



**APEX-04**

**SpaceX-10**

**Shawn Stephens – ESC Project Lead**

# APEX-04 Team

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- **Investigators – University of Florida**
  - Anna-Lisa Paul, Ph.D.; Robert Ferl, Ph.D.
- **NASA PM**
  - Trent Smith (KSC)
- **NASA Project Scientist**
  - Howard Levine, Ph.D. (KSC)
- **PIM**
  - Mickie Nagy, Ph.D.; Michelle Gray (MSFC)
- **Ops Lead**
  - Linda Gibson (MSFC)
- **PARC**
  - Chris Traylor (MSFC)
- **Crew Rep**
  - Isaac Ozinga (JSC)
- **KSC Team**
  - Anne Marie Campbell, Gerard Newsham, Chuck Spern, Colleen Huber

# APEX Overview

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- **APEX (Advanced Plant Experiments on orbit) is a series of investigations which focus on fundamental plant biology**
- **Multiple investigations have flown to ISS under the APEX name**
  - APEX-Cambium – STS-129, 130, 131
    - *Salix babylonica* (willow)
  - APEX-TAGES – STS-129, 130, 131
    - *Arabidopsis thaliana*
  - APEX-02 – SpaceX-3
    - *Saccharomyces cerevisiae* (yeast)
  - APEX-03 – SpaceX-5
    - *Arabidopsis thaliana*
- **APEX has utilized multiple hardware facilities on ISS**
  - Advanced Biological Research System (ABRS)
  - NanoRacks
  - Veggie

# APEX-04 Key Concepts

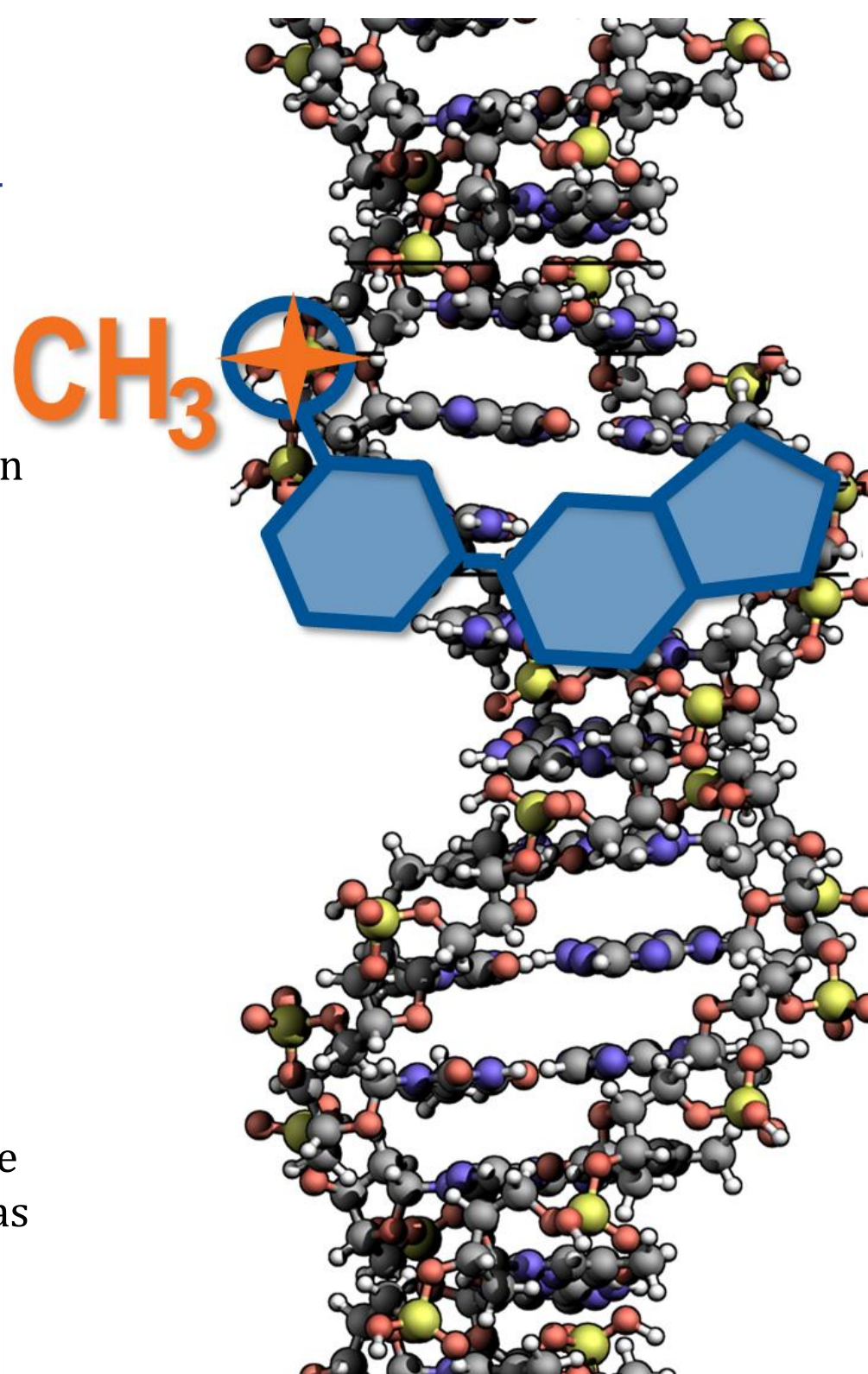
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One feature of the epigenome is characterized by the chemical-groups - such as methyl-groups - that decorate the primary sequence of the genomic DNA.

