

International Space Life Sciences Working Group

Space Life Sciences Flight Experiments Information Package

2004

A Companion Document to
Agency Solicitations in Space Life Sciences

Issued by the International Space Life Sciences Working Group



Canada



France



DLR
Germany



Europe



Japan



USA



Ukraine

ISLSWG

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Introduction

This supplement is a companion to the 2004 research solicitations released by agency members of the International Space Life Sciences Working Group (ISLSWG): the Canadian Space Agency (CSA), France's Centre National d'Études Spatiales (CNES), Germany's Deutsches Zentrum für Luft-und Raumfahrt (DLR), the European Space Agency (ESA), the Japan Aerospace Exploration Agency (JAXA), the United States' National Aeronautics and Space Administration (NASA), and the National Space Agency of Ukraine (NSAU). The various sections of this supplement provide a common basis for proposal preparation and submission by any eligible scientist, regardless of the country of origin.

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Individuals submitting responses to agency solicitations should be aware that a notice of intent (NOI) to propose is requested by March 2, 2004, and the proposal submission deadline for Research Opportunities for Flight Experiments in Space Life Sciences Research Announcement 2004 is May 5, 2004. Please see section 5 for directions for NOI and proposal submission.

1.0 Anticipated Flight Opportunities for Space Life Sciences

In general, resources such as crew time, electrical power, and refrigeration/freezing will be extremely limited during this period. Thus, it is expected that implementation will be limited to experiments that require minimal crew training, simple and limited experiment procedural steps, minimal energy, and minimal thermal-conditioned storage of samples. In principle, within the guidelines described in detail in Section 1.1, experiments requiring up to 190 days of low Earth orbit may be accommodated. Information for research involving human subjects is provided in

Section 2.1. Opportunities for experiments using the model organisms *S. cerevisiae*, *C. elegans*, *Arabidopsis*, and *Brassica* as subjects are described in detail in Section 2.2.

Flight experiment opportunities are limited and constrained in a number of ways. Proposals that require resources beyond the capabilities described in this section should NOT be submitted.

Given the limited availability of flight opportunities, flight experiments will be the most competitive area within Space Life Sciences for selection in fiscal year 2005. Flight experiment proposals must represent mature studies strongly anchored in previous or current ground-based or flight research. Ground-based research may, and usually must, represent one component of a flight experiment proposal. For a flight experiment proposal, ground-based research should be limited to activities that are essential for the final development of an experiment for flight, such as definition of flight procedures and control activities for the flight experiment. In this case, only one (flight) proposal needs to be submitted.

Flight experiment proposals must clearly define the actual experiment duration and all requirements and conditions required to successfully complete the experiment. The investigator should allow for flexibility in the selection of the best hardware to be used to accomplish the experimental goals. Descriptions and websites of the functional capabilities of hardware available to support human and nonhuman experiments are included in Sections 2 and 3 of this document. This information should be used to develop an understanding of the available capabilities. Investigators should use this information as a guide for developing experiment requirements and procedures *rather than selecting specific hardware items*.

Some investigators may wish to develop their own special experiment hardware to work in conjunction with the facilities and functional capabilities of existing hardware. Development of experiment-unique equipment will require additional funding, and individual agencies may negatively factor such cost into their overall assessment. Design, construction, and flight of major experiment-unique equipment hardware items or facilities usually require the commitment of large quantities of resources (power, crew time, volume). In the event that such items are proposed, they should be clearly identified. Proposals for major hardware items or facilities to be developed by the investigator will not be considered.

Flight experiments should only be proposed if they can realistically be implemented in a timeframe compatible with their assignment of a first flight opportunity between mid 2004 and late 2006 for model organisms. With the Definition and Development Periods generally requiring approximately one to two years, experiments that cannot be conducted within this time should not be submitted.

It is expected that the majority of experiments selected from proposals in response to this announcement will be performed on the International Space Station (ISS). A small number of flight opportunities may become available for experiments that do not require ISS resources and can be accommodated on the Space Shuttle. Because this prospect is uncertain, proposals for research appropriate for the ISS will have the highest priority for selection and funding. Pre- and post-mission studies that involve tests of the astronaut crew before and upon return from their

space flight may also be submitted (see Section 1.2 and 1.3 for specific constraints on pre- and post-flight astronaut participation).

Multiple flight opportunities may be provided when required to meet scientific objectives. However, proposals that request only one flight to meet their proposed research goals will have a higher probability of selection. Careful consideration and discussion of subject requirements in the proposal is highly recommended.

1.1 Flight Experiments

There are, in principle, two kinds of flight experiments possible: 1) experiments in the Space Shuttle with typical flight durations of 8 to 11 days, and 2) experiments on the ISS with potential flight durations of up to 190 days. Serious consideration should be given to both active and passive phases of the proposed experiment (e.g., reagent and specimen storage time and conditions) in order to define adequately the experiment requirements, procedures, and flexibility.

1.1.1 ISS Flight Experiments

Research opportunities will be available on a limited basis during the construction phase of the ISS. The research will be accomplished during Space Shuttle missions when the Shuttle visits the ISS and during the times between the Space Shuttle missions when the ISS crew will act as experiment operators and, if necessary, as subjects. The duration of microgravity exposure can, in theory, be indefinite, with periodic disturbances of up to 5 days every 30 days caused by U.S. and Russian transportation vehicle docking activities.

It is expected that transport frequency, power during transport, and mass of transported items will all be severely constrained throughout the ISS assembly period. The primary opportunities to transport scientific equipment, supplies, and samples will be on the periodic logistic flights of the Shuttle to the ISS specifically dedicated to this purpose. In addition, modest capabilities for research-related deliveries and sample returns may be available on the Shuttle flights dedicated to assembly of the ISS. It is expected that for the anticipated flight opportunity time period, Shuttle flights to the ISS will occur approximately 4 to 5 times per year. Refrigerated and frozen transport of samples on the Shuttle will be very limited, and during certain timeframes, refrigerated and frozen storage may not be available on the ISS. Power outages may also be experienced during the assembly of the ISS. Experiments with few and/or simple crew-supported in-flight activities have the greatest potential for selection during this timeframe due to limitations on crew time and crew training. Samples or specimens from experiments may be returned to Earth only periodically. Depending upon the duration of the active phase of the experiment, storage of samples up to 190 days must be possible, and up to 365 days for cold stowage must be possible. There is a minimum storage period of several days before starting an ISS experiment, since the Shuttle must travel to and dock at the ISS and the experiment must be transferred to its ISS facility (see Figure 1). The requirements necessary to preserve the integrity of an experiment during these storage periods must be described on the Space Flight Experiment Requirements Summary (Form F).

The availability of the crew for specifically timed science operations and as subjects of research will also be constrained during ISS assembly. On average, a total of approximately twenty crew hours per week will be available for all research. A subset of this crew time will be available to support life sciences research. However, ISS crewmembers have indicated an interest in science tasks that can be performed on a time-available basis and proposers are strongly encouraged to identify objectives that can be achieved in this manner. Estimates of crew time required to complete the experiment must include the time required for crewmembers to both operate an experiment and serve as subjects. Moreover, crew time for data collection before and after flight is extremely limited and consideration of current exercise countermeasure protocols is strongly recommended (see Section 2.1.3, and Goals of Exercise Countermeasures Program on the International Space Station at http://hrf.jsc.nasa.gov/checs_hw/CheCS_Exercise_HW_Operational_Use.ppt). There is no assurance that all crewmembers will agree to participate as subjects in experiments. See section 2.1 for more information regarding the use of crewmembers as subjects and the assumptions to be made in planning these types of experiments.

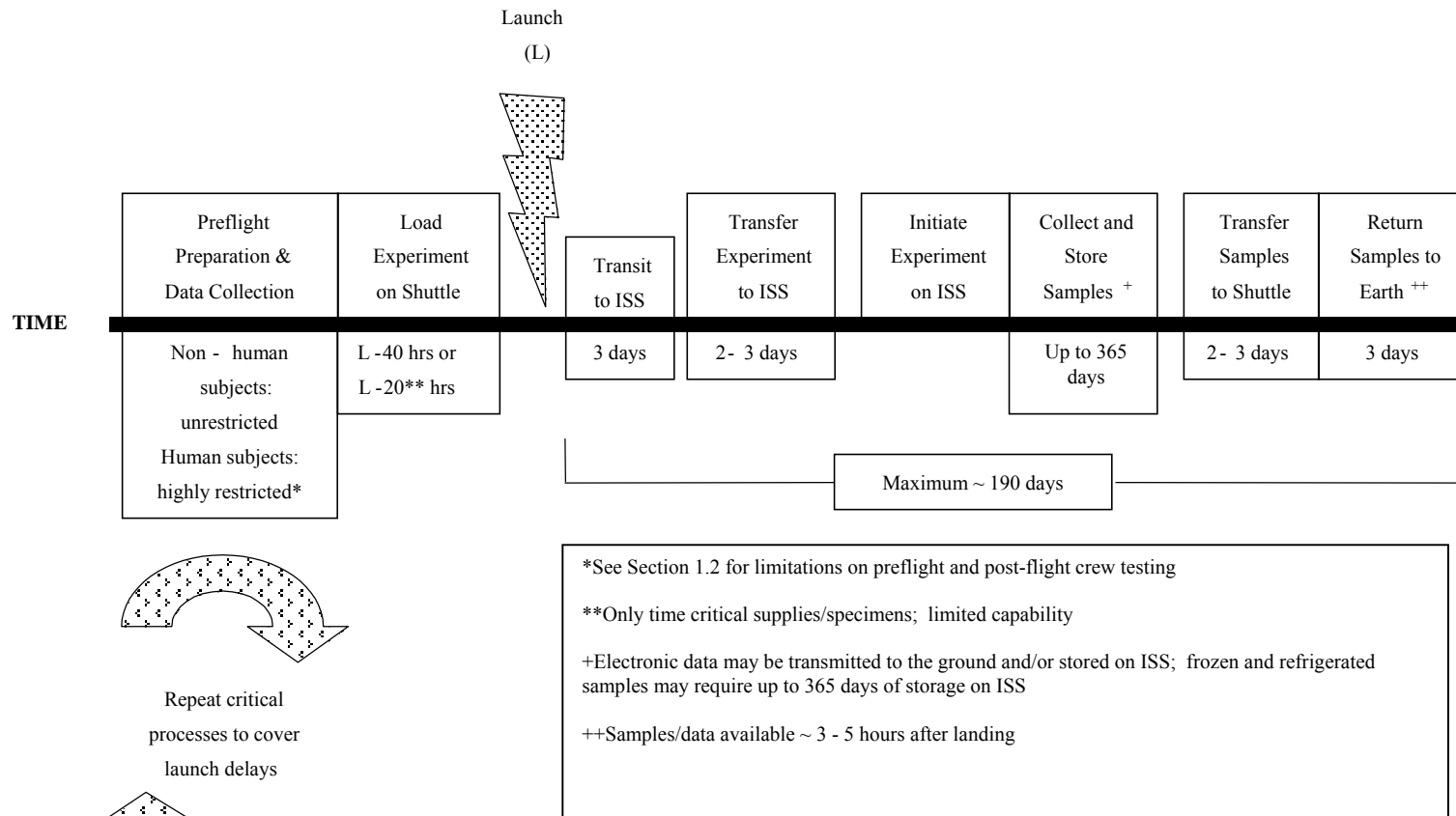
1.1.2 Short Duration Flight Experiments

Short duration experiments may be accommodated on the Shuttle for approximately 11 days of microgravity exposure. The experiments themselves must require only limited crew training and involvement to execute. Experiment hardware that occupies or requires a large volume to operate will not likely be accommodated. Experiments that do not require Shuttle power will be more easily accommodated. Descriptions of the functional capabilities of hardware available to support human and nonhuman experiments are included in Section 2.0 of this document. Section 2.0 also lists websites that contain more information about the hardware. This information should be used to develop an understanding of the available capabilities and investigators should use this information as a guide for developing experiment requirements and procedures *rather than selecting specific hardware items*.

Equipment and supplies that do not have a shelf life may be loaded onto the Shuttle days or weeks before launch. It is possible to arrange for late preflight installation (approximately Launch minus 20 hours) and early post-flight recovery (Landing plus 3 hours) of equipment, supplies, and data that have time- or temperature-critical sensitivities. Note that there are periods of time before flight and after landing when no access to the experiment is possible and maintenance of the experiment/data integrity must be assured. The requirements necessary to preserve the integrity of an experiment during these storage periods must be described on Form F.

As many as four to seven Shuttle crewmembers per flight will support flights during this time period. The number of crew subjects available to perform short duration human studies will be restricted due to the limited amount of crew time available for such experiments, and there is no assurance that all crew members will agree to participate as subjects in experiments. The availability of Shuttle resources for experiments that require animal subjects will also be extremely limited for short duration experiments (see Section 2.2).

