Cell Science 2 (CS-02) Payload Overview

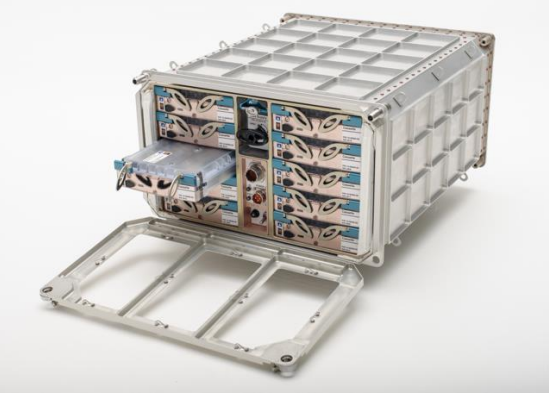
October 2017

POIWG #42

Kevin Sims

Ames Research Center (ARC)

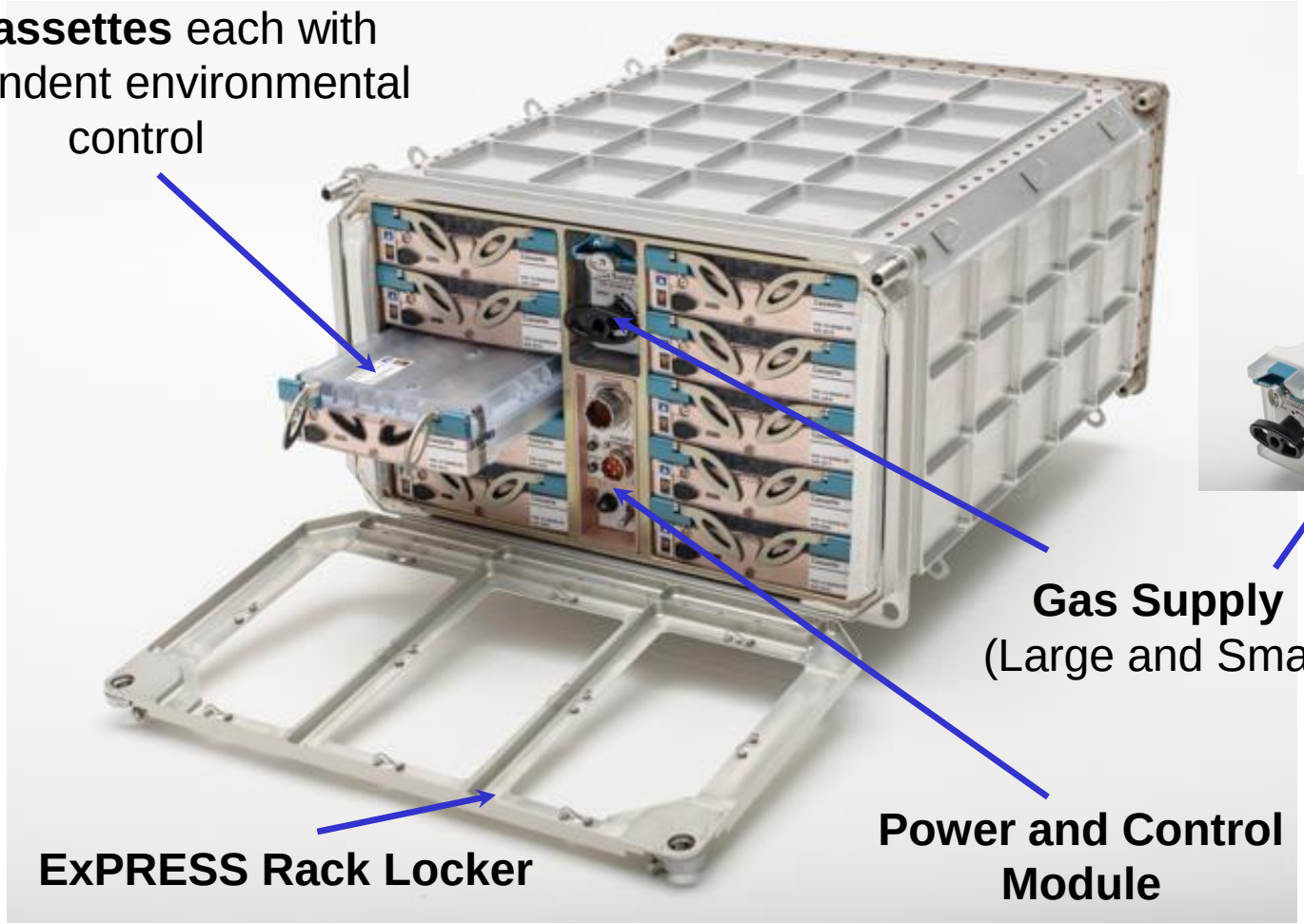
Description of the Bioculture System



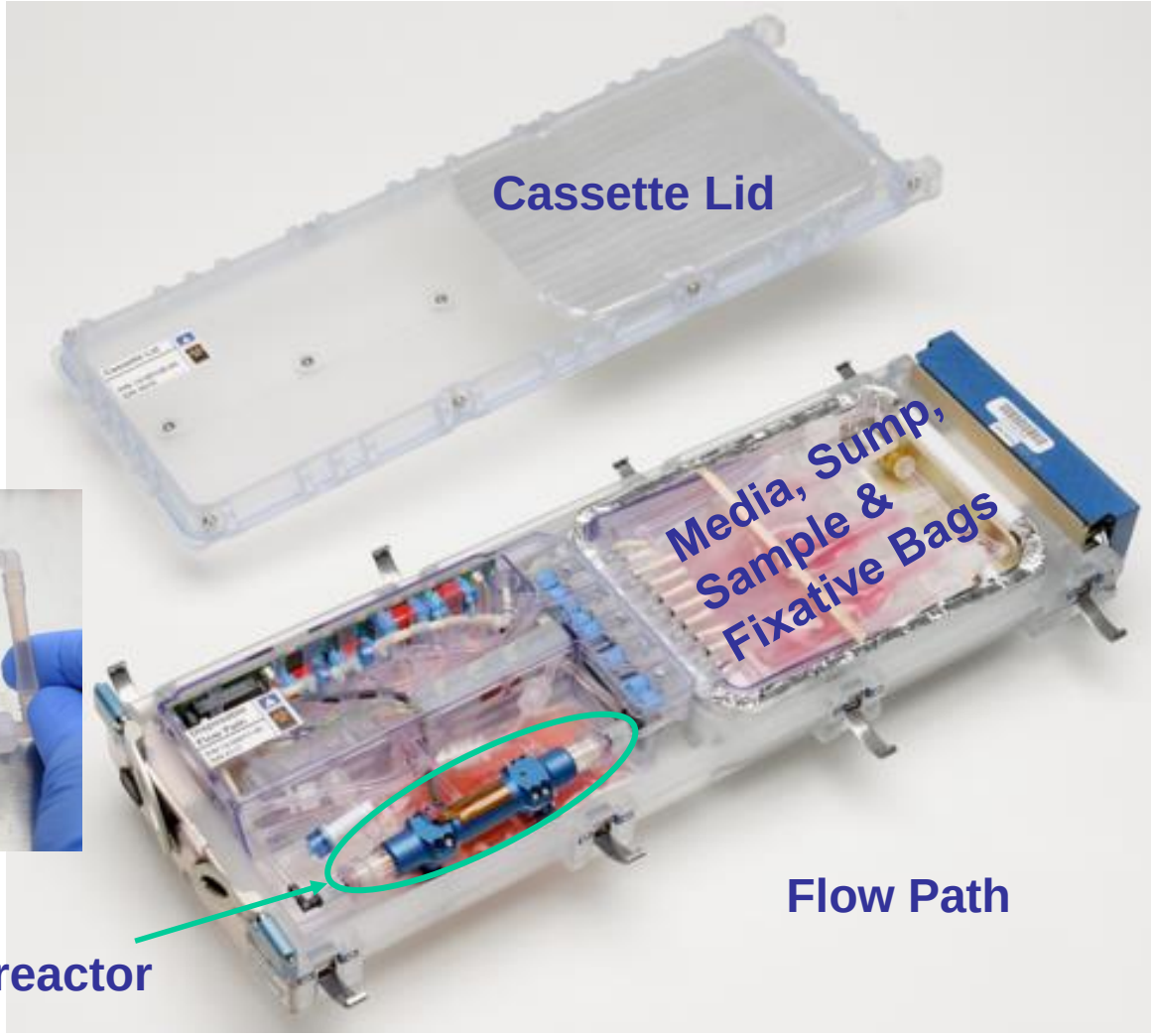
- Automated cell biology system for laboratory and International Space Station (ISS) National Laboratory research.
- Enhanced cell culture platform that provides undisturbed culture maintenance, including feedback temperature control, medical grade gas supply, perfusion nutrient delivery and removal of waste, and automated experiment manipulations.
- Programmable manipulations include: media feeds/change out, injections, fraction collections, fixation, flow rate, and temperature modification within a one-piece sterile barrier flow path.
- Cassette provides 3 levels of containment and allows Crew access to the bioculture chamber and flow path assembly for experiment initiation, refurbishment, or sample retrieval and preservation.

