

Space Biology

Space Life and Physical Sciences Division

Human Exploration and Operations Mission Directorate

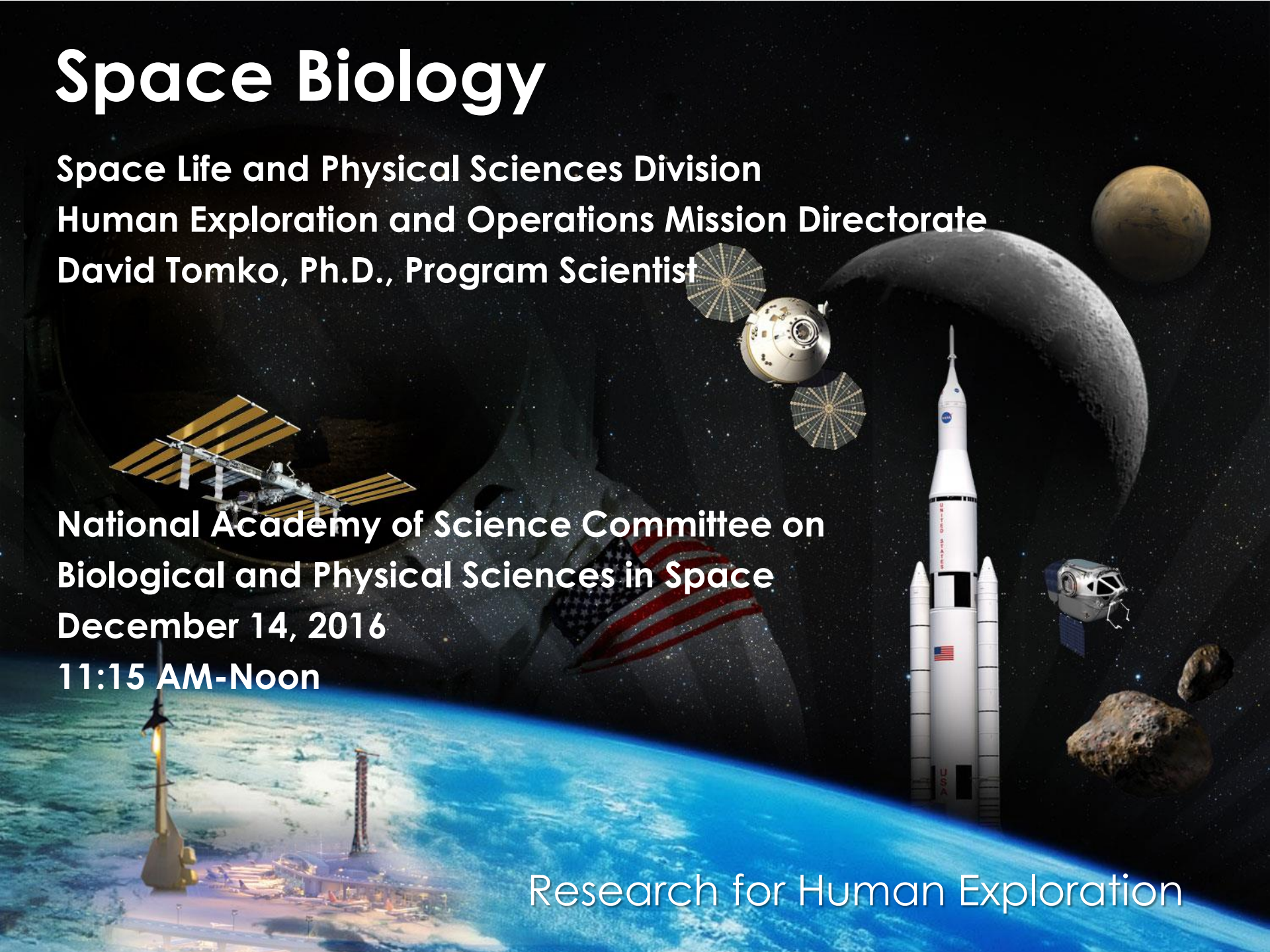
David Tomko, Ph.D., Program Scientist

National Academy of Science Committee on
Biological and Physical Sciences in Space

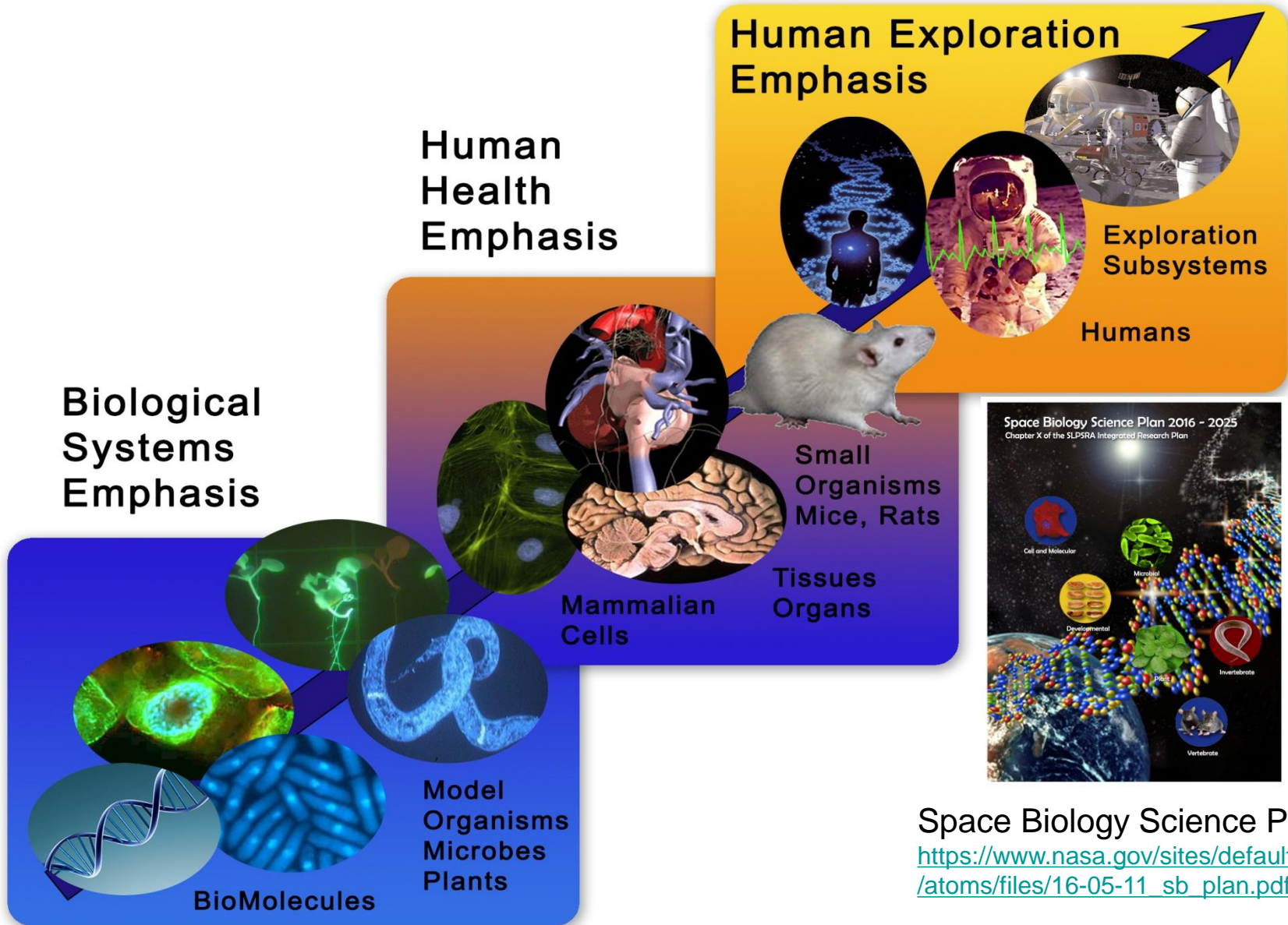
December 14, 2016

11:15 AM-Noon

Research for Human Exploration



Space Life Sciences Research Continuum



Space Biology Science Plan
https://www.nasa.gov/sites/default/files/atoms/files/16-05-11_sb_plan.pdf 2

What is Translational Research by Design?



- 2011 NRC Decadal Survey Recommended Horizontal Integration and Vertical Translation within the NASA Life Sciences Portfolio
 - **Horizontal Translation:** increase integration of efforts within each level of research, toward conduct of cross-disciplinary, focused translational research and development with applications to human space risks and related areas supporting human space exploration.
 - **Vertical Translation:** Research teams to create “meaningful interactions among basic, preclinical, and clinical scientists to translate fundamental discoveries into improvements in health and well-being of crew members in space and their re-adaptation to gravity.”
- **For Space Biology and HRP management** - Identify a knowledge gap that basic research can potentially fill, and solicit research to fill that gap.
 - This can either be “pull”, where the program with the gap asks for the research or “push”, where the basic science program sees a potential solution to an applied problem and solicits research to fill it.
 - Either way, the solicitation of the fundamental research is “**designed**” to fill a particular gap, or answer a particular question.

Space Biology and the Human Research Program



Basic Research

Medical Operations

Space Biology (SB)

How does life respond, adapt, develop, interact and evolve in spaceflight across gravity levels?

- Microbiology
- Cell & Molecular Biology
- Plant and Animal Biology
- Developmental, Reproductive & Evolutionary Biology
- Systems Biology & Omics (GeneLab)

Science exploring the unknown

Synergies

SB and **HRP** coordinate to define research priorities and identify opportunities to facilitate countermeasure development

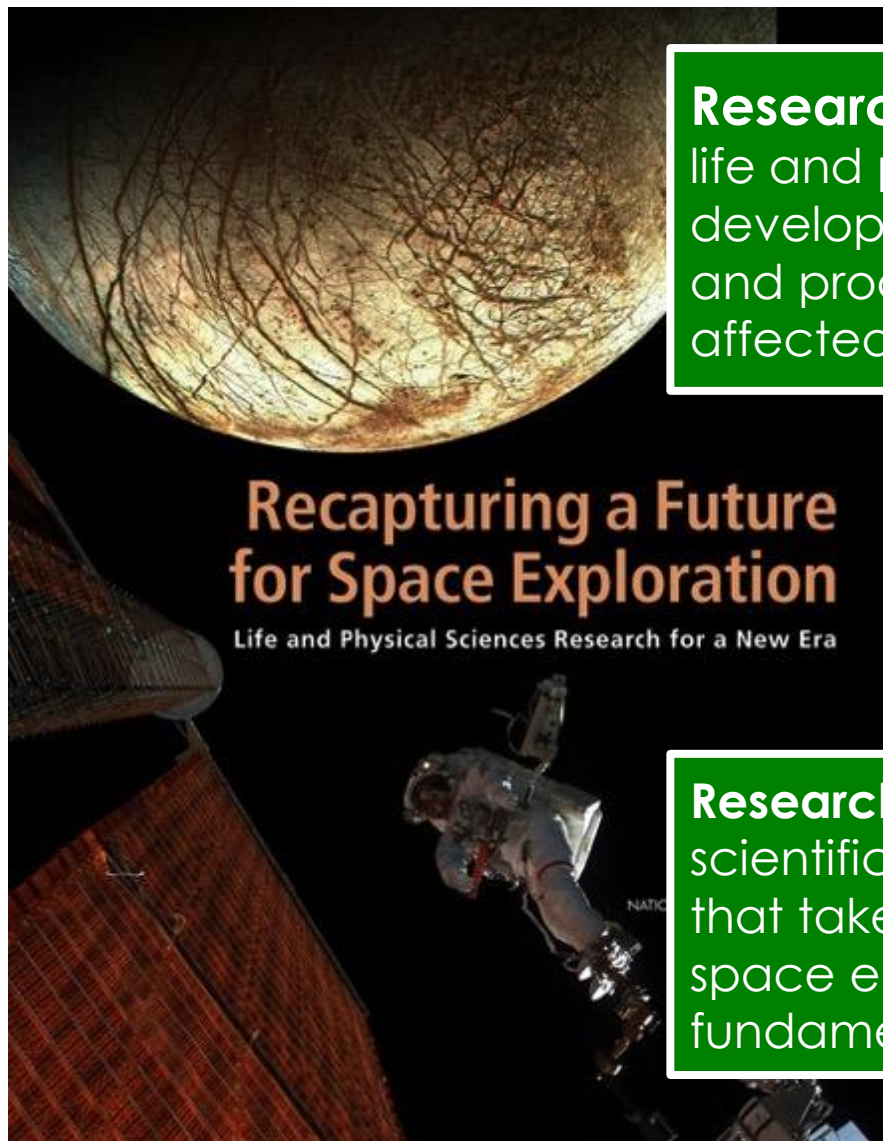
- Cell, Tissue & Animal Studies
- Immunology
- Wound healing & fracture repair
- Radiation/Microgravity interactions
- Oxidative Stress and Damage
- Microbe-Host Interactions
- Visual Impairment Syndrome
- Artificial Gravity/ Gravity as a Continuum

Human Research Program (HRP)

Identify, characterize, and mitigate human health & performance risks in spaceflight

- Exercise Countermeasures
- Physiological Countermeasures
- Space Radiation Biology
- Behavioral Health and Performance
- Space Human Factors and Habitability
- Exploration Medical Capability

Science addressing known risks



Research that enables space exploration: life and physical sciences research needed to develop advanced exploration technologies and processes, particularly those profoundly affected by operation in a space environment.

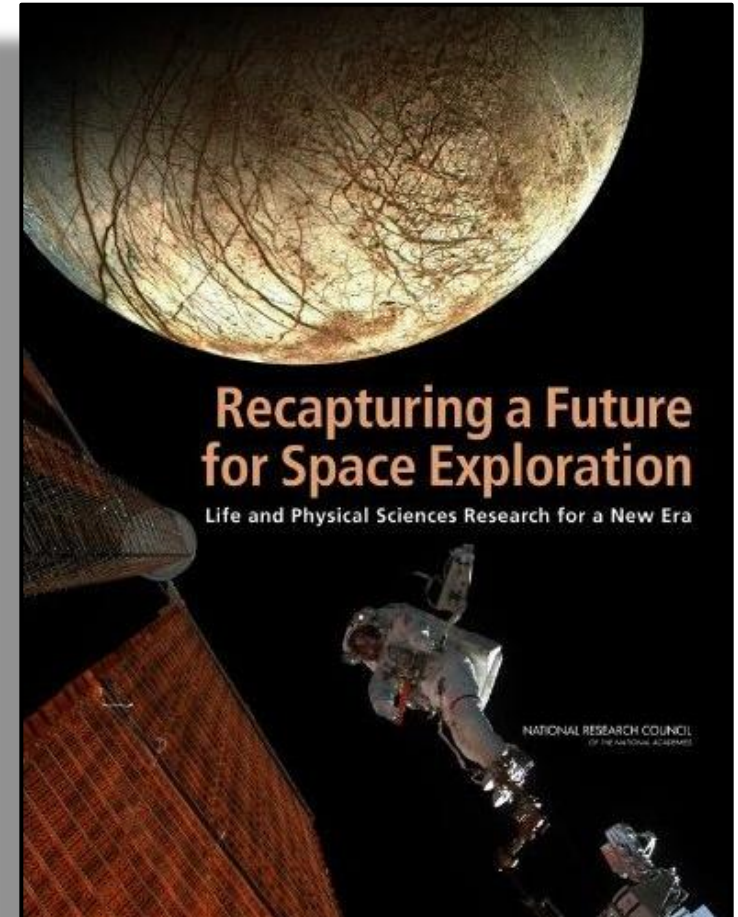
SLPS' purpose is two-fold:
Enable exploration and Pioneer scientific discovery

Research enabled by access to space: scientific research in life and physical sciences that takes advantage of unique aspects of the space environment to significantly advance fundamental scientific understanding

Space Life Sciences Recommendations for 2010-2020: Using the 2011 NRC Decadal Study



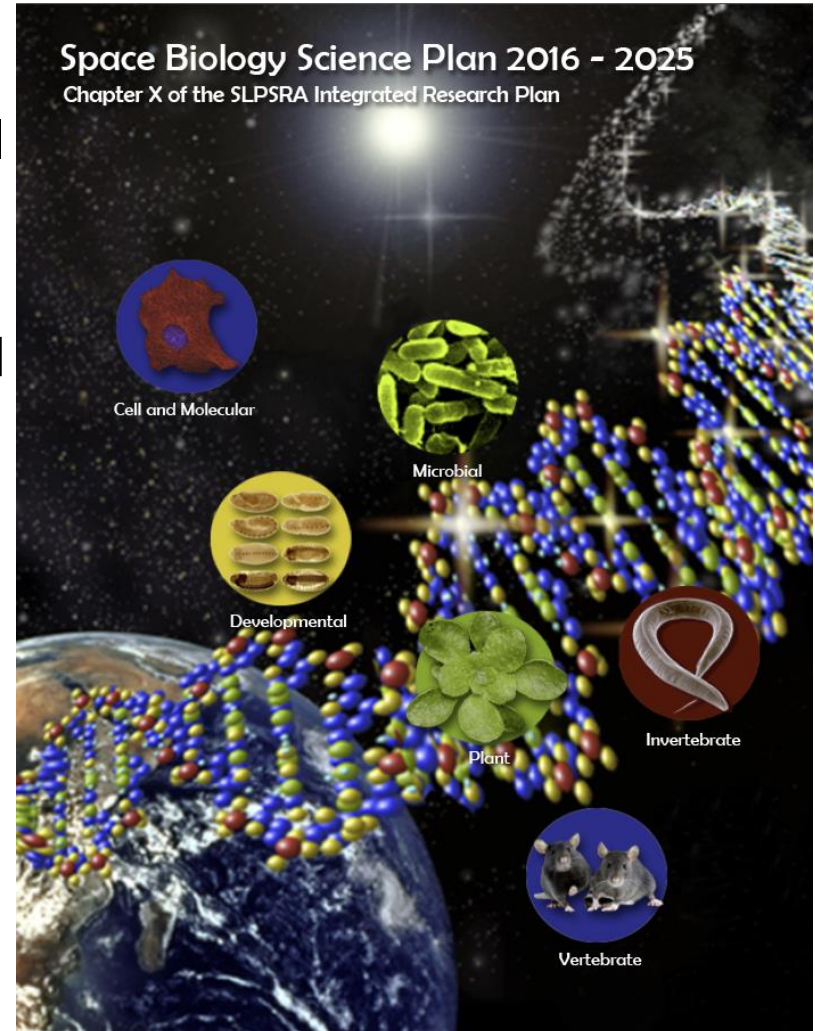
- Plant and Microbial Biology
 - Multigenerational studies
 - Responses to spaceflight
 - Plants and microbes in closed-loop life support
- Animal and Human Physiology
 - Bone and muscle studies
 - Drug/countermeasure evaluations
 - Vascular and interstitial pressure changes during spaceflight
 - Orthostatic intolerance
 - Deposition of aerosols in lung
 - T-cell and immune system studies
 - Multi-generation and early development
- Cross-Cutting Issues for Humans in Space
 - Artificial-G as a countermeasure
 - Animal studies to assess radiation risks
 - Cellular studies to define biomarkers for radiation toxicity
 - Understanding sex differences in adaptation to spaceflight



http://www.nap.edu/catalog.php?record_id=12944

Vision and Goals

- Create new knowledge of how different gravity levels affect biological systems important to human space exploration
- Build Links between Space Biology and Human Research
- Perform translational research by design from DNA and RNA to clinical medicine
- Leverage & amplify Space Biology findings using state-of-the-art omics, molecular/systems biology tools, & open access GeneLab data base
- Train and inspire a new generation of Space Biologists



