Cell Science-03 (CS-03) Payload Overview

POIWG #44

October 25, 2018

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Ames Research Center (ARC)

Approved for public release, unlimited distribution

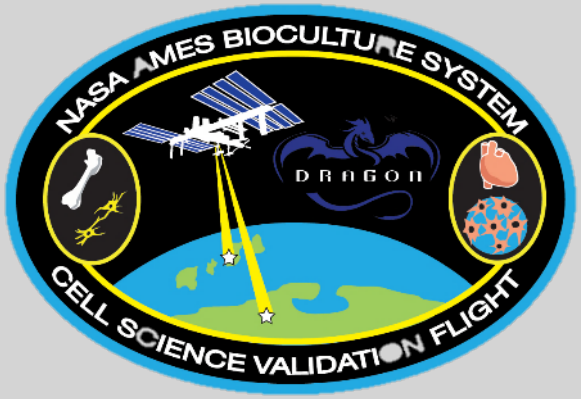
Thank you!

NASA SILICON VALLEY

AMES RESEARCH CENTER

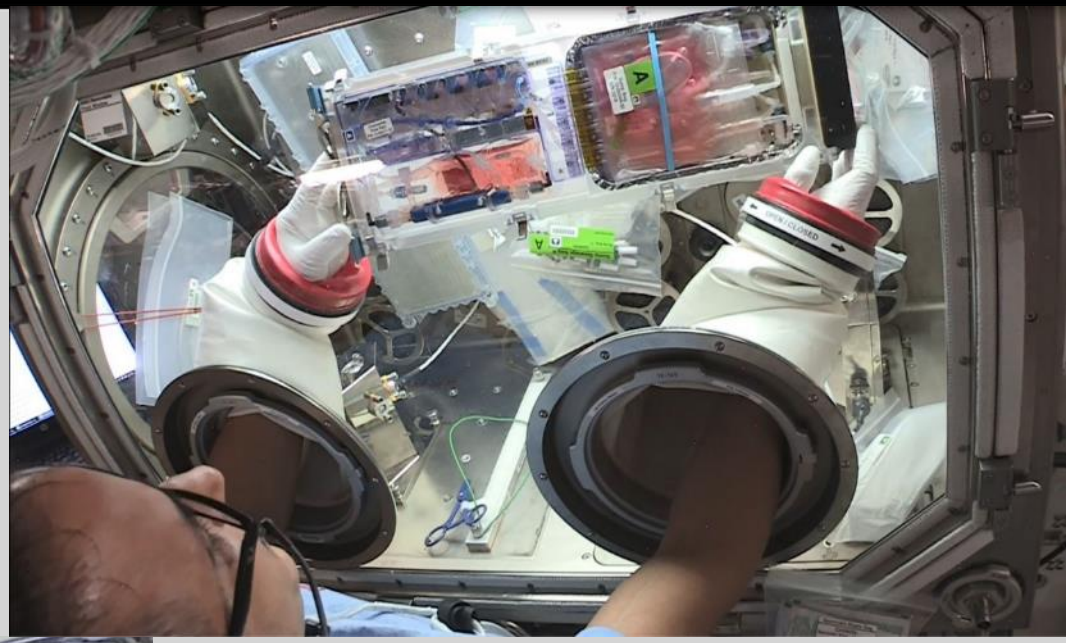
Cell Science Validation (CS-V)

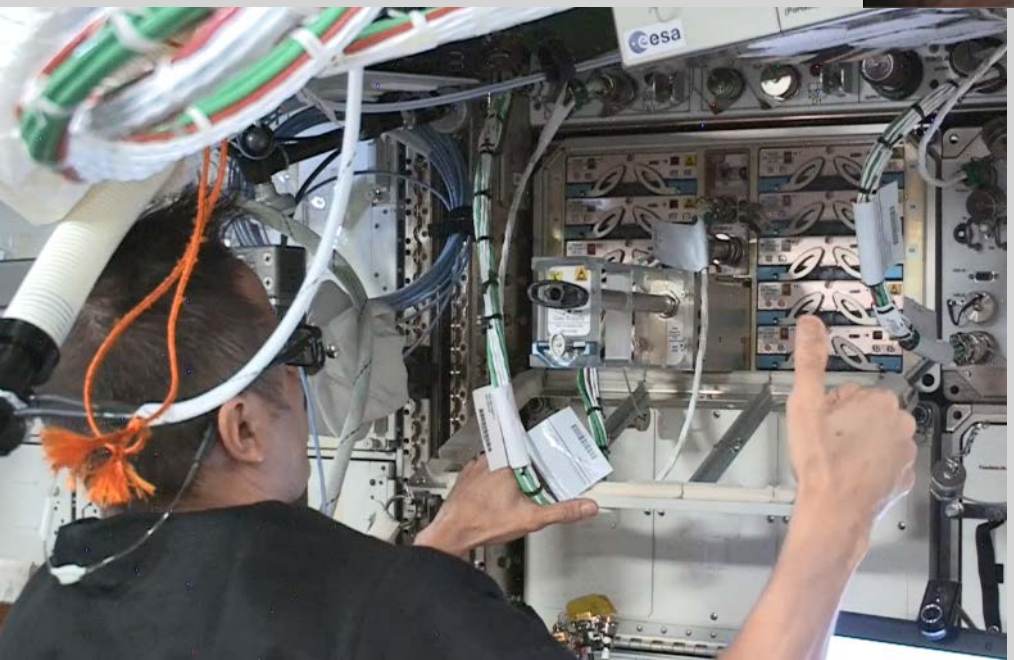
- **Vehicle**: SpX-13
- **Dates**: December 15th, 2017 – January 13th, 2018
- **Crew**:
  - Joe Acaba performed the majority of CS-V operations
  - Maker (Scott Tingle) performed bioreactor removal
  - Nemo (Norishige Kanai) performed transfer to ISS
- **Results**:
  - 100% Science Return (8/8 Bioreactors and 4/4 Samples)
- Received positive feedback on hardware in crew debriefs