Figure 1: Flight Experiment Implementation Flow



1.2 Pre- and Post-mission Studies

Opportunities will be available to perform experiments, collect samples, and take physiological measurements of the astronaut crew both before their space mission and following their return to Earth. Such proposals are considered flight experiments and should specify the desired activities, the timeframe in which these activities must be performed prior to and following the mission, and the required mission duration (i.e., prior to and following a short duration Shuttle mission versus a longer duration ISS mission). Access to long duration ISS crews for pre- and post-mission studies will be extremely limited. There is no assurance that all crewmembers will agree to participate as subjects in experiments. Access to the crew immediately before and upon return is extremely limited (availability of astronauts for research tests on the day of return to Earth, or the day after, may be as little as one hour per day total. See Section 1.3).

1.3 Transportation

Within the timeframe identified in this document, Shuttle launch capabilities will be augmented by additional payload launch vehicles. ESA is providing the Automated Launch Vehicle (ATV). Russia's Progress vehicle will continue to provide limited transportation opportunities to the ISS. Investigators should remain aware of the capabilities and requirements associated with each of the carriers. Progress and the ATV provide logistics support to the ISS but provide no return capability.

1.3.1 Space Shuttle

The Space Shuttle provides the bulk for upmass and downmass capability for ISS outfitting and utilization. Within the Shuttle, the primary payload transportation capabilities are the Shuttle Middeck, the Multi-purpose Logistics module (MPLM) that is carried in the Shuttle cargo bay and the SpaceHab module, an additional cargo bay carrier system.

1.3.2 Progress

The Russian Progress cargo module is similar in construction to the Soyuz orbital module. The cargo module carries pressurized cargo that the crew transfers into the station through the docking hatch. After the cargo module is unloaded, trash, unwanted equipment, and wastewater can be loaded into the Progress for disposal when the spacecraft leaves the Station.

1.3.3 Automated Transfer Vehicle (ATV)

The ESA ATV is a new vehicle designed to provide additional logistics support to the ISS Program. Pressurized cargo is soft or hard mounted within rack structures mounted in the ATV.

1.4 Difficult Experimental Requirements to Implement during the Assembly Phase of the ISS

There are certain experimental procedures that, while not impossible to perform, are difficult to implement during the assembly of the ISS. Those requirements that may be difficult to accommodate include:

1. Experiments requiring Shuttle Middeck volume, with a special emphasis on those requiring powered Middeck resources.
2. The need for a large allocation of in-flight crew time (experiment procedures will take more than 3 hours per week).
3. Measurements to be made on long duration crew members within their first days on-orbit, which implies that the measurements have to be made on the Shuttle before docking with the ISS or on the return trip.
4. Intensive Early Flight Activities (Flight Day 0 to Flight Day 15): Operations that require more than 1 hour per subject per day for more than 2 days during this period are considered intensive operations.
5. Baseline Crew Data Collection on the two days after landing: Recovery+0 to Recovery+2 (R+0 to R+2).
6. Baseline Crew Data Collection during the 30 days before launch (L-30 to Launch).
7. Excessive Crew Training (more than 10 hours to familiarize a novice with the procedure).
8. A large number of crew subjects (more than 6).
9. Complex or invasive inflight procedures on the crew, such as indwelling catheters, multiple hardware items that must be integrated or synchronized, precise requirements for when an experiment must be performed, and complex skills required (e.g., inflight biopsies, microneurography, etc.).
10. Large Upmass/Volume: Volume on the Space Shuttle is usually measured in "Middeck Locker Equivalents." A Middeck Locker can hold a volume with dimensions of 44.0 x 25.3 x 51.6 cm) (17.337 x 9.969 x 20.320 in.) and can hold a total of 27.2 kg (60 lbs). A request of more than three of these dedicated to a single experiment on a single mission would be difficult to accommodate.
11. Upmass capabilities and footprints for payloads using Progress and ATV as logistics carriers.
12. Procedures on nonhuman specimens on the day of launch (unless automated).
13. Procedures that require crew time before docking on the ISS or on the day of landing.
14. Complex in-flight procedures on nonhuman specimens, such as surgeries or dissections.
15. Experiments that require more than one flight to meet objectives.

2.0 Flight Research Capabilities

2.1 Research Involving Human Subjects

The amount of time it takes to complete a study is based on the required number of subjects and crewmember participation. Investigations selected under this solicitation will be flown while there are three crew members on board the ISS, and it should be assumed that two Increment crews will be flown every year for a total of six potential subjects a year. In order to account for variations in subject participation and suitability, it should be assumed that two subjects per Increment will participate, for a total of four subjects per year. Therefore, if an investigation requires a minimum of six crewmember subjects, it will take a minimum of three ISS Increments (1.5 years) to complete the in-flight data collections. Investigations requiring short duration crewmembers as subjects should assume flight data could be collected on six subjects per year.

Due to the limited resources (e.g., crew time, on-orbit experimental supplies, temperature-controlled sample storage) available for the conduct of ISS research, ISLSWG is pursuing the intentional formation of teams of investigators whose experiments will leverage resources by addressing different facets of the same question. ISLSWG anticipates that such intentional teaming arrangements will result in better utilization of available resources to resolve specific questions. ISLSWG strongly encourages individual investigators submitting applications in response to this solicitation to consider identifying such collaborations between individual proposals as part of the development of their individual proposals and to identify this pre-coordination in their submissions.

All use of human subjects for research must comply with [NASA Policy Directive NPD 7100.8D, Protection of Human Research Subjects](#). Informed consent of human subjects must be obtained before carrying out any study in space, and potential applicants should be aware that obtaining such informed consent will involve a uniform process regardless of the country of origin of the applicants. The availability of consenting subjects may affect the probability of achieving experiment objectives within the expected timeframe.

There are many research tools available to investigators who wish to conduct human physiological research on the ISS. The ISS Human Research Facility (HRF) is a suite of hardware that provides core capabilities to enable research on human subjects. HRF consists of instruments mounted in two racks located in the US Lab, as well as separate equipment kept in stowage and brought out as needed. HRF Rack 1 is currently on-orbit. Some additional HRF hardware items are still in development but will be available for use in the near future.

A complementary set of hardware is provided via the European Physiology Modules Facility (EPM), a multi-user facility supporting human studies. The EPM rack is outfitted with an initial complement of instruments. Due to the modular design, this initial configuration can be easily complemented and/or modified with instruments still under development or to be developed, according to the scientific needs.

Once all hardware is available on the ISS, it is planned to place both HRF1 and 2 with EPM in the Columbus laboratory to allow for combined experiments.

A complete list of hardware in the HRF and EPM inventories, and a web site reference for design details is provided in Table 1. A general description of HRF and EPM core capabilities is provided below.

In addition to HRF and EPM equipment specifically intended for research, the Crew Health Care System (CHeCS) is also potentially available to ISS researchers. CHeCS is a suite of hardware used to maintain and monitor the crew's health onboard the ISS. CHeCS hardware can be used for research but this must be closely coordinated with the flight surgeons and cannot interfere with planned operational use. A partial list of CHeCS hardware is included in Table 1 and a general description of CHeCS capabilities is provided below.

2.1.1 Physiological Monitoring

- **Blood Pressure:** Capabilities include noninvasive monitoring and collection of blood pressure data, both extended duration and intermittent, on human subjects. The data can be collected by manual or automated methods during periods of rest or exercise.
- **ECG/EMG/EEG:** Acquisition of human physiological data such as ECG, EMG, EEG, temperature, and skin galvanic responses is possible. Multichannel data (16 differential channels) can be collected by means of portable, crew-worn devices over extended periods of time (24 hours), or via rack-mounted devices.
- **Pulse/Blood Oxygen:** A pulse oximeter will be available to monitor the percentage of hemoglobin oxygen saturation in the blood.
- **Metabolic Activity/Pulmonary Physiology:** Two gas analyzers are available: one based on the use of mass spectrometry and the other on infrared gas analysis techniques. Combined with ancillary equipment, including gas supplies for supplying special respiratory gas mixtures, the following measurements are possible:
 1. Breath-by-breath measurements of VO_2 , VCO_2 , and VE
 2. Diffusing capacity of the lung for CO
 3. Expiratory reserve volume
 4. Forced expired spirometry
 5. Functional residual capacity
 6. Respiratory exchange ratio
 7. Residual volume
 8. Total lung capacity
 9. Tidal volume
 10. Alveolar ventilation

11. Vital capacity
12. Volume of pulmonary capillary blood
13. Dead-space ventilation
14. Cardiac output
15. Fractional inspiratory and expiratory volumes, F_{IO_2} and F_{EO_2} , F_{ICO_2} , and F_{ECO_2}
16. Numerous other specialized tests of pulmonary function

- **Ultrasound/Doppler:** An ultrasound system is available to perform medical imaging and to measure flow rates. The system uses hand-held probes and performs functions to support cardiac, abdominal (deep organ), vascular, muscle and tendon, and transcranial ultrasound.
- **Mass Measurement:** A mass measurement device is available to measure real-time on-orbit body mass with a +/- 0.5 lb accuracy.

2.1.2 Sample Collection and Storage

Blood, urine, and saliva samples may be collected from crew subjects before, during, and after flight. NASA can provide blood, urine, and saliva collection kits for the collection, preservation, and storage of samples. NASA can also provide tracer kits to provide oral ingestion, bolus injection over a short period of time, or infusion over a designated period of time of metabolic tracers and/or other pharmaceuticals. A refrigerated centrifuge capable of biological sample separation under controlled temperature (4°C) conditions is also available for use.

2.1.3 Exercise

The primary suite of equipment from the CheCS inventory available to researchers is the crew exercise equipment. Several exercise devices are available for research including a cycle ergometer, a resistive exercise device, and a treadmill. Use of this equipment will require coordination with Flight Medicine to ensure appropriate and proper usage.

The **cycle ergometer** provides workload, driven by the hands or feet, which is controlled by manual or computer adjustment. It operates with the subject seated or supine, and provides time-synchronized data compatible with other complementary analyses. The data output consists of work rates in watts and pedal speed (rpm) for use with a data acquisition system.

The **treadmill** may be used for walking and running exercise. The device employs various strategies to simulate, as closely as possible, 1 g skeletal loading during exercise bouts. The treadmill will measure and display the loads exerted on the subject by restraint harnesses before, during, and after the exercise bout. The restraint system provides stabilization of the user and load distribution on the body in a weightless environment. The treadmill can be motor-driven or passively operated. As with the cycle ergometer, the treadmill provides data compatible with other complementary analyses.

An **interim resistive exercise device (iRED)** is installed on the ISS and consists of two canisters, each containing a series of "flex packs" that can be dialed in sequentially to add greater resistance to a cable. The cable is wrapped around a pulley in each canister, and each pulley is connected to a shaft that runs through the center of the flex packs in such a way that, as the cable is extended, the "elastomer" straps of each flex pack are stretched and this creates resistance at the cable. There is a variety of human-machine interface devices (e.g., handgrips, straps, curl bars, ankle cuffs, squat harness, etc.) that permit a variety of exercises to be performed by the astronauts. The design of the hardware is such that the forces imposed upon a muscle group during an eccentric muscle action are less than the maximum concentric force that can be generated by the user.

2.1.4 Evaluation of Muscle Strength and Exercise Capacity

A **Flywheel Exercise Device (FWED)** that can be used for evaluation of exercise capacity regarding strength and fatigue. The flywheel provides resistance when the wheel is accelerated (concentric phase) and subsequently decelerated (eccentric phase). A variety of exercises can be performed including both the upper and lower body. This device provides higher load during the eccentric than in the concentric phase. Continuous measurements of torque, force and, and knee joint angle can be recorded. In combination with HRF it is possible to record EMG while using the FWED. Evaluation of the FWED as a Countermeasure Device, regarding resistive exercise, is also feasible.

A **Muscle Atrophy Research and Exercise System (MARES)** can also be used to evaluate muscle strength and exercise capacity (available no earlier than 2006). The MARES provides active resistance (concentric and eccentric) that can be fully programmed as motion profiles.

MARES supports the following capabilities:

- Measurement of the (bidirectional) torque, position, and velocity generated during programmable tests on the agonist and antagonist muscle groups of the trunk and extremity joints including ankle, knee, hip, wrist, elbow, shoulder, trunk, whole leg, and whole arm
- Measurement of these parameters during submaximal and maximal exercises throughout the entire range of motion (except for shoulder) in the isometric, isokinetic (concentric and eccentric), and isotonic (concentric and eccentric) modes
- Simulation of ideal elements: spring, friction and inertia
- Parameter control following predefined pattern: position control, velocity control, torque/force control, power control
- Quick release of free motion
- Complex combinations of the previous modes

- Bilateral torque and angular position/velocity measurements and training on the flexion and extension of the knee, ankle, trunk, hip, shoulder, elbow and wrist, and on the supination/pronation, radial/ulnar deviation of the wrist.
- Bilateral force and linear position/velocity measurements and training on the following multi-joint linear movements:
 - i Arm press (front, overhead and intermediate trajectories)
 - ii Leg press (front, down and intermediate trajectories)
- The displays available to the subject are highly programmable, i.e., display of peak torque vs. joint angles, and average torque at specific joint angles as well as torque-velocity throughout the entire range of motion, etc. curves).
- The motion and experiment profiles are highly programmable (e.g., programming of variable and quantifiable velocities and resistances during training exercises, assessment of fatigue over serial contractions)

Currently, there are already several additional instruments available for:

- Measurement of hand grip strength or pinch strength as a function of time
- Local noninvasive muscle stimulation on human subjects using a high current stimulator that provides trains of pulses up to 0.8 amps, according to pre-programmed protocols. It can be connected to MARES.
- Portable, ambulatory measurement of full range of motion in either 1 or 2 degrees of freedom in selected joints.