Implementation Strategies



- Scientific Investigations
 - Traditional PI-led
 - Team-led
 - Science Definition Teams
 - Co-Principal Investigator Teams
- Disseminate results through databases
 - Timely data deposition
 - With space flight metadata
 - With unique analysis tools
 - Supported by GeneLab, Physical Science Informatics, MaterialsLab, etc.
- Partner to ensure value and increase capability
 - Exploration customers (AES, HRP, STMD)
 - Potential adopters (CASIS, NIH, NIST, NSF, OGAs; international partners; industry)





Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- Challenges
- Genelab Update



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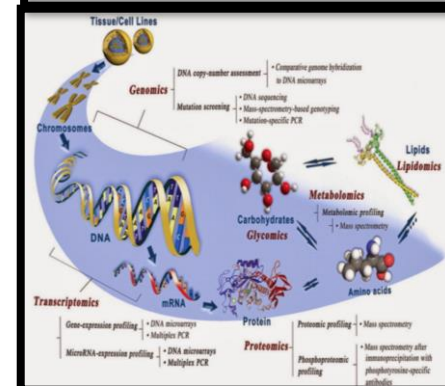
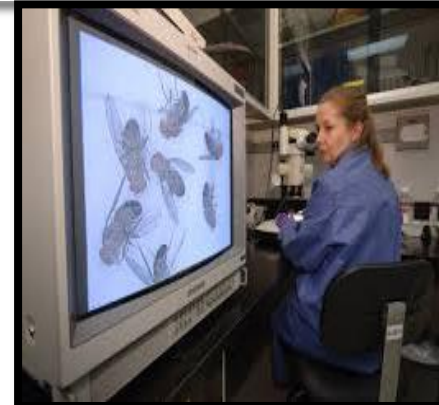
Current Ground Grant Status:

• 16 Ground NRA Space Biology grants

PI Last Name	Project Title
Bloomfield	Iron Overload and Oxidative Damage: Regulators of Bone Homeostasis in the Space Environment
Delp	Disuse Osteopenia: A Potential Vascular Coupling Mechanism--NNX14AQ57G
Foster	Impact of Microgravity on the Cell-Cell Interactions between a Mutualistic Bacterium and Its Animal Host
Haswell	Mechanosensitive Ion Channels in Plants: Genetic, Computational and Systems-Level Approaches to Underst
Hoffman	Gravity-Induced Plasticity in Mammalian Utricular Hair Cells: Intrinsic or Multisensory?
Hogan	Pre-Treatment Approaches for Improving the Response of Bone to Disuse and Recovery
Judex	Genes that Predict the Loss of Bone during Weightlessness
Lawler	Redox Regulation of nNOS Translocation and Muscle Atrophy During Mechanical Unloading
Mao	Role of Oxidative Stress in Mediating the Effects of Combined Exposure to Simulated Microgravity and Radiatic
Mehta	Determination of Roles of Microgravity and Ionizing Radiation on the Reactivation of Epstein-Barr Virus
Monshausen	Defining the Role of the Receptor Like Kinase FERONIA in Plant Architecture Development Under Mechanical L
Rojas-Pierce	The Role of Vacuole Membrane Fusion in Plant Gravity Perception
Sabanayagam	Epigenetic and Protein Expression Pattern Profiling of Caenorhabditis elegans Exposed to Time-Varying Gravita
Sams	Integration of Mechanotransduction and T-cell Activation Thresholds: Understanding of the Effects of Mechani
Ferl	Hypobaric Plant Biology - Molecular Responses of Arabidopsis to the Low Atmospheric Pressures of Spaceflight
Roux	Rapid Signaling Changes Induced by Gravity in Cells of the Fern Ceratopteris richardii

• 2 Space Biology/HRP Artificial Gravity grants

- 15-15 NASA AG-0013 "Partial-Gravity Dose Response: Roles of vestibular input & sex in response to AG" - Charles Fuller (University Of California, Davis) Team Alwood, Hoban-Higgins, Ronca
- 15-15 NASA AG-0005 "Musculoskeletal response to partial-gravity analog in rats: structural, functional & molecular alterations" - Seward Rutkove (BETH ISRAEL DEACONESS) Co-I Boussein



Space Biology Ground Research Status - II



6 GeneLab Innovation grants awarded October 2016

PI Last Name	Organization	Title	Experimental or Tool Development	Model System	NEW SB PI or Early Career Investigator?	Center Mgmt
Allen	University Of Florida, Gainesville	Microgravity effects on co-cultured vascular cells types	Experimental	Cocultures of endothelial and circulating stem cells	Yes (Young Investigator)	ARC
Gilroy	University Of Wisconsin, Madison	Genelab: Revealing Spaceflight- and Gravity-Response Networks in Plants	Experimental and Tool Development	Arabidopsis	No	KSC
Keller	University Of Wisconsin, Madison	Omics data mining of the ISS Aspergillus fumigatus strains in elucidating virulence characteristics	Experimental	Aspergillus	Yes (New)	ARC
Meyerowitz	Cal Tech	Using GeneLab Data to identify novel gravity sensory components in Arabidopsis	Experimental	Arabidopsis	Yes	KSC
Nicholson	University Of Florida, Gainesville	Comparative evaluation of microbial transcriptomic responses to spaceflight stress: elucidating underlying molecular mechanisms	Experimental and Tool Development	B. subtilis and other bacterial strains	No	KSC
Porada	Wake Forest University	Effects of microgravity on the risks of space radiation-induced leukemogenesis	Experimental	Human Hematopoietic Stem Cells	Yes (New)	ARC

Space Biology Upcoming Payloads Status



Flown

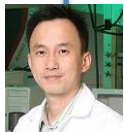
SpX-8



WetLab-2
Validation



Micro-9
Hammond



Micro-10
Wang



MT-1C
Venkateswaran

SpX-9



PRR
Perera



BRIC-23
Process Val



RR-3
BSP

Falcon 9

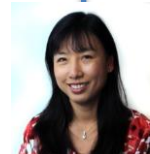


EcAMSat
Matin

SpX-10



APEX-4
Paul

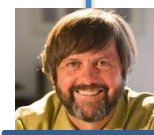


MT-2
Jaing



RR-4
BSP

SpX-11



FFL-2
Bodmer



SG-3
Medina (ESA)
Kiss (NASA)



APEX-02-2
Hammond

SpX-12



JRR-1
TBD



BRIC-22
Brandizzi



Micro-12
Hogan



EMCS-2
Wolverton

34 other PIs waiting for flight assignment...

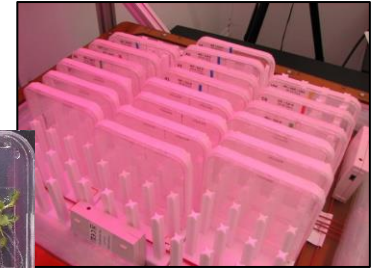
Space Biology Upcoming Payloads Status



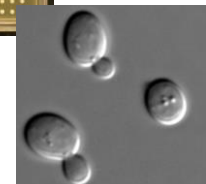
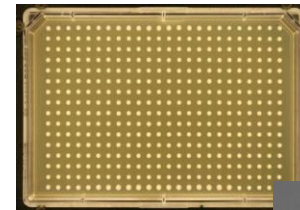
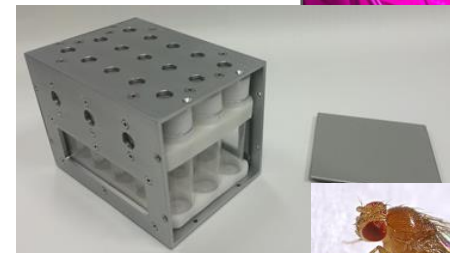
- *Non-ISS Free Flyer: EcAMSat NET January 2017 (Formosat-5/SHERPA)*
- SpaceX-10 (January 2017)
 - APEX-04 (PI: Paul) PI/GeneLab collaborative experiment with *Arabidopsis* will be the first spaceflight experiment to directly investigate how the plant methylome contributes to space adaptation.
 - MT-2 (PI:Jaing) Pre-position kits
- SpaceX-11 (March 2017)
 - Fruit Fly Lab-2 (PI: Bodmer) PI/translational GeneLab collaborative study of cardiac genes in the fruit fly that relate to human heart health and arrhythmia
 - Seedling Growth-3 (PIs: Kiss & Medina) an ESA-led experiment with NASA and ESA PIs using the EMCS to study gravity and light sensing and control in *Arabidopsis*
 - APEX-02-02 (PI:Hammond) APEX-02R research investigates *S. cerevisiae* growth and physiological responses to the multiple stimuli encountered in spaceflight environments.



Arabidopsis after 12 days growth.



COTS *Arabidopsis* plate holder for APEX



Space Biology Upcoming Payloads Status

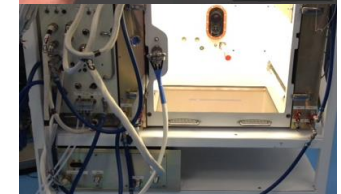
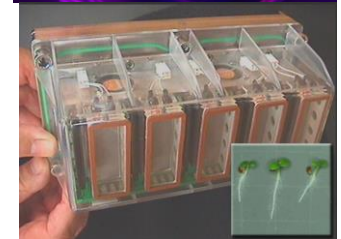
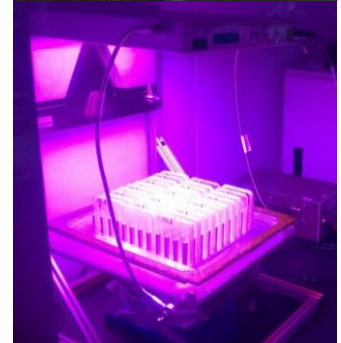


- SpaceX-12 (June 2017)

- JRR-1 (US and Russian PI Team) First ISS based collaborative US/Russian Rodent Research Payload (Space Biology team led by Delp and including Mao and Willey)
- BRIC-22 (PI:Brandizzi) Expand upon results from the BRIC-18 spaceflight experiment pertaining to the regulation of stress responses in plants exposed to microgravity conditions by the AtIRE1 protein (a master regulator of transcription).
- Micro-12 (PI:Hogan) : Investigating the Physiology and Fitness of an Exoelectrogenic Microorganism Under Microgravity Conditions
- BRIC-24 LED SDT Mission and Hardware Validation
- Veggie SN0001

- SpaceX-13 (September 2017)

- APEX-05 (PI:Gilroy) This experiment will determine how responses seen on orbit can be explained by the development of long-term hypoxia linked to the microgravity environment as compared to plants grown on the ground
- Plant Gravity Perception (PI: Wolverton) Characterize the primary statolith-based gravity sensing mechanism, including the threshold acceleration force responsible for activation and capacity for modulating growth regulation.
- Plant Habitat-01 (PI:Lewis) Multi-omics team based investigation to modulate lignin content and vascular apparatus performance by manipulation of the arogenate dehydratase multi-gene family.





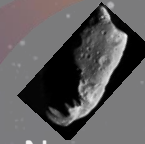
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- **Planning**
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Space Biology Research Planning

Understand biological processes to support humans on voyages beyond LEO to the Moon or Mars

GREAT UNKNOWN
NASA
BioSentinel



Near Earth Object



Mars
36 million mi



Moon
240,000 mi



ISS &
free flyers in LEO
180-300 mi



62 mi

BECOMING KNOWN

LITTLE KNOWN

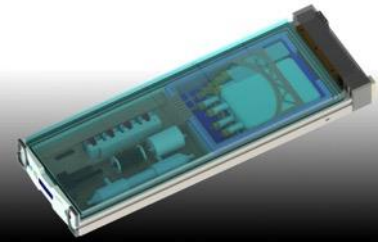
Mission Duration

Minutes-
12.5 Days

12 Months

3 Years

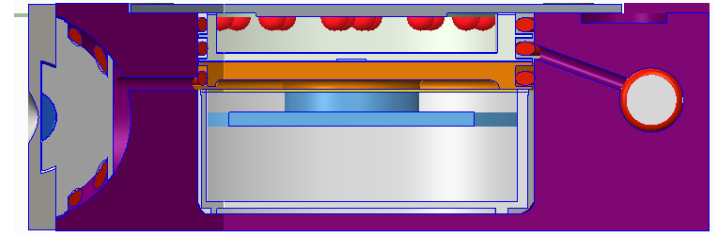
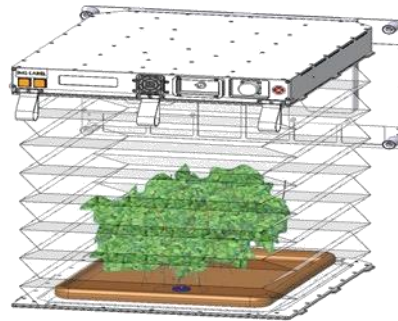
Space Biology Research Planning New Hardware to Enable Research



Bioculture System
(SpaceX-13: Sept 2017)



Veggie Unit #1 on ISS,
Veggie Unit #2 arrives on
SpaceX-12 (6/1/17)



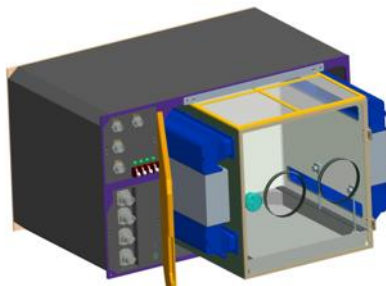
BRIC LED
(SpaceX-12: 6/1/17)



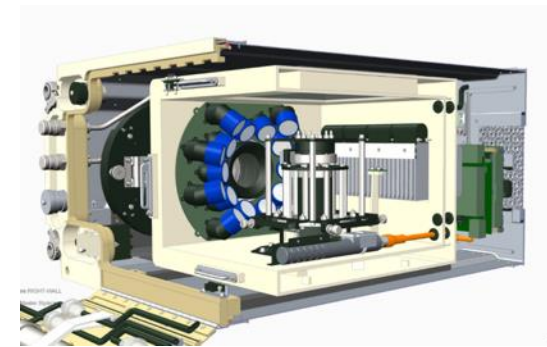
WetLab 2
(On ISS, ready for use)



Advanced Plant Habitat
(OA-7: Feb 2017)

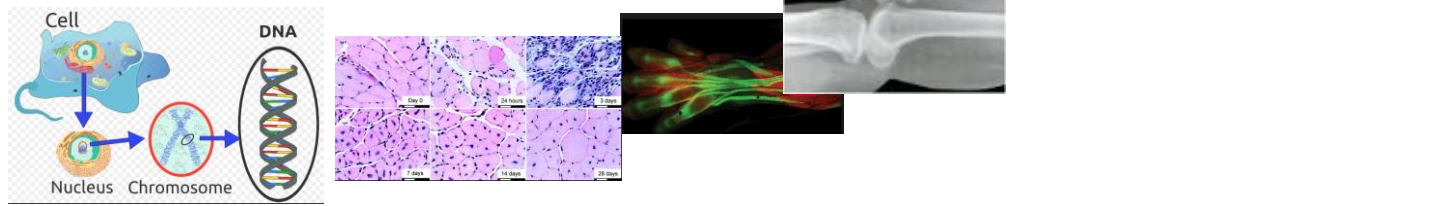


MultiSpectral Fluorescent Imager/Spectrum
(SpaceX-15: April 2018)



The Gravity Dose Response Curve: Threshold or Continuum?

