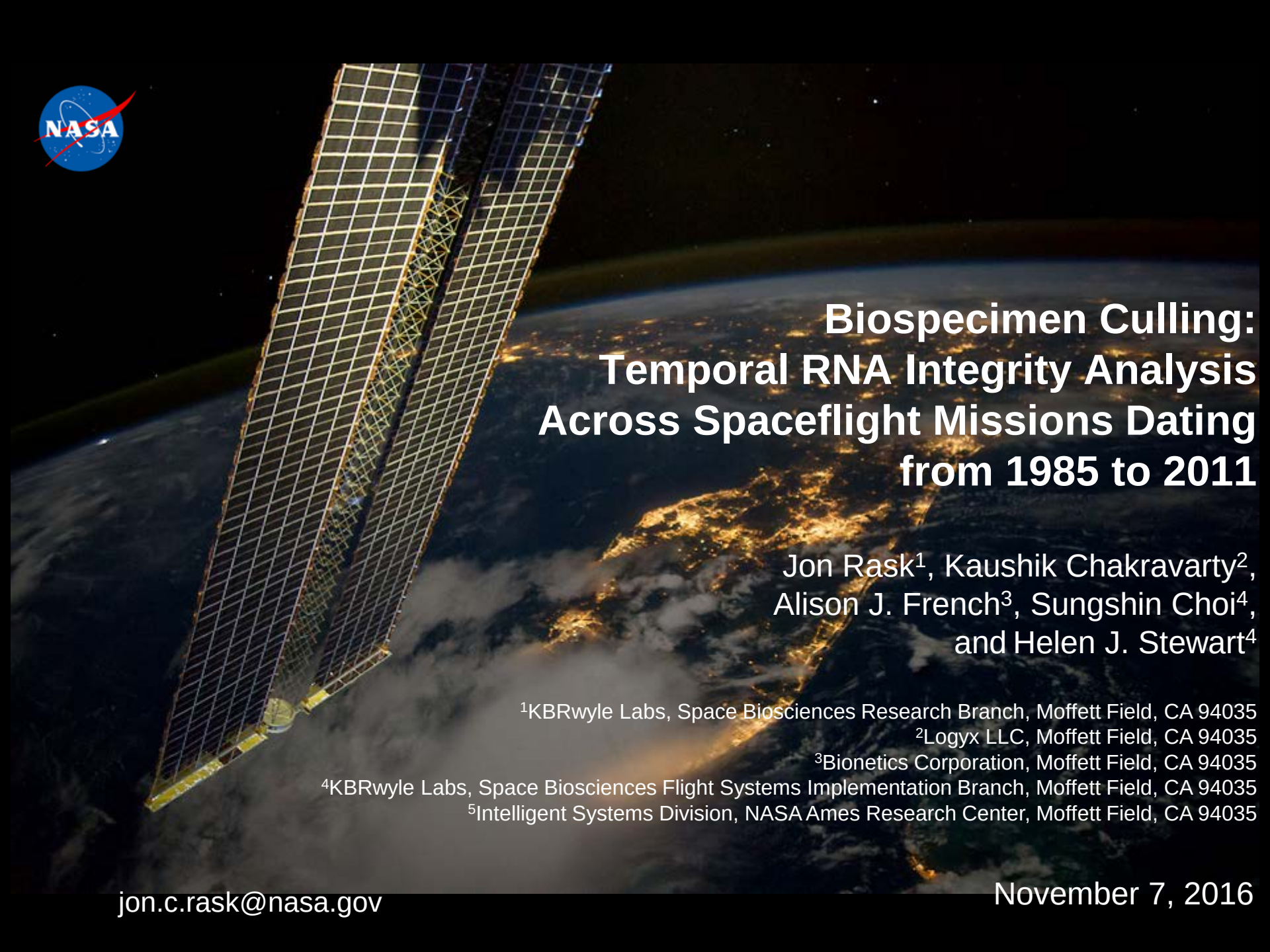


Biospecimen Culling: Temporal RNA Integrity Analysis Across Spaceflight Missions Dating from 1985 to 2011

Jon Rask[1], Kaushik Chakravarty[2], Alison J. French[3], Sungshin Choi[4], and Helen J. Stewart[4]

[1]KBRwyle Labs, Space Biosciences Research Branch, Moffett Field, CA 94035
[2]Logyx LLC, Moffett Field, CA 94035
[3]Bionetics Corporation, Moffett Field, CA 94035
[4]KBRwyle Labs, Space Biosciences Flight Systems Implementation Branch, Moffett Field, CA 94035
[5]Intelligent Systems Division, NASA Ames Research Center, Moffett Field, CA 94035

jon.c.rask@nasa.gov

November 7, 2016

Outline

- **Introduction**
  - Ames Life Science Data Archive (ALSDA)
  - Biospecimen Storage Facility (BSF)
  - Institutional Scientific Collection (ISC)
- **What is Biospecimen Culling?**
- **Objectives**
- **Approach**
- **Results**
- **Summary**
- **Future Work**

Ames Life Science Data Archive

NASA Ames Research Center project node of the NASA Space Life Sciences Data Archive

Responsible for the capture, preservation and dissemination of life science data and biospecimens from NASA Ames-managed flight and ground experiments.