CS-V Imagery

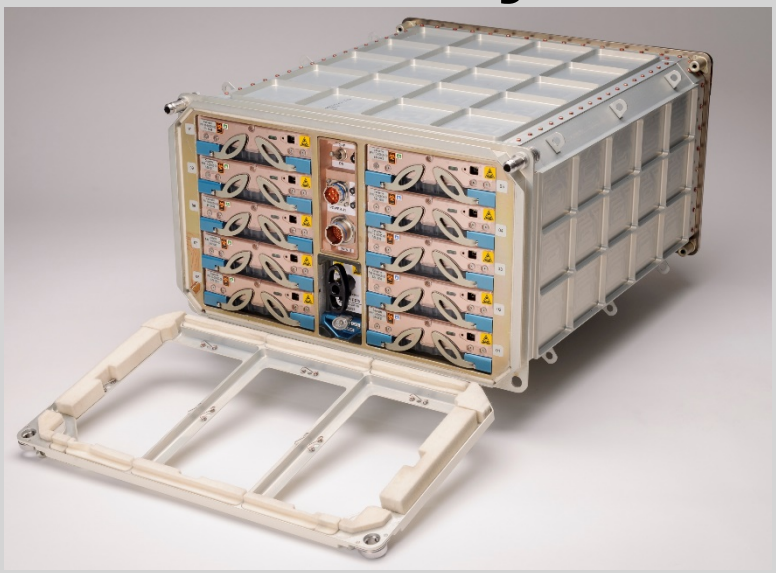
Bag Change Out in MSG





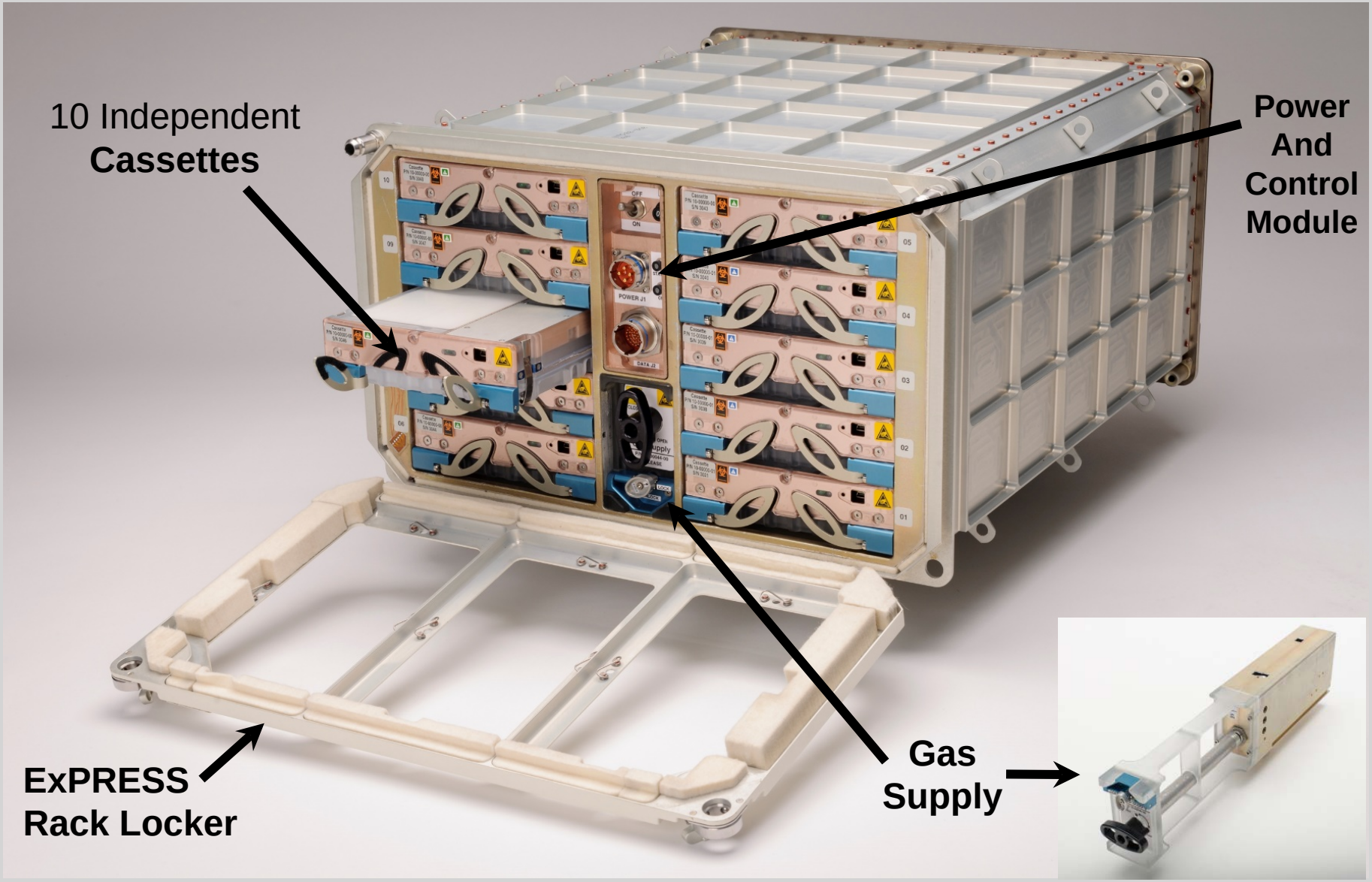
Gas Supply Change Out

Bioculture System



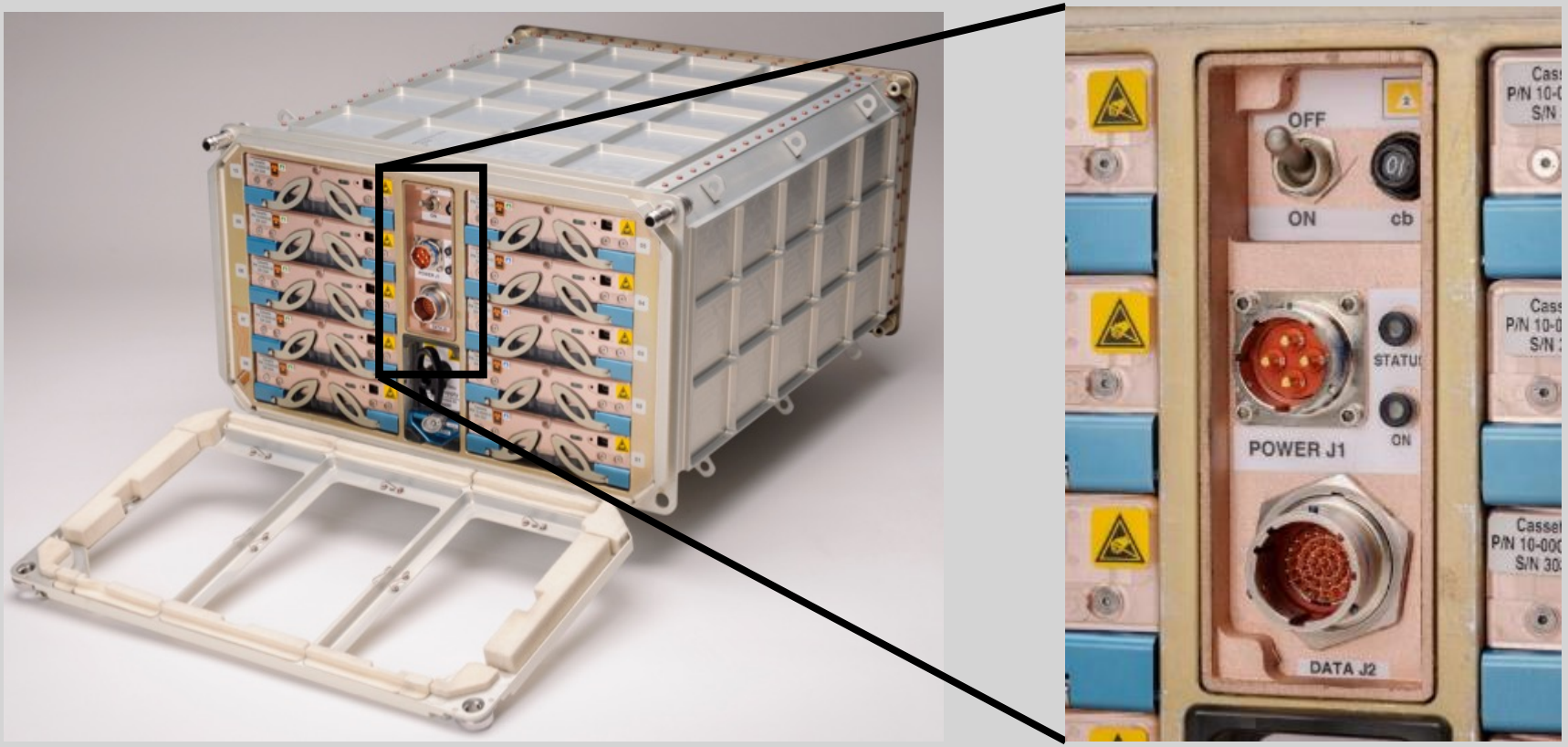
- Automated cell biology system with 10 independent cassettes
- Programmable manipulations include:
  - Media flow rate and feeding schedule
  - Adjustable gas dosing
  - Sample collection
  - Fixative injection
  - pH measurements
- Cassettes will be removed and opened in Life Sciences Glovebox (LSG) to allow initiation of cell cultures, media change out, sample retrieval and preservation.
- Hardware is ESD sensitive