We know that changes in these decorations can alter gene expression, and further, that plants use this approach to physiologically adjust to adverse environments on Earth.

For the APEX-04 spaceflight experiment we will re-sequence the genomes to see how changes in DNA-methylation contribute to the response.

These experiments will provide a key piece of the puzzle of how plants adjust their physiology to meet the needs of growing in a place outside their evolutionary experience. And the more complete our understanding, the more success we will have in future missions as we take plants with us off planet.



# APEX-04 Science Overview

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- **APEX-04 will specifically address the role of epigenetic DNA methylation in guiding the adaptive responses of *Arabidopsis thaliana* seedlings to the spaceflight environment**
- **The investigation consists of the following genotypes:**
  - (1) wild type Col-0
  - (2) Col-0 line deficient the gene encoding *elp2*
  - (3) Col-0 line deficient the gene encoding *met1*
- **APEX-04 builds upon several previously flown investigations**
  - Transgenic Arabidopsis Gene Expression System (TAGES)
  - APEX-03 (SpaceX-5)
  - Plant Growth Investigations in Microgravity (PGIM) (STS-93)
  - Biological Research in Canisters (BRIC)-16 (STS-131)

# APEX-04 Operations Overview

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- APEX-04 will launch with 30 wrapped Petri plates in cold stowage (+4<sup>0</sup>C)
- Petri plates are removed from stowage, unwrapped, and placed in Veggie to initiate seed germination and seedling growth
- Photos taken during growth period to document plant development (4 & 8 days) of first column only (A1, B1, C1)
- After the required growth period (11 days), the crew removes all Petri plates from Veggie to be photographed and harvested
- Plants are placed into Kennedy Space Center Fixation Tubes (KFTs) containing RNAlater as a chemical preservative and stowed at -80<sup>0</sup>C or colder (MELFI)
- Samples are returned to the ground in cold stowage for post-flight analysis

# Science Hardware

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- **30 total Petri plates with science contained in 3 kits**
  - 27 Primary plates, 3 spares (1 per genotype)
  - *Arabidopsis thaliana* seedlings placed on Phytigel media
  - Petri plates wrapped in black cloth to prevent light intrusion
  - One kit will contain two autonomous HOBO data loggers
- **27 Kennedy Space Center Fixation Tubes (KFTs) with RNAlater**
  - Contained in three Nomex kit bags
  - Harvest requires 24 KFTs during nominal operations (3 spares)
  - Photo plates (A1, B1, C1) will be harvested if KFTs are available
- **Harvest kit**
  - Short and long forceps to remove plants from media and assist in placement in KFT
  - Cryogenic pens to mark GMT and plate number on KFT
  - Actuator handle to assist in KFT actuation