ARC Life Sciences Institutional Scientific Collection: The Biospecimen Storage Facility (BSF)

- **The BSF maintains fixed and frozen biospecimens from Space Shuttle and International Space Station missions (1985 to current).**
- **Approved for storage of tissues with radioactive isotopes.**
- **Seven -86ºC Freezers, ~0.6 m^3 each, one is a back-up)**
- **Fail-safe power backup; connected to emergency generator; all units alarmed and monitored 24/7.**
- **Inventory management with secure database**

  -species, tissue type, fixation, treatment, location, other metadata, and chain-of-custody information

What is culling?

- **Sampling of biospecimens in the Ames Life Sciences Institutional Scientific Collection to determine characteristics for disposition.**

- **Dispositions include:**

  (1) Continue to store sample

  (2) Disposal

Objectives

1. **Identify candidate biospecimens for RNA integrity analysis.**
2. **Perform extraction and purification of RNA.**
3. **Collect RNA integrity data from archived space flight tissues.**
4. **Prepare a NASA white paper and/or a manuscript**
5. **Support and guide NASA policy on best practices for curation of biological collections (NPD 7100.xx Scientific Collections Directive), addressing storage duration and temperature, sample testing cycle and frequency.**

Approach

Phase 1

Create and sort biospecimen list derived from the ALSDA tissue tracking system that contains tissues from 1985 to 2011

- Sort information on mission, launch date, payload, kind of organism, tissue type, number of tissue samples, radioactive tracers, storage temperature, and fixative used (if any).
- Develop standard operating procedures

Phase 2

Preparation and processing of selected biospecimens

- Determine RNA Integrity Number (RIN) of selected biospecimens

Phase 3

Support development of NASA scientific collections directives, perform additional sample analyses, draft manuscripts, outreach, draft requests for proposals

