Biological Imaging Capability in the ABRS Facility on ISS

David R. Cox
ASGSB 2010
11/7/10
Agenda

• ABRS Background
• APEX-Cambium Background
• ABRS Green Florescent Protein Imaging System
• GIS Development (for TAGES)
• GIS Demonstrated Capability (w/ TAGES)
• GIS Availability
• GIS Enhancements?
ABRS Background

- The Advanced Biological Research System (ABRS) was developed as an environmental control chamber for ISS as an EXPRESS Sub-Rack Facility.
- ABRS has two independently controlled Experiment Research Chambers (ERCs) with temperature, relative humidity, carbon dioxide.
Biological Flight Systems Hardware Development

Key Projects

• **ABRS Highlights**
  - Single Middeck Locker Equivalent (MLE)
  - Capable of launching and returning live plants
  - STS Middeck and ISS EXPRESS Rack compatible
  - Designed for extended duration on ISS with ORU refurbishment and external water addition
  - First flight will include GFP imaging capability
  - Provides programmable LED lighting, Temperature, Relative Humidity and Carbon Dioxide control, ethylene scrubbing and water recycling
ABRS is a third generation plant growth system based on PGU and PGF knowledge.

ABRS uses half (or less) of the mass, volume and power of other plant growth systems.
Advanced Biological Research System (ABRS) Sustaining Engineering / Maintenance

WBS: TBD

PI: N/A
PS: N/A
PM: David R. Cox
Engineering Team: Life Sciences Services Contract (LSSC) at KSC

Objective:
♦ Provide on-going maintenance and sustaining engineering for ABRS flight and ground units
♦ Ensure enhancements made to on-orbit unit(s) are successful and are mimicked in the ground unit(s)
♦ Provide Certificate of Flight Readiness (CoFR) and Flight Safety documentation for all ISS Stage reviews

Relevance/Impact:
♦ Maintain ABRS at full capability to enable continued use of microgravity and other characteristics of space to enhance understanding of fundamental biological processes
♦ Retain ABRS demonstrated scientific return through return of biological specimens as well as via telescience using the Green Fluorescent Protein (GFP) Imaging System (GIS)
♦ Ensure research integrity is preserved through proper use of ground controls

Development Approach:
♦ Use generally accepted engineering practices and research standards to maintain ABRS flight and ground hardware and software
♦ Follow ISS Payloads Office (OZ) templates to meet required CoFR and Flight Safety deliverables

ISS Resource Requirements

<table>
<thead>
<tr>
<th>Accommodation (carrier)</th>
<th>EXPRESS Rack 2 on ISS</th>
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<tr>
<td>Uppmass (kg) (w/o packing factor)</td>
<td>N/A</td>
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<tr>
<td>Volume (m³) (w/o packing factor)</td>
<td>ABRS Facility 1 MLE &amp; ABRS / APEX Stowage 1 CTB</td>
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<td>Power (kw) (peak)</td>
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Project Life Cycle Schedule

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<th>Milestones</th>
<th>CDR</th>
<th>Safety</th>
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<th>Ops</th>
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<td>STS-129/ULF3</td>
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</tbody>
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Revision Date: 05/6/2010
Advanced Plant EXperiments on ISS – Cambium (APEX-Cambium)

WBS: 825080.04.05.20.05

Pls: CSA: Rodney Savidge, Ph.D., Univ. of New Brunswick,
Jean Beaulieu, Ph.D., Natural Resources of Canada
NASA: Robert Ferl & Anna-Lisa Paul, Ph.Ds., Univ. of Florida
PS: Howard G. Levine, Ph.D., NASA-KSC
PM: David R. Cox, NASA-KSC
Engineering Team: Life Sciences Services Contract (LSSC) at KSC

Objectives:
♦ Launch and execute the APEX-Cambium payload operations in conjunction with the
Canadian Space Agency (CSA) and International Space Life Sciences Working
Group (ISLSWG) agreements.
♦ Cambium: Determine the role of gravity in Cambium wood cell development.
♦ Transgenic Arabidopsis Gene Expression System (TAGES): Demonstrate non-
destructive reporter gene technology & investigate spaceflight plant stress.