# Science Ground Processing



1) Populated plate with blackout cloth



2) Wrapped plates from PI team



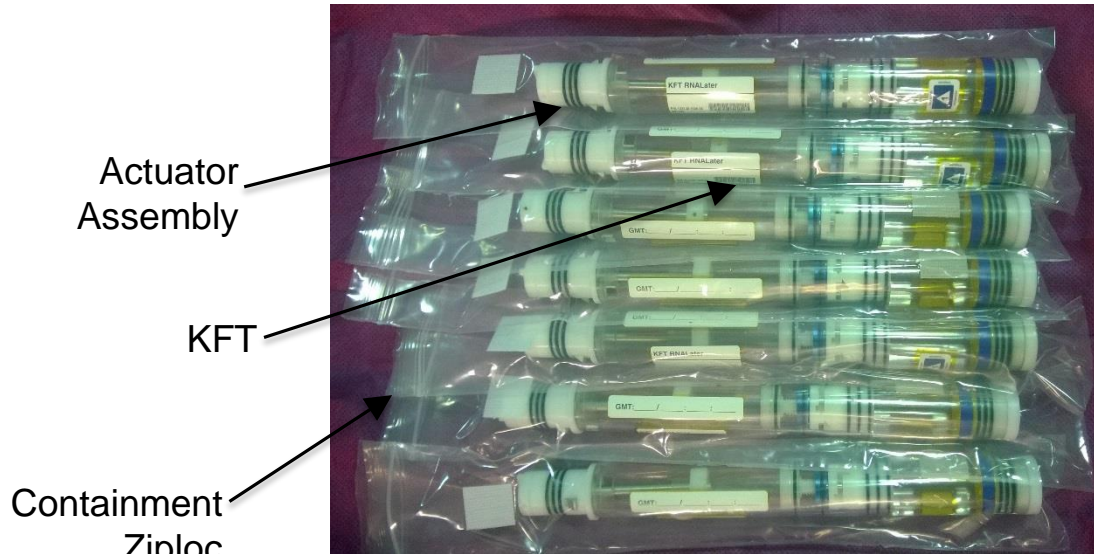
3) Plates bundled with Velcro in Nomex bag



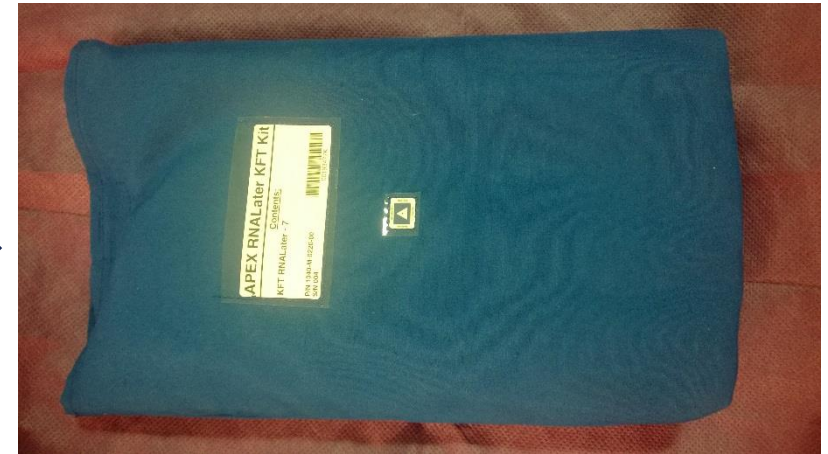
4) Completed Science Kit ready for turnover



# Science Ground Processing



KFTs



APEX RNAlater KFT Kit



Harvest Kit Contents

KFT Actuator Handle

Cryogenic Pen

Forceps

# Ascent and On-orbit Stowage

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- **Cold storage items**
  - Science kits are maintained at +4<sup>0</sup>C during integration, ascent, and prior to Veggie insertion
  - Ascent in Double Cold Bag (DCB)
  - Transferred to powered asset after docking (i.e. MERLIN)
- **Ambient items**
  - Harvest kit and RNAlater kits are late-stowed at ambient
  - Harvest and RNAlater kits transferred to ISS & remain ambient
- **APEX-04 operations begin 3-5 days after docking**

# Operations

- **Science kits are removed from cold stowage**
- **Veggie facility configured for APEX operations**
  - Light levels set per procedure
  - Data loggers from Science Kit 1 placed in Veggie
  - 3 Petri Plate Holders installed in Veggie (on-orbit; APEX-03)
- **Petri plates are placed in Veggie**
  - Plates are inspected for evidence of contamination, damage, or early germination of seedlings; replaced with spare if required