Approach: Criteria to Identify Candidate Biospecimens for RNA Analysis

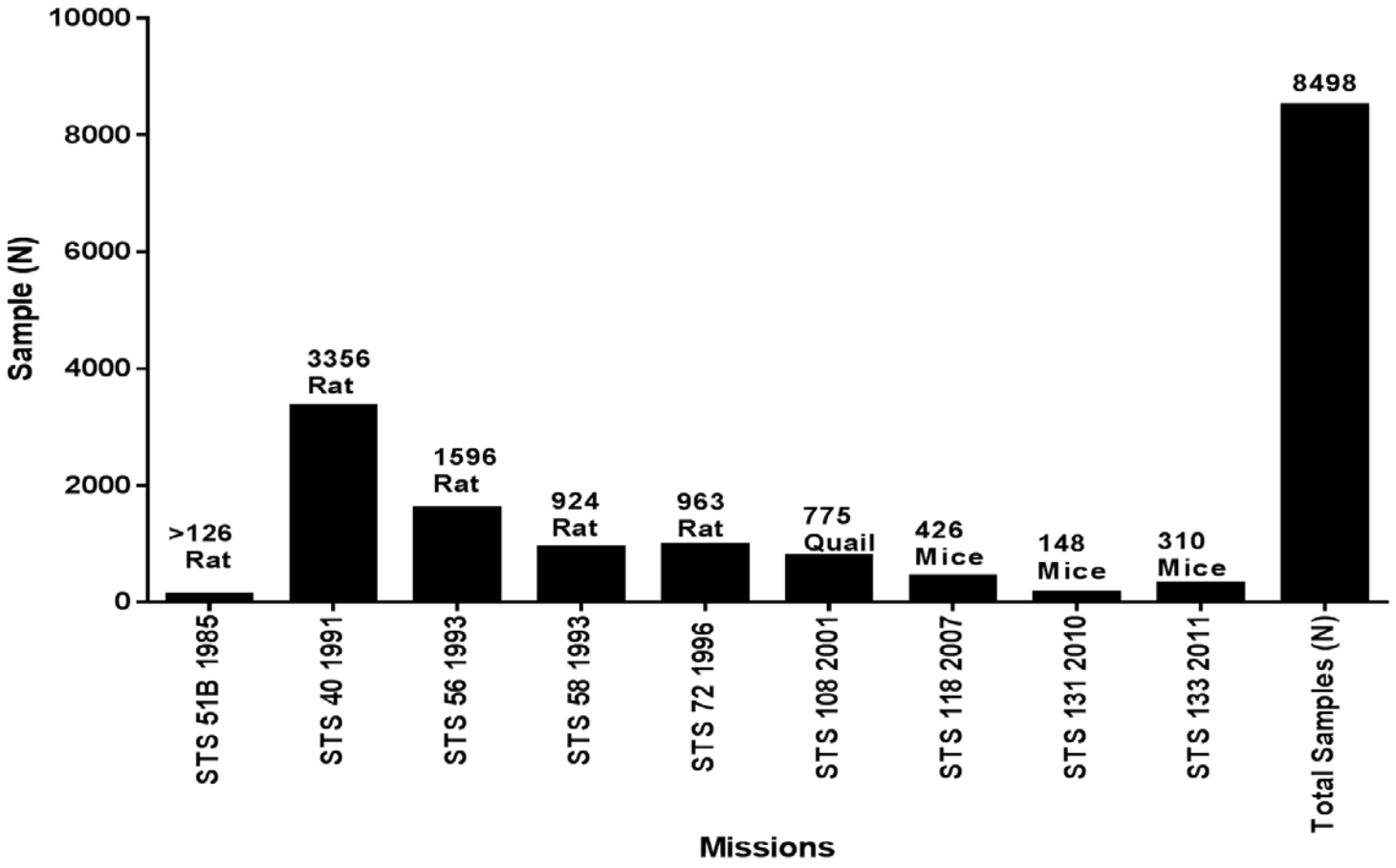
- **Select in***
  - stored at -70˚C or colder
  - Preserved in RNALater
- **Select out****
  - stored at 4˚C/ambient or in aqueous buffer without fixative.
  - Dehydrated samples, stored at room temp, samples that have experienced freeze thaw cycles, or appear deteriorated.
  - Radioactive samples containing 3H-Thymidine and 3H-Proline (half life = 12.3 years).
  - Unlabeled

*~89 biospecimens identified as candidates RNA Analysis
**All select out specimens will be stored for future assessment

Results: Biospecimen Culling List Summary

Date	Mission	Payload	Organism	# of Biospecimens	# of Samples
4/29/85	STS-51B	SL-3	Rat	9	>126
6/5/91	STS-40	SLS1	Rat	58	3346
4/8/93	STS-56	PARE.03	Rat	23	1615
10/18/93	STS-58	SLS2	Rat	56	>999
7/13/95	STS-70	NIH.R2	Rat	2	127
1/11/96	STS-72	NIH.R3	Rat	23	1022
12/5/01	STS-108	ADF	Quail	24	729
8/8/07	STS-118	CBTM2	Mouse	9	426
4/5/10	STS-131	Immune_STL	Mouse	4	180
2/24/11	STS-133	MI2	Mouse	8	>302
Totals	**10**	**10**	**3**	**216**	**>8872**

Summary List



Results: RNA Analysis Candidates

Date	Mission	Payload	Organism	# of Biospecimens	# of Samples
4/29/85	STS-51B	SL-3	Rat	8	>126
6/5/91	STS-40	SLS1	Rat	31	1376
10/18/93	STS-58	SLS2	Rat	40	>667
7/13/95	STS-70	NIH.R2	Rat	2	127
12/5/01	STS-108	ADF	Quail	5	135
8/8/07	STS-118	CBTM2	Mouse	8	384
2/24/11	STS-133	MI2	Mouse	3	168
Totals	**7**	**7**	**3**	**97**	**>2983**

Results: Common Tissue Types
in the RNA Analysis Candidates

Date	Mission	Payload	Organism	Liver	Lungs	Bone	Kidneys	Stomach	Adrenals
4/29/85	STS-51B	SL-3	Rat	No	No	No	No	No	No
6/5/91	STS-40	SLS1	Rat	Yes	Yes	No	Yes	Yes	Yes
10/18/93	STS-58	SLS2	Rat	Yes	Yes	Yes	Yes	Yes	Yes
7/13/95	STS-70	NIH.R2	Rat	No	No	No	No	No	Yes
12/5/01	STS-108	ADF	Quail	Yes	Yes	Yes	Yes	No	No
8/8/07	STS-118	CBTM2	Mouse	No	No	Yes	No	Yes	No
2/24/11	STS-133	MI2	Mouse	No	No	No	Yes	Yes	Yes