Bioculture System

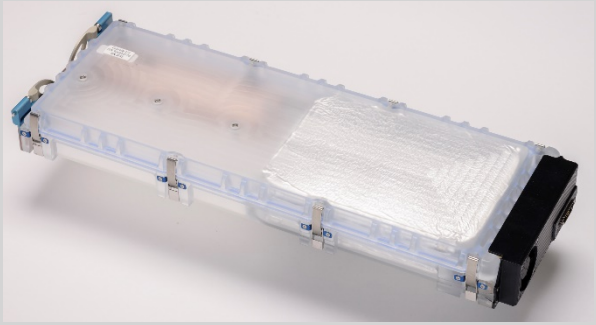


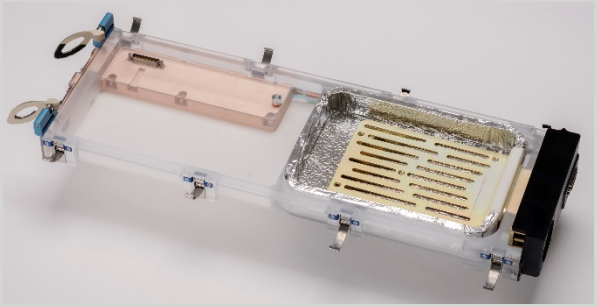
Power and Control Module

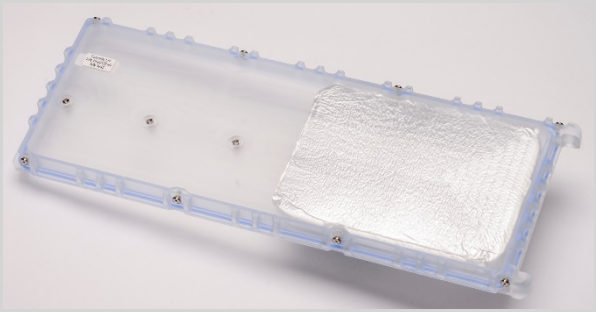
The Power and Control Module takes 28V Rack voltage, converts it to 12V, and uses standard interfaces for both power and data to the Rack. The Power and Control Module contains an On/Off toggle switch, a circuit breaker, Data Connector, Power Connector, and two LEDs for Power/Fault Status.