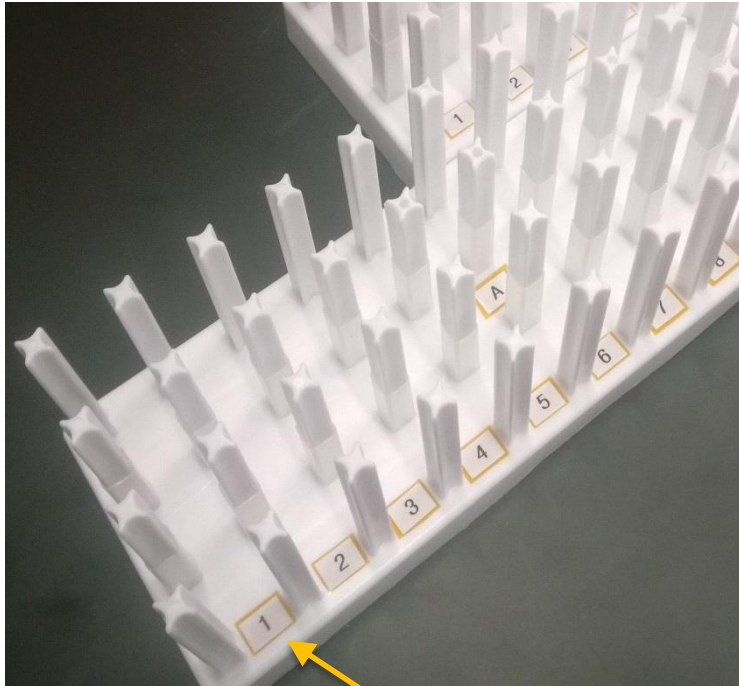


Petri plates in holder



Veggie Facility  
with APEX  
Science

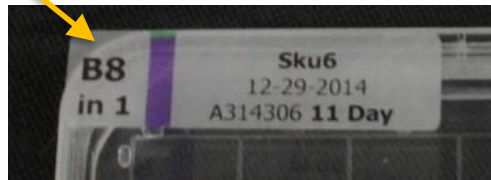
# APEX/Veggie Layout



	1	2	3	4	5	6	7	8	9	10
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	

Label Designates Position in Veggie

Labels on Petri Plate Holders



APEX-04 Veggie Configuration

# Photos and Harvest

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- **Photos taken of front and back of plates A1, B1, and C1 to document plant development during growth period (4 & 8 days)**
  - Photos taken during growth period and harvest will use several on-orbit assets
    - GFE Nikon D4, Nikon 105-mm lens, and SB-810 flash
    - MWA Work Surface Area, MWA Utility Kit, and ABRS Photogrid
- **Harvest operations also require the APEX Harvest Kit and RNAlater KFT Kits (Day 11)**
- **Plates are removed from Veggie, photographed, and harvested**
  - Photos taken front, back, then with lid off prior to harvest
  - Plant material from each plate is harvested into a single KFT
  - KFTs will remain at ambient temperature for 12-24 hours after actuation to allow fixative to perfuse then transferred to -80°C cold stowage until return on Dragon

# APEX-03 Operations – Butch Wilmore

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1) Petri plate photographed prior to harvest



2) Plants harvested from media using forceps

# APEX-03 Operations – Butch Wilmore

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3) Plants inserted into sample tube of KFT



4) Plants preserved during KFT actuation

# APEX-03 Operations – Butch, Samantha

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5) KFTs returned for post-flight analysis



# End of On-orbit Operations

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- **Veggie Facility**
  - Petri plate holders are removed from Veggie and stowed on ISS
  - Data loggers stowed at ambient until return
  - Veggie is powered-down
- **Trashed items**
  - Petri plates
  - Nomex kit bags
  - APEX-03 Harvest Kit – APEX-04 Harvest Kit to remain on orbit
- **Descent**
  - KFTs with contained samples return in -20°C DCB
  - Data loggers return soft-stowed at ambient

# Future Missions in Veggie

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- **Veg-03**
  - Tech Demo, NASA
  - Chinese Cabbage, Red Romaine Lettuce, plant pillows
- **Ceres**
  - Thomas Pesquet, ESA, Educational Outreach
  - Inserted into Veggie after APEX-04
  - Technical understanding in work
- **Veggie S/N 001 to ISS**
  - SpaceX-12 Launch
- **APEX-05**
  - Dr. Simon Gilroy, University of Wisconsin – Madison
  - *Arabidopsis thaliana*, Petri plates

# Future Missions in Veggie

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- **APEX-06**
  - Dr. Patrick Masson, University of Wisconsin – Madison
  - *Brachypodium*, Petri plates or Magenta Jars
- **Veg-04**
  - Dr. Gioia Massa, NASA
  - Chinese cabbage, plant pillows, two Veggie units
- **Veg-05**
  - Dr. Gioia Massa, NASA
  - Dwarf tomato, plant pillows, two Veggie units

# Lessons Learned

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- **Dynamic launch schedule**
  - APEX-04 Crew Training – Tim Peake, Jeff Williams
  - APEX-05 Crew Training – Shane Kimbrough, Peggy Whitson
  - APEX-04 operations most likely performed by Shane Kimbrough
- **ISS Ambient Conditions**
  - Veggie facility uses ISS environment
  - ISS environment can vary from 65°F to 80°F (18°C to 27°C)
  - Plant growth can be affected by environmental variations
  - Growth duration may require adjustment due to environment

# Questions?

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