- Gravity induces biological responses at the gene expression, cellular, systems and whole organism level
- The dose response curve of any of these responses is not fully characterized



- It is not known if responses are a continuum or are based on reaching thresholds
- Its is not known if responses require continuous or intermittent exposures
- It is not known if the sensitivity/dose response changes during development

Gravity as a Continuum ToolBox - ground & flight research on a variety of organisms to define dose response curve & adaptation mechanisms from 0 to >2+g

Ground-based Centrifuges

In-flight Centrifuges (EMCS, KUBIK, JAXA mouse centrifuge, Free-flyers)

Parabolic Flight

Ground-Based Fractional G Simulators (Clinostats, RPMs, HARVs, etc)

Partial Unloading in Animals and Humans

Space Biology Planning: Gravity as A Continuum

Example – Body mass and bone density

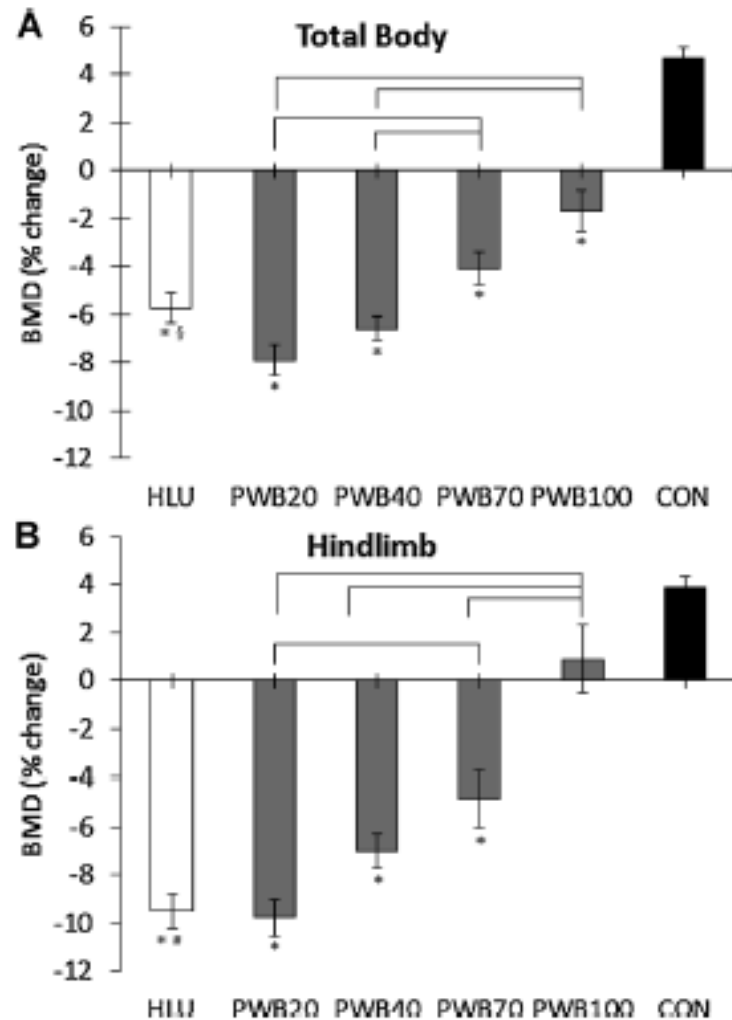


Fig. 5. Percent change in (A) total body and (B) hindlimb bone mineral density from baseline to day 21 (mean \pm SEM). * = Different from CON ($p < 0.05$), § = HLU different from PWB20, PWB100 ($p < 0.05$), # = HLU different from PWB70, PWB100 ($p < 0.05$), brackets = pairwise differences between PWB groups (ANOVA with Scheffé post hoc, $p < 0.05$).

Partial Reductions in Mechanical Loading Yield Proportional Changes in Bone Density, Bone Architecture, and Muscle Mass - Rachel Ellman, Jordan Spatz, Alison Cloutier, Rupert Palme, Blaine A Christiansen, and Mary L Buxsein *Journal of Bone and Mineral Research*, Vol. 28, No. 4, April 2013, pp 875–885

Space Biology Planning: Upcoming Solicitations



Early 2017

- **NRA NNH16ZTT001N Appendix C: “Space Biology Experiment aboard the BION M2 Biosatellite”** TBD after further discussions with our Russian Collaborators
- **NRA NNH16ZTT001N Appendix: “Space Biology Research Using Parabolic and Suborbital Flight Campaigns”** Solicit research to use parabolic or suborbital flight to conduct investigate biological effects of shortn micro- or partial-gravity on bio-systems
- **NRA NNH16ZTT001N Appendix: “Space Biology Research Using Antarctic Balloon Flight Campaigns”** Solicits proposals to use balloon campaigns to investigate biological effects of long duration space radiation exposure on living organisms
- **NRA NNH16ZTT001N Appendix: “Space Biology Research Using Microgravity Simulation Devices”** Solicits research to use microgravity simulators to test specific hypotheses regarding effects of altered gravity on biological systems
- **NRA NNH16ZTT001N Appendix: “Plant and Microbial Biology Research”** Solicits flight & ground plant & microbe research to answer Space Biology questions & maximize ISS use

Mid to Late 2017

- **NRA NNH16ZTT001N Appendix: “Space Biology Ames Life Science Data Archive Biospecimen Sharing”**
- **NRA NNH16ZTT001N Appendix: “Space Biology Beyond Low Earth Orbit (Orion EM-1)”**
- **NRA NNH16ZTT001N Appendix: “Gravity as a Continuum”**



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Space Biology Research Accomplishments: Growing Plants in Low Earth Orbit



Gioia Massa



VEGGIE

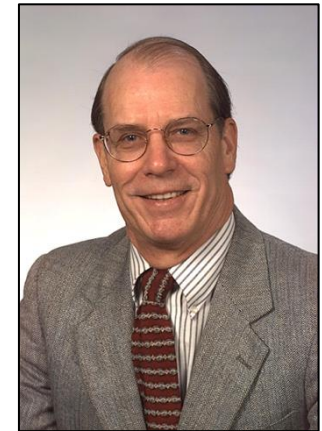
Astronauts Grow, Harvest, and Consume Fresh Vegetables from ISS Garden

The Kennedy Space Center (KSC) Vegetable Production System (Veggie) “Outredgeous” lettuce experiment (Veg-01b) demonstrated the capability to grow edible vegetables in space - from seed to mature plant. The International Space Station (ISS) crew enthusiastically harvested and consumed the lettuce on orbit. Live media broadcasts captured the vegetable harvest and consumption. The remaining lettuce samples were returned to Earth on SpaceX -8 in May 2016 for analysis by KSC scientists. The Veg-01 experiments revealed acceptable seed viability for long-term seed storage (approximately 1.5 years on ISS). The Veggie flight experiments received high praise from the ISS crew and NASA top management and became a hot-topic in the media.

Space Biology Research Accomplishments: Microbial Tracking Experiment Identifies New Bacterial Species Aboard the ISS and Confirms Fungal Virulence



Researchers on the Microbial Tracking-1 experiment announce the discovery of a new species of bacteria sampled from two different locations aboard the International Space Station. *Enterobacter piersonii* have been named after Dr. Duane Pierson (at right) at Johnson Space Center to commemorate his many years of service to NASA in the field of microbiology. The Space Biology-funded flight investigation involved researchers from NASA's Jet Propulsion Laboratory and is led by Dr. Kasthuri Venkateswaran. These eight strains of bacteria are most closely related to *E. cloacae* – a known, opportunistic human pathogen. As a member of the genus *Enterobacter* these newly identified strains are also likely to be resistant to drugs and more work is needed to study them in a model organism host.



Duane Pierson

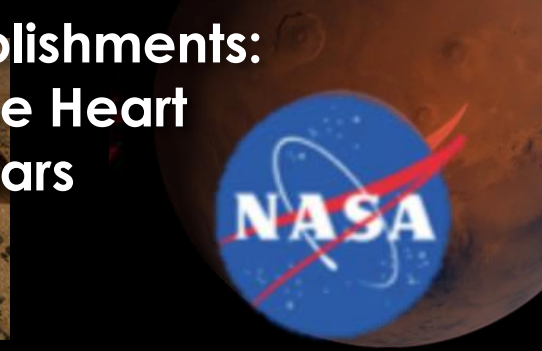
Unique Fungus Discovered on the International Space Station by Microbial Tracking-1 Investigators Found to be More Virulent than Known Clinical Strains in

a Zebrafish Model. Analysis of samples from the first of the three flights found that a unique strain of the fungus *Aspergillus fumigatus* was collected from the ISS. This fungus was characterized and compared to well-established strains isolated in experiments in Earth laboratories. Assessment of the fungal growth characteristics, secondary metabolite production, and susceptibility to chemical stresses revealed no differences between the ISS and clinical strains that would suggest special adaptation to life aboard the International Space Station. Virulence of the unique fungus was assessed using a zebrafish model, which revealed that the ISS isolates were significantly more lethal compared to clinical strains.



Kasthuri Venkateswaran

Space Biology Research Accomplishments: Deep Space Radiation May Cause Heart Problems for Future Travelers to Mars



Michael Delp

July 28, 2016 – 47 years after humans first landed on the moon space biology researchers have found an increased risk of mortality from cardiovascular disease (CVD) in astronauts who traveled deep into space. Apollo astronauts experienced nearly five times greater risk of death from CVD than astronauts who've traveled to low Earth orbit.

Researchers first compared mortality rates of Apollo astronauts with other astronauts who either never flew in orbital missions or only flew in low Earth orbit. They found a concerning trend in rates of cardiovascular disease that warranted further investigation.

To test the effects of spaceflight on the cardiovascular system directly, investigators subjected mice to simulated conditions of zero-gravity, cosmic radiation, or both combined. After half a year (the equivalent of 20 human years), they found that only the mice that had been exposed to radiation had sustained damage to their blood vessels. In particular, the researchers found damage to the lining of the blood vessels, which is typically the first indication of long-term heart disease leading to a heart attack or stroke. Based on further research in mice, they suggest that the cause of cardiovascular disease in these astronauts may have been deep space radiation.

Principal Investigator for this Space Biology-funded study, Dr. Michael Delp, acknowledges shortcomings of the mortality study of the astronauts, but cautions that their findings indicate the need for more research on radiation effects on cardiovascular function in astronauts. This study was funded in part by the National Space Biomedical Research Institute.



Space Biology Research Accomplishments: New Study Finds Liver Damage in Animals From Two Weeks in Space



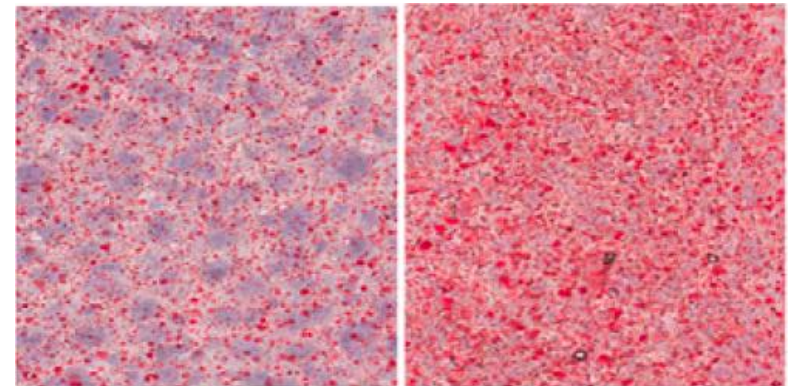
Michael Pecaut

A recently published study found troubling signs of fatty liver disease in mice after only two weeks in space aboard the STS-135 shuttle. These findings have contributed important information about potential risks to liver function where little is known and the results could have implications for long-duration missions to Mars.

- Mice flown in space lost weight, but redistributed lipids to their livers showing approximately 3.5 times more areas of fat storage (lipid droplets) than ground controls
- Other changes observed in the mouse livers indicate a stress response, which, if left unchecked, could lead to irreversible damage from fibrosis
- Transcriptomic and metabolomic data collected and analyzed in this study have been entered into the GeneLab data system and are publicly available for further analysis by the scientific community

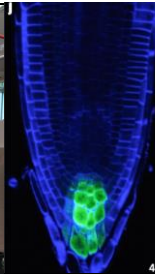
Source article: [Spaceflight Activates Lipotoxic Pathways in Mouse Liver.](#)

Jonscher KR, Alfonso-Garcia A, Suhalim JL, Orlicky DJ, Potma EO, Ferguson VL, Bouxsein ML, Bateman TA, Stodieck LS, Levi M, Friedman JE, Gridley DS, Pecaut MJ. PLoS One. 2016 Apr 20;11(4):e0152877. doi: 10.1371/journal.pone.0152877. eCollection 2016.



Above: Magnified (40x) photos of liver sections from a ground control mouse (left) and one flown for two weeks aboard the STS-135 shuttle (right). Dark red spots in the images are areas of fat storage.

Space Biology Research Accomplishments:



Glow-in-the-dark Science:
Fluorescent genes help botanists visualize gravity sensing in space grown plants

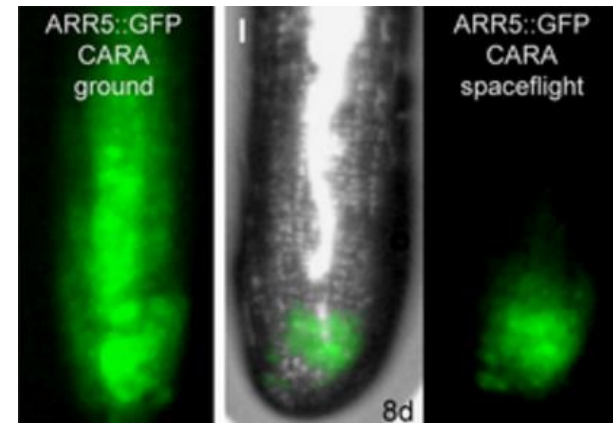
Anna Lisa Paul Robert Ferl

Space Biology researchers Rob Ferl and Anna-Lisa Paul recently published the results of a series of spaceflight experiments where Green Fluorescent Protein (GFP) tagged genes were used to identify the activity of growth hormones in plants grown on the ISS. Their findings established the plant hormones sensitive to gravity are located in the root tips, instead of in the primary root.

- The use of fluorescent reporter genes allowed investigators to visually identify the presence and distribution of plant growth hormones in the roots
- They found the distribution of fluorescent-tagged growth hormones in the *primary roots* of plants grown on the ISS was identical to that of the ground controls, but found a more restricted distribution in the root tips of the space plants
- Their findings confirm that the establishment of the auxin-gradient system, the primary guide for gravity signaling in the root, is gravity independent and that root responses to gravity are sensed in the tips

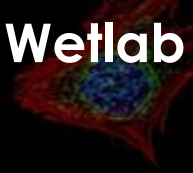
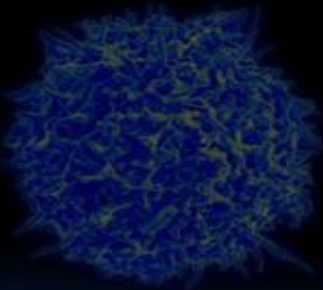
Results of these experiments reveal important information about which cellular parts of a plant respond to gravity. Research into how terrestrial biology responds to spaceflight will both refine our understanding of what it takes to explore space and help define how conditions on Earth have shaped biology here.

Source article: The effect of spaceflight on the gravity-sensing auxin gradient of roots: GFP reporter gene microscopy on orbit. npj Microgravity. Ferl RJ, Paul A-L. 2016 Jan 21;2:15023.



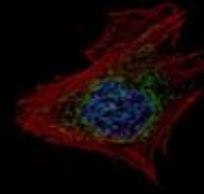
Above: Fluorescent-tagged genes 'report' their location within the plant root by glowing green. The root at left shows a normal distribution of growth hormones while the two right images of space grown plants show a restricted distribution with the hormones concentrated in the root tips.

Space Biology Research Accomplishments: Mission Success for Wetlab 2



WetLab-2 is a research platform for conducting real-time quantitative gene expression analysis aboard the International Space Station. The WetLab-2 validation experiment has been successfully completed, and, in short – it worked! The team reports that full mission success criteria have been met. April 29, astronaut Jeff Williams tested the system's Sample Prep Module for the first time in space to extract and purify RNA from cells before performing the qPCR analysis. The WetLab-2 team at Ames received data from the

space station that clearly shows that Williams isolated RNA in microgravity achieved reverse transcription—conversion of RNA to DNA—and got amplification of that DNA. More details about the WetLab-2 validation experiment from project manager Julie Schonfeld are on the [WetLab-2 validation mission webpage](#). The experiment was featured in the [April 29th edition of the "Weekly Recap From the Expedition Lead Scientist"](#).



Space Biology Research Accomplishments: First GeneLab Reference Mission Test

Wayne Nicholson



GeneLab

Open Science
for Exploration

First GeneLab Reference Mission Test

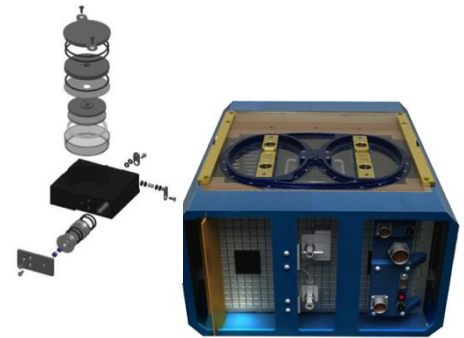
Kennedy Space Center developed and flew the first experiment designed to test the cradle-to-grave process for collecting spaceflight reference mission data for the NASA GeneLab Data System. The BRIC -23 experiment, titled **GeneLab Process Verification Test Using Microorganisms *Bacillus subtilis* and *Staphylococcus aureus***, was designed by KSC Project Scientist Dr. Howard Levine and Science Consultant Dr. Wayne Nicholson, University of Florida. The microorganisms were allowed to dry on Petri dishes, integrated into the BRIC-PDFU hardware, and flown to the ISS on SpaceX-9. The ISS crew initiated growth of the organisms by injecting nutrient solution into the Petri dishes. After the organisms reached the desired growth stage, they were frozen at -80°C then returned to Earth on SpaceX-9 for GeneLab “omics” analyses.