2.1.5 Cardiovascular Loading

A **lower body negative pressure (LBNP)** device that encloses the lower abdomen and lower extremities to maintain a controlled pressure differential below ambient during periods of extended weightlessness will be available. This device may be used in conjunction with the physiological monitoring capabilities described above. It will provide pressure applications to the lower body in a range from ambient to -60 mm Hg. It allows performance of a continuous decompression to -60 mm Hg at a range of 10 seconds to 10 minutes (i.e., rapid to slow decompression).

An adjustable foot support, removable saddle, and knee fixation within the device provides skeletal “loaded” and “unloaded” LBNP. The decompression device is available not only for cardiovascular research, but also for any other physiological research.

2.1.6 Posture

Single axis loads between the foot and the supporting surface can be measured during any activity in which a crewmember engages. In addition to the measurement of total force between the foot and the surface, regional force values may also be measured. Selective regional

measurements of the loads applied to the rear foot, mid foot, medial metatarsal head, lateral metatarsal heads, hallux, and lesser toes can also be made.

2.1.7 Activity Monitoring

Measurements indicative of the crew's activity level can be made using a small wrist- or ankle-worn device that can detect movement and light levels. The device is used to evaluate sleep/wake adaptation, circadian cycles, sleep quality, sleep onset, hyperactivity, and other daily routines of human activity. The device can be battery operated for up to 150 hours. Sampling rates of accelerations and light intensity are programmable.

2.1.8 Eye Movements

A 3-dimensional **Eye Tracking Device (ETD)** for the recording of eye movements will be available. This device may be used to measure horizontal, vertical and/or torsional eye positions by means of digital processing of the recorded eye image sequences. Furthermore, head movements will be measured by means of three orthogonally arranged angular rate sensors and three orthogonally arranged linear accelerometers. This encompasses all three degrees of freedom of eye movement (in the head) and all six degrees of freedom of head movement in space.

2.1.9 European Physiology Modules Facility (EPM)

The initial instrument complement to be accommodated includes:

MEEMM (Multi-Electrode EEG Mapping Module). Designed for supporting brain and muscle activity studies by measuring EEG/EMG and evoked potentials. The main features of the MEEMM are:

- Supporting acquisition of up to 128 EEG channels (maximum sampling frequency 2.2 kHz, 0.01-580 Hz maximum bandwidth)
- Supporting acquisition of up to 32 EEG channels (maximum sampling frequency 40 kHz, 1.5 Hz-10 kHz maximum bandwidth)
- Supporting acquisition of up to 32 surface EMG channels (64 electrodes) (maximum sampling frequency 40 kHz, 1 Hz-10 kHz maximum bandwidth)
- External triggering digital signal acquisition (8 bit digital interface)

PORTEEM (Portable EEG). Modular instrument for ambulatory/sleep EEG measurements, initial configuration :

- 12 EEG channels (0.3-70 Hz maximum bandwidth)
- 2 EMG channels (1-150 Hz maximum bandwidth)
- 1 ECG channel (1-150 Hz maximum bandwidth)
- 1 strain gauge respiratory signal (0.3-30 Hz maximum bandwidth)

BAM (Bone Analysis Module). Evaluation of the mineralization state of the calcaneus using ultrasound. The BAM produces an ultrasonic image of the calcaneus. The BAM allows for the determination of “Speed Of Sound (SOS)” and “Broad-Band Ultrasonic Attenuation (BUA)”.

CARDIOLAB. (Cardiovascular Laboratory). CARDIOLAB consists of a central data management system providing services to a complement of instruments (sensors and stressors), including :

- **CARDIOPRES:** Continuous acquisition of blood pressure (finger and arm cuffs), ECG from 1 to 7 leads derivations, thoracic and abdominal breathing patterns.
- **HLTE:** ECG Holter (24 hours ECG full stripes recording)
- **HLTA:** Arm-cuff blood pressure Holter (Systolic, Diastolic and Mean Blood Pressure measurements)
- **PDOP:** Portable ultrasound doppler instrument (Main arteries blood velocities measurements up to three channels at a time with 2Mhz, 4Mhz and 8Mhz pulsed wave probes).
- **APLT:** Air plethysmography, providing limb volume variations against venous occlusion.
- **LVMD:** Limb volume measurement device, reconfigurable for body position determination.
- **PBAD:** Portable Blood Analysis Device (ISTAT blood analyser) providing main electrolyte parameters analyses depending on specific cartridge sets.
- **HEMO:** Hemoglobinometer; measurement of hemoglobin by azide methemoglobine method; control of the status of whole blood.
- **HEMC:** Hematocrit Centrifuge (determination of the whole blood hematocrit by centrifugal separation of blood cells from plasma).
- **CMAS:** Continuous Measurement Ambulatory Device .
- **CWPG:** Cold/Warm pressure glove. Application of thermal stress on the forearm in a range from -5°C to 40°C with a regulation in the case of positive temperature of 0.5°C
- **LACS:** Leg/arm occlusion cuff system. Application at the level of the limbs of an occlusive stress in a range from 0mm of Hg to 300 mmHg (two different level/profiles of pressure on the arms and on the legs).

SCK (Sample Collection Kit). Stowage of medical and clinical equipment for blood, saliva and urine sample collection and disposal and management of used medical/biohazard items.

NASA Drawer. NASA plans to launch within the EPM initial configuration following stowage instruments : Handgrip Dynamometer (HGD) and Pinch Force Dynamometer (PFD)

Table 1: Hardware Available to Support Human Subject Research

Hardware Available to Support Human Subject Research	Shuttle-Based	ISS-Based	Agency	Website
X = on-orbit/ready to fly, Y = requires development and/or certification				
Physiological Monitoring				
Blood Pressure/Electrocardiograph	X	X	NASA	http://hrf.jsc.nasa.gov/chees_hw/CHeCS_Exercise_HW_on_ISS.ppt
Automatic Blood Pressure Cuff	X	X	NASA	http://hrf.jsc.nasa.gov/chees_hw/CHeCS_Exercise_HW_on_ISS.ppt
Continuous Blood Pressure Device	Y	X	NASA	http://hrf.jsc.nasa.gov/cbpd.htm
Pulmonary Function System		X	NASA/ESA	http://hrf.jsc.nasa.gov/pfs.htm http://www.spaceflight.esa.int/users/file.cfm?filename=fac-iss-othfac-pfs
Gas Analyzer Mass Spectrometer		X	NASA	http://hrf.jsc.nasa.gov/gasmap.htm
Ambulatory Data Acquisition System (analog to digital recorder)	Y	X	NASA	http://hrf.jsc.nasa.gov/adas1.htm
Holter Monitor	X	Y	NASA	
Pulse Oximeter	Y	X	NASA	http://hrf.jsc.nasa.gov/pulseox.htm
Ultrasound Doppler		X	NASA	http://hrf.jsc.nasa.gov/ultrasound.htm
Portable Clinical Blood Analyzer		X	NASA	
Space Linear Acceleration Mass Measurement Device		X	NASA	http://hrf.jsc.nasa.gov/SLAMMD.htm
Sample Collection and Stowage				
Human Sample Collection Kits	X	X	NASA	
Refrigerated Centrifuge		X	NASA	http://hrf.jsc.nasa.gov/rc.htm
Exercise				
Cycle Ergometer	X	X	NASA	http://hrf.jsc.nasa.gov/chees_hw/CHeCS_Exercise_HW_on_ISS.ppt
Treadmill	X	X	NASA	http://hrf.jsc.nasa.gov/chees_hw/CHeCS_Exercise_HW_on_ISS.ppt
Interim Resistive Exercise Device		X	NASA	http://hrf.jsc.nasa.gov/chees_hw/CHeCS_Exercise_HW_on_ISS.ppt
Flywheel		X	ESA	http://spaceflight.esa.int/users/file.cfm?filename=fac-iss-fwed
Muscle Strength, Torque, and Joint Angle				
Muscle Atrophy Research and Exercise System		Y	NASA/ESA	http://www.spaceflight.esa.int/users/file.cfm?filename=fac-iss-othfac-mares
Percutaneous Electrical Muscle Stimulator		X	NASA/ESA	http://www.spaceflight.esa.int/users/file.cfm?filename=fac-iss-othfac-pems
Hand Grip/Pinch Force Dynamometer	Y	X	NASA/ESA	http://www.spaceflight.esa.int/users/file.cfm?filename=fac-iss-othfac-hg
Joint Excursion System		X	NASA	http://hrf.jsc.nasa.gov/jes.htm
Cardiovascular Loading				
Lower Body Negative Pressure	Y	Y	DLR	http://hrf.jsc.nasa.gov/lbnp.htm http://www.dlr.de/struktur_strategie/raumfahrtmanagement/RD-JW/projekte-uebersicht
Posture				
Foot-Ground Interface	Y	Y	NASA	

Hardware Available to Support Human Subject Research	Shuttle-Based	ISS-Based	Agency	Website
X = on-orbit/ready to fly, Y = requires development and/or certification				
Lower Extremity Load Measurement				
Total Force Foot-Ground Interface	Y	X	NASA	http://hrf.jsc.nasa.gov/tf-fgi.htm
Activity Monitoring				
Actilight Watch	X	Y	NASA	http://hrf.jsc.nasa.gov/am.htm
Eye Movements				
3 D Eye Tracking Device	Y	X	DLR	http://hrf.jsc.nasa.gov/etd.htm http://www.dlr.de/struktur_strategie/raumfahrtmanagement/RD-JW/projekte-uebersicht
European Physiology Modules				
Multi Electrode EEG Mapping Module		X	ESA	http://spaceflight.esa.int/users/file.cfm?filename=fac-iss-col-epm
Portable EEG (PORTEEMM)		X	ESA	http://spaceflight.esa.int/users/file.cfm?filename=fac-iss-col-epm
Bone Analysis Module		Y	ESA	http://spaceflight.esa.int/users/file.cfm?filename=fac-iss-col-epm
Sample Collection Kit (SCK)		X	ESA	http://spaceflight.esa.int/users/file.cfm?filename=fac-iss-col-epm
CARDIOLAB		X	ESA	http://spaceflight.esa.int/users/file.cfm?filename=fac-iss-col-epm

2.2 Research Involving Nonhuman Subjects

2.2.1 Yeast Model Specimen Flight Opportunities

S. cerevisiae is an excellent model organism for studying the effects of space flight on living organisms because: (1) it is a well studied eukaryotic organism; (2) its complete genome has been sequenced; (3) many genes in yeast have significant mammalian homologs; and (4) it replicates quickly and multiple generations can be grown in space.

The earliest opportunity is a Biospecimen Sharing Opportunity that seeks to maximize the scientific yield from the previously selected and currently manifested Yeast Group Activation Packs (Yeast GAP) experiment, and solicits proposals for the scientific utilization of samples that will be available after completion of approved procedures for this experiment. Due to mission constraints, and to minimize interference with the approved Yeast-GAP experiment objectives, the experiment design, the on-orbit processing and temperature conditions are limited to the on-orbit processing described. The other opportunities are described below - Effects of Microgravity on Model Yeast Specimens (EMMYS-1, and EMMYS-2) and Model Yeast Cultures On Station (MYCOS). These opportunities will follow the Office of Biological and Physical Research model specimen approach. For both EMMYS and MYCOS investigations, this approach will offer the opportunity for submission of full proposals that fit within the described capabilities and constraints described, and it will involve investigating fundamental space biology on the ISS.

Yeast-GAP Biospecimen Sharing Program Opportunity

The Yeast-GAP Biospecimen Sharing Program (BSP) Opportunity seeks to maximize the scientific yield from the Yeast-GAP experiment and solicits proposals for the scientific utilization of samples that will be available after completion of approved procedures for the Yeast-GAP experiment. Samples of deletion series of *Saccharomyces cerevisiae*, grown in microgravity at ambient conditions and then stabilized at ambient conditions, in RNALaterII supplemented with Nystatin, will be available upon return from the ISS.

The experiment approach was developed by the Yeast GAP team and involves the use of a mixture deletion series *S. cerevisiae*. At approximately launch minus (L-) 75 days, the yeast deletion strain mixture was dried down, spotted onto filter paper, and placed into 8 Fluid Processing Apparatus (FPA) from BioServe Space Technologies. The FPAs were then loaded into a Group Activation Pack (GAP) from BioServe Space Technologies. During shipment, launch, and while on-orbit, the experiment will be maintained at ambient temperature conditions.