Relevance/Impact:
♦ APEX-Cambium payload will provide NASA and the ISS community a permanent
controlled environment capability (the Advanced Biological Research System
(ABRS)) to support growth of various organisms (i.e. whole plants). Also, as a result
of the TAGES experiment Green Florescent Protein (GFP) imager development, ISS
partners will benefit from a modern biological analysis capability that can provide real
time non-destructive gene expression data which can ultimately optimize ISS
microgravity biological experimentation and greatly reduce required specimen
downmass.
♦ The Cambium experiment will provide the pulp & paper and construction industries
insight into the fundamental mechanisms of wood cell formation.
♦ The TAGES experiment will demonstrate non-destructive GFP reporter gene
technology and improve the understanding of spaceflight stresses on terrestrial
organisms.

Development Approach:
♦ APEX-Cambium completed the Advanced Biological Research System (ABRS) final
ISS verifications, assembly, Launch and initial on-orbit operations.
♦ Design, develop and test a Green Florescent Protein (GFP) imaging system for use in
ABRS.

Project Life Cycle Schedule

<table>
<thead>
<tr>
<th>Milestones</th>
<th>CDR</th>
<th>Ph3 FSR</th>
<th>PVT</th>
<th>Launch</th>
<th>Ops</th>
<th>Return</th>
<th>Final Report</th>
</tr>
</thead>
</table>
Advanced Plant EXperiments on ISS – Series (APEX-02+)

Pls: TBD from targeted NRA or ILSRA
PS: Howard G. Levine, Ph.D., NASA-KSC
PM: David R. Cox, NASA-KSC
Engineering Team: Life Sciences Services Contract (LSSC) at KSC

Objectives:
♦ Perform plant research on ISS in support of the Fundamental Space Biology (FSB) Strategic Plan.
♦ Leverage International Space Life Sciences Working Group (ISLSWG) and National Lab partner ISS resources to optimize ISS utilization efficiencies and maximize scientific return to the research communities.
♦ When appropriate, utilize the NASA Advanced Biological Research System (ABRS) EXPRESS Sub-rack Facility on ISS including its Green Florescent Protein (GFP) Imaging System.

Relevance/Impact:
♦ The APEX payload series is designed to study the fundamental cellular, molecular and whole plant sensing mechanisms and reactions to changes in gravity.
♦ In addition, the APEX payload series can help determine the viability of multi-generation developmental responses to micro and fractional gravity.
♦ The knowledge gained in plant systems will have direct application to closing the Water, Air and Carbon loops in long duration human space travel.

Development Approach:
♦ Solicit peer-reviewed research in support of the FSB Strategic Plan and Decadal Studies through NASA Research Announcements (NRAs) and International Life Sciences Research Announcements (ILSRAs).
♦ Partner with International Space Life Sciences Working Group (ISLSWG) and National Lab partners.
♦ Identify opportunities for Translational Teaming wherever possible.

Clockwise from Top Left: Canadian Astronaut Bob Thirsk harvesting Willow trees, Spruce trees on ISS, Transgenic Arabidopsis Gene Expression System (TAGES) Green Florescent Protein (GFP) image, Advanced Biological Research System (ABRS) GFP Imaging System, Astronaut Jeff Williams preserving Arabidopsis in a Kennedy Fixation Tube (KFT).

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<tr>
<td>(w/o packing factor)</td>
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Launch/Increment | TBD

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<td>Actual/ Baseline</td>
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<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>TBD</td>
<td>Return + 12m</td>
</tr>
</tbody>
</table>

Revision Date: 05/03/10
TAGES – Transgenic Arabidopsis Gene Expression System

Principal Investigators: Dr. Robert J. Ferl, Professor  
Dr. Anna-Lisa Paul, Research Associate Professor

- Using biosensor technology and telemetric imaging to capture molecular responses to spaceflight (The ABRS GIS hardware).
- Using state of the art molecular and genomic tools to evaluate changes in gene expression in returned samples.
TAGES Technology is Scalable for Telemetric Data Collection in a Variety Exploration Platforms

- GFP (Green Fluorescent Protein) reporters make a fluorescent gene product - gene responses captured in real-time with digital cameras.
- Digital images can be transmitted from the site of the experiment to the site of the researcher.
- Totally autonomous data collection, minimal to no crew needed:
  - Orbital (ISS) / Planetary landers / Small satellite

TAGES plants on vertical agar plates. Plants engineered to respond to reduced oxygen levels.