Space Biology Research Accomplishments



Flight Experiments Completed:

- BRIC-21, VEG-01b, VEG-01c
- [SpaceX-8 \(4/8/16\)](#): VEG-03 Tech Demo, MT-1c, Micro-9, Micro-10, WetLab-2 Validation
- [SpaceX-9 \(7/18/16\)](#): BRIC-23 GeneLab Process Verification Test, Rodent Research-3 Biospecimen Sharing



Recent Space Biology Publications - Top 20+1

1. Sofronova, Tarasova, Gaynullina, Borzykh, Behnke, Stabley, McCullough, Maraj, Hanna, Muller-Delp, Vinogradova, Delp. Spaceflight on Bion-M1 biosatellite alters cerebral artery vasomotor & mechanical properties in mice. *J Appl Physiol* (2015,118;830-8).
2. Checinska, Probst, Vaishampayan, White, Kumar, Stepanov, Fox, Nilsson, Pierson, Perry, Venkateswaran. Microbiomes of dust particles collected from ISS & Spacecraft Assembly Facilities. *Microbiome*. (2015,3;50).
3. Delp, Charva, Limoli, Globus, Ghosh. Apollo lunar astronauts show higher cardiovascular disease mortality: Possible deep space radiation effects on vascular endothelium. *Sci Rep*. (2016,28;29901).
4. Grigoryan, Anton, Poplinskaya, Aleinikova, Domaratskaya, Novikova, Almeida. Signs of Müller cell gliotic response found in retina of newts exposed to real & simulated microgravity. *Adv in Space Research* (2012,49;1465-1471).
5. Jonscher, Alfonso-Garcia, Suhaim, Orlicky, Potma, Ferguson, Bouxsei, Bateman, Stodieck, Levi, Friedman, Gridley, Pecaut. Spaceflight activates lipotoxic pathways in mouse liver. *PLoS One*. (2016,11; e0152877).
6. Vandenbrink, Herranz, Medina, Edelmann, Kiss, A novel blue-light phototropic response is revealed in roots of *Arabidopsis thaliana* in microgravity. *Planta*.(2016,244;1201-1215).
7. Fajardo-Cavazos, Nicholson. Cultivation of *Staph epidermidis* in human spaceflight environment leads to alterations in frequency & spectrum of spontaneous rifampicin-resistance mutations in *rpoB* gene. *Front Microbiol*. (2016,28; 999)
8. Hughes-Fulford, Chang, Martinez, Li. Spaceflight alters expression of microRNA during T-cell activation. *FASEB J*. (2015,29; 893-900).
9. Blaber, Finkelstein, Dvorochkin, Sato, Yousuf, Burns, Globus, Almeida, Microgravity reduces the differentiation and regenerative potential of embryonic stem cells. *Stem Cells Dev*. (2015,24;2605-21).

Space Biology Research Accomplishments



Recent Space Biology Publications - Top 20+1 (CONTINUED)

10. Ghosh, Stabley, Behnke, Allen, Delp. Effects of spaceflight on the murine mandible: Possible factors mediating skeletal changes in non-weight bearing bones of the head. *Bone*. (2016,83;156-61)
11. Wang, Singh, Benoit, Keyhan, Sylvester, Hsieh, Thathireddy, Hsieh, Matin. Sigma S-dependent antioxidant defense protects stationary-phase *Escherichia coli* against the bactericidal antibiotic gentamicin. *Antimicrob Agents Chemother*. (2014,58;5964-75).
12. Chowdhury, Seetharam, Wang, Liu, Lossie, Thimmapuram, Irudayaraj. A study of alterations in DNA epigenetic modifications (5mC and 5hmC) & gene expression influenced by simulated microgravity in human lymphoblastoid cells. *PLoS One*. (2016,11;e0147514).
13. Evans, Choi, Gilroy, Morris. A ROS-assisted calcium wave dependent on AtRBOHD and TPC1 propagates the systemic response to salt stress in *Arabidopsis* roots. *Plant Physiol*. (2016,171;1771-84).
14. Chang, Spurlock, Candelario, Grenon, Hughes-Fulford. Spaceflight impairs antigen-specific tolerance induction in vivo and increases inflammatory cytokines. *FASEB J*. (2015,29;4122-32).
15. Ferl, Koh, Denison, Paul. Spaceflight induces specific alterations in proteomes of *Arabidopsis*. *Astrobiol*. (2015,15;32-56)
16. Gridley, Mao, Tian, Cao, Perez, Stodieck, Ferguson, Bateman, Pecaut. Genetic and apoptotic changes in lungs of mice flown on the STS-135 mission in space. *In Vivo*. (2015,29;423-33).
17. Kwon, Sparks, Nakashima, Allen, Tang, Blancaflor. Transcriptional response of *Arabidopsis* seedlings during space-flight reveals peroxidase & cell wall remodeling genes associated with root hair development. *Am J Bot*. (2015,102;21-35).
18. Nislow, Lee, Allen, Giaever, Smith, Gebbia, Stodieck, Hammond, Birdsall, Hammond. Genes required for survival in microgravity revealed by genome-wide yeast deletion collections cultured during flight. *Biomed Res Int* (2015;976458).
19. Singh, Blachowicz, Checinska, Wang, Venkateswaran. Draft Genome Sequences of Two *Aspergillus fumigatus* Strains, Isolated from the International Space Station. *Genome Announc*. (2016,4;e00553-16).
20. Crabbé, Liu, Sarker, Bonenfant, Barrila, Borg, Lee, Weiss, Nickerson. Recellularization of decellularized lung scaffolds is enhanced by dynamic suspension culture. *PLoS One*. (2015,10;e0126846).
21. Andreev-Andrievskiy, Popova, Boyle, Alberts, Shenkman, Vinogradova, Sychev. Mice in Bion-M 1 space mission: training and selection. *PLoS One*. (2014,9;e104830).

Space Biology - Pioneering Scientific Discovery

Reference Mission Team Experiments



Effects of Spaceflight on Gastrointestinal Microbiota in Mice: Mechanisms & Impact on Multi-System Physiology. Lead - Fred Turek, (Northwestern) Team members - Green, S (Univ Ill), Keshavarzian, A (Rush Univ Med Ctr), Forsyth, C (Rush Univ Med Ctr), Vitaterna, M (Northwestern)

Examine potential role(s) of disruption of microbial communities in gut (dysbiosis) in mammalian adjustment to space environment. Challenges to mammalian physiological homeostasis & immune, inflammatory, & metabolic changes in spaceflight may be attributable in part to dysbiosis. A series of experiments in mice will define mechanisms by which time in space, diet, & host genotype interact to impact the gut microbiota, & how dysbiosis relates to gene expression (by RNA-seq) & physiology in serum, colon, ileum, spleen, liver, & fat as well as the sleep/wake cycle & feeding behavior.

ISS Microbial Observatory of Pathogenic Virus, Bacteria, & Fungi (ISS-MOP) project. Lead - Crystal Jaing, (Lawrence Livermore) Team members - Mehta, S (Wyle), Pierson, D (NASA JSC), Smith, D (NASA ARC), Venkateswaran, K (NASA JPL) In this ISS-MOP project a cultivation-based assay (Aim 1) provides a valid estimation of microbial presence, but offers a limited assessment of the phylogenetic/pathogenic diversity & physiological breadth of the microbial population present. So, the team will use novel & comprehensive LLMDA microarray specifically designed to elucidate the pathogenic viral & microbial diversity profiles of complex samples to at least the species level (Aim 2). In addition, they will characterize virulence & antibiotic resistance mechanisms associated with these ISS pathogens by developing a new Virulence & Antibiotic Resistance gene array & analyze the samples on this array. The final deliverable of the proposed project to NASA will be the ISS-MOP dataset, a compilation of all of the genomic sequences & genetic information of viruses & microbes encountered on & within the ISS habitat (Aim 3).

Space Biology - Pioneering Scientific Discovery

Reference Mission Team Experiments



Integrated Omics Guided Approach to Lignification & Gravitational Responses: The Final Frontier Lead - Norman Lewis (Washington State) Team members - Davin, L (Washington State), Hanson, D (Univ NM), Lipton, M (Battelle Memorial Inst), Sayre, R (NM Consortium), Starkenburg, S (Los Alamos Natl Lab) Arabidopsis multi-omics study (metabolomics, transcriptomics, proteomics, phenomics) melded with integrated computational biology (ICB) approach. Plant lines include mutants with different lignin amounts & lines enhanced in C assimilation capacity. Will investigate gene/metabolic network relationships & adaptations resulting from varying lignin & C assimilation levels on photosynthesis, C allocation, water use efficiency (WUE), plant growth/development, vasculature performance, auxin transport & gravitational adaptations. integrated omics analyses of factors controlling lignin will provide new insights into global effects on plant biological processes at both 1g & in micro-g.

High dimensional biology to understand the functional response of Salmonella to long-term multigenerational growth in the chronic stress of microgravity Lead - Cheryl Nickerson, Arizona State Team members - Abu-Ali, G (Harvard) Barrila, J (Arizona State), Huttenhower, C (Harvard), Ott, M (NASA JSC), Travisano, M (U Minnesota) Investigators propose that long-term growth in spaceflight culture will induce heritable genomic & epigenetic changes in micro-organisms with adverse phenotypic outcomes that could have a profound impact on risk to crew health & vehicle system performance. They hypothesize that long-term multigenerational culture of *S. Typhimurium* in flight will result in stress-induced mutations & genomic instability as reflected in genomic, epigenetic, transcriptomic & select phenotypic characteristics changes (including virulence) that reflect a central role for Hfq as a global regulator of these heritable responses. They will characterize the impact of long term spaceflight culture (~300 generations) of an *S. Typhimurium* wild type & hfq mutant on genomic, epigenetic, transcriptomic, virulence & pathogenesis-related characteristics.

Space Biology - Pioneering Scientific Discovery Partnership and Collaboration with Physical Sciences



Characterization of Biofilm Formation, Growth, & Gene Expression on Different Materials & Environmental Conditions in Microgravity – PI Luis Zea (Univ Colorado)

Characterize impact of long duration spaceflight on Biofilm Mass, Thickness, Morphology, & microbial gene expression of Uropathogenic *Pseudomonas aeruginosa* and *Penicillium chrysogenum* to form and maintain biofilms on different surfaces commonly used in spacecraft, cellulose, aluminum 6061, titanium alloy, polycarbonates, silicon, stainless steel and carbon fibers. Biofilm formation and risk of causing equipment malfunction and human illnesses is a problem that needs to be addressed to enable safe long-term human space exploration. Experiments will elucidate biomechanical & transcriptomic mechanisms involved in formation of “column-and canopy” biofilm architecture observed in space & assess gene expression associated with conferring resistance to oxidative stress, acidity, & antimicrobials on microorganisms in biofilms.

Polymicrobial biofilm growth & control during spaceflight - Robert McLean (Texas State - San Marcos)

Microorganisms grow as surface-adherent biofilm communities in spaceflight. Organisms in biofilms are resistant to traditional antimicrobial chemicals & can foul water treatment filters. Biofilms on ISS include many species, but studies to date have only tested single-species biofilms. Experiments will characterize how two bacterial species (*E. coli* and *P. aeruginosa*) interact, and how interactions impact biofilm's ability to adhere to surface, antimicrobial resistance, & ability to degrade a metallic surface. Characterize biological mechanisms by which these microbes interact with each other, such as changes in quorum-sensing and other secreted proteins. Transcriptomic analyses & changes in microbial gene expression will provide insights into effects of spaceflight on microbial physiology.



NASA Postdoctoral Program (NPP)

- Provides early-career and more senior scientists the opportunity to share in NASA's mission
- Fellows work on 1-3 year assignments at NASA centers and institutes
- Fellows contribute to our national scientific exploration, confirm NASA's leadership in fundamental space research, and complement the efforts of NASA's partners in the national science community.

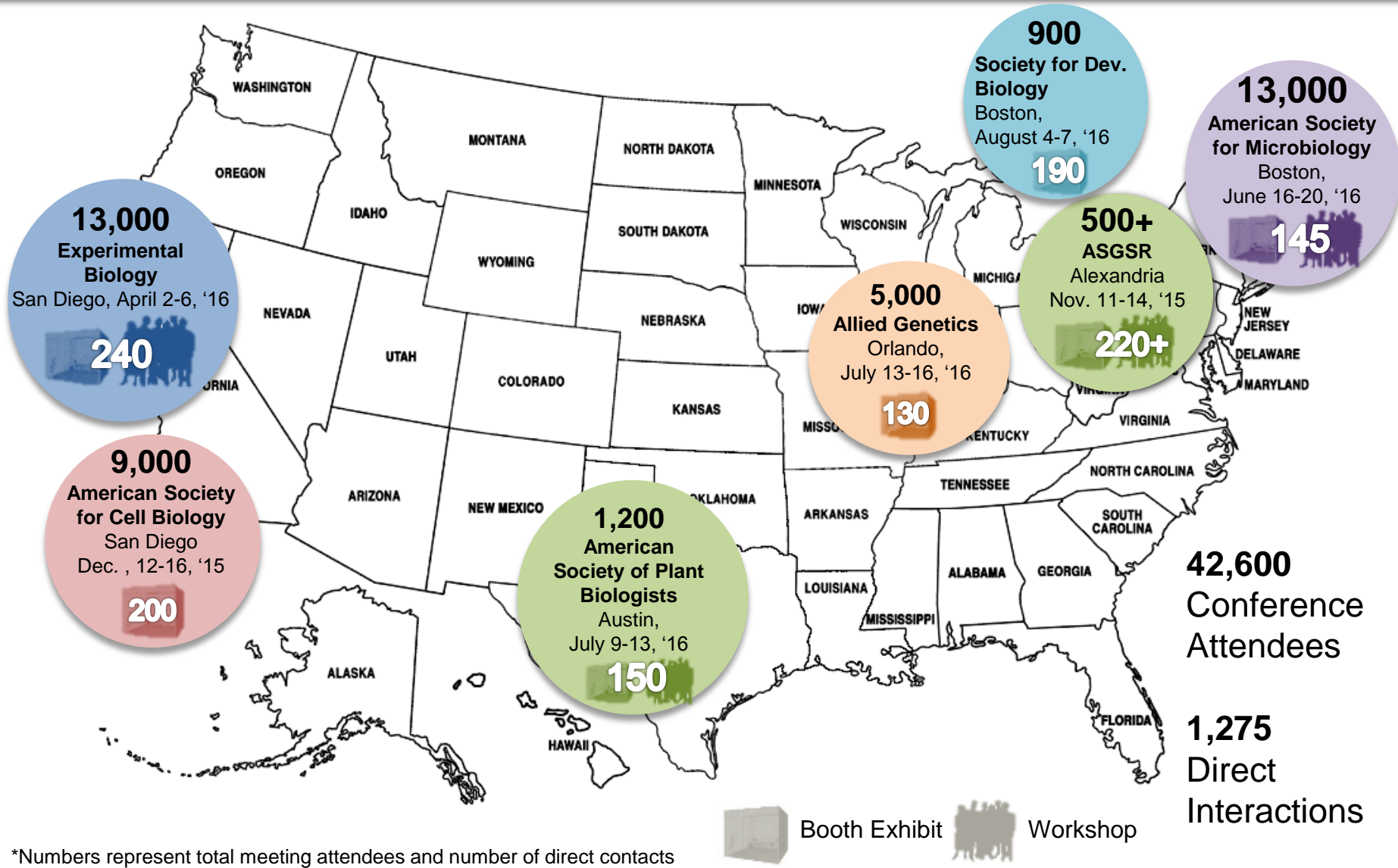


Space Life Sciences Training Program (SLSTP)

- Trains our next generation of scientists and engineers and enable NASA to meet future research and engineering challenges in the space life sciences
- Students conduct hands-on research, attend technical lectures, develop professional & project management skills, perform a team project and submit an abstract to a professional scientific or engineering organization for presentation

Space Biology October 2015 – September 2016

Scientific Exhibits & Workshops



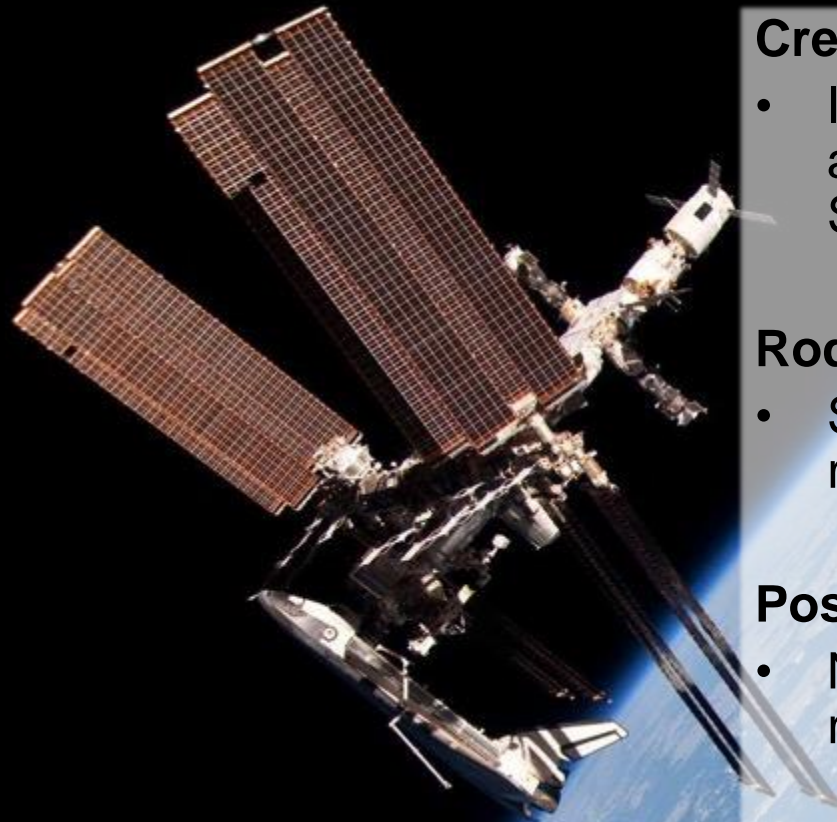
*Numbers represent total meeting attendees and number of direct contacts



Topics to be covered today

- Status
- Planning
- Research findings/Accomplishments
- **Challenges**
- Genelab Update