This opportunity is targeted for launch in early 2004. Dried down deletion series *S. cerevisiae* will be launched and stored on the ISS at ambient conditions. The yeast will be activated in microgravity by introduction of Yeast extract, Peptone, Dextrose media (YPD) and incubated at ISS ambient temperature for 60 - 72 hours. Following incubation, the cultures will be stabilized in flight with RNALaterII supplemented with Nystatin. The stabilized cultures will be maintained at ISS ambient temperature until return from flight. Quantities of approximately 1 ml of the flight and ground control sample populations will be made available to the PIs.

Effects of Microgravity on Model Yeast Specimens (EMMYS)

The EMMYS flight opportunities will help delineate how the space environment affects life processes at the molecular and cellular levels and how the space environment affects organisms throughout their lives. Results from these flight opportunities will lay the foundation for determining the role that the space environment plays on regulating *S. cerevisiae* gene expression and other cellular responses and will address how the space environment affects *S. cerevisiae* development and maturation. Data from these flight opportunities can be subsequently linked to the wealth of information from ground-based studies on Earth obtained from *S. cerevisiae*, which is widely used in biomedical research as a model for human genetics and disease. This combined information will also contribute to our understanding of how the space environment affects organisms at the molecular level and will provide insight to potential countermeasures necessary to prepare humans for long-duration exploratory missions.

The EMMYS flight opportunities represent early opportunities to study *S. cerevisiae* as a model specimen and the information described here is intended as a framework upon which respondents can propose. Due to the timeframe of the EMMYS experiments, hardware that has been previously approved and used for space flight will be required for EMMYS-1 and, although not a requirement, will have distinct advantages for EMMYS-2. Specific details related to final selection of hardware and details of the experimental design will be dependent upon input provided by the PIs, but must fall within the constraints of the flight opportunity described.

Principal Investigator Participation: PIs will participate in 1) identifying specific yeast strains, 2) identifying storage and experiment initiation conditions, 3) determining the incubation and storage thermal requirements, 4) identifying hardware [for EMMYS-2], 5) ground testing studies, and 6) defining specimen processing and collaborative efforts. The consideration for each of these items must fit within the constraints of the EMMYS opportunities. Depending on the processing requirements, PIs may be involved in pre-and postflight sample processing operations at the launch site processing facility and at the prime and secondary landing facilities. PIs may also be involved in ground communication activities.

EMMYS-1 Experiment Design

The EMMYS-1 opportunity will provide the opportunity to study cultures of wild type and/or mutant strains of *S. cerevisiae* over multiple generations utilizing techniques such as Green Fluorescent Protein (GFP) tagged organisms. This opportunity is targeted for launch in 2004.

Wild type *S. cerevisiae* (BY4743 [ATCC 4040005] homozygous diploid) and cultures of a mixture of GFP tagged *S. cerevisiae* and/or mutant strains that will have grown in space over multiple generations will be utilized. The cultures will be stabilized on-orbit in a flight-qualified fixative and/or stabilizer. Specimens will be available for postflight molecular and morphological analyses.

Due to the timeframe of the EMMYS-1 experiments, hardware that has been previously approved and used for space flight must be utilized for this experiment. Current plans call for the use of the FPA and GAP hardware described in the Yeast-GAP opportunity.

EMMYS-1 Objective: Determine the effects of space flight on wild type and/or mutant strains and GFP tagged *S. cerevisiae* at its most fundamental levels, from the gene to the cell.

EMMYS-1 Protocol: The wild type and/or mutant strains and GFP tagged *S. cerevisiae* will be lyophilized before flight, and flown in the FPA/GAP hardware described in the Yeast-GAP opportunity. Specimens will be prepared at around 9 weeks before flight. Specimens will be cultured on-orbit, under static conditions, to early/mid log phase and to mid/late log phase. Samples will be fixed/stabilized on-orbit for postflight molecular and morphological analyses. Provided that nominal activation and incubation and fixation occur on-orbit, wild type *S. cerevisiae* grown and stabilized in microgravity, will be available postflight for molecular analysis and GFP tagged *S. cerevisiae*, grown and fixed in microgravity, will be available postflight for morphological analysis. Synchronous ground controls will be performed using culture and media from the same stock used for flight. Asynchronous ground controls will be performed post mission following temperature conditions recorded during all mission phases. A control utilizing a clinostat or rotating wall vessel may also be available. The flight and control samples will be distributed postflight to PIs.

EMMYS-2 Experiment Design

The EMMYS-2 opportunity will establish continuous cultures of *S. cerevisiae* during space flight. This opportunity would utilize static and possibly stirred cultures, as well as on-orbit passaging capabilities.

EMMYS-2 Objective: This opportunity will allow investigation into the links between changes observed in the genome, gene expression, and development from samples cultured under static conditions from Yeast-GAP and EMMYS-1 compared to samples cultured with perfused media under both static and possibly stirred conditions in microgravity. The on-orbit passaging capability will allow for the extended multi-generational comparisons.

EMMYS-2 Protocol: Wild type, select mutants, or deletion series *S. cerevisiae* will be cultured in microgravity with perfused media under static and possibly stirred conditions. Portions of the yeast populations, at multiple sampling time points, will be stabilized on-orbit for postflight genetic analyses and for analyses of the effects of microgravity on mass transport in yeast. Hardware that could be considered for this opportunity are the Single Loop Cell Culture (SLCC) hardware described below in “MYCOS Experiment Design” and Table 4, or the SHOT Advanced Separation (ADSEP) hardware described below in CEMMS experiments and Table 7. Synchronous ground controls will be performed using culture and media from the same stock used for flight. Asynchronous ground controls will be performed post-mission utilizing temperature conditions recorded during all mission phases. The flight and control samples will be distributed postflight to PIs.

Model Yeast Cultures on Station (MYCOS)

This announcement solicits proposals for participation in the Fundamental Space Biology-1 (FSB-1) Model Yeast Cultures on Station (MYCOS) experiment. In this experiment, yeast (*S. cerevisiae*) will be cultured on the ISS using the Space Station Biological Research Project (SSBRP) Incubators. These incubators have temperature control (+4 °C to +45 °C), temperature and humidity sensors, air circulation, telemetry, data, video pass-through, and commanding capabilities.

MYCOS will follow the Office of Biological and Physical Research model specimen approach to fundamental space biology on the ISS and will review and select experiment proposals that are meritorious and fit within the described constraints of the FSB-1 MYCOS scenario and protocol (see below and Table 2). Proposed experiments that do not conform to the scenario and constraints of the protocol framework will not be considered. NASA strongly encourages the teaming of projects and sharing of specimens and resources.

MYCOS Experiment Design

MYCOS is designed to support experiments directed at investigating how the space flight environment affects the cellular, molecular, and biochemical processes of *S. cerevisiae*. In particular, experiments that can be supported by MYCOS include mass transport, radiation biology, and the process of pseudohyphal growth.

MYCOS Objective: Provide early science return for the use of the SSBRP Incubator with the model organism *S. cerevisiae*.

MYCOS Protocol: The yeast specimens, which are in growth and metabolic stasis, will be delivered to the ISS. For ascent, the yeast specimens will be stored in a thermally conditioned carrier that is located in the ascent Orbiter Middeck. After docking and then transfer to the ISS, the yeast will be stored in an ISS cold storage unit that supports the maintenance of the stasis condition, until required hardware operations are completed. Within two to three weeks of the docking of the descent Orbiter, the yeast experiment will be initiated. Two SSBRP Incubators will be used to culture the yeast specimens. At the end of the incubation period, the yeast will be placed under conditions that return the specimens back into stasis for the remainder of the ISS phase. The yeast will be returned to Earth in a thermally conditioned carrier (+4 °C or lower).

Data on the environmental conditions of the Incubator will be downlinked regularly. The Passive Dosimeter System (PDS, see Table 14) will be used to provide data on space radiation exposure during the on-orbit duration of the experiment.

Principal Investigator Participation: PIs will participate in 1) identifying specific yeast strains, 2) identifying storage and experiment initiation conditions, 3) determining the incubation and storage thermal requirements, 4) identification of growth chamber type, 5) ground testing studies, and 6) defining specimen processing and collaborative efforts. The consideration for each of these items must fit within the constraints of the MYCOS protocol. Depending the processing requirements, PIs may be involved in pre-and postflight sample processing operations at the launch site processing facility and at the prime and secondary landing facilities. PIs may also be involved in ground communication activities.

Accessory Hardware: The MYCOS protocol can support the use of OptiCells, Petri dishes, and the Single Loop Cell Culture (SLCC) system. Each SLCC contains one 30 ml Cell Specimen Chamber. The capacity of the Incubator allows for a the use of 1) forty-two OptiCells, 2) a minimum of forty 100 mm Petri dishes, 3) a minimum of sixty 60 mm Petri dishes, or 4) two SLCC systems. Please refer to Table 4 for more information on the SLCC and OptiCells. In addition, gloveboxes will be available for operations that require containment.

Table 2. MYCOS Protocol Framework Summary

<i>Organism</i>	<i>S. cerevisiae</i>
Incubator	2 SSBRP Incubators
Duration On-Orbit	30 to 110 days
Experiment Duration	Incubation period ¹
Ascent Thermal Requirement	+4 °C live specimen storage
ISS Thermal Requirement	
- Pre-incubation	+4 °C live specimen storage (approx. 14 to 80 days)
- Incubation period	+20 °C to +30 °C
- Post-incubation	+4 °C (approximately 14 to 21 days)
Descent Thermal Req.	+4 °C live specimens
Culturing Chamber	OptiCell
Medium	YPD ²

Radiation Monitoring	Passive Dosimeter System
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¹Duration dependent on strains selected and ground studies

²Medium used is dependent on strains selected

Table 3. Yeast Model Specimen Research Questions and Objectives

Critical Research Questions	Flight & Date	# of Days	Environment & Hardware	Media & Conditions of Specimens	Fixation and/or Freeze	Perfused Media	Objectives (Analyses)
<p>How does life respond to gravity and space environments? (Supports strategic goal #4)</p> <p>How do space environments affect life at molecular and cellular levels?</p>	<p>Yeast GAP 13P Progress</p> <p>Targeted for launch in 2004</p>	72 hrs for experiment incubation	<p>Passive 16 Fluid Processing Apparatus/ 2 Group Activation Packs (FPA/GAP)</p> <p>Ambient temperature conditions throughout experiment</p>	Dried down yeast, YPD media, <i>S. cerevisiae</i> deletion series	<p>1 fixative, 1 time point. 16 replicates.</p> <ul style="list-style-type: none"> • Stabilized in flight in RNALaterII supplemented with Nystatin. • Experiment is maintained at ambient conditions throughout. • Experiment could remain on the ISS for up to 1-year post stabilization. 	No	<p>Yeast GAP Objectives: Determine the effects of space flight on <i>S. cerevisiae</i> deletion series (diploid homozygous and heterozygous) at its most fundamental levels, from the gene to the cell. PIs will perform various molecular post flight analyses.</p>
<p>How does life respond to gravity and space environments? (Supports strategic goal #4)</p> <p>How do space environments affect life at molecular and cellular levels?</p> <p>How do space environments affect organisms throughout their lives?</p>	<p>EMMYS-1 15P Progress</p> <p>Targeted for launch in 2004</p>	<p>Two time points for incubation</p> <p>(early to mid log phase and mid to late log phase)</p>	<p>Passive 64 Fluid Processing Apparatus/ 8 Group Activation Packs (FPA/GAP)</p> <p>Ambient temperature conditions throughout experiment with the possibility of 22°C for incubation</p>	<p>Lyophilized yeast, YPD media</p> <p>wild type and/or mutant and GFP tagged <i>S. cerevisiae</i></p>	<p>Cultured in flight under static conditions fixed/stabilized in flight for post flight morphologic and molecular or genetic analysis.</p>	No	<p>EMMYS-1 Objectives: Determine the effects of space flight on wild type and/or mutant and GFP tagged <i>S. cerevisiae</i> at its most fundamental levels, from the gene to the cell. PIs will propose specific experimental design and will perform various molecular and morphological post flight analyses.</p>
<p>How does life respond to gravity and space environments? (Supports strategic goal #4)</p> <p>How do space environments affect life at molecular and cellular levels?</p> <p>How do space environments affect organisms throughout their lives?</p> <p>Can life be sustained and thrive in space across generations?</p>	<p>EMMYS-2</p> <p>Targeted for launch in 2005</p>	Passaging and multiple sampling time points	<p>Passive Single Loop Cell Culture (SLCC) system or SHOT ADSEP fluid processing cassette</p> <p>Ambient temperature conditions throughout experiment with the possibility of 22°C for incubation</p>	<p>Lyophilized yeast, YPD media & passage</p> <p>Wild type, select mutant, or deletion series <i>S. cerevisiae</i></p>	<p>Wild type, select mutant, or deletion series <i>S. cerevisiae</i> will be cultured under static and/or stirred conditions and with perfused media on the ISS. At multiple sampling timepoints, portions of the yeast populations can be fixed/stabilized on-orbit for postflight genetic analysis and to look at the effects of microgravity on mass transport.</p>	Yes	<p>EMMYS-2 Objectives: This opportunity will allow investigation into the links between changes observed in Yeast GAP and EMMYS-1 compared to perfused and passaged culture conditions in microgravity. PIs will propose specific experimental design and will perform various molecular and morphological post flight analyses.</p>