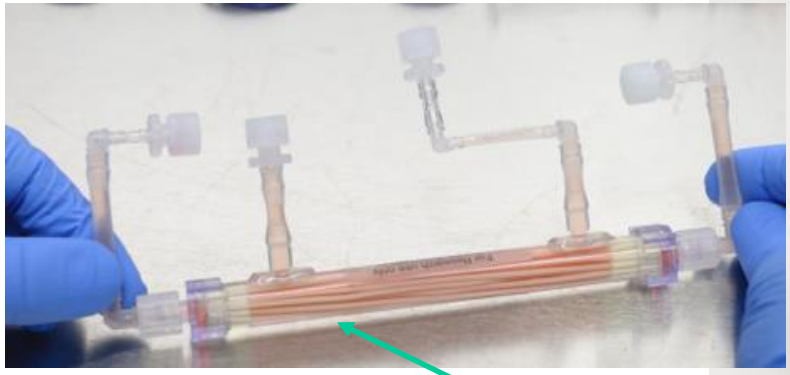
Bioculture System

10 **Cassettes** each with independent environmental control



Cassette with Flow Path





Bioreactor

CS-02 Project Team (Hammamieh/Kacena)

Payload Title: Cell Science-02 (CS-02) Tissue Regeneration

Experiment Title: Pan-Omics Approach to Characterize the Factors Involved in Mammalian Tissue Regeneration in Microgravity

Principal Investigator: Rasha Hammamieh, Ph.D.
Director, Integrative and Systems Biology Program
US Army Center for Environmental Health Research (USACEHR)

Principal Investigator: Melissa Kacena, Ph.D.
Professor, Department of Orthopaedic Surgery
Indiana University School of Medicine

Co-Investigator: Nabarun Chakraborty
Integrative and Systems Biology Program
US Army Center for Environmental Health Research (USACEHR)

CS-02 Hypothesis and Objectives (Hammamieh/Kacena)

Hypothesis: Nearly all experiments investigating bone loss in microgravity have focused on studying bone physiological and morphological changes and identifying which cellular and molecular mechanism are affected. This tissue regeneration space flight experiment will investigate the ability of two different bone stimulating factors, Bone Morphogenic Protein-2 (BMP-2) and Thrombopoeitin (TPO), to stimulate the growth, differentiation and related cellular functions of osteoblast cells in culture. This study hypothesizes that:

1. BMP-2 and TPO will differentially impact osteoblast differentiation in 1g versus microgravity conditions and
2. Microgravity-specific biomarkers/networks associated with bone differentiation could have significant relevance in the astronaut therapeutic program

Objectives: The objective of this study is to determine how BMP-2 and TPO factors differentially affect osteoblasts in the different gravity conditions and to define alterations in genomics, transcriptomics, proteomics, and metabolomics patterns and networks.

- **Specific Aim 1:** Determine whether osteoblast lineage cells treated with BMP-2 or TPO have altered multi-omics signatures.
- **Specific Aim 2:** Determine how gravity manipulates the multi-omics signatures of osteoblast lineage cells treated with BMP-2 or TPO.

CS-02 Project Team (Blaber)

Payload Title: Cell Science-02 (CS-02) Tissue Regeneration

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.

Principal Investigator: Elizabeth Blaber, Ph.D.
NASA Ames Research Center

CS-02 Hypothesis and Objectives (Blaber)

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:

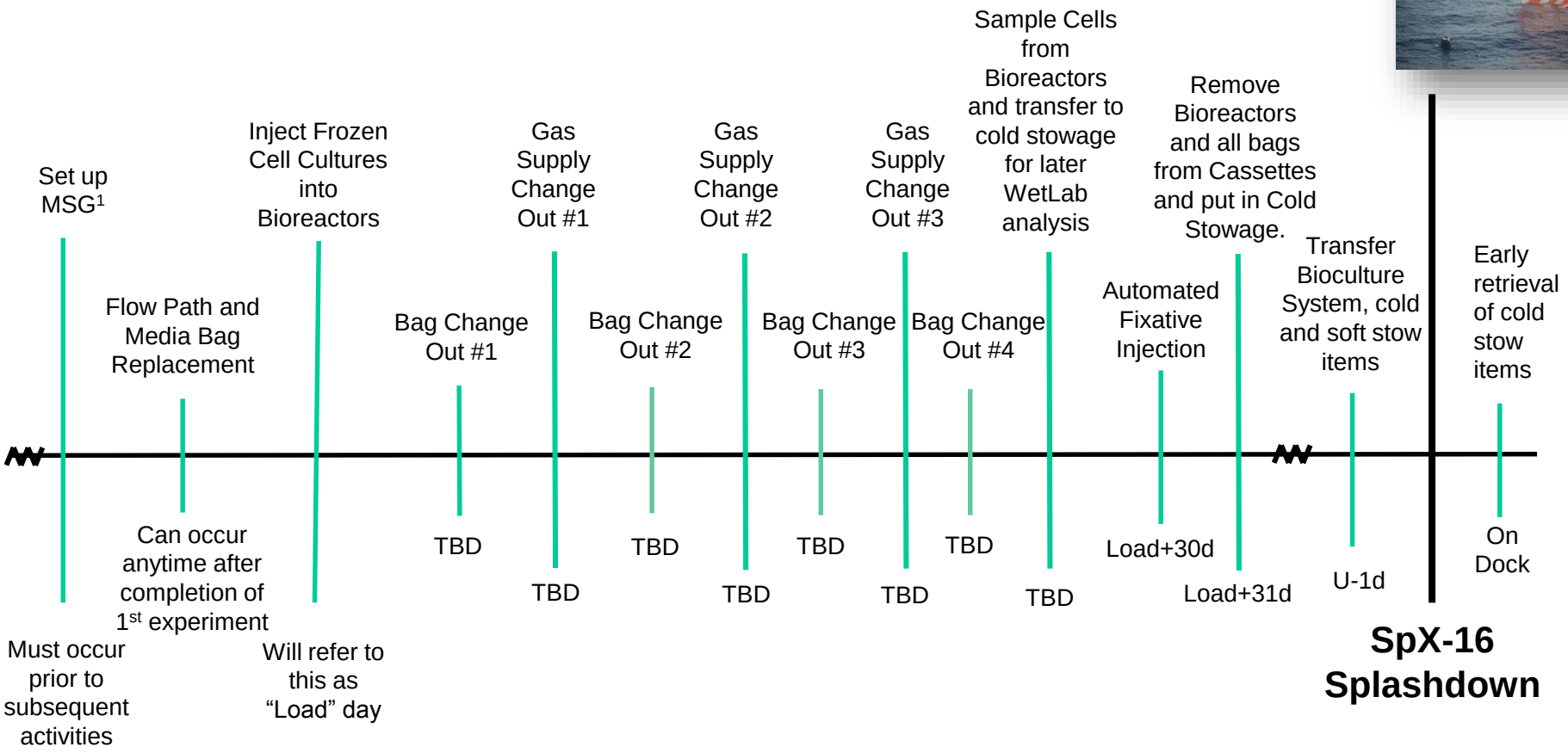
- **Specific Aim 1:** Assess the in-vitro proliferation, differentiation, and mineralization capacity of bone marrow mesenchymal stem cells isolated from CDKN1a/p21-null mice compared to wild-type animals in microgravity versus 1g controls.
- **Specific Aim 2:** Determine cellular mechanisms associated 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.

CS-02 Timeline (Hammamieh/Kacena)

- Ship soft stow items to JSC — L-9w
- Turnover cold stowage items (media bags and factors) for use on Orbit — L-3d
- Turnover Powered Bioculture System[1] — L-24h
- **SpX-15 Launch**
- Set up MSG[2] — NLT L+6d
- **Dragon Berth**
- Transfer Bioculture System from Dragon to EXPRESS[1] — Berth +24 to 48h
- Bag Change Out #1 — L+6d[3]
- Gas Supply Change Out #1 — TBD
- Bag Change Out #2 — L+12d[3]
- Gas Supply Change Out #2 — TBD
- Bag Change Out #3 — L+16d[3]
- Gas Supply Change Out #3 — TBD
- Bag Change Out #4 — L+20d[3]
- Automated Fixative Injection — L+21d[3]
- Remove Bioreactors and all bags from Cassettes and put in Cold Stowage. — L+22d[3]
- Transfer cold stow items — U-1d
- **SpX-15 Splashdown**
- Early retrieval of cold stow items on dock — U+2d

[1] = No more than 15 minutes powered off during transfers
[2] = Assumption - leave MSG set up through bioreactor removal
[3] = Activity is tied to number of days since cell loading, which will nominally occur at L-1

CS-02 Timeline (Blaber)



[1] = Assumption - leave MSG set up through bioreactor removal

Operations Flight Overview

Manifested for SpX-15 Launch (Jun 2018), return of cold stow samples from Hammamieh/Kacena's experiment on SpX-15 (July 2018) and return of Bioculture System and cold stow samples from Blaber's experiment on SpX-16 (Dec 2018)

Powered internal payload, operational for first 21 days after SpX-15 launch, then another 30 consecutive days sometime before SpX-16 return.