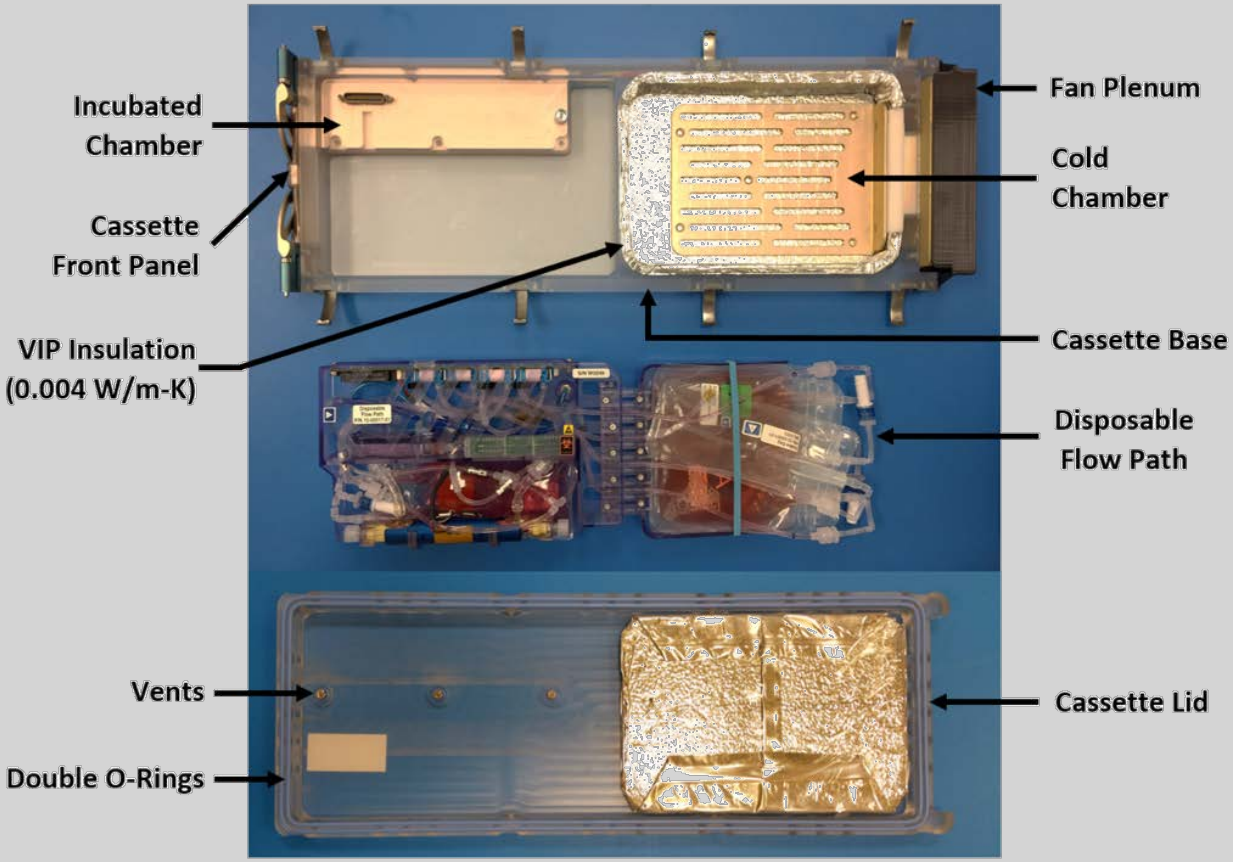


Durable Cassette with Disposable Flow Path

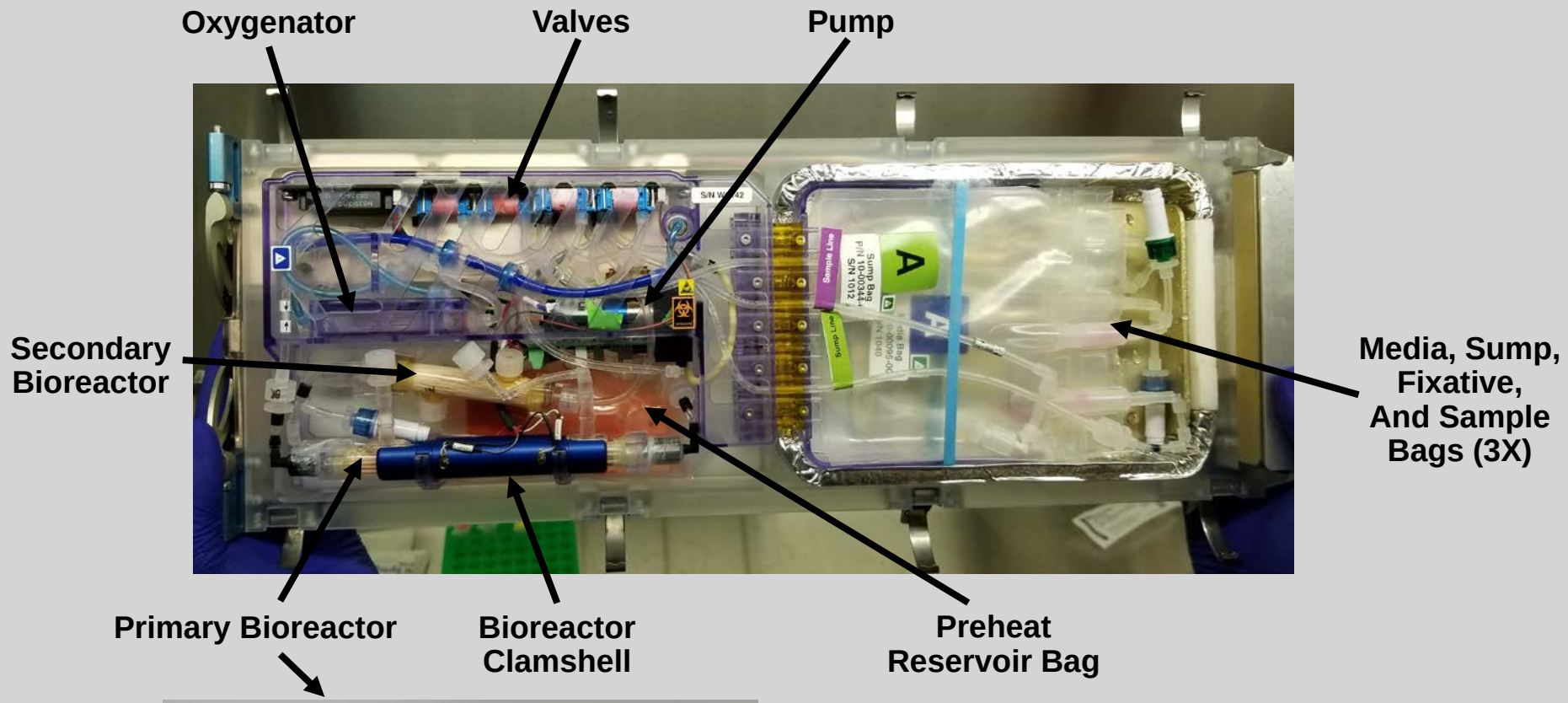








Disposable Flow Path



*NOTE: Photo depicts current configuration of the Disposable Flow Path, including a secondary bioreactor containing growth factor beads. Testing is in-work and is required before final configuration will be determined.

CS-03 Experiment Summary

Principal Investigators	Elizabeth Blaber, Ph.D. Universities Space Research Association/ NASA Ames Research Center
Co-Investigators	Eduardo Almeida, Ph.D NASA Ames Research Center
Sponsor	SLPSRA – Space Biology Program
Funding Authority	NASA / Human Exploration & Operations / ISS NASA / Human Exploration & Operations / Space Life and Physical Sciences
Experiment Title	Osteogenic Differentiation of Somatic Stem Cells in Space: A Study Investigating the Role of CDKN1a/p21 on Mesenchymal Stem Cell Proliferation, Differentiation, and Regeneration in Microgravity.
Experiment Duration	30 Days – Initiated on orbit
Cell Type	Mouse bone marrow mesenchymal stem cells
Independent Variable	Cell Type: Mesenchymal stem cells are isolated from 2 separate groups: Wildtype (WT) vs CDKN1a/p21 knockout (KO) mice