Space Biology Research Challenges



Crew Time:

- ISS crew time is oversubscribed; little is available for Space Biology and Physical Science

Rodent Research:

- Severely limited ability to execute rodent research on orbit

Post-ISS Exploration:

- NASA will not be the “anchor tenant” for research in low Earth orbit

New Platforms:

- Continuing research activities on ISS while developing opportunities to move beyond LEO and utilize alternative platforms

Addressing Space Biology Research Challenges



Crew Time:

- Focus near-term on ISS payloads that can fly using reserve crew time
- Focus on automating ISS flight experiments to reduce/eliminate crew time
- Partner with CASIS, Roscosmos, HRP, STMD and other Programs to co-sponsor payloads of joint interest in exchange for prime crew time from those Programs
- Target alternative vehicles to increase flight opportunities and go beyond LEO

Rodent Research:

- Implement a BSP, in collaboration with CASIS, for every Rodent Research Mission to maximize utilization of tissues returned from space and obtain tissues for Space Biology PI research
- Collaborate with JAXA and Russia for BSP and Joint Rodent Research Missions to obtain tissues for Space Biology PI research
- Seek new opportunities for Rodent Research in Space (e.g. Bion)

Post-ISS Exploration and New Platforms:

- Working to develop plans to enable increased Life Beyond Low Earth Orbit Activities
- Working to fly investigations on all NASA Exploration missions and increase utilization of new space flight carriers



Topics to be covered today

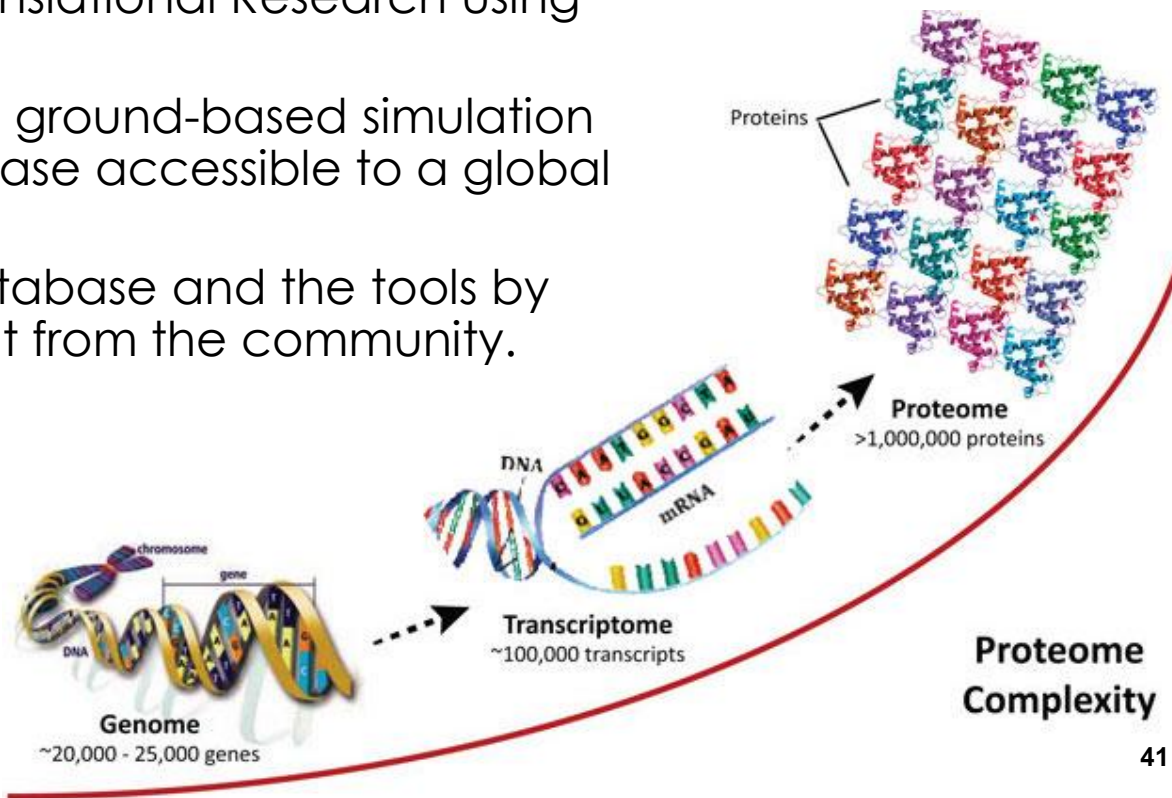
- Status
- Planning
- Research findings/Accomplishments
- Challenges
- **Genelab Update**

Space Biology - Pioneering Scientific Discovery

Space Flight Data to Knowledge – Genelab Goals



- Serve as a resource for researchers to convert spaceflight Life Sciences data to new knowledge to maximize use of ISS Life Science research results .
- Collect and archive spaceflight genomic, transcriptomic, proteomic, and metabolomic data for discovery use.
- Support research community exploration of molecular network responses to spaceflight by maintaining access and tools for performing Translational Research using Genelab data.
- Make all SB spaceflight and ground-based simulation data in the Genelab database accessible to a global researcher network.
- Continually improve the database and the tools by seeking feedback and input from the community.



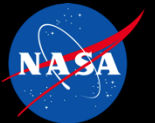


- The GeneLab Data System (genelab.nasa.gov) currently houses 81 datasets which are meticulously curated and fully accessible to the public: 47 datasets are from Spaceflight experiments, and 33 from relevant ground experiments.
- Averaging more than 1000 file downloads per month, with a data volume of roughly 3 terabytes per month, available worldwide.
- Newsletter updates are currently sent to 4,479 subscribers.
- Collaborated with recent missions (including CASIS) to obtain either tissue samples or data for subsequent omics analyses: Rodent Research-1, Rodent Research-3, Micro-9, Micro-10, Microbial Tracking-1c, BRIC-19, BRIC-20, and the BRIC-23 Process Verification Test.
- Space Biology issued an Innovation Awards Research solicitation (NRA NNH16ZTT001N-GL) in March 2016 - 6 of 34 proposals received were funded after scientific merit and technical feasibility reviews.

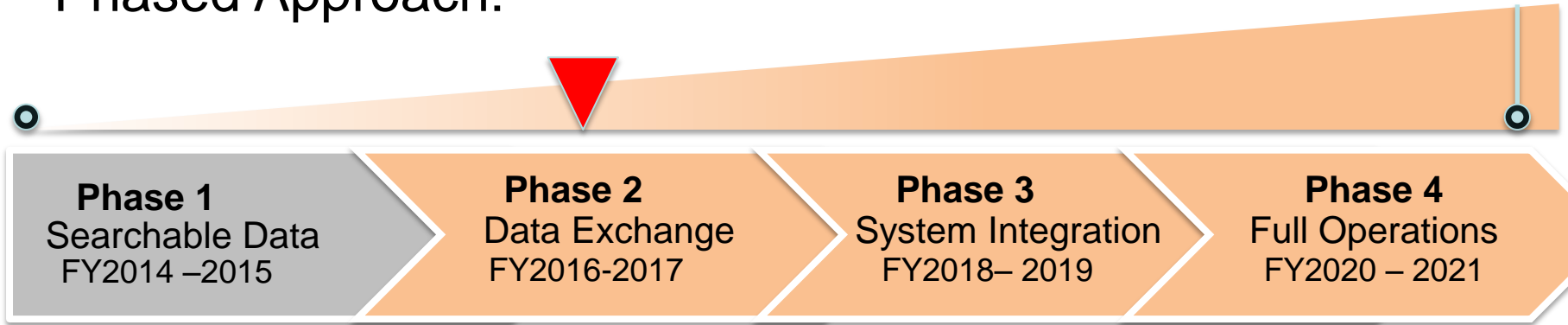


Space Biology - Pioneering Scientific Discovery

Space Flight Data to Knowledge – Genelab Planning



Phased Approach:



Spaceflight Experiments

- ✓ At least two Collaborative Missions (BRIC19 and BRIC20)
- ✓ Establish PI Data Agreements
- ✓ Data analysis validation

Spaceflight Experiments

- ✓ Standards for samples
- ✓ Standards for metadata
- ✓ NRA for Data analysis from initial studies
- ✓ Participate in multiple missions/year
- ✓ Legacy Data
- Minimum Data Set

Spaceflight Experiments/Analysis

- Continue ground controls and process enhancement
- Engage with Scientists external to NASA as part of Outreach Program
- Multiple flight experiments every year

Full Operations

- Full science community engagement
- Analysis and modeling
- Expanding catalog of flight experiments

Data System

- ✓ Public Website
- ✓ Searchable Data Repository
- ✓ Top Level Requirements
- ✓ New Data and Legacy Data

Data System

- Link to Public Databases via Data Federation.
- Integrated Search (e.g., data mashup)

Data System

- Integrated Platform across model organisms
- Build Community via collaborative science analysis & modeling

Full Operations

- Provide access to biocomputational tools for omics analysis
- Provide collaboration framework and tools

Space Biology - Pioneering Scientific Discovery

Space Flight Data to Knowledge – Genelab Results



GeneLab
Open Science
for Exploration

Home Repository Data Data Mining Tools Submit Data Contact Us Q

81 Studies
and Growing

<https://genelab-data.ndc.nasa.gov/genelab/projects/>

Page 1 of 1 (Total Studies: 80)
Studies Per Page:



GLDS-83

Comparative Transcriptomic Analysis of Adult Medaka Tissues Sampled after Adaptation to a Space Environment

Organisms	Factors	Assay Types	Release Date	Description
Oryzias latipes	Spaceflight	transcription profiling	15-Oct-2015	To understand how humans adapt to space environments, many experiments can be conducted on astronauts while they work aboard the Space Shuttle or the International Space Station (ISS). We also need animal experiments that can apply to human...



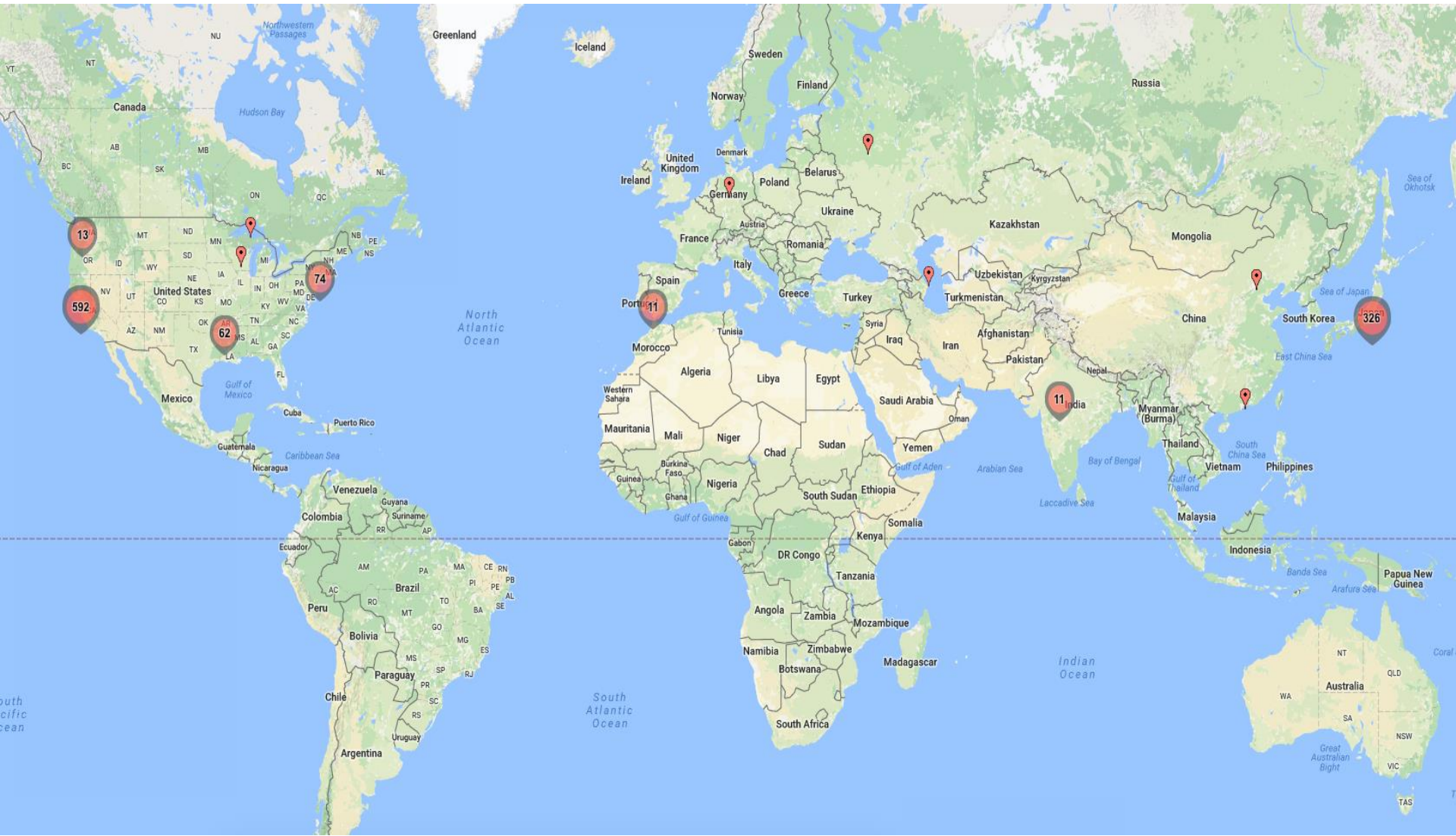
GLDS-81

Bacillus subtilis strains at low-pressure: 5 kPa versus 101 kPa growth

Organisms	Factors	Assay Types	Release Date	Description
Bacillus subtilis	strain pressure	transcription profiling	14-Mar-2014	Comparing the transcriptional responses of Bacillus subtilis strains WN624 and WN1106 at 5 kPa and 101 kPa. WN1106 is a 5 kPa-evolved strain with increased fitness compared to ancestor-WN624 strain at 5 kPa. This experiment probed the diffe...

Space Biology - Pioneering Scientific Discovery

Space Flight Data to Knowledge – Genelab Access

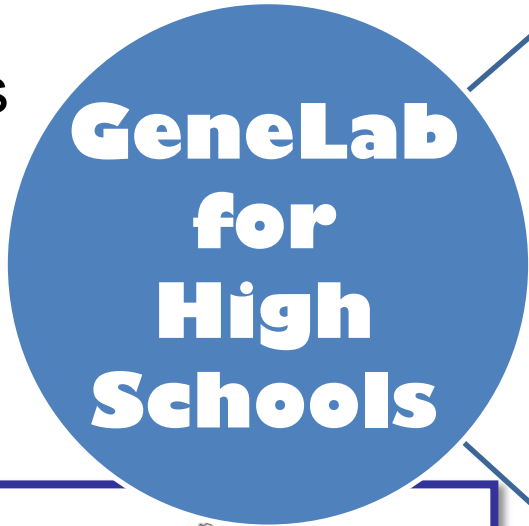


Space Biology - Pioneering Scientific Discovery

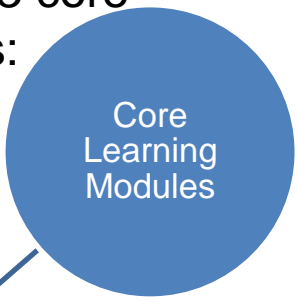
Space Flight Data to Knowledge – Genelab Outreach



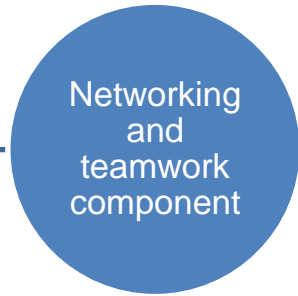
- New Space Biology project
- Targets high school students
- Pilot program begins Summer 2017



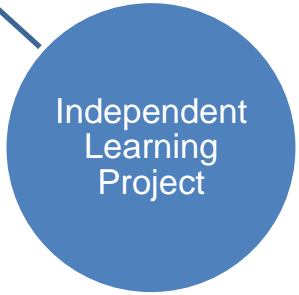
Consists of 3 core components:



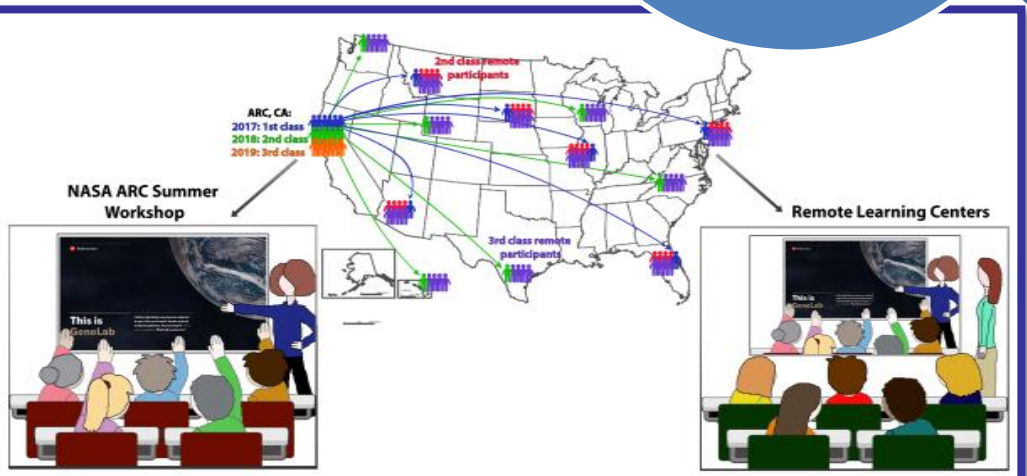
- Introduction to GeneLab Data Platform and 'omics datasets and analysis tools
- Introduction to model organisms and analysis of model organism datasets



- Guest lectures by University Professors and Industry experts
- Networking receptions and field trips (University core facilities and Industry)



- Analysis of dataset of choice
- Development of hypothesis and proposal of research project



Elizabeth Blaber, Ph.D.
Development and Organization Lead



Common Threads

- NASA Space Biology objectives and research projects are well aligned with 2010 Decadal Survey priority recommendations
- Every Space Biology research task is required to comply with President Obama's Executive order "Making Open and Machine Readable the New Default for Government Information", enabled through the NASA GeneLab Project
- Potential for more collaboration with other NASA programs, CASIS, or international partners
- In-flight centrifugation is required across all elements and species to determine gravity thresholds, responses to fractional Earth gravity such as will be experienced on Mars or moon, and to provide 1g controls in flight.
- Ability to accomplish goals & recommendations of NRC Decadal Survey is diminished by reduced access to ISS & reduced crew time availability for implementing NASA science
- Need for ground-based research and alternative spaceflight platforms.
- Systems Biology Approach at some level present in 50-70% of all Space Biology tasks.
- Need to actively solicit users of the open science GeneLab database to develop new knowledge from spaceflight omics data.
- Attempt in each element to enlarge NASA's research community by outreach to major scientific professional societies (e.g., Experimental Biology, American Society for Microbiology, American Plant Biology Society, American Society for Cell Biology.)