Table 3 continued. Yeast Model Specimen Research Questions and Objectives

Critical Research Questions	Flight & Date	# of Days	Environment & Hardware	Media & Conditions of Specimens	Fixation and/or Freeze	Perfused Media	Objectives (Analyses)
<p>How does life response to gravity and space environments? (Supports strategic goal #4)</p> <p>How is life affected by the space environment at the molecular and cellular level?</p> <p>How does the space environment affect organisms throughout their lives?</p> <p>How does the space environment affect organisms throughout their lives?</p>	<p>FSB-1</p> <p>Targeted for launch in 2006</p>	<p>Incubation duration is approximately 72 hours</p>	<p>SSBRP Incubator</p> <p>+4 °C or lower during ascent flight, ISS stowage, and descent flight. +20 °C to +30 °C during incubation</p>	<p>Growth medium and growth chambers</p> <p>Wild-type and mutant <i>S. cerevisiae</i> strains</p>	<p>Yeast strains in stasis will be stored at +4 °C or lower during delivery to the ISS. After transfer to the ISS, the yeast will be stored at +4 °C or lower until the SSBRP facility class hardware installation and checkout is completed. Initiation of experiment will be on-orbit and incubation in the SSBRP Incubator. The experiment will be started within 2 -3 weeks of the docking of the return Orbiter. Post-incubation, samples will be returned to +4 °C or lower for the remainder of the ISS duration and then returned on the Orbiter in +4°C or lower storage.</p>	<p>No</p>	<p>FSB-1 Objectives</p> <p>Provide a protocol framework that will support the Fundamental Space Biology Model Specimen Approach, early science return for the use of the SSBRP Incubator. PIs will be involved in development of the specific experiment design for MYCOS and team collaboration and specimen distribution agreements.</p>

Note: The Cell Culture Unit is not included in this solicitation and will be included in a future solicitation

Table 4. Hardware Available to Support Research on *S. cerevisiae*

	Shuttle-Based	ISS-Based	Agency	Website
Fluids Processing Apparatus (FPA)	X	X	NASA	http://www.colorado.edu/engineering/BioServe/spaceflight.html
Group Activation Pack (GAP)	X	X	NASA	http://www.colorado.edu/engineering/BioServe/spaceflight.html
ADvanced SEParations Processing (ADSEP) Vented Fluid Processing Cassette		X	NASA	http://www.shot.com/Products.htm
SSBRP Incubator		X	NASA	http://brp.arc.nasa.gov/
KUBIK		X	ESA	http://spaceflight.esa.int/users/file.cfm?filename=fac-iss-kubik
Single Loop Cell Culture system (SLCC)		X	NASA	http://brp.arc.nasa.gov

2.2.2 *C. elegans* Model Specimen Flight Opportunities

C. elegans is an excellent model organism for studying the effects of space flight on living organisms for the following reasons: (1) it is an extensively utilized eukaryotic organism in the fields of developmental biology, genetics, neurology, and aging; (2) its complete genome has been sequenced and many well characterized mutants are available; and (3) its hardiness, short life cycle, and small size make it easy to maintain for long periods without complex hardware. These flight opportunities will help delineate the requirements for long term survival and will provide significant baseline data regarding development and genetic and cellular responses.

The following opportunities are described below: *C. elegans* Model Specimen in Space (CEMSS), and the Fundamental Space Biology (FSB) Incubator Experiment using *Caenorhabditis elegans* (FIERCE). These opportunities will follow the Office of Biological and Physical Research model specimen approach and will offer the opportunity for submission of full proposals that fit within the described capabilities and constraints described.

Results from these flight opportunities will provide a basic understanding of culture conditions and sample processing, and will lay the foundation for determining the role that the space environment plays on regulating *C. elegans* morphology, gene expression, development, and behavior. Precise development of *C. elegans* as a model specimen in space will allow the discovery of genes, gene products, and signaling pathways that are responsive to the environment of space. Data from CEMSS can be subsequently linked to the wealth of information from ground-based studies on Earth obtained from *C. elegans*, which is widely used in biomedical research as a model for human development, genetics, aging, and disease. This combined information will contribute to our understanding of how the space environment affects organisms at the most fundamental levels and will provide insight to potential countermeasures necessary to prepare humans for long-duration exploratory missions. Additional information on how the different flight opportunities with *C. elegans* relate to critical research questions is summarized on Table 6 “*C. elegans* Model Specimens in Space Research Questions & Objectives”.

Experimental Design and Specimens Available

Three different experimental designs will be flown to progressively answer critical questions regarding how the space flight environment effects *C. elegans* culture, growth, development, behavior, and gene expression.

CEMSS-1

The first experiment (CEMSS-1) will establish long-term cultures of *C. elegans* in space aboard the ISS for a period up to 8 months. Live specimens returned from space flight will be processed postflight at the landing site and distributed to PIs for postflight molecular and morphological studies. The experiment is targeted for launch in the timeframe between March and June 2004.

CEMSS-1 Objectives: Determine the effects that long-term exposure to the space environment has on *C. elegans* over multiple generations in space.

CEMSS-1 Protocol: Duplicate sets of a mixed population of worms will be prepared at approximately Launch minus (L-) 5 weeks. The experiment will be passively stowed in the launch vehicle and the specimens will be at ambient vehicle temperatures during launch. The specimens will be transferred to the ISS and maintained at temperatures between 18 and 26 °C. While on-orbit, a small portion of the worm population from each set will subsequently be passaged to a fresh media bag approximately every 4 to 8 weeks for 4 to 8 months on board the ISS. Worms will be maintained in liquid axenic media, passaged three to six times, and the resultant worm population (duplicate 20 ml cultures; approximately 25-45 generations after launch) will be returned alive at ambient Shuttle (or Soyuz) temperatures. Synchronous ground controls will be conducted using cultures and media from the same stock used for flight. Asynchronous ground controls will be conducted postflight utilizing the temperature conditions recorded during all mission phases. Upon return to Earth at the landing site, at approximately Recovery plus (R+) 6 hours or sooner, a portion of the worm cultures will be frozen slowly to keep them alive for production of clonal populations from single organisms and subsequent genetic and morphologic analysis. Additional portions of the worm cultures will be immediately fixed in formaldehyde and will be available for morphology/antibody staining analysis or frozen for subsequent molecular preparation and analysis.

In addition, worm cultures in the upstream bags will be available. However, upon return, these cultures will be approximately 3, 5, and 7 months old without fresh media exchange and, therefore, the scientific value of each bag will decrease dramatically for the older cultures. It is possible that sheath length determinations may be obtained from the 3 and 5 month old cultures.

CEMSS-2

The second opportunity (CEMSS-2) has two components. The first component (CEMSS-2.a) will establish axenic *C. elegans* cultures from staged dauer larvae grown in space for a relatively short period of time and then fixed on-orbit for postflight molecular and morphological/antibody staining analyses. This experiment is targeted for a 10 to 16 day mission. The second component (CEMSS-2.b) will provide long-term axenic cultures of *C. elegans* over multiple generations that are fixed in space aboard the ISS for postflight RNA, morphological, and antibody staining analyses. This opportunity is targeted for approximately 4 months on-orbit. These opportunities are scheduled to launch in the 9/2004 to 12/2005 timeframe. These opportunities will provide short- and long-term data addressing the question: How does space affect life at its most fundamental levels, from the gene to the cell?

CEMSS-2 Objectives: 1. (CEMSS-2.a) Determine short-term effects of space on *C. elegans* at its most fundamental levels, from the gene to the cell. 2. (CEMSS-2.b) Determine long-term effects of the space environment on *C. elegans* at its most fundamental levels, from the gene to the cell. The objectives of CEMSS-2.b are similar to CEMSS-2.a. However, since the opportunity is designed to maintain the population on-orbit for several months, a mixed population of worms will be fixed on-orbit for gene expression, morphology and various cellular protein level changes by antibody staining. These opportunities are designed to allow the characterization of novel signal transduction pathways used for life in the space environment.

CEMSS-2.a Protocol: Dauer worms will be launched and cultured on-orbit. This synchronized population will be subsequently passaged to a growth bag containing fresh media, releasing them from the dauer stage. The worms will be grown for approximately one generational cycle at ambient temperatures. Quadruplicate 20 ml *C. elegans* cultures will allow two of the cultures to be fixed in flight for RNA analysis and two of the cultures to be fixed in flight for morphology/antibody staining. Portions of the worm populations will be terminated on-orbit for postflight RNA analysis and for morphological and antibody staining analyses by transferring the worms to subsequent bags containing the appropriate fixatives. Live worms will be recovered from residual fluid in the growth bag for cloning purposes. These samples will be distributed postflight to PIs. Synchronous ground controls will be performed using culture and media from the same stock used for flight. Asynchronous ground controls will be performed after the mission, utilizing temperature conditions recorded during all mission phases.

CEMSS-2.b Protocol: Mixed stage worms will be cultured on the ISS for approximately 8 weeks. A small portion of the worm population will be subsequently passaged to a fresh media bag and allowed to grow for another 8 weeks. Portions of the worm populations will be stabilized on-orbit for postflight RNA analysis and portions will be fixed for morphological and antibody-staining analyses by transferring the worms to subsequent bags containing the appropriate fixatives. Live worms will be recovered from residual fluid in the growth bag for cloning purposes. These samples will be distributed to PIs. Synchronous ground controls will be performed using culture and media from the same stock used for flight. Asynchronous ground controls will be performed after the mission, utilizing temperature conditions recorded during all mission phases.

Upon return, the samples from CEMSS-2.a and CEMSS-2.b will be provided to PIs to determine how microgravity alters morphology, protein production, and gene expression. By fixing cultures on-orbit, this design eliminates potentially confounding re-adaptation responses that may occur upon return to a 1g environment. Postflight, specimens can also be collected from the residual worms in the non-fixative bags and processed for clonal analyses of mutations and to determine sheath lengths, as discussed for CEMSS-1.

CEMSS-3

The third opportunity (CEMSS-3) will establish long-term cultures of *C. elegans* during space flight. This opportunity will also allow investigation into the links between changes observed in the genome, gene expression, and development from CEMSS-1 and CEMSS-2 compared to behavior observed over long-term space flight. The experiment is designed for a 70–180 day mission on board the ISS, targeted for launch in 2005. This opportunity will provide data focused on addressing the question: How does the space environment affect the life cycle and behavior of *C. elegans*?

CEMSS-3 Objectives: This opportunity will utilize information from the previous experiments to correlate changes observed in gene expression and development with changes in behavior during long-term space flight. Long-term exposure to the space environment will likely produce multiple changes in gene expression that will lead to developmental and behavioral changes.

The focus of this opportunity will be analysis of development, behavior, morphology, and gene expression over multiple generations in space.

CEMSS-3 Protocol: Cultures of *C. elegans* will be launched, transferred to the ISS, and allowed to grow over multiple generations. Behavioral data will be captured by video imaging at designated intervals. Periodically, samples will be fixed and/or frozen for molecular and morphological analyses. The cultures flown will be based upon results from the CEMSS-1 and CEMSS-2 opportunities and may contain mutants or cultures labeled for specific analyses. Synchronous ground controls will be performed using culture and media from the same stock used for flight. Asynchronous ground controls will be performed post mission utilizing temperature conditions recorded during all mission phases. Upon return, video images, specimens fixed in flight, and specimens fixed postflight for RNA analysis and morphological/antibody staining analysis will be provided to approved investigators to provide links between alterations in gene expression and developmental and behavior changes in the organism.

Fundamental Space Biology Incubator Experiment using C. elegans (FIERCE)

This NRA solicits proposals for participation in the Fundamental Space Biology (FSB) Incubator Experiment using *Caenorhabditis elegans* (FIERCE). The experiments will be conducted on the ISS, utilizing the SSBRP Incubator. The incubators have temperature control (+4 °C to +45 °C), temperature and humidity sensors, air circulation, telemetry, data, video pass-through, and commanding capabilities.

FIERCE will follow the OBPR model specimen approach to fundamental space biology on the ISS and will review and select proposed experiments that are meritorious and fit within the described constraints of the FIERCE scenario and protocol (see below and Table 5). Proposed experiments that do not conform to the scenario and constraints of the framework will not be considered.

Experiment Design

FIERCE Objective: The FIERCE objective is to provide a protocol framework that will support the Fundamental Space Biology Model Specimen Approach and early science return for the use of the SSBRP Incubator.

FIERCE Protocol: The FIERCE protocol includes 1) four separate incubation periods, 2) video recording sessions, 3) three subculture sessions, 4) on-orbit preservation of specimen aliquots by freezing (-80 °C) or immersion in Trizol, and 5) return of live specimens. A total of 18 (10 ml) OptiCells will be inoculated preflight, with *C. elegans* in axenic medium (Lu, N. C; Goetsch K. M. *Nematologica* 39(3): 303-311). The *C. elegans*-OptiCells will be stored at +20 °C during delivery to the ISS.