Launch

Schedule

CS-03 is currently scheduled for a SpX-18 launch (May 2019)

Duration of SpX-18 is 30 days from time of berth:

- Berth (5/9) – Unberth (6/8)

Duration

CS-03 experiment duration is 30 days. The experiment will not be completed prior to SpX-18 unberth, so PD will request samples and hardware to be stowed for return on SpX-19.

New Crew Operations

Development of new crew operations will be finalized once the following documents and tests are completed:

- **Experiment Requirements Document (ERD)**
- **Science Verification Test (SVT)**
- **Experiment Verification Test (EVT)**

New Crew Operations

Disposable Flow Paths:

- Current plan is the Disposable Flow Paths will be flown separately and the crew will install into the BCS Cassettes on-orbit.

Wet Lab RNA SmartCycler (Wet Lab-2):

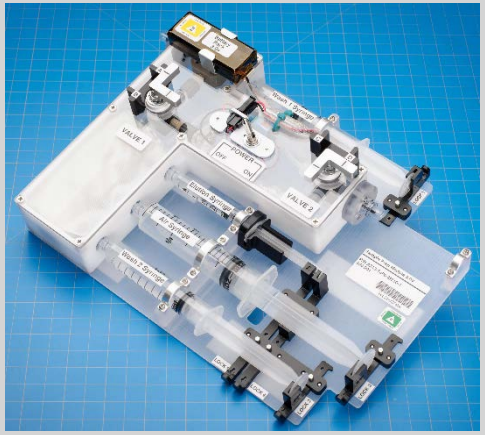
- System will be used at two time points to verify expression of culture growth genes in sampled cells and finalized time of fixation.

Bioreactor Injections:

- Frozen cells derived from two types of mice will be injected into the Bioreactors to initiate cultures.