Peer-Reviewed Research. University based and funded by NASA:
Current Funding Sources - NASA Flight (NNX07AH27G);
NASA Fundamental Space Biology (NNX09AL96G); NASA MMAMA (NNX09A078G)
Background

- APEX-Cambium payload includes the NASA sponsored Transgenic Arabidopsis Gene Expression System (TAGES) experiment.
- TAGES plants are genetically modified Arabidopsis utilizing a Green Florescent Protein (GFP) [naturally found in jellyfish] as a non-destructive plant stress reporting mechanism.
- Based on the TAGES requirements, a piece of Experiment Unique Equipment (EUE) was designed to fit inside one ABRS Experiment Research Chamber (ERC) to capture, store and transmit GFP images of the TAGES plants to the ground in near real time (telemetric science!)
- GIS worked flawlessly on ISS Stage ULF3.
Advanced Plant EXperiments on orbit—Cambium

**PROPOSAL NUMBERS**
CSA: ILSRA-04-122
NASA: 98-HEDS-02-299

**SCIENCE TEAM**
Rodney Savidge, Ph.D.
Univ. of New Brunswick
Robert Ferl, Ph.D.
Anna-Lisa Paul, Ph.D.
Univ. of Florida
Jean Beaulieu, Ph.D.
Natural Resources Canada

**RESEARCH OBJECTIVES**
There are two plant experiments in the APEX-Cambium payload: Cambium and the Transgenic Arabidopsis Gene Expression System (TAGES). TAGES will use Arabidopsis thaliana with sensor promoter-reporter gene constructs that render the plants as biomonitors of their environment using real-time nondestructive Green Fluorescent Protein (GFP) and traditional postflight analyses. Cambium Run 1 seeks definitive evidence that gravity has a direct effect on the cambial cells which contribute to reaction wood formation in trees, e.g., willow (Salix babylonica). Cambium Run 2 is an investigation of tree response to microgravity and the adaptation of white spruce (Picea glauca) to the space environment. Both experiments are conducted in the NASA developed Advanced Biological Research System (ABRS) ISS facility.

**POINTS OF CONTACT**
CSA Program Manager
Luc Lefebvre
NASA Project Manager
David R. Cox

**Hardware**
- ABRS: 1 MLE replacement middeck/EXPRESS facility. Uses mdk rear breathing and EXPRESS AAA, MTL, Ethernet, video, commanding. ABRS to remain on ISS.
- Approximately 1 MLE of ambient stowage per ascent (include both experiment & support hardware, some items limited life).
- Cold stow required for all ascents and descents.

**Facility/Interfaces**
Advanced Biological Research System (ABRS)

**Late access**
<1-28 hrs
ABRS, and limited life stow items.

**Pre-flight**
N/A

**In-flight: # of sessions**
On 3 flights: Runs 1, 2 & 3
38 days continuous operation for each run.

**Post-flight**
N/A

**Early Retrieval**
R+6 hrs
KFTs for Run 1, 2 & 3.

**Target Subjects**
US and CSA astronauts as operators only.

**Total # of Subjects Required**
Short-term
N/A
Long-term
N/A

**Total # of Subjects Collected so far**
Short-term
N/A
Long-term
N/A

**Ground reference**
Yes
Asynchronous at KSC SLSL
Advanced Plant EXperiments on orbit-Cambium (APEX-Cambium) Payload

PROPOSAL NUMBERS
CSA: ILSRA-04-122
NASA: 98-HEDS-02-299

SCIENCE TEAM
Rodney Savidge, Ph.D.
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David Cox