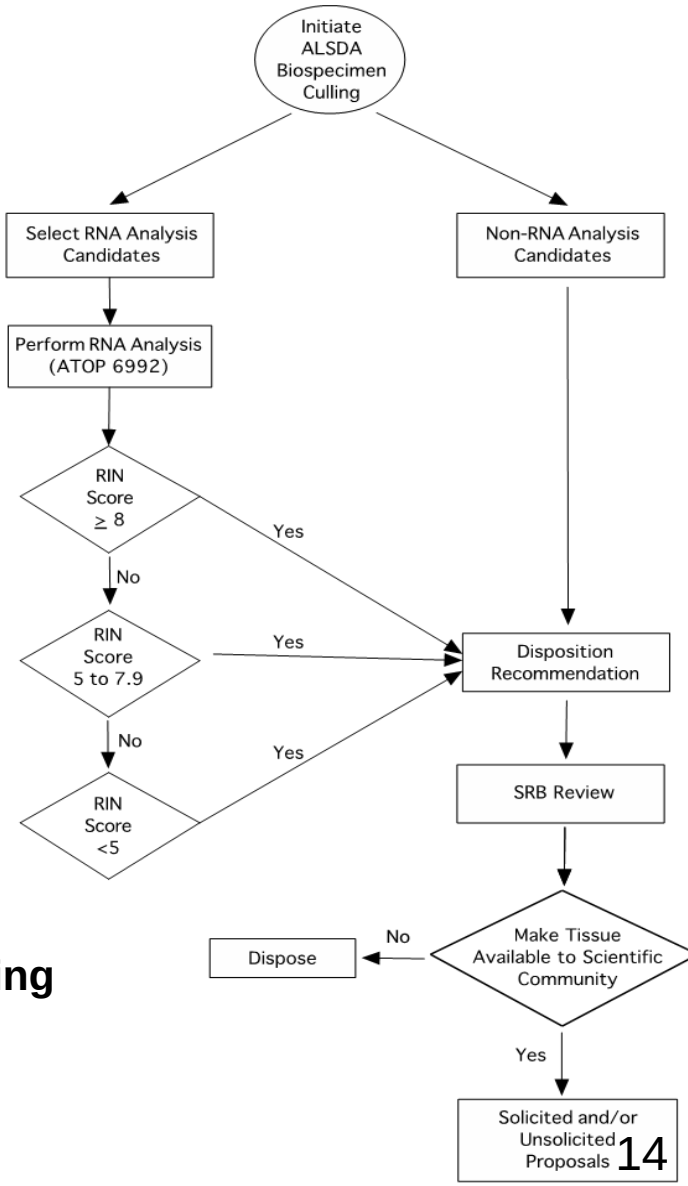
Assumptions for RIN Data

8 or above:	**Viable for future omics analysis**
5 to 7.9:	**Viable for qPCR.** **Samples may still have viable DNA and could be used for genomics or immunohistochemistry.**
Less than 5:	**Considered non-viable for RNA analysis.** **The samples may still have viable DNA and could be used for genomics or immunochemistry.**

RIN data will be made available to the science community

Summary

- **Developed an RNA-Integrity culling methodology, project documentation, and supporting operating procedures.**
- **Identified candidate biospecimens for analyses.**
- **Established Science Review Board to review disposition recommendations.**
- **Future sample analyses will assess storage duration and temperature, sample testing cycle and frequency**
- **Created scientific investigation opportunities, including temporal assessment of tissue viability**



For More Information

NASA Life Science Data Archive (LSDA):
http://lsda.jsc.nasa.gov

Biospecimen Requests:
http://lsda.jsc.nasa.gov/common/datarequest.aspx

NASA Ames Life Science Data Archive (ALSDA):
https://www.nasa.gov/ames/research/space-biosciences/data-archive-project

NASA Ames Space Biosciences
https://www.nasa.gov/ames/research/space-biosciences

NASA Ames Institutional Science Collection: Contact Helen Stewart:
helen.j.stewart@nasa.gov

Acknowledgements

NASA Ames Research Center

Elizabeth Taylor
Helen Stewart
Mike Skidmore
Jon Rask
Kaushik Chakravarty
San-Huei Lai
Alison French
Rick Chen
Sungshin Choi
Oana Marcu
Frances Donovan
Alan Wood
Matt Lera
Science Review Board

NASA Kennedy Space Center

Jeff Smith

Funding from NASA Space Biology Project