Once on the ISS, the *C. elegans*-OptiCells will be transferred to the SSBRP Incubator (+20 °C): four of them will be placed into the Incubator video system holders. Periodically during each incubation period, the *C. elegans* in each video system holder will be video recorded. The video

data will be downlinked at the earliest opportunity. At the end of each incubation period, all of the *C. elegans*-OptiCells will be subcultured, at which point aliquots will be sampled for freezing at -80°C and for nucleic acid and protein preservation in Trizol. The Trizol-treated samples will be stored at -20°C or colder. For the descent phase of the experiment, the *C. elegans* from the fourth incubation period will not be processed on-orbit, and the specimens will be returned alive. The -80°C samples will be returned in a gaseous nitrogen Dewar (GN2 Dewar), and the Trizol-treated samples will be returned at $+4^{\circ}\text{C}$ or colder. A ground control experiment that mirrors the flight experiment will be conducted.

The Passive Dosimeter System (PDS) will be used to provide data on space radiation exposure during the on-orbit duration of the experiment. The PDS is designed to measure and record the biologically active space radiation dose at experimenter-defined locations. The PDS consists of a two-component assembly and a Reader (see Table 14 for details). The assembly contains one Pille Thermoluminescent Detector (TLD) system and three stacks of Plastic Nuclear Track Detector (PNTD) system inserted into a PDS holder. The PNTD component of the PDS will be used to measure dosage during the ascent and descent flights. The scenario for use of the PDS is as follows. A PDS holder containing the PNTDs will be placed into the transport container, which is carrying the *C. elegans*-OptiCells for ascent. Once on the ISS, a TLD will be inserted into the holder, and the PDS assembly will be co-localized at all times with the *C. elegans*-OptiCells. At the end of the mission, the PNTD will be returned in the same transport container as the *C. elegans*-OptiCells, and the Pille memory cards will be stowed in the Middeck. The team at the Hungarian Space Agency will analyze the data from the Pille memory cards and then the data will be delivered to the PNTD processing team. The PNTD processing team will analyze the PNTD stacks and then combine this data with the TLD data to determine the corrected total dose, dose equivalent, and average quality factor. The PDS report will be provided to PIs.

Principal Investigator Participation: PIs will participate in 1) identifying specific *C. elegans* strains (including deletion mutant series) and incubation thermal requirements, 2) ground testing studies, and 3) defining specimen processing and collaborative efforts. The consideration for each of these items must fit within the constraints of the FIERCE protocol. Depending upon the processing requirements, PIs may be involved in pre- and postflight sample operations at the launch site processing facility and primary and secondary landing facilities. PIs may be involved in ground communication activities.

Table 5. FIERCE Protocol Framework Summary

Organism	<i>C. elegans</i>
Habitat	1-2 SSBRP Incubators
On-Orbit Duration	30 to 110 days
Ascent Thermal Requirement	+ 20°C Incubation
ISS Thermal Requirement	+ 20 °C incubation + 4 °C media storage ≤-20 °C Trizol sample storage -80 °C sample storage
ISS Flight H/W	Glovebox
Descent Thermal Req.	+ 20 °C live specimens ≤+4 °C sample storage ≤-80 °C sample storage
Culturing Chamber	OptiCell
Medium	Axenic Liquid Medium* <i>C. elegans</i> maintenance medium
Incubation Period Duration	17 to 28 days
Subculture	3 sessions
Sampling	Concurrent with subculture sessions
Specimen Video Sessions	7 to 14 day intervals (4 <i>C. elegans</i> -OptiCells)
Radiation Monitoring	Passive Dosimeter System

*Lu, N.C., and Goetsch, K.M. 1993, Nematologica 39 (3):303-311

Table 6. *C. elegans* Model Specimen in Space Research Questions & Objectives

Critical Research Questions	Flight & Date	# of Days	Environment & Hardware	Media & Conditions Specimens	Fixation and/or Freeze	Video Image	Objectives (Analyses)
<u>Long Term Survival</u> : How does the unique environment of space affect <i>C. elegans</i> over multiple generations and its ability to acclimate to this new niche?	CEMSS-1 Targeted for launch in 2004	180 - 245	Passive ADSEP Vented Fluid Processing Cassette (VPC)	Liquid axenic Passaged Mixed Population	<u>Fixed Post Flight</u> -Quick Freeze (LN2) -Slow Freeze with glycerol for clonal analyses -*Formaldehyde / β -mercaptoethanol -Residual worms will be processed for analysis of sheath lengths, indication of general health, and population numbers	No flight Ground only	Live specimens and fractions returned from space flight will be fixed or frozen postflight at the landing site and distributed to PIs for various molecular and morphological analyses
<u>Short Term Survival & Flourish</u> : How do space environments affect life at the molecular and cellular levels? (Determine short-term effects of space on <i>C. elegans</i> at its most fundamental levels, from the gene to the cell.)	CEMSS-2.a Targeted for launch in 2004	10 - 12	Passive ADSEP Vented Fluid Processing Cassette (VPC) Passive Kennedy Space Center Fixation Tube	Liquid axenic Staged Liquid NGM vs. Axenic media	<u>Fixed In Flight</u> -RNALater /BME -*Formaldehyde /BME -Residual worms will be processed for clonal analyses of mutations, sheath lengths, general health, and population No fixative (KFTs)	No flight Ground only	Fractions of samples fixed in flight will be distributed postflight to PIs for molecular and morphological analysis, media comparison, and behavioral analyses.
<u>Long Term Survival & Flourish</u> : How do space environments affect life at the molecular and cellular levels? (Determine long-term effects of space on <i>C. elegans</i> at its most fundamental levels, from the gene to the cell.)	CEMSS-2.b Targeted for launch in 2004 - 2005	180 - 145	Passive ADSEP Fluid Processing Cassette (FPC)	Liquid axenic & passage Staged. Mutants	<u>Fixed In Flight</u> -RNALater /BME -*Formaldehyde/BME -Residual worms will be processed for clonal analyses of mutations, sheath lengths, general health, and population numbers	No flight Ground only	Fractions of samples fixed in flight will be distributed postflight to PIs for molecular and morphological analyses

Table 6 continued: *C. elegans* Model Specimen in Space Research Questions & Objectives

Critical Research Questions	Flight & Date	# of Days	Environment & Hardware	Media & Conditions Specimens	Fixation and/or Freeze	Video Image	Objectives (Analyses)
<u>Long Term Survival & Flourish</u> : Can life be sustained and thrive in space across generations?	CEMSS-3 Targeted for launch in 2005	90 to 240	Controlled thermal carrier BRIC-60, GN2 freezer Opticells, CBOSS, ARTIC being considered ADSEP FPC	Liquid axenic & passage Staged mutants	Cultures will be allowed to grow over multiple generations. Behavioral data will be captured on-orbit by video at designated intervals. Periodically, samples will be fixed/frozen for molecular and morphological analysis.	Yes, flight video	Developmental comparisons: lifespan, Upon return. video images and fixed samples will be distributed to PIs for behavioral, molecular anatomical and physiological analyses.
<u>Long Term Survival & Flourish</u> : How do space environments affect life at the molecular and cellular levels? How does the unique environment of space affect <i>C. elegans</i> over multiple generations and its ability to acclimate to this new niche?	FIERCE Targeted for launch in 2006	30 to 110	Thermal control carrier, SSBRP Incubator, Incubator Video System, OptiCell, PDS, CONTEX	Liquid axenic and subculture Wild-type or the PIs will select <i>C. elegans</i> strain	Cultures will be grown over multiple generations. Movement and behavioral data will be collected by video at specific time points. Periodically, samples will be subcultured and sampled for preservation by -80 °C freezing or treatment with Trizol	Yes, flight and ground video	Live and preserved samples will be returned. The PIs will develop postflight sample distribution and processing activities. The on-orbit video recordings will be downlink periodically during flight. The flight and ground video recordings will be provided to the PIs.

Table 7. Hardware Available to Support Research on *C. elegans*

	Shuttle-Based	ISS-Based	Agency	Website
ADvanced SEParations Processing (ADSEP) modified Vented Fluid Processing Cassette	X	X	NASA	http://lsda.ksc.nasa.gov/Hardware/GetSpecificHardware.pl?hdw=bric
Space Station Biological Research Project (SSBRP) Incubator		X	NASA	http://brp.arc.nasa.gov/

2.2.3 Plant Model Specimen Flight Opportunities

During the time period for which these solicitations apply, plant research is limited to experiments using *Arabidopsis thaliana* and *Brassica rapa*. Proposals using these plant organisms will be considered if their use is justified for specific scientific investigations and fits within the constraints listed below. Proposals must include appropriate previous ground research data/results that show why a flight experiment is required. Two hardware platforms are available, the European Modular Cultivation System (EMCS) and the Advanced Biological Research System (ABRS). Either hardware is compatible with experiments starting from seeds and both will support experiment durations up to 130 days. ABRS is capable of launching live plants, which can remain in ABRS hardware or transported to EMCS on-orbit. The crew can access plant chambers on-orbit in both pieces of hardware. Additionally, on-orbit chemical fixation and freezer hardware will be available for harvested tissue. Specifics of the hardware capability are provided in the matrix below and at the websites listed in Table 8. Requirements for use of available hardware and resources will need to be addressed in Form F.

Table 8: Hardware Available to Support Research on Plants

	Shuttle-Based	ISS-Based	Agency	Website
European Modular Cultivation System (EMCS)		X	ESA	http://www.spaceflight.esa.int/users/file.cfm?filename=fac-iss-dest-emcs
Advanced Biological Research System (ABRS)		X	NASA	http://lsda.ksc.nasa.gov/Hardware/GetSpecificHardware.pl?hdw=abrs

Table 9: Plant Growth Chamber Capabilities

	ABRS	EMCS
Growing area	2 chambers @275 cm ²	8 chambers @ 36 cm ²
Maximum plant height	19.0 cm	16.0 cm
Illumination	max 300 μmol/m ² /s	180-250 μmol/m ² /s
Photoperiod	User adjustable	User adjustable
Temperature	10-35 °C controlled	18-40 °C controlled
Water/nutrient addition	Automated	Automated
Atmospheric separation	2 chambers, using cabin air with filtration and ethylene removal	8 chambers, using bottled gases mixed to provide user-required atmospheric composition
Atmospheric CO ₂ control	0.03%-ambient controlled	0.01-0.2% & 0.2-5.5% controlled
Atmospheric ethylene removal	25 ppb maximum	Purafil cartridge
Atmospheric relative humidity	60-80%, individually controlled for each growth chamber	50-90%, individually controlled for each growth chamber, 30% dry mode
Downlink	Data and video	Data and video
Imaging	Visible light	Visible and IR light

Gravity level	0-g	0-g, 10^{-3} g – 2.0 g
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It is the intent of the model specimen approach to solicit and select appropriate meritorious experiments which fit within defined space flight constraints. It is anticipated that this approach will facilitate rapid implementation of selected experiments and increase flight opportunities. These flight opportunities will occur on the ISS; it is expected that this approach will aid in maximizing ISS science productivity. Proposed experiments which do not fit within the experiment scenarios and constraints listed below will not be considered for selection. It should be noted that teaming and sharing of specimens and resources across investigations is highly desired.

Many critical questions regarding plant gravitational biology need to be answered. For example, what is the gravitational threshold for normal and productive plant growth and development? Can plants go from seed to seed in the space flight environment? Can we grow plants reliably and repeatedly in space? Are biological (microbial) communities stable in the space environment, in particular plant-associated microbial communities growing under closed environmental conditions?

Experiment Scenarios:

Description: The experiment can be initiated from either seeds or live plants. Flight opportunities for seeds will occur at a greater frequency than those with live plants. Plant growth in a controlled environment can be accommodated at microgravity and variable artificial gravity conditions while on the ISS. Two hardware platforms are available, the European Modular Cultivation System (EMCS) and the Advanced Biological Research System (ABRS). See Tables 8 and 9 for information about the functional capabilities of these hardware systems. Candidate proposals must include explicit justification for the need to launch live plants, return live plants, and/or utilize on-orbit centrifugation. Requirements for use of available hardware/resources will need to be addressed in Form F.

Mission Scenarios:

Experiment operations, vehicle constraints, and operational activities vary during different phases of a mission and are dependant on the physical location of the payload hardware and/or biological samples. The following phases address capabilities and limitations during those periods.

Late Access/Ascent:

Payload hardware and biological specimens are loaded into the Shuttle Middeck or into the Multi-Purpose Logistics Module (MPLM) in the shuttle payload bay before launch. Loading of the MPLM typically occurs one to two months prior to launch with limited access approximately 4 days before launch. Access to the Middeck can be as late as 17 hours prior to launch on a limited basis.

Passive thermal carriers, ambient temperature, and soft stowage are also available for transport of biological specimen. Opportunities for launch of live plants to the ISS will

be available but less frequent than launches of dry seeds. Proposals must address the experimental requirement to launch live plants versus seeds.