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<th>Pre-flight</th>
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<th>In-flight: # of sessions</th>
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<tr>
<td>On 3 flights: Runs 1, 2 &amp; 3</td>
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<td>38 days continuous operation for each run.</td>
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<th>Post-flight</th>
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<td>KFTs for Run 1, 2 &amp; 3.</td>
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<td>Long-term</td>
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<td>Long-term</td>
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<tr>
<th>Ground reference</th>
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<tbody>
<tr>
<td>Yes</td>
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<tr>
<td>Asynchronous at KSC SLSL</td>
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**APEX-Cambium – Increments 21/22, 23/24 scenario**

### Run 1

<table>
<thead>
<tr>
<th>Day</th>
<th>Activity</th>
<th>Comments</th>
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<tbody>
<tr>
<td>L+1 d</td>
<td>Transfer to ISS</td>
<td>Transfer time not charged to PL</td>
</tr>
<tr>
<td>L+5 d</td>
<td>TAGES harvest 1A</td>
<td></td>
</tr>
<tr>
<td>L+5 d</td>
<td>Cambium lcps/hoisting 1A</td>
<td></td>
</tr>
<tr>
<td>L+6 d</td>
<td>Water reuply</td>
<td></td>
</tr>
<tr>
<td>L+12 d</td>
<td>Water reuply</td>
<td></td>
</tr>
<tr>
<td>L+16 d</td>
<td>Filter changeout</td>
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</tr>
<tr>
<td>L+36 d</td>
<td>TAGES harvest 1B</td>
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7.38 Total crew time

*Approximation of planning, not actual execution time

- Å = MWA Setup
- ¥ = Still Camera setup

### Run 2

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<tr>
<th>Day</th>
<th>Activity</th>
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</tr>
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<tbody>
<tr>
<td>L+2 d</td>
<td>Crew Payload Review</td>
<td>Run 2 begins with launch of plants on 19A</td>
</tr>
<tr>
<td>L+4 d</td>
<td>TAGES harvest 2</td>
<td></td>
</tr>
<tr>
<td>L+5 d</td>
<td>Cambium harvest 2A</td>
<td></td>
</tr>
<tr>
<td>L+6 d</td>
<td>Water reuply</td>
<td></td>
</tr>
<tr>
<td>L+12 d</td>
<td>Water reuply</td>
<td></td>
</tr>
<tr>
<td>L+16 d</td>
<td>Filter changeout</td>
<td></td>
</tr>
<tr>
<td>L+20 d</td>
<td>Water reuply</td>
<td></td>
</tr>
<tr>
<td>L+26 d</td>
<td>Filter changeout</td>
<td></td>
</tr>
<tr>
<td>L+32 d</td>
<td>TAGES harvest 2B</td>
<td></td>
</tr>
<tr>
<td>L+36 d</td>
<td>MELFI insertion</td>
<td></td>
</tr>
<tr>
<td>L+36 d</td>
<td>TAGES harvest 2C</td>
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11.92 Total crew time

*Approximation of planning, not actual execution time

- Å = MWA Setup
- ¥ = Still Camera setup

### Run 3

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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>L+4 d</td>
<td>Reinitialize ABRS</td>
<td>TAGES Run 3 began</td>
</tr>
<tr>
<td>L+6 d</td>
<td>TAGES harvest 3</td>
<td>with launch on 20A</td>
</tr>
<tr>
<td>L+5 d</td>
<td>Filter installation</td>
<td></td>
</tr>
<tr>
<td>L+17 d</td>
<td>TAGES plate rotation/implant</td>
<td></td>
</tr>
<tr>
<td>L+29 d</td>
<td>TAGES plate rotation/implant</td>
<td></td>
</tr>
<tr>
<td>L+41 d</td>
<td>TAGES plate removal</td>
<td></td>
</tr>
<tr>
<td>L+41 d</td>
<td>Filter removal</td>
<td></td>
</tr>
<tr>
<td>L+42 d</td>
<td>ABRS shutdown</td>
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</tr>
<tr>
<td>L+30 d</td>
<td>Transfer to STS</td>
<td></td>
</tr>
</tbody>
</table>