Veg-03A Ready for science harvest (50%) 12/12/16
Ready for crew salad harvest (100%) 12/16/16





Supplementary Slides for the Committee

Pioneering Scientific Discovery

Space Biology: External Partnership and Collaboration



- International Space Life Sciences Working Group: Forum for developing life science collaborations between NASA, ESA (CSA, CNES, DLR, ASI) and JAXA: ISS experiments, Research Announcements, and GeneLab
- JAXA: OP3 Framework: Rodent Experiments, Rodent Centrifuge and Aquatic Habitat
- U.S./Russian Joint Working Group: Forum for developing life science collaborations with Russia: ISS experiments, Bion, Foton, and GeneLab
- CASIS: collaboration on ISS based experiments with translational relevance



New Platforms



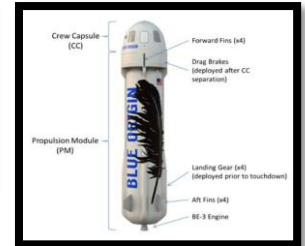
NASA and Other Governmental Opportunities

- Orion: Space Biology Pathfinder Mission
- Flight Opportunities Program
- Advanced Exploration Systems: BioSentinel technology for future LBLEO missions
- Air Force: X37B



International

- JAXA future Mouse Habitat BSP and Aquatic Habitat for GeneLab
- Bion-M2 Free Flyer Mission



Commercial

- Blue Origin
- SpaceX: Falcon and Red Dragon
- CASIS Rodent Biospecimen Sharing

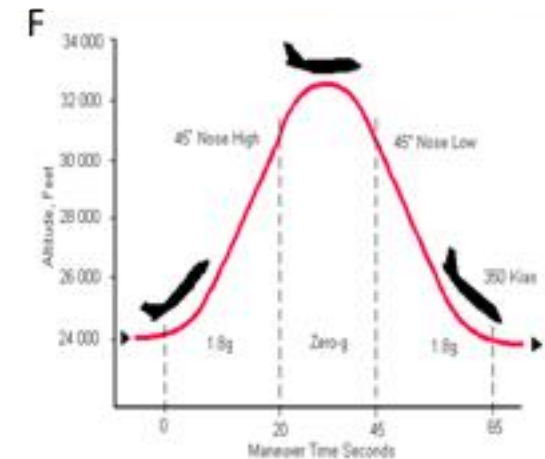


Parabolic Flight Campaigns for Microgravity and Partial Gravity Life Science Experiments



Objectives:

- Establish a **yearly Parabolic Flight Campaign** for Life Scientists that will provide opportunities for short duration (**20-25 sec**) **micro-g** and **partial-g** investigations.
- Work towards the expansion of this capability to **90 sec micro-g and partial-g exposures** based upon future modifications to **Commercial F104 jets**.
- A number of conventional (but modified) aircraft are capable of flying a series of parabolas that provide 20-25 seconds of micro-g (or partial-g) at the top of the parabolas.
- Typically, 40 parabolas are flown each flight, and four consecutive flight days can be provided per campaign.



Some Commercial Parabolic Flight Providers. A. Starfighters Aerospace F104. B. Starfighters Aerospace Falcon 20. C. Swiss Space Systems (S3) Airbus A310. D. T-6 Texan WWII Warbird. E. Zero Gravity Corp Boeing 727. F. Parabolic Flight Profile.

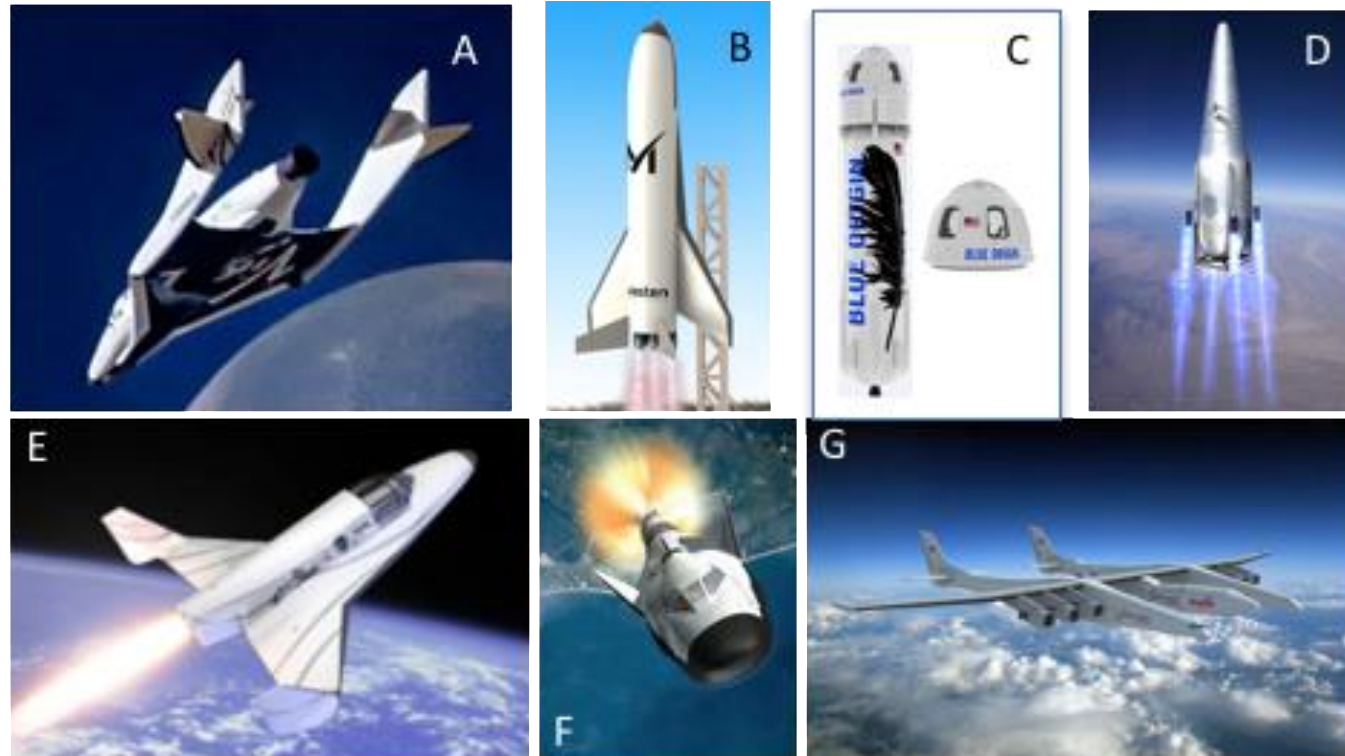
Suborbital Flight Campaigns for Microgravity Life Science Experiments



Objective: Establish yearly Suborbital Flight Campaigns that will provide 200-300 sec micro-g and partial-g exposures for life science investigations.

Suborbital Flight Microgravity Durations
Microgravity ends when the vehicle re-enters the atmosphere. The duration of microgravity is primarily a function of the maximum altitude which is reached during the flight. A few examples:

<u>Altitude</u>	<u>Micro-g Duration</u>
100 km	3:10 min
200 km	5:45 min
400 km	9:10 min



Some Commercial Suborbital Launch Vehicles: A. Virgin Galactic SpaceShip Two. B. Masten Space Systems Experimental Spaceplane XS-1. C. Blue Origin New Shepard. D. Armadillo Aerospace Next Generation Rocket. E. XCOR Aerospace Lynx. F. Sierra Nevada Dream Chaser. G. Stratolaunch Systems Carrier Aircraft.

Antarctic Balloon Campaigns for Long-Term Low Dose Deep Space Life Science Radiation Studies

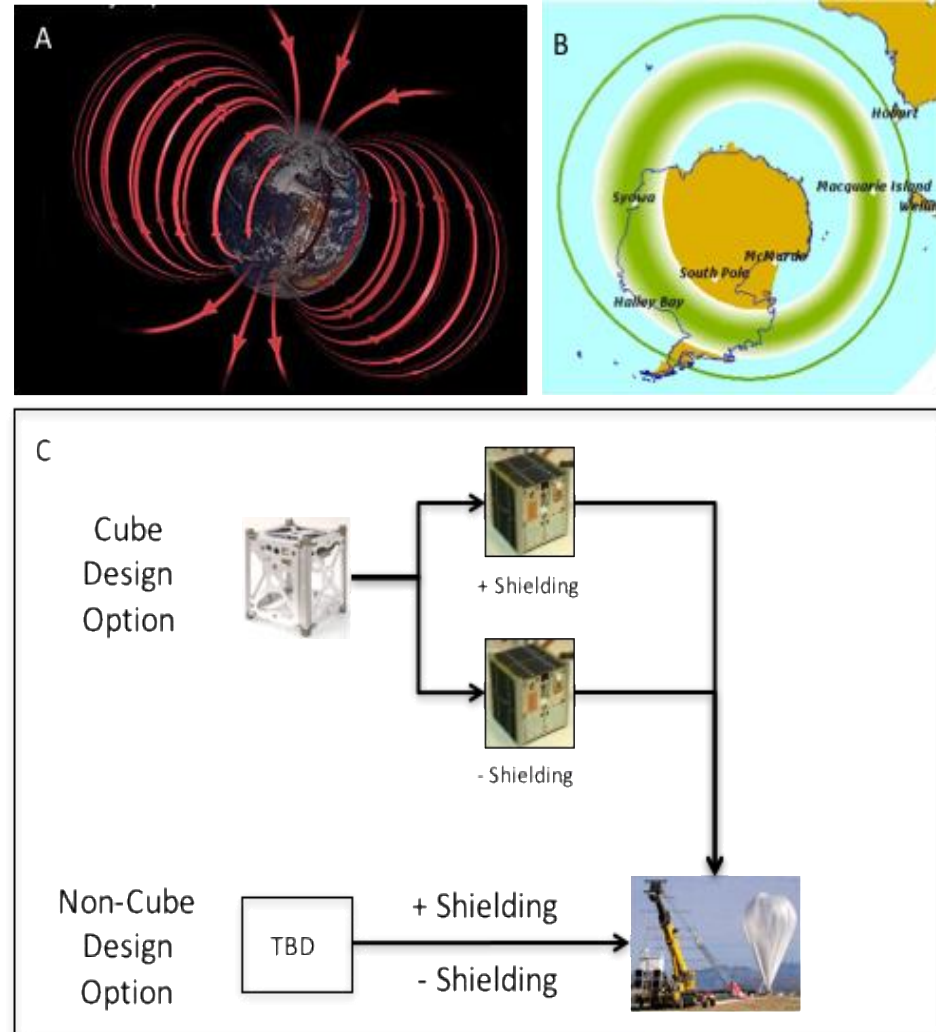


Objective: Facilitate Deep Space Radiation High Altitude 1-3 month life science investigations.

Earth's Magnetic Field Protects us from Space Radiation:

- Most dangerous particles don't hit the Earth's surface because they are forced by **the Earth's magnetic field** to move around the Earth.
- Particles do enter at the magnetic north and south poles where the magnetic field points directly into the ground, so in those areas **particles from deep space** are free to rain in.
- The result is a **Space Radiation environment comparable to what the crew will experience on the way to Mars.**

A. Depiction of the magnetic field lines of the Earth showing polar region areas of deep space radiation penetration. B. View of Auroral Zone's ring shaped region representing the area of deep space radiation penetration with a 2500 km radius around the Earth's magnetic pole (about 2000 km away from the geographic pole). C. Investigators will develop hardware (either Cube-based or TBD) that piggyback on the primary science payload's gondola.



Microgravity Simulators for Ground-Based Gravitational Research

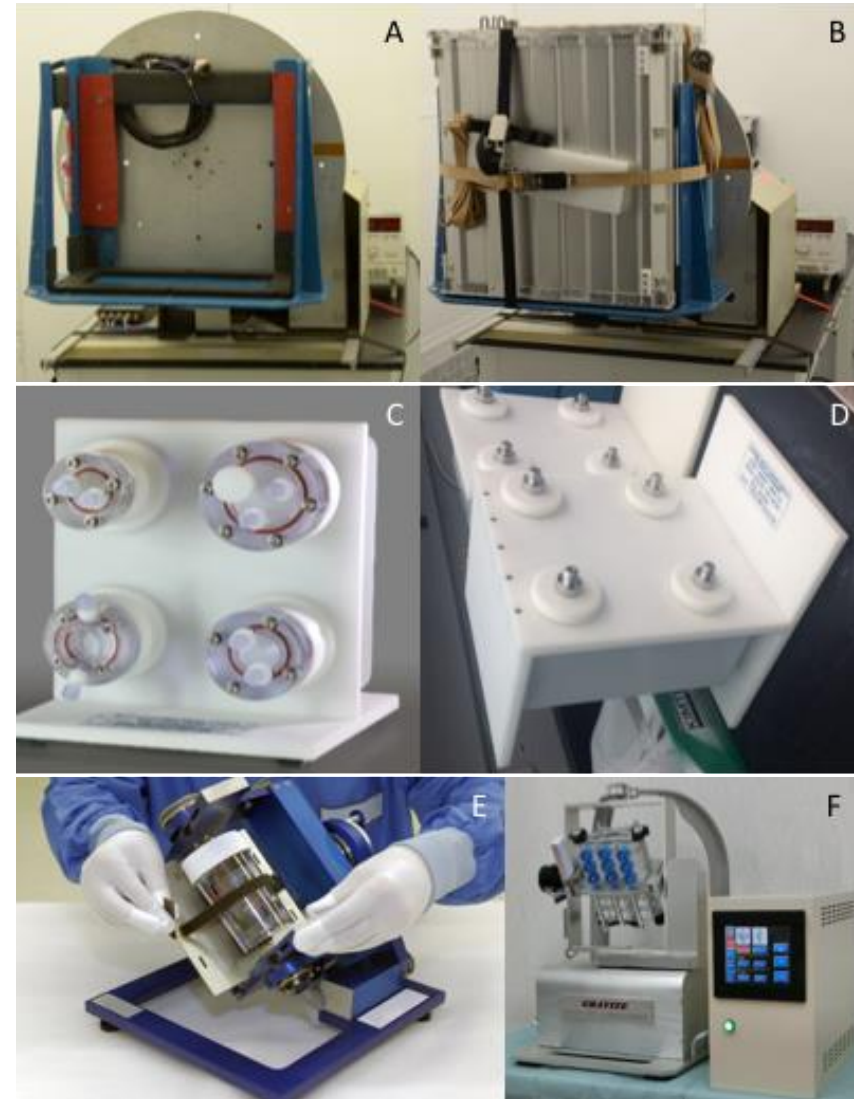


Objective:

Provide the U.S. Space Biology research community a **ground-based micro-g & partial-g simulation capability** composed of devices that negate the directional influence of the “g” vector (e.g. 2D & 3D Clinostats, Random Motion Machines, Rotating Wall Vessels).

Clinostats currently available for use.

- A. KSC Slow Rotating Clinostat for large containers (up to 32 kg).
- B. KSC Slow Rotating Clinostat in ISS stowage locker configuration.
- C. Rotating Wall Vessel (Micro-g configured with HARV's attached).
- D. Rotating Wall Vessel holder in 1g “control” configuration.
- E. Airbus RPM 2.0 configured with experimental vessel.
- F. Space Bio-Laboratories, Inc. GRAVITE RPM.



Bion-M2 Free Flyer Mission: Russian/US Collaboration

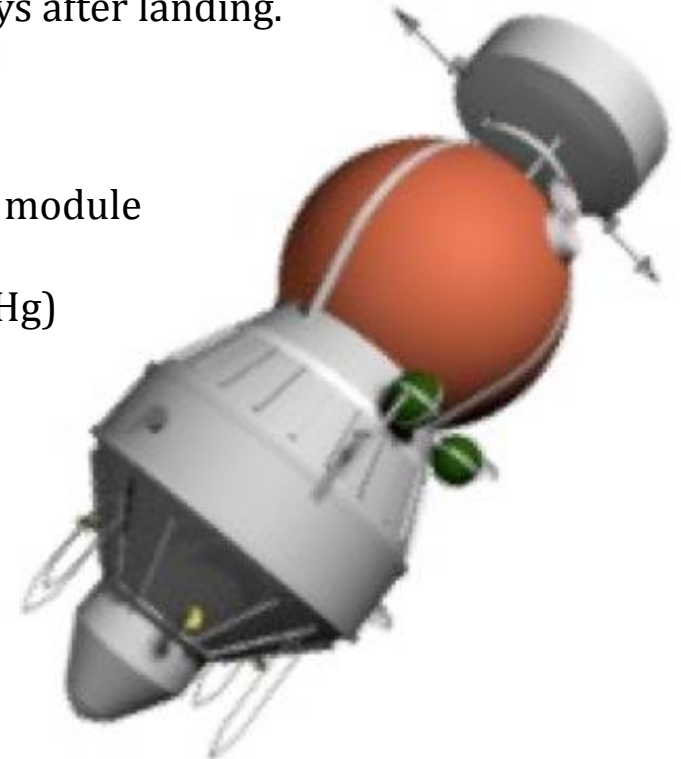


Goal: To investigate systemic, cellular and molecular mechanisms underlying adaptation of mammals to the combined effects of microgravity, cosmic radiation and other spaceflight factors as well as their readaptation to the terrestrial environment upon return to Earth.