During the ascent phase, limited crew procedures may be performed on the experiment/hardware before Shuttle/ISS docking (launch + three days). Proposals must provide explicit justification for crew-mediated operations during this flight phase. Items that are stowed in the MPLM will not be accessible during this period.

Docked Operations:

During docking operations (docking operations are defined as launch + 3 days through launch + 10 days), experiment hardware/stowage will be transported from the Middeck and MPLM to the ISS. During this phase, it is highly unlikely that the flight crew will be available for performing experiment operations.

ISS Operations:

The duration of the proposed experiments on the ISS may range from days to weeks. Given the constraints and priority of the ISS assembly requirements, it is advantageous if the proposed experiments are flexible regarding initiation, termination, and operational performance. Plant growth can be accommodated at various gravity levels during ISS operations using EMCS.

If biological samples are to be collected during the experiment and/or at its end, the proposal must address how the samples will be stored. Chemical fixation will be possible. Refrigerator or freezer storage of fixed or unfixed samples will be available but limited. Proposals that utilize sample storage at ambient temperature are preferable for this solicitation.

Descent/Early Access:

Limited opportunities are available for the return of live plants. During the descent phase, crew operations on plants (i.e. harvesting, fixation) will be highly limited. Limited freezer volume will also be available. The proposal must address the need for the return of live plants, the descent crew operations required, and the justification for fixation/freezing.

Early access (within 3 hours) to the hardware/samples following landing is available for items stowed in the Middeck. Items that are stowed in the MPLM will be accessible within 2-3 days following landing.

3.0 General Support Capabilities

3.1 Temperature-Controlled Storage

There are a number of hardware systems and methods for the maintenance of specific temperatures for specimens or preserved samples:

- Ambient Storage (approximately 20°C – 28°C)
- Refrigeration (+4°C)
- Freezing (-20°C to -196°C)

General information on cold storage capabilities is available to NASA computer users at <http://iss-www.jsc.nasa.gov/ss/issapt/payofc/OZ2/ColdStow.html>. Others may obtain the information by contacting Yeun Cheung at (281) 226-6999 or yeun.s.cheung@boeing.com. Experiment operational requirements, hardware availability, and sample volumes dictate which system or combination of systems is used to accommodate specific experiment objectives.

Table 10: Hardware Available for Temperature-Controlled Storage

	Shuttle-Based	ISS-Based	Agency	Website
Incubators	X	X	NASA	http://brp.arc.nasa.gov/
Passive Freezers	X		NASA	
Minus Eighty Degree Life Sciences Freezer		X	NASA/ESA	
GN ₂ Freezers (single/double locker)	X	X*	NASA	http://lsda.ksc.nasa.gov/Hardware/GetSpecificHardware.pl?hdw=kscgn2
BRIC – Passive Cooler	X*	X*	NASA	http://lsda.ksc.nasa.gov/Hardware/GetSpecificHardware.pl?hdw=bric
Passive Thermal Cooling Unit (PTCU)	X	X*	ESA	

*To be used for thermal transport up/down, not for extended storage.

3.2 Chemical Fixation

Several options are available to chemically preserve specimens prior to return to Earth for analysis. Fixation cocktails would need to be tested in the specific hardware for biocompatibility. Previous flights have allowed chemical fixation with glutaraldehyde- and formaldehyde-based cocktails. The investigator is encouraged to suggest less toxic chemical fixatives to decrease the use of hazardous materials.

Table 11: Hardware Available for Chemical Fixation

	Shuttle-Based	ISS-Based	Agency	Website
KSC Fixation Tube (KFT)	X	X	NASA	http://lsda.ksc.nasa.gov/Hardware/GetSpecificHardware.pl?hdw=kft
Petri Dish Fixation Unit (PDFU)	X	X	NASA	http://lsda.ksc.nasa.gov/Hardware/GetSpecificHardware.pl?hdw=pdfu
Fluid Processing	X	X	BioServe	http://www.colorado.edu/engineering/

Apparatus/Group Activation Pack (FPA/GAP)			Space Technologies	BioServe/spaceflight.html
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3.3 Mass Measurement

The ISS will have the capability to measure the mass of the human body.

Table 12: Hardware Available to Measure Mass

	Shuttle-Based	ISS-Based	Agency	Website
Body Mass Measurement Device (human)		X	NSA	http://hrf.jsc.nasa.gov/SLAMMD.htm

3.4 Computers

Laptop computers outfitted with mass storage devices, communication adapters, power supplies and cables, and custom-built software are available for use. These laptops support software compatible with a Microsoft Windows operating system.

A computer workstation is available that is capable of providing high capacity data collection and mass storage, display of high resolution graphics, video processing, and real-time data processing. The workstation is compatible with a wide variety of operating systems, including DOS/Windows, UNIX/X-windows, OS/2, Windows NT, and Mac OS. The workstation will also be capable of uploading and downloading software and data and be capable of multichannel equal interval sampling and precise reaction time measurement.

Table 13: Computers Available

	Shuttle-Based	ISS-Based	Agency	Website
Laptops	X	X	NASA	
Human Research Facility Portable Computer		X	NASA	http://hrf.jsc.nasa.gov/pc.htm
Human Research Facility Computer Workstation		X	NASA	http://hrf.jsc.nasa.gov/r2ws.htm

3.5 Radiation Monitoring

A passive dosimeter system will be available on the ISS to determine the space radiation dose for payloads at specific locations within the ISS. It uses thermoluminescent detectors (TLDs) to accumulate exposure and a reader/annealer to measure that exposure on-orbit. TLD sensitivity varies depending on the energy spectrum of the radiation present. Therefore, it is necessary to use plastic nuclear track detectors (PNTDs) to determine the energy spectrum of the radiation absorbed by the TLDs. The PNTDs will be co-located with TLDs during dose accumulation. The PNTDs will be returned to the ground to be processed and analyzed in a laboratory to obtain the linear energy transfer (LET) spectrum. The LET spectrum is then combined with the dose information from the TLDs to determine a corrected total dose. This system can provide dose information for periods as short as 10 minutes or as long as one year.

Three active dosimeter systems will be available on the ISS: the Real-Time Radiation Monitoring Device (RRMD), a tissue equivalent proportional counter (TEPC), and two charged particle directional spectrometers (CPDSs). Incidences of charged particles detected by RRMD will be monitored from the ground in real time. Small chambers for biological specimens and passive dosimeters may be attached to the RRMD sensor unit. The TEPC will be moved around the pressurized volume of ISS. The CPDSs have limited real-time data collection capability. One will be housed inside the Habitation Module (not available until late 2004), and the other, a triple CPDS with 3-axis sensitivity, is located outside on the S0 truss. The intravehicular CPDS will be moved from module to module to conduct surveys. Initially, the instruments' first priority will be to support operational measurements, including contingencies. Eventually, the data is expected to become available for payload users.

Table 14: Radiation Monitoring Tools

	Shuttle-Based	ISS-Based	Agency	Website
Tissue Equivalent Proportional Counter	X	X	NASA	
Charged Particle Directional Spectrometer	X	X	NASA	
Passive Dosimeter System	X	X	NASA	http://brp.arc.nasa.gov/GBL/Lab_Eqpmnt/measure.html
Passive Dosimeters	X	X	NASA	
Small-Size Passive Dosimeter Package	X	X	JAXA	http://jem.tksc.jaxa.jp/kibo/kibomefc/index_e.html
Real-Time Radiation Monitoring Device	X	X	JAXA	http://jem.tksc.jaxa.jp/kibo/kibomefc/index_e.html

3.6 Video Imaging

Activities may be documented using video (8 mm camcorder) and still cameras (35 mm, positive and negative). Most habitats for nonhuman specimens provide both data and video downlink.

Various image data taken by video or digital cameras inside of experiment hardware will be accepted by the Image Processing Unit (IPU) through the ISS data network. IPU will encode or edit the image data. NTSC video image inputs will be digitized into MPEG2. Still images will be compressed to TIFF/LZW format and downlinked. The IPU also has capability to store images in digital videotapes or removable hard disks.

Table 15: Video Imaging

	Shuttle-Based	ISS-Based	Agency
Cameras	X	X	Various
Incubator Video System Tray		X	NASA
Image Processing Unit		X	JAXA

3.7 Centrifuges

In addition to the centrifuges that are built into various habitats and facilities and the EPM hematocrit centrifuge, a refrigerated centrifuge will be available for processing of biological samples such as blood and saliva.

Table 16: Centrifuges

	Shuttle-Based	ISS-Based	Agency	Website
HRF Refrigerated Centrifuge		X	NASA	http://hrf.jsc.nasa.gov/rc.htm

3.8 Gloveboxes and Specimen Manipulation

Gloveboxes provide an enclosed environment to conduct manipulations of specimen, chambers, other materials, and the science support equipment necessary to conduct experiments in orbit. These gloveboxes have been designed to isolate the crew from potentially hazardous materials used during experiment operations (such as fixations, injections, waste removal, and dissections) while maintaining an internal environment suitable for specimen manipulation. There are also a large number of tools, surgical instruments, and kits designed for a wide range of applications in support of on-orbit biomedical and fundamental biology investigations.

Table 17: Gloveboxes and Specimen Manipulation

	Shuttle-Based	ISS-Based	Agency	Website
Life Sciences Glove Box		X	NASA	http://brp.arc.nasa.gov/
CONTEX		X	NASA	http://www.bradford-space.com/

3.9 Microscopes

The ISS will provide advanced microscopy capabilities for specimen manipulation and observation with the Light Microscopy Module (LMM). Information on the LMM is available at <http://microgravity.grc.nasa.gov/6712/lmm.html>. This microscope is a modified commercial research imaging light microscope with laser-diagnostic software and interfaces. Objective lenses include 10X (NA 0.3), 40X (NA 0.85), 50X (NA 0.55), 63X (NA 0.7), and 100 X (1.40). Other features include color video microscopy, brightfield, darkfield, phase contrast, differential interference contrast (DIC), spectrophotometry (400 nm to 700 nm), confocal microscopy combined in a single configuration, and laser tweezers for sample manipulation. Confocal imagery is accomplished using a Nipkow disk and fluorescence excitation at 532 nm. Wide field fluorescence imaging is supported using the fiber-coupled output of the Nd:YAG laser operating at 532 nm, the 437 nm line of a mercury arc, or appropriate narrow-band filter of the FIR provided metal halide white light source. The LMM provides an enclosed work area (Auxiliary Fluids Container, AFC) with gloveports and a configurable Equipment Transport Module for transfer of specimens to the LMM. The AFC is sealed to provide a clean working environment and one level of containment.

4.0 Flight Proposal Evaluation Process

This section describes the evaluation and selection process that will be used for flight experiment proposals submitted to any member agency of the International Space Life Sciences Working Group (ISLSWG) in reply to the coordinated 2004 Space Life Sciences Research Announcements.

Each research proposal must be a complete response to the appropriate individual space agency's official solicitation. In that solicitation, an agency may define a number of critical constraints that proposals must satisfy to be considered for selection. For example, an agency may not accept proposals for work in certain discipline areas. Proposals to these agencies to carry out work that is not responsive to their solicitation will be returned without further review. For this reason, individuals are advised to communicate with their agency officials before submission if there is any doubt of the acceptability of a proposal by the agency in question.

Compliant proposals submitted in response to the Space Life Sciences Research Announcements will undergo an intrinsic scientific or technical merit review. Proposals that receive a passing score in this review will then undergo additional review(s) as follows:

- Flight feasibility review
- Relevance to the programs of the soliciting agencies
- Cost (applicable to proposals submitted to NASA, JAXA, and CSA only)

Proposals will undergo the following three-tiered review process to assess these factors.

4.1 Scientific or Technical Merit Review

The first review will be a merit review by a panel of international scientific or technical experts. The number and diversity of experts required will be determined by the response to this research announcement and by the variety of disciplines represented in the proposals. The merit review panel will assign a **score from 0 to 100** or a designation of "not recommended for further consideration" based upon the intrinsic scientific or technical merit of the proposal. This score will reflect the consensus of the panel.

The score assigned by this panel ***will not be affected by the cost of the proposed work, nor will it reflect the programmatic relevance of the proposed work.*** However, the panel will have the opportunity to include in their critique of each proposal any comments they may have concerning the proposal's budget and relevance.

The following will be used to determine the merit score:

- **Significance:** Does this study address an important problem? If the aims of the application are achieved, how will scientific knowledge or technology be advanced? What will be the effect of these studies on the concepts, methods, or products that drive this field?

- **Approach:** Are the conceptual framework, design, methods, and analyses adequately developed, well integrated, and appropriate to the aims of the project? Does a flight proposal build upon a successful foundation of ground studies? Is the proposed approach likely to yield the desired results? Does the applicant acknowledge potential problem areas and consider alternative tactics?
- **Innovation:** Does the project employ novel concepts, approaches, or methods? Are the aims original and innovative? Does the project challenge existing paradigms or develop new methodologies or technologies?
- **Investigator:** Is the investigator appropriately trained and well suited to carry out this work? Is the work proposed appropriate to the experience level of the Principal Investigator and any Co-investigators? Is the evidence of the investigator's productivity satisfactory?
- **Environment:** Does the scientific environment in which the work will be performed contribute to the probability of success? Do the proposed experiments take advantage of unique features of the scientific environment or employ useful collaborative arrangements? Is there evidence of institutional support?