7.5 Total crew time

*Approximation of planning, not actual execution time

**Cambium Run 3 en hold. Implementation expected in 2-3 years.**

---

**US module:**

- **Run 1:** ULF3, 16 Nov 09
- **Run 2:** 20A, 7 Feb 10
- **Run 3:** 19A, 5 April 10
- **Cambium Run 3 TBD Increment:** ULF4, 14 May 10

**Mass up:**
- Inc-21/22: 66.19 kg
- Inc-23/24: 7.61 kg

**Mass down:**
- Inc-21/22: 7.61 kg
- Inc-23/24: 11.55 kg
TAGES Replant

• The initial TAGES 3A grow-out will use only a single Petri Plate installed into the imaged position (position 1) of the Green Fluorescent Imager as the primary objective of this experimental run is to obtain fluorescent images.

• Imager positions 2 – 6 will not be populated with Petri Plates at this time.
Airflow Duct Install

- Kapton Tape covering gap (to seal off undesired airflow)
- Foam Strip being installed on outer (lower) frame corners
- Airflow Duct (in proper position)
- Green Fluorescent Imager Lens
Airflow Duct Install

Kapton Tape covering outside corners

Kapton Tape covering both outside corners, tucked in and not exposed from GFI frame

Kapton Tape covering outside slotted frame on the 3, 4 side
Stage 20A Operations

- Docked Operations:
  - ABRS Activation (only ERC 1)
  - ICL Flush (using improved procedure and kit H/W)
  - Airflow Duct installation into Green Florescent Protein (GFP) Imaging System (GIS) to prevent condensation on imaged surface of the Petri plate
  - Initiate Transgenic Arabidopsis Gene Expression System (TAGES) experiment Replant 3A in ERC 1
Stage 20A Operations

• Post-docked Operations after the conclusion of the TAGES 3A 12-day grow-out:
  - ABRS ICL Fuse Replacement with new fuse design
  - Potential ICL Pump Replacement as required
  - ICL Rear Flush using rear ICL QD (best possible flush)
  - Additional TAGES 3B and 3C 12-day grow-outs

NOTE: TAGES Run 3 science data is completely thru downlinked images from the ABRS GFP Imaging System and does not require harvested tissue; growth duration ≥12 days
Summary

- ABRS new EXPRESS sub-rack facility performing extremely well except for temperature control on ERC 2
- Thorough analysis performed and refurbishment plan developed in time for Stage 20A all while maintaining/maximizing the TAGES science return
- ABRS will be ready for CSA’s next Cambium experiment on Stage 19A!
Advanced Plant Experiments on Orbit – Cambium (APEX-Cambium)

CSA PI: Dr. Rodney Savidge, University of New Brunswick
U.S. PI: Drs. Robert Ferl & Anna-Lisa Paul, Univ. of Florida
PS: Dr. Howard G. Levine, NASA KSC
PM: David R. Cox, NASA KSC
Engineering Team: The Bionetics Corporation

Objective:
• Meet International agreements as an active member of the International Space Life Sciences Working Group (ISLSWG) and utilize U.S. flight hardware to support a Canadian Space Agency (CSA) investigation on reaction wood cell formation.
• Augment the CSA experiment with a U.S. investigator studying spaceflight stress effects on genetically modified plants using remote sensing and telemetric analysis techniques.

Relevance/Impact:
• APEX-CSA will maintain active U.S. participation in the ISLSWG as well as provide fundamental knowledge about reaction wood cell formation which will be extremely useful in the construction and paper pulp industries world wide.
• The U.S. investigation will demonstrate remote sensing biometric techniques for stress detection in living organisms during spaceflight. This knowledge will be extremely useful for future transit and surface missions to the Moon and Mars.

Development Approach:
• Fly the NASA developed Advanced Biological Research System (ABRS) flight hardware on ISS. Perform Arabidopsis plant manipulations and harvests by the crew.
• Downlink images of the genetically modified Arabidopsis plants using the specialized Green Florescent Protein (GFP) imager integrated into ABRS.
• Preserve and return harvested tissue for ground analyses.