- Bion-M2 experiments will be performed on space-flown C57BL/6 mice as well as ground controls housed in flight habitats and exposed to a simulated flight environment (and vivarium controls).
- Biosamples will be collected to detect acute spaceflight effects 1) approximately 2-3 hours after landing; 2) 14-17 hours after landing; 3) 3 days and 4) 7 days after landing.

Bion Characteristics

- 2.5 m Sphere used for spaceflight experiments
- 700 kg of payload mass (4 cubic meters) within recoverable module
- 800-1000 km circular orbit for 30 days
- Internal Pressure: 660-960 mm Hg (typically 720-760 mm Hg)
- pO_2 : 140-180 mm Hg
- pCO_2 : <7 mm Hg (typically <1 mm Hg)
- Relative Humidity: 30-80%
- Capsule Temperature: 18-28C (targets 25 +/- 0.3C)
- Average Power: 350+ Watts
- Inclination Angle: 62.8° or 82.3°
- Period: Approximately 90 minutes
- Flight Duration: 21-60 days

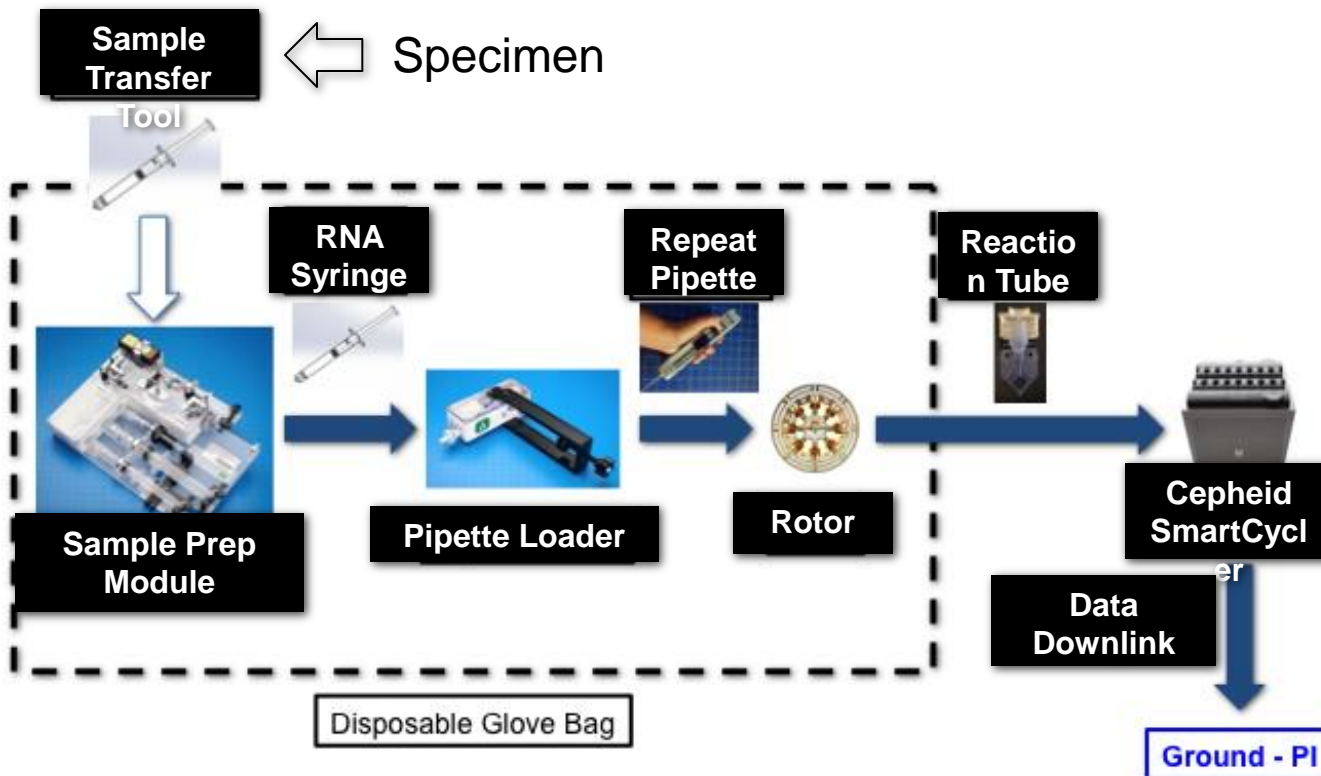


Wetlab-2 (Validated on ISS during SpX-8)



WetLab-2 System was developed to provide an on-orbit nucleic acid analytical capability. WetLab-2 is a research platform for conducting real-time quantitative gene expression and DNA genetic analyses. The COTS Cepheid SmartCycler and its PCR Reaction Tube were adapted for use on ISS in microgravity. The full system provides the ability to process samples on-orbit, isolate DNA and RNA, reverse transcribe RNA, and perform qPCR. The isolate nucleic acid is amplified and analyzed using the Cepheid SmartCycler. The data is downlinked for analysis within 2 hours of run completion. Purified RNA and DNA may be returned to the PI.

WetLab-2 Suite of Equipment and Workflow



Specifications:

- Cepheid SmartCycler
 - 16 qPCR in parallel
 - 4 optical channels to measure fluorescence
- Reaction Tube
 - Carries reaction solutions and PCR primers
 - Up to quadplex reactions (currently validated for triplex reactions on-orbit)
- Sample Prep Module:
 - Capable of processing microbes, cells, and tissues
 - Sample lysis and extraction and purification of RNA and DNA
- Pipette Loader
 - Provides sample de-bubbler
- Rotor
 - Used with ISS Drill
 - Draws sample down to reaction solutions and primers in the Reaction Tube
- Thermal protocols can be uplinked from ground

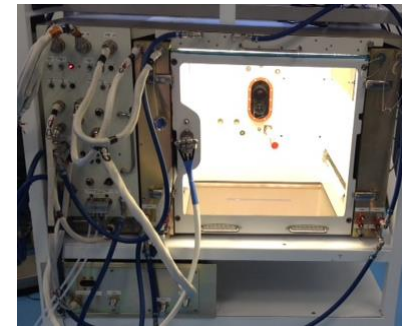
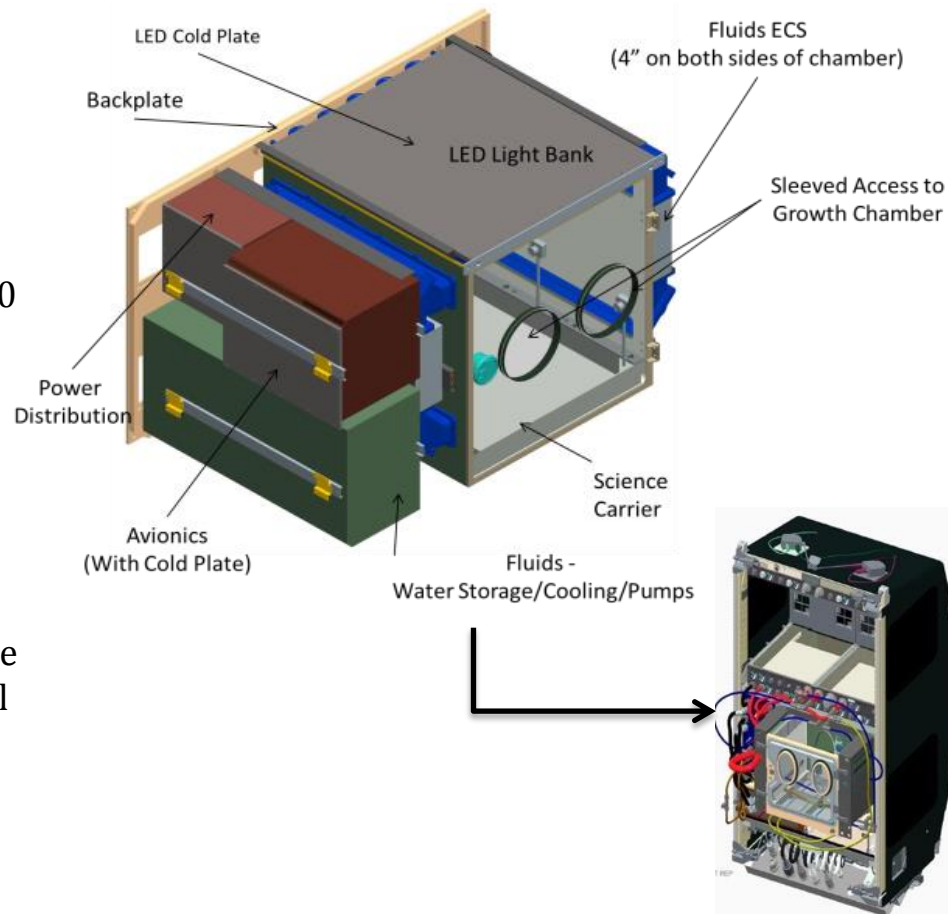
Advanced Plant Habitat (OA-7: Feb 2017)



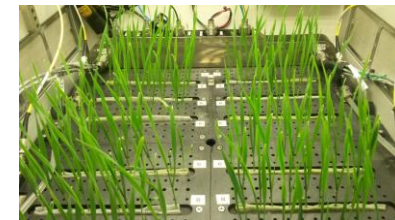
Advanced Plant Habitat (APH) = a large growth volume plant habitat capable of hosting multi-generational studies with environmental variables tracked and controlled in support of whole plant physiological testing (up to 135 days) and Bioregenerative Life Support System investigations.

Specifications:

Max. Shoot Height 45 cm; Root Zone Height 5 cm, Growth Area 1,700 cm²; Growth Volume 112,500 cm³; Light Intensity <math><1000 \mu\text{moles}</math> [Red 630-660 nm, Blue 450 ± 10 nm, Green 525 ± 10 nm, Far Red 730 nm, White]; Temperature 18-30 C; Relative Humidity 50-90%; Condensate Recycled; CO₂ Controlled (400-5000 ppm); Air Flow @ 0.3-1.5 m/s; Ethylene Scrubbed to ≤ 25 ppb; Air Sampling Ports; Water Sampling Ports; Leaf Temperature Sensor; Root Zone Moisture Level Monitored; O₂ Sensors in both Shoot & Root Zone.



APH Engineering Design Unit (EDU).



Dwarf Wheat Dwarf Wheat Grown within the APH EDU.

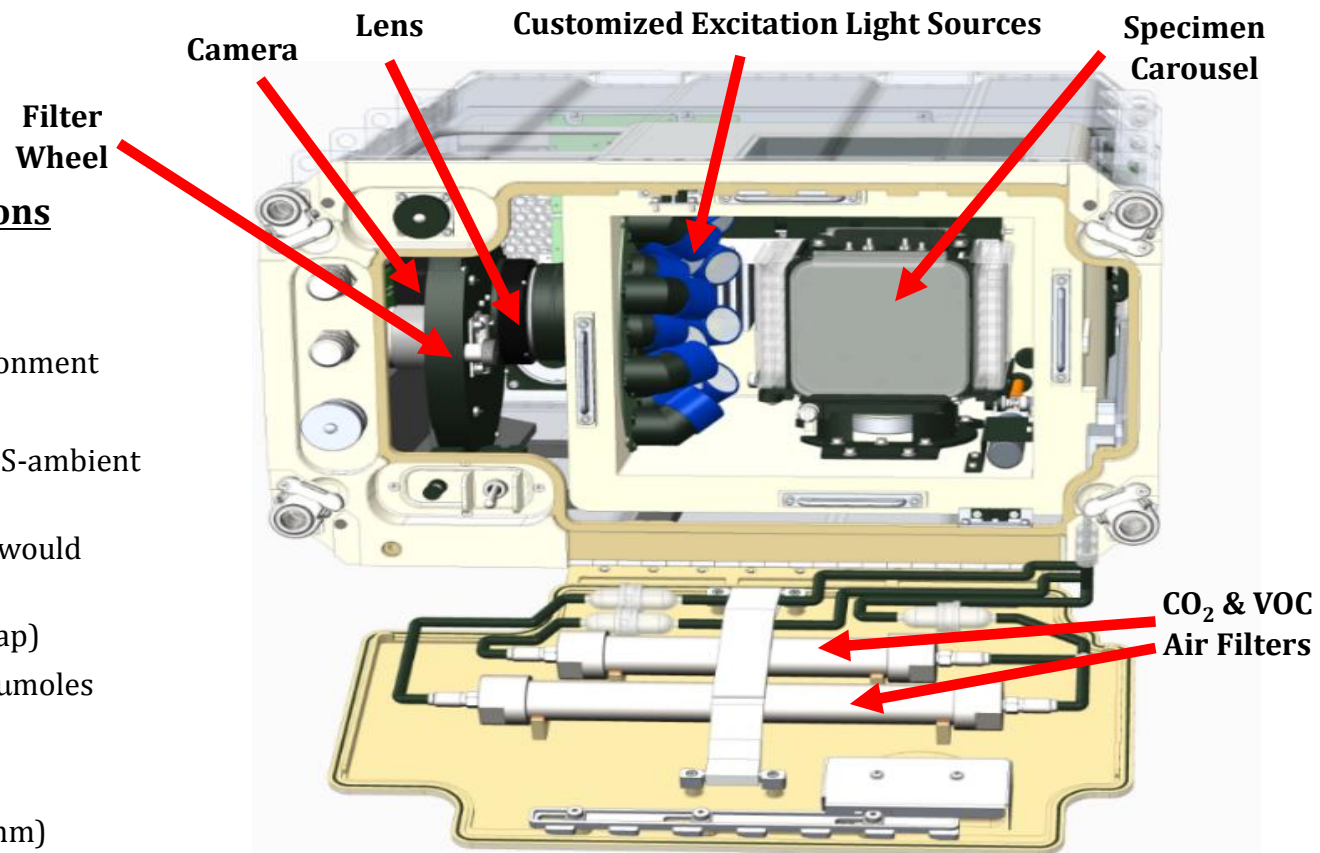


Science Carrier.

Spectrum: Multispectral Fluorescent Imager (SpaceX-15: April 2018)



- Images *in vivo* reporter genes using fluorescent proteins incorporated into model organisms.
- Accommodates 10 cm x 10 cm Petri plates, multi-well culture plates, & other custom containers.
- Capable of capturing high-resolution images with dissection scope level magnification.
- Data collection, storage & downlink retrieval for near-real time evaluation by the investigator team.



Schematic displaying front view of Spectrum.

Internal Environmental Conditions

- Programmable Temperature: 18-37C
- Programmable Light Cycles
- Relative Humidity: Ambient ISS environment
- Ethylene scrubbing (< 25 ppb)
- CO₂ control (between 400 ppm and ISS-ambient levels in units of 100 ppm)
- Airflow to prevent condensation that would interfere with imaging.
- Lighting (at 10 cm beneath the light cap)
 - Broad-spectrum white light 0-100 μ moles (400-750 nm)
 - Darkness: <1 μ moles
 - Red light: 0-100 μ moles (630-660 nm)
 - Blue light: 0-50 μ moles (400-500 nm)
 - Green light 0-30 μ moles (520-530 nm)

Biological Research In Canisters (BRIC) Light Emitting Diodes (LED) (SpaceX-12: 6/1/17)



BRIC-LED Hardware Objective: Provide discrete illumination to biological specimens contained in 60mm Petri dishes that are subjected to a microgravity environment.

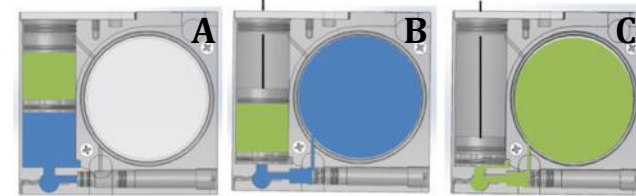
Petri Dish Fixation Units

Single or Dual Fluid Injections: 17mL total fluid volume for injection of Growth media and/or liquid treatments/preservatives/fixatives.

- N=36 60mm Petri dishes available per mission
- Capable of >150 μ moles of light
- >10% intensity resolution control for each wavelength
- 4 discrete LEDs types currently ranging from 430-750nm (blue, red, white and far-red)
- Customizable wavelengths
- Programmable lighting schedule with 1 sec resolution
- >70% light uniformity when using 4 discrete wavelengths
- Light tight from external sources

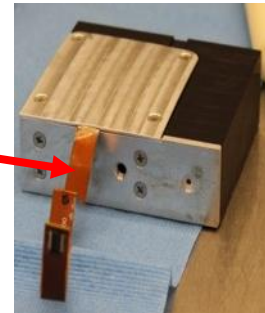
Locker & Tray

- Resides in US Lab on ISS
- Holds 6 BRIC Canisters
- Canisters Travel up/down
- KSC Ground Station controlled
- Commanding start/stop of expt.
- Real time telemetry of Tray and Canister temperatures & LED status
- Forced air cooling to reject heat
 - 1.5C between Canisters
 - 3C from EXPRESS AAA air
- Internal Canister Pressure Logged
- Temperature Sensors on Canister Lid Boards
- One 3-Axis Accelerometer

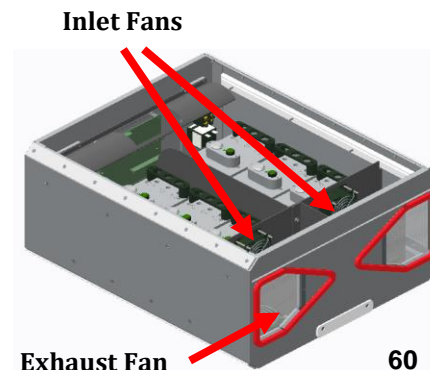
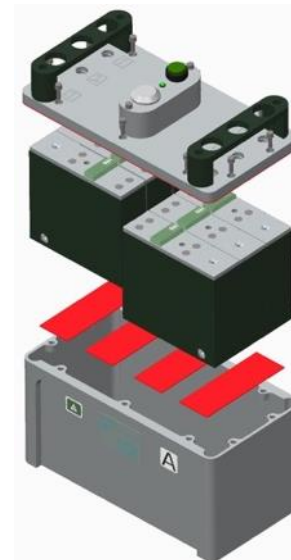


A. Pre-Actuation: Filled with 2 Fluids. B. 1st Actuation: Fluid 1 Dispensed. C. 2nd Actuation: Fluid 2 Dispensed.

PDFU LED
Flex Circuit



BRIC-LED PDFU Lid showing LEDs.