MinION:

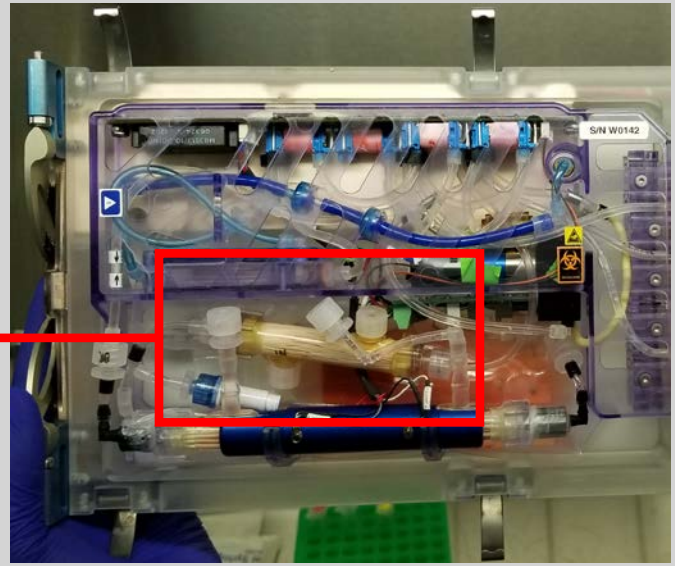
- Portable real-time device for DNA and RNA sequencing.



New Crew Operations

Growth Factor Beads Bioreactor Installation:

- An additional smaller secondary bioreactor with growth factor beads will be installed into each Disposable Flow Path prior to cell injection into the primary bioreactor.



*NOTE: Photo depicts current configuration of the Disposable Flow Path, including a secondary bioreactor containing growth factor beads. Testing is in-work and is required before final configuration will be determined.

EdU Injection:

- 5-ethynyl-2'-deoxyuridine (EdU) will be injected into the primary bioreactor to label cellular DNA.

PFA Exchange:

- Cells will be sampled from the primary bioreactor and culture media from the sample will be replaced by paraformaldehyde (PFA).

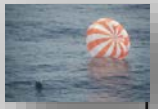
PBS Exchange:

- PFA in syringes containing fixed cells will be replaced by phosphate buffered saline (PBS).

RNAprotect:

- Cells will be sampled from the primary bioreactor and mixed with RNAprotect prior to hand over for Wet Lab-2 ops.

CS-03 Timeline



Turnover Unpowered Bioculture System (BCS)

Ship soft stow items to JSC

Turnover cold stowage items (media bags, and frozen cells) for use on Orbit

*Turnover Disposable Flow Paths

L-9w | L-3d | L-2d

L-6w

Set Up LSG

Transfer BCS from Dragon to ExPRESS

Bag Change Out, Culture media bag install, GF Bioreactor install

*Disposable Flow Path Install, Priming Media Bag Install & Wash

NLT L+4d | L+5d & 6d | L+7d

Dragon Berth

SpX-18 Launch

Thaw Frozen Cell Cultures and Inject into Bioreactors

Will refer to this as "Initiation" day

Sample Cells from Bioreactors for Wet Lab-2 analysis #1, Sample Bag Removal

Bag Change Out | Gas Supply Change Out #1 | TBD

Initiation +10d

Bag Change Out | Gas Supply Change Out #2 | TBD

Bag Change Out | Gas Supply Change Out #3 | TBD

Sample Cells from Bioreactors for Wet Lab-2 analysis #2, Sample Bag Removal

Initiation +20d

SpX-18 Splashdown

Remove Bioreactors and all bags from Cassettes and put in Cold Stowage, Cell samples washed (PBS)

EdU Injection

Initiation +27d

Sample Cells from Bioreactors & fix in PFA

Initiation +28d

Automated Fixative Injection into Bioreactors

Initiation +30d

Initiation +30d

Transfer Bioculture System, cold and soft stow items to Dragon

Tear Down LSG

Flexible | TBD

SpX-19 Splashdown

Priority Return of Soft-stowed BCS to ARC

Retrieval of cold stow items

TBD | TBD

Purple Text indicates the activity occurs in the LSG

* Ongoing testing will determine future crew operations with Cassettes/Disposable Flow Paths stowage and install operations

Integration Overview

- **Bioculture System will be operational for 34 days after it is installed into the ExPRESS Rack**
- **Bioculture System Facility Payload Integration Agreement (PIA) Letter contains the following unique agreements:**
  - Bioculture System shall remain on the pad no longer than 48 hours after turnover, after which the medium shall be changed out
  - Nominal turnover of unpowered Bioculture System
  - Use of LSG for crew operations
  - Use of SSPF labs at KSC pre-launch

Integration Overview (Continued)