ISS Resource Requirements

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<td>Volume (m³)</td>
<td>2 MLE (CSA Allocation)</td>
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<td>(w/o packing factor)</td>
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<tr>
<td>Power (kw)</td>
<td>0.2 max (CSA Allocation)</td>
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<tr>
<td>(peak)</td>
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<td>Crew Time (hrs)</td>
<td>TBD (~15-30 CSA + ~5-10 NASA)</td>
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<th>Return</th>
<th>Final Report</th>
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<td>8/07</td>
<td>10/07</td>
<td>3/08</td>
<td>1st Q FY09</td>
<td>ISS</td>
<td>L+4-6 months</td>
<td>Return + 12m</td>
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Revision Date: 02/15/07
ERC

- Cooling Units
- Environmental Control Panels (ECP)
- ERC Housing
- MTL Lines
- ERC Root Trays
ABRS Overview

- Single middeck locker replacement facility plus stowage (filters, kits, etc.)
- Shuttle and EXPRESS compatible
- Easy specimen access and manipulation
- Can be used as a primary growth facility or up/down specimen transportation
- Two large, independent Environmental Research Chambers (ERCs)
- Condensate recovery and automated water delivery
ABRS Environmental Research Chamber (ERC)

- Temperature: 23-26 °C (8 °C below ambient)
- PAR: 50-300 μmol/m²/sec
- Ethylene removal to below 25 ppb
- CO₂: controllable
- VOC removal
- RH: 60-90%
- Atmospheric humidity and root zone moisture control
- Volumetric air flow rate 6-15 cfm depending on RH
- Generic plant imaging
- GFP imaging capable
ABRS LED Light Module (LLM)

- LED growth lighting for improved efficiency
- Twin independent light banks
- Red, blue, green and white LEDs for full spectrum coverage
- Intensity of individual colors can be adjusted
ABRS Thermal Management

- Electronics Control Unit (ECU) located at the rear for heat rejection
- ERC assembly includes insulated sandwich-panel construction for chamber thermal isolation
- Growth chambers cooled by internal water loops—one loop per chamber
- Cooling unit TECs reject heat via rear-breathing (shuttle) and/or MTL (EXPRESS/ISS)
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TAGES (Transgenic Arabidopsis Gene Expression System)
Principle Investigators: Dr. Robert J. Ferl and Dr. Anna-Lisa Paul
University of Florida - Horticultural Sciences / Plant Molecular and Cellular Biology

Arabidopsis plant engineered with the Green Fluorescent Protein (GFP) gene reporters. The older plant at left is expressing Adh/GFP gene reporter in response to hypobaric (10 kPa) stress. This picture was featured on the cover of the Journal *Plant Physiology* (January, 2004) to illustrate our research article in that issue. The seedlings at right show plants expressing CaMV35s/GFP and Adh/GFP on the surface on an agar nutrient plate.

An Arabidopsis plant engineered with the Adh/GFP gene reporter. The left hand panel shows the plant photographed in white light and the right hand panel shows the same plant photographed in short-wave blue light to illuminate GFP expression. The actively growing apical meristem region of the plant and the trichome hairs are very sensitive to changes in the environment.
Acronyms

AAA Avionics Air Assembly  KFT KSC Fixation Tube  
ABRS Advanced Biological Research  LED Light-Emitting Diode  
System  LLM LED Lighting Module  
APEX Advanced Plant Experiments on Orbit  MTL Medium Temperature Loop  
CSA Canadian Space Agency  NASA National Aeronautics and Space  
Administration  
ECU Electronics Control Unit  OCA Orbiter Communications Adapter  
EIA EXPRESS Integration Agreement  PAR Photosynthetically Active  
ERC Environmental Research  Radiation  
Chambers  ppb parts per billion  
EXPRESS Expedite the Processing of  PVP Payload Verification Plan  
Experiments to Space Station  RH Relative Humidity  
GFP Green Fluorescent Protein  STS Space Transportation System  
GUS 8 Glucuronidase  TEC Thermo-electric Cooler  
IDD Interface Definition Document  VOC Volatile Organic Compounds  
ISLSWG International Space Life Sciences Working  
Group  
ISS International Space Station