Inlet Fans
Exhaust Fan
60

Veggie Unit #2 (SpaceX-12: 6/1/17)



Veggie is an easily stowed, high growth volume, low resource facility capable of producing fresh vegetables and supporting science experiments on ISS. It also provides real-time psychological benefits for the crew, and facilitates outreach activities.

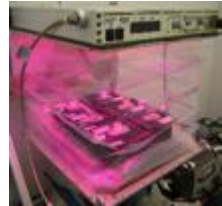
Veggie Configured for Growth of Vegetables



Veggie Light Bank



Veggie Plant Pillow



Veggie + 6 Plant Pillows



Veggie on ISS



Astronaut Steve Swanson harvesting Lettuce.

Specifications:

Light:

- 100-500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ PPF of Red (630 nm), Blue (455nm) and Green (530 nm)

Cabin Air Fan Settings:

- Low / High / Off

Baseplate Footprint:

- 29.2 cm x 36.8 cm

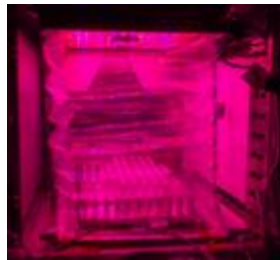
Max. Height:

- 47.0 cm empty
- 41.9 cm with root mat

Veggie Configured for Petri Plate Science Experiments



Petri plate holder with *Arabidopsis* petri plates inserted.



Petri plate holder containing up to 30 *Arabidopsis* plates in Veggie with bellows closed.

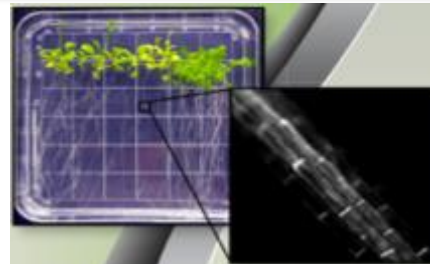
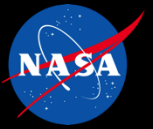


Image of *Arabidopsis* root taken in the Light Microscopy Module (LMM).

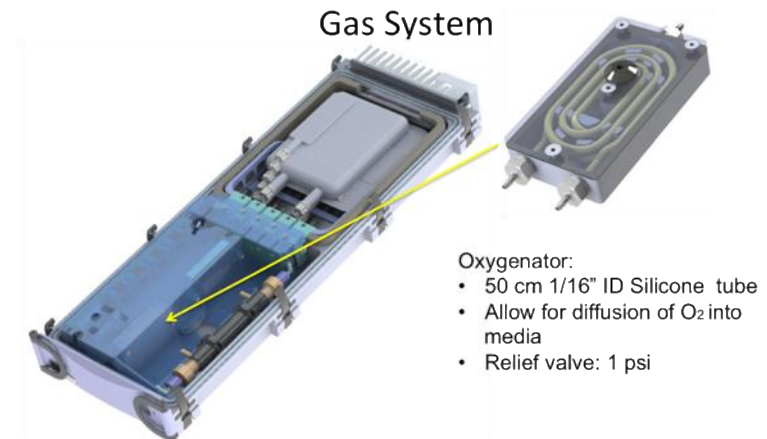
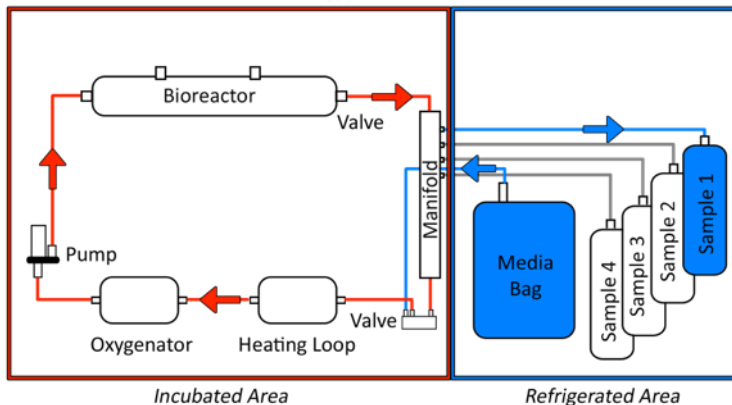
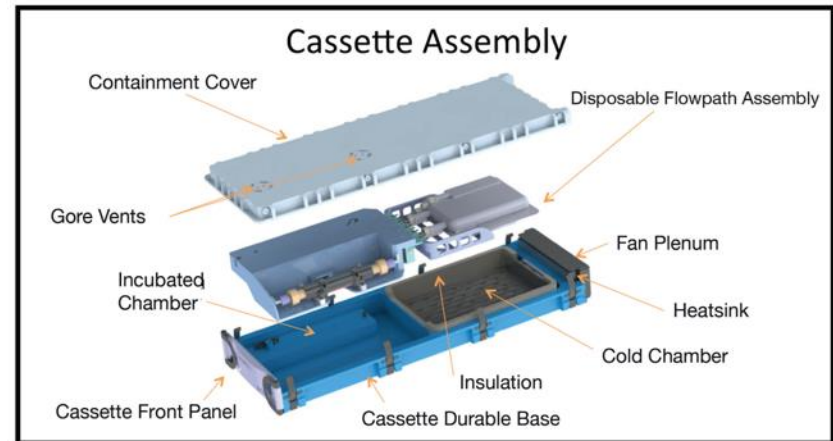
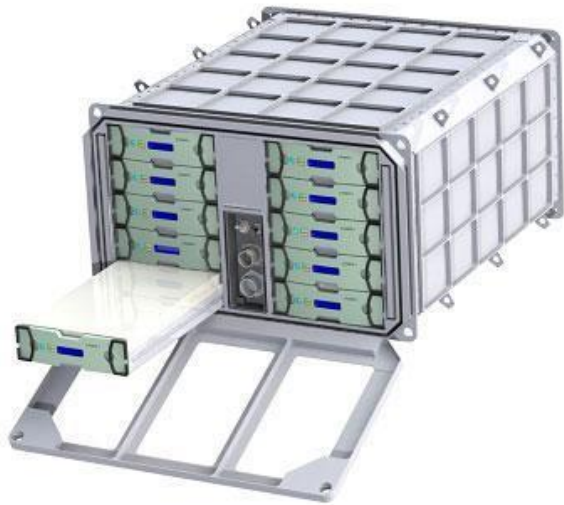


Astronaut Butch Wilmore fixing plants on the ISS using a Kennedy Space Center Fixation Tube (KFT).

Bioculture System (SpaceX-13: Sept 2017)



The Bioculture System is a perfusion culture system. The primary component is the hollow fiber bioreactor, designed to efficiently deliver nutrients and remove waste via multiple, tightly packed perfusion fibers. The hollow fiber system is particularly suited for microgravity cell culture where nutrient transfer is limited to diffusion. The increased surface area and low shear perfusion ensures that cells are receiving sufficient nutrients and gas to grow unabated.



Space Biology: Recent/Current Solicitations



- **NRA NNH16ZTT001N Research Opportunities in Space Biology (ROSBio)** (Release 3/24/16)
 - ROSBio-2016 is an Omnibus Research Announcement that covers all aspects of basic and applied research and technology supporting Space Biology
 - Specific research/funding opportunities announced through Appendices that solicit proposals for experiments to address Space Biology's primary objectives
 - Appendices will be released until Dec. 31st, 2017
- **NRA NNH16ZTT001N-GL Appendix A: "GeneLab Innovation Awards for Translational Systems Biology & Informatics Research Using GeneLab Data System"** (Release 3/24/16)
 - Solicited research projects to: 1) perform ground-based experiments to test novel hypotheses derived from analysis of data in GeneLab Database, or 2) develop novel computational tools to enhance usability & value of GeneLab. **Total value ~\$1.4M**
 - Selected 6/32 proposals: 4 New and 2 Veteran Space Biology Investigators
- **NRA NNH16ZTT001N-MoBE Appendix B: "Research Opportunities for Post-Doctoral Fellowships in Space Biology to Study Microbiome of ISS as a Built Environment"** (Release 9/15/16 - Close 11/30/16)
 - Solicits research from post-doctoral fellows to conduct studies to characterize microbial populations isolated from ISS
 - Released in parallel with Sloan Foundation solicitation for same purpose
 - NASA and Sloan expect to grant 2 awards each with a duration of 2 years

Space Biology: Gravity as A Continuum

Example – Gravitational responses of plant roots

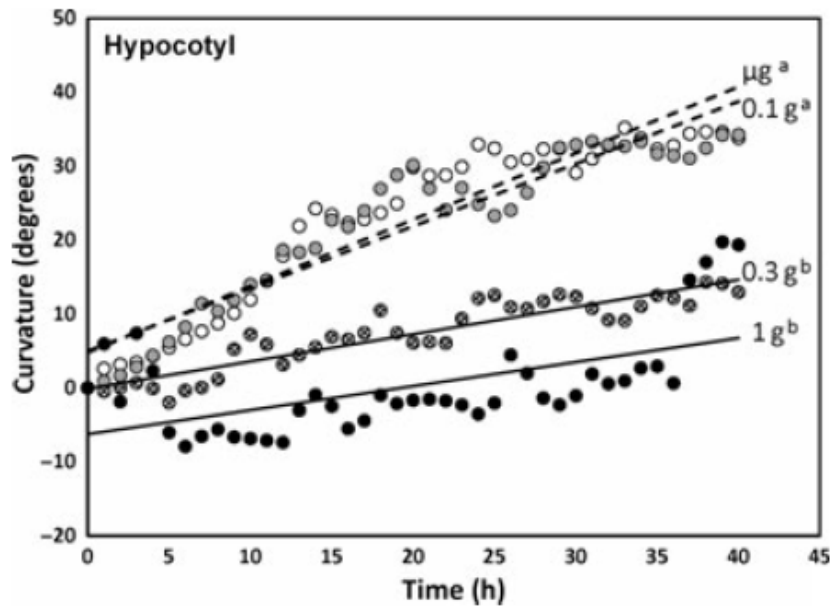


Fig. 3. Time-course studies of positive phototropic curvature in hypocotyls of *Arabidopsis* seedlings in response to red light during the TROPIC-2 spaceflight experiment. White circles indicate micro-*g*, grey circles indicate 0.1 *g*, hatched circles indicate 0.3 *g* and black circles indicate 1 *g*. The plots represent the mean, and different letters indicate significant differences among the first-order regression plots. Note the magnitude of the positive response at micro-*g* and 0.1 *g*. The response at 0.3 *g* was not significantly different from the value of the 1 *g* control, and there was attenuation of red light phototropism at 0.3 *g* and 1 *g*.

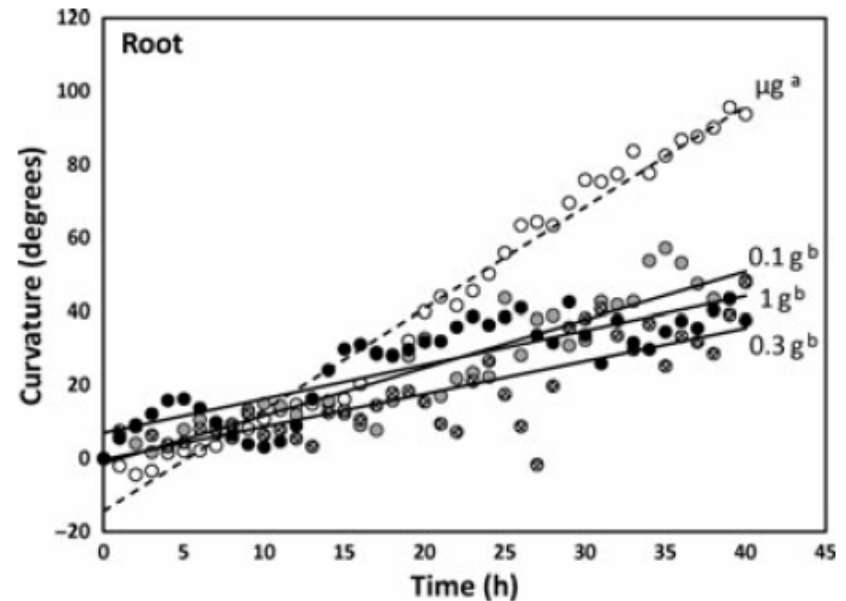


Fig. 4. Time course studies of positive phototropic curvature in roots of *Arabidopsis* seedlings in response to red light during the TROPIC-2 spaceflight experiment. White circles indicate micro-*g*, grey circles indicate 0.1 *g*, hatched circles indicate 0.3 *g* and black circles indicate 1 *g*. Note the large magnitude of the positive response at micro-*g*. The responses at 0.1 *g* and 0.3 *g* were not significantly different from the value of the 1 *g* control, and the former values were attenuated compared to the robust response at micro-*g*.



Integrated Impact of Diet on Human Immune Response, Gut Microbiota, & Nutritional Status During Adaptation to Spaceflight. Lead - Grace Douglas (NASA JSC) Team members - Crucian, B (NASA JSC), Lorenzi, H (J Craig Venter Inst), Ploutz-Snyder, R (USRA), Smith, S (NASA JSC), Zwart, S (USRA) Compare current spaceflight diet to "enhanced" diet, with complete dietary tracking, on immune function, gut microbiota composition, & nutritional status of crew in ground-control, & on ISS. Hypothesis: Increasing consumption of fruits, vegetables & bioactive compounds (omega-3 fatty acids, lycopene, flavonoids) & enhancing overall nutritional intake will improve human immunological profiles, taxonomic profile of the gut microbiota, & nutritional status biomarkers. Will determine diet effects on immune dysregulation, including leukocyte distribution, inflammatory cytokine profiles, T cell function, & other relevant immunological markers, the taxonomic & metatranscriptomic profile of the gut microbiome, & nutritional biomarkers & metabolites at selected intervals, in spaceflight & ground-control data.

Pick-&-Eat Salad-Crop Productivity, Nutritional Value, & Acceptability to Supplement ISS Food System. Lead - Gioia Massa (NASA KSC) Team members - Douglas, G (NASA JSC) Hummerick, M (Qinetiq NA) Mitchell, C (Purdue) Morrow, R (Orbital Technologies) Wheeler, R (NASA KSC) Young, M (NASA JSC) Williams, T (Wyle) Growing nutritious, palatable food for crew consumption during spaceflight can provide health promoting, bioavailable nutrients, enhance dietary experience, & reduce launch mass for longer-duration missions. The "Veggie" vegetable-production system on ISS offers an opportunity to develop a "pick-&-eat" fresh vegetable component as a step to bioregenerative food production. Growing salad plants in Veggie during spaceflight will determine effects of light quality & fertilizer formulation on crop morphology, edible biomass yield, microbial food safety, organoleptic acceptability, nutritional value & behavioral health benefits of fresh produce. Phase A flight tests use leafy greens and Phase B dwarf tomato. Deliverable will be the development of growth protocols for these crops in a spaceflight vegetable production system.

Pioneering Scientific Discovery - Gravity as a Continuum Partnership between SB and HRP



15-15 NASA AG-0013 “Partial-Gravity Dose Response: Roles of vestibular input & sex in response to AG” - Charles Fuller (University Of California, Davis) Team Alwood, Hoban-Higgins, Ronca - Study male, female & labyrinthectomized rats, who no longer have macular vestibular input. Studies will adapt rats to 2G using chronic centrifugation. Our previous research has shown that animals exposed to chronic centrifugation establish a new steady-state in physiology & behavior. We will then return the animals to 1G Earth gravity, subjecting them to a 1G step down in ambient force. All animals will be studied for 60 days post-centrifugation. Tissues will be collected for endocrine, histological & proteomic analysis. Body temperature, activity & sleep-wake parameters. Body mass, & food & water consumption will be monitored. Body composition & bone structural analysis will be measured & video recordings will be used for behavioral phenotyping.

15-15 NASA AG-0005 “Musculoskeletal response to partial-gravity analog in rats: structural, functional & molecular alterations” - Seward Rutkove (BETH ISRAEL DEACONESS) Co-I Bouxsein One countermeasure to negative consequences of space travel is artificial gravity (AG) via centrifugation. Lower levels of force may be effective for preventing muscle & bone atrophy. This study will evaluate AG for prevention of muscle & bone atrophy & weakness by studying rats using partial unloading developed previously for mice. Musculoskeletal responses of adult male rats will be studied following exposure to 0.2, 0.4, & 0.7G as well as 1 G for 1, 2 & 4 weeks. Measures will include histological analyses, force of muscle contraction, & bone mass, microarchitecture, & strength. Serological & tissue molecular analyses will be used to understand mechanisms and time course of changes. At the conclusion, we will have defined the potential impact of partial artificial gravity for prevention of musculoskeletal dysfunction & deterioration to inform future application of AG in human research & space exploration.