- **Pre-flight specimen and hardware processing in the KSC SSPF**
- **On-orbit operations**
  - Majority of crew procedures already developed and executed on-orbit for CS-V (SpX-13).
  - Updates for new operations for CS-03 are in work with POIC cadre
  - Training Strategy: OBT
  - Real-time support at ARC: Telemetry monitoring and commanding
  - Requesting PD enablement and over-the-shoulder video during operations in LSG
- **Use of ISS facilities:**
  - ExPRESS Rack
  - Life Science Glovebox (LSG)
  - MELFI (and Cold Stowage on Dragon – Polar and/or DCB)
  - Wet Lab RNA SmartCycler
  - MinION (TBD)
- **Asynchronous Ground Control at ARC**
- **Post-flight science recoveries at PI laboratories.**

Stowage Overview: Ascent on SpX-18

Unpowered Locker

- Bioculture System
  - Containing 10 Cassettes with no live cells

Cold Stowage

- Cell Loading Kit (TBD)
  - -80°C or colder
  - Each 3mL syringe contains frozen cells
- Media Stowage Bag (x11)
  - +4°C
  - Each Media Stowage Bag contains five Media Bags (total of 45)
- Priming Media Stowage Bag (x4)
  - +4°C
  - Each Priming Media Stowage Bag contains five Media Bags (total of 20)
- Cell Sampling PFA Kit (x2)
  - -20°C or colder
  - Each contains five syringes
- EdU Kit (x2)
  - -20°C or colder
  - Each contains five syringes
- PBS Kit (x2)
  - +4°C
  - Each contains five syringes
- Growth Factor Beads Bioreactor Kit (TBD)
  - +4°C
  - Each contains five Bioreactors

Soft Stowage

- Disposable Flow Paths (x10)
- Cassettes (x2)
  - Spares
- Bag Changeout Kit (x4)
  - Contains empty Sump Bags, Hemostats, Wipes, and other accessories
  - One kit contains supplies for 10 cassettes.
- Bioreactor Removal Kit (x1)
  - Contains Tube Cutter, two hard-sided Bioreactor Containers, and stowage bags
- Sample Bag Removal Kit (x3)
  - Tube cutter and Sample Stowage Bags
- Gas Supply (x3)
  - Replacement Gas supplies to be installed during gas supply changeout
- Setup Kit (x1)
  - Items for setting up LSG (wipes, O-rings)
- Accessories Kit (x1)
  - Spare syringes, hemostats, caps, wipes, etc
- Cell Sampling RNAprotect Kit (x2)
  - Syringes with RNAprotect

Stowage Overview: Descent on SpX-19

Cold Stowage

- Bioreactor Container (x2)
  - -80°C
  - Each container will have five individually bagged Bioreactors
- Sample Stowage Bag (TBD)
  - -80°C
- Return Bag (TBD)
  - -80°C (Media Bags)
- Return Bag (TBD)
  - -80°C (Sump Bags)
- Wet Lab-2 Sample Return Vial (x12)
  - -80°C
- MinION Sample Return Bag (TBD)
  - -80°C
- PFA Preserved Sample Return Bag (TBD)
  - +4°C

Soft Stowage

- Bioculture System
  - Will be unpowered, removed from ExPRESS Rack, and packed in foam following conclusion of the experiment.
  - Will require oversize bag
  - Needs to be priority return to ARC
- Gas Supply (x3)
  - Used gas supplies for refurbishment
- Cassettes (x2)
  - Spares returned

Backup Slides

CS-03 Hypothesis and Objectives

Hypothesis: Based on PI's previous results, CDKN1a/p21 may be a key molecular mechanism in the control of stem cell based tissue regeneration and is therefore a key candidate for stem cell-based tissue regenerative therapies and investigation in microgravity. This study hypothesizes that CDKN1a/p21 inhibits the proliferation and differentiation of mesenchymal stem cells into bone forming osteoblasts in space environment. Therefore, bone marrow mesenchymal stem cells from the CDKN1a/p21-null mice are expected to show unrestrained proliferation and differentiation in microgravity.

Objectives:

- **Specific Aim 1:** Assess the in-vitro proliferation, differentiation, and mineralization capacity of bone marrow mesenchymal stem cells isolated from CDKN1a/p21-null mice compared to wild-type animals in microgravity versus 1g controls.
- **Specific Aim 2:** Determine cellular mechanisms associated with alterations in osteoprogenitor differentiation potential in CDKN1a/p21-null mice versus wild-type controls
- **Specific Aim 3:** Investigate the signal transduction pathways, specifically NFκB, MAPK, and Pi3K signaling, which are responsible for activation of CDKN1a/p21 in microgravity and therefore inhibition of in vitro bone formation in space.