Payload Integration Agreement (PIA) Letter contains the following unique agreements:

- Late load of L-24 hours or later
- Cells shall remain on the pad no longer than 48hrs after turnover, after which the biology and medium shall be changed out
- 150 W powered on Dragon - Power interruption of no more than 15 minutes at any time
- Early retrieval cold stow samples at Recovery dock
- Soft stowed items returned at JSC
- Standard health and status data, and non-standard data - cabin temperature, total pressure, relative humidity, ppCO2, ppO2
- Powered locker pre-flight (JSC)

Operations Flight Overview, cont'd

Pre-flight specimen and hardware processing in the KSC SSPF

- Require ESD controlled area

On-orbit operations

- Crew procedures already developed and to be executed on-orbit for CS-V (Dec '17)
- On-orbit Training videos for crew, in place of hands-on training
- Real-time support at ARC

Simultaneous Ground Control at KSC

Post-flight science recovery at ARC laboratory

Use of ISS facilities:

- ExPRESS Rack
- Microgravity Science Glovebox (MSG)
- MELFI (and Cold Stowage on Dragon – GLACIER or DCB)
- Wetlab Items (gloves, biocide wipes, absorbent pads)

Cold Stowage Overview (Hammamieh/Kacena)

Launch – L-3 Turn Over

- Media Stowage Bag (x4)
  - +4°C
  - Each Media Stowage Bag contains ten Media Bags (total of 40)
- Factor Injection Kit (x4)
  - -20°C or colder
  - Each kit consists of ten 3mL syringes containing growth factors
  - Each kit contains 3 types of growth factors: TPO (x3), BMP-2 (x3), Saline (x4)

On-Orbit Usage

- Bag Changeout and Factor Injection (x4)
  - L+5, 11, 17, 21 days
  - Remove one Factor Injection Kit (<-20°C) and one Media Stowage Bag (+4 °C) from cold stowage each execution
  - Add 10 Sump Bags (<-80°C) and 10 Media Bags (+4°C) to cold stowage each time
  - For L+17 Bag Changeout, remove 10 Sample Bags from cassettes and stow at <-80°C
- Bioreactor Removal (x1)
  - Remove 10 Bioreactors, 10 Sump Bags, 10 Fixative runoff bags, and 10 Sample Bags and place in cold stowage (<-80°C)
  - Remove 10 Media Bags and place in cold stowage (+4°C)

Return on SpX-15

- Stowed at <-80°C:
  - 10 Bioreactors
- Stowed at <-20°C:
  - 50 Sump Bags
  - 20 Sample Bags
  - 10 Fixative Runoff Bags
- Stowed at +4°C:
  - 50 Media Bags

Cold Stowage Overview (Blaber)

Launch – L-3 Turn Over

- Cell Loading Kit (x1)
  - -80°C or colder
  - Contains ten 3mL syringes containing frozen cell lines
  - Two cell types: Wild-Type (WT) and CDKN1a/p21 knockout (KO) – five syringes of each
- Media Stowage Bag (x4)
  - +4°C
  - Each Media Stowage Bag contains ten Media Bags (total of 40)
- Factor Injection Kit (x4)
  - -20°C or colder
  - Each kit consists of ten 3mL syringes containing Ascorbic Acid

On-Orbit Usage

- Flowpath R&R
  - Remove one Media Stowage Bag (+4 °C) from cold stowage and use to set-up cassettes
- Cell Loading
  - Remove Cell Loading Kit (<-80°C) and use to load bioreactors
- Bag Changeout and Factor Injection (x4)
  - Load + TBD days
  - Remove one Factor Injection Kit (<-20°C) and one Media Stowage Bag (+4 °C) from cold stowage each execution
  - Add 10 Sump Bags (<-80°C) and 10 Media Bags (+4°C) to cold stowage each time
- Bioreactor Removal (x1)
  - Remove 10 Bioreactors, 10 Sump Bags, 10 Fixative runoff bags, and 10 Sample Bags and place in cold stowage (<-80°C)
  - Remove 10 Media Bags and place in cold stowage (+4°C)

Return on SpX-16

- Stowed at <-80°C:
  - 10 Bioreactors
- Stowed at <-20°C:
  - 50 Sump Bags
  - 20 Sample Bags
  - 10 Fixative Runoff Bags
- Stowed at +4°C:
  - 50 Media Bags

Thank you!