4.2 Flight Feasibility Review

A second review will be an evaluation of the feasibility of the proposed work using available facilities on a space platform. The flight feasibility review will be conducted for each flight experiment proposal that receives a scientific merit score greater than a threshold score agreed upon by the ISLSWG Steering Committee. An international team of engineers and scientists experienced in the development of space flight experiments will conduct this review. For this reason, experimental requirements and procedures should be clearly and succinctly explained in terms that a layperson can understand.

In addition to the actual proposal, the information requested in Form F is essential to the flight feasibility review. Flight experiment proposals submitted without the information requested in Form F will not be evaluated.

It is important to note that during this early utilization phase of the ISS, resource constraints on the shuttle and ISS will favor selection of proposals with simple requirements and procedures including experiment equipment mass, volume, power, crew training, and crew subject/operating time. Of particular concern regarding the feasibility of a proposal is the identification of risk factors which could affect the implementation of an otherwise meritorious proposal. Therefore, the feasibility of implementing the proposal and associated risks will be evaluated using the following technical criteria:

- **Functional Requirements:** Will the planned flight and ground hardware meet the requirements of the experiment? What experiment-unique hardware will be required, and can it be developed in time for projected flight opportunities? Are the number of subjects or specimens required attainable within a reasonable period of time (1-2 years for non-

humans, 2-3 years for human subjects) considering projected flight opportunities and other competition for those flight opportunities?

- **Operational Feasibility:** How complex are the experimental procedures? Will the crew have sufficient time to be trained to perform the experiment? Will they have sufficient time in their schedule to perform the experiment? Are the requirements for launch vehicle loading and unloading of the experiment specimens compatible with the capabilities of these vehicles? Can requirements for data collection on human subjects be accommodated in the preflight and postflight schedules for the astronauts? Has the experimental protocol taken into account the unavoidable period of time between the launch of an experiment and the actual initiation of the experiment? Will the experiment requirements for crew time, experiment volume, mass, power, or other features of on-orbit operations (such as temperature-controlled storage) affect the completion of this or other experiments? What other impacts will the experiment have on activities or experiments planned for the same mission?
- **Environmental Health and Safety:** Are there elements of the proposed ground or flight activities that pose concerns for the health and safety of personnel and/or the environment? For experiments that utilize the crew as research subjects, could the implementation of these experiments, even if considered safe, lead to an impact on their performance with respect to their other crew duties? Is it possible that specific restrictions on the human subjects (such as diet, exercise, etc.) will interfere with their other activities?

Using the risk factors identified in the evaluation, a score will be assigned to indicate this level of uncertainty. The risk assessment score categories are:

Low Risk: minimal risk to the successful achievement of objectives

Medium Risk: moderate risk to the successful achievement of objectives

High Risk: extreme risk to the successful achievement of objectives

The Principal Investigators will not be provided the risk assessment score, but in cases where the decision to not select a proposal is based in part on the technical evaluation, a description of the identified risk factors will be provided.

4.3 Evaluation of Programmatic Relevance and Cost

A third review will evaluate the programmatic relevance and cost of proposals that meet scientific/technical merit and flight feasibility criteria. This review will be conducted independently by program scientists and managers from each soliciting agency for proposals submitted to their specific solicitations. Programmatic relevance is determined by the contribution of the proposed work to the balance of scientific and technical issues identified by agencies in their research announcements.. Review of cost is applicable to proposals submitted to only CSA, JAXA, and NASA. Evaluation of cost will also be performed for proposals submitted to other agencies that include a component requiring CSA, JAXA, or NASA funding.

Evaluation of the cost of a proposed effort will include consideration of the realism and reasonableness of the proposed cost and the relationship of the proposed cost to available funds.

4.4 Recommendation for Selection for Further Definition

The results of the three levels of review will be used to prepare a recommendation for selection for further definition developed by each of the soliciting agencies. This recommendation will be based on:

1. The numerical merit score from the peer review panel
2. The results of the flight feasibility review
3. The programmatic relevance
4. Cost (applicable as described in Section 5.9)

A high merit score does not guarantee selection. A proposal must also be feasible to implement, have programmatic relevance, and have reasonable projected costs to be selected. The members of the ISLSWG will meet to ensure appropriate coordination of all their selections to optimize science return and resource utilization. For example, the composite selection will not greatly exceed the projected flight opportunities. In addition, it may be more efficient or effective to form international teams of researchers requiring similar resources to address overlapping questions than to have individuals competing for the use of the same specimens or test subjects. Such teams are best formed at the time of selection and early in the experiment definition period, rather than later during the flight experiment development process.

Following this coordination meeting of the ISLSWG, each agency will finalize and announce its own selections.

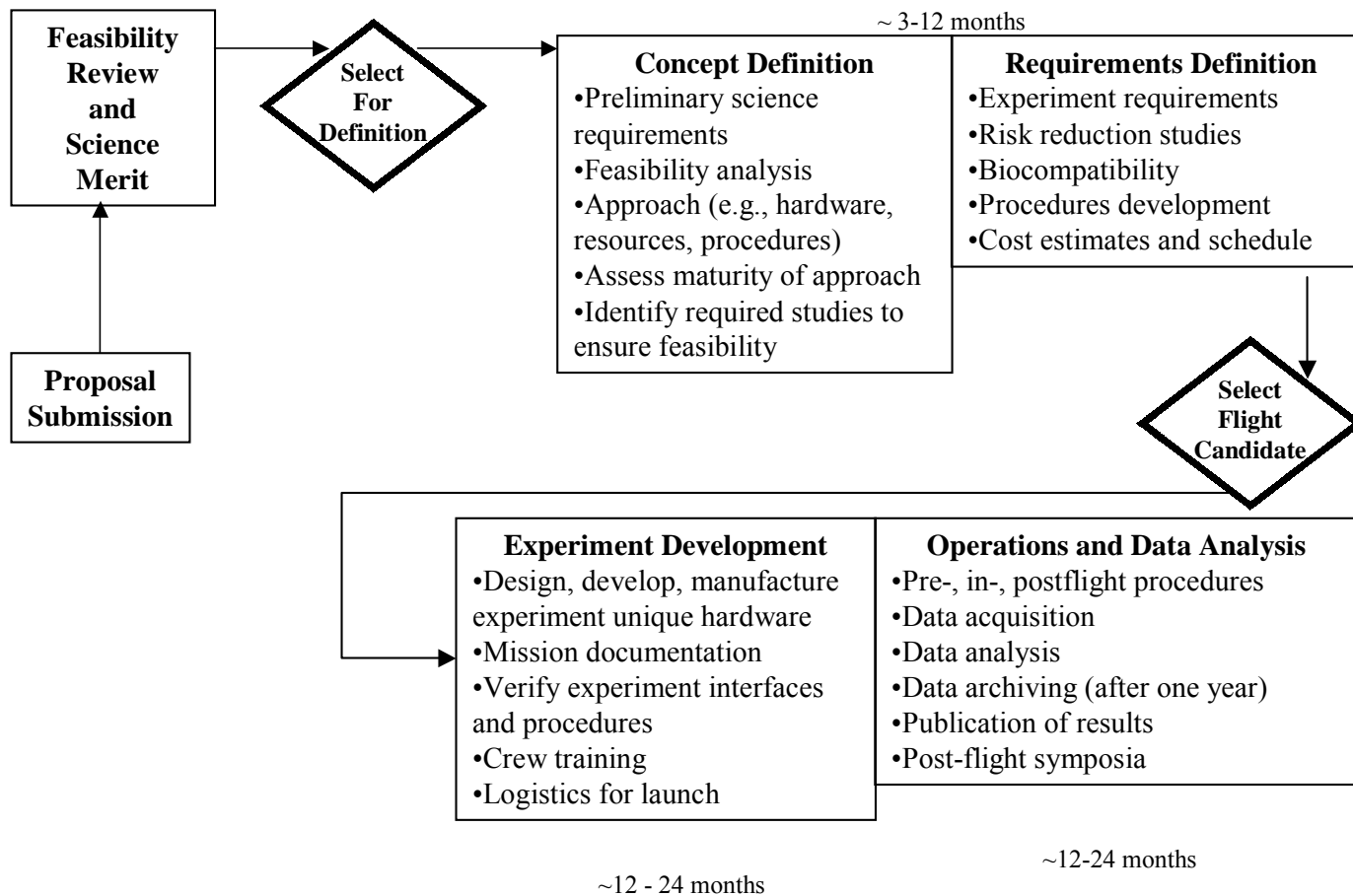
4.5 Flight Experiment Implementation

Applicants should be aware that flight experiment implementation is a multi-step process (Figure 2). Following the complete review of flight proposals, successful investigators will receive a letter informing them that their experiment has been selected for entry into a definition period. During the definition period, the agency with management responsibility for the experiment will interact with the investigator to determine specific hardware and operational requirements needed to achieve the proposed objectives. Identification of issues that will affect implementation of the space flight experiment and refinement of the funding requirements are key components of the definition period. After successful completion of the definition period, the experiment will be selected for flight and will enter a development period, leading eventually to implementation on a space mission. Detailed budgets will be refined or negotiated for each flight experiment during each period. The flight experiments selected will be reviewed every year and may be deselected based on the policy of each agency for deselection. One or more of the following conditions may warrant deselection:

1. Definition activities have indicated that the experiment is technically infeasible or so high risk that successful completion is unlikely.

2. Ground-based studies conducted as part of the definition period, or related research in the field, produce results that demonstrate the hypothesis of the flight experiment to be flawed.
3. The projected costs of the experiment, as determined during definition, are significantly greater than anticipated funding levels will support.
4. The investigator does not maintain a reasonable publication record in peer-reviewed journals in the specific research area to which the flight experiment is directed or with the results from previous flight experiments.
5. The experiment has been in the definition period for three or more years, due to either the lack of flight opportunities or the failure on the part of the investigator to complete definition activities.
6. Weaknesses identified in the scientific evaluation of the original proposal were not addressed during the definition period.
7. Funding limitations require reduction in the flight program. In such cases, the original proposal and critiques, the cost of the investigation, the ongoing publication record, and the length of time the investigator has been in definition will be considered in determining which experiments will be deselected.

Figure 2: Experiment Definition and Selection for Flight Process



5.0 International Application Forms and Instructions for Proposal Preparation

This section contains the general instructions for submission of a notice of intent, proposal preparation, and the specific forms required by individuals responding to agency solicitations for flight experiments in the Space Life Sciences for 2004. *Applicants from U.S. institutions are referred to the companion NASA Research Announcement “Research Opportunities for Flight Experiments in Space Life Sciences”, NRA 04-OBPR-01, for instructions.*

The following forms are included at the end of this section:

- Checklist for International Proposers
- International Cover Page
- International Proposal Abstract
- Biographical Sketch (Form B)
- Summary Budget Form/Budget Justification (Form C)
- Detailed 12-Month Budget (Form D)
- Other Support (Form E)
- Space Flight Experiment Requirements Summary (Form F)

The required forms are available in the International Forms Package at:

http://research.hq.nasa.gov/code_u/nra/current/NRA-04-OBPR-01/index.html

Applicants from U.S. institutions should use the NASA Forms Package available at the same site.

5.1 Notice of Intent

A notice of intent (NOI) to propose is requested by March 2, 2004. NOIs should be submitted via email to:

noi@hq.nasa.gov

The subject heading of the e-mail message should read “Notice of Intent-ILSRA2004.” NOIs should include the following information:

Principal Investigator’s name, email, phone number, and institution
Co-Investigators’ names and institutions
Project Title
Project Summary
Funding Agency
Science Area/Organism

If you do not have access to e-mail, you may submit an NOI by U.S. Postal Service or commercial delivery to the address listed below. Proposals and NOIs mailed through the U.S. Postal Service by express, first class, registered, or certified mail are to be sent to the following address:

NASA Peer Review Services
SUBJECT: ILSRA 2004 Flight Experiments in Space Life Sciences
500 E Street, SW
Suite 200
Washington, DC 20024-0001

Proposals and NOIs that are hand delivered or sent by commercial delivery or courier services are to be delivered to the above address between 8:00 AM and 4:30 PM. The telephone number, 202-479-9030, may be used when required for reference by delivery services. NPRS cannot receive deliveries on Saturdays, Sundays, or Federal holidays. Upon receiving a proposal, NPRS will send notification to the investigator confirming its arrival; however, there will not be a response from OBPR.

General Instructions for Proposal Preparation

The information contained in these instructions is specific to the research solicitations and repeats or supplements the general guidance provided in agency specific announcements.

All international proposals should include one copy of each of the International Checklist for Proposers, International Cover Page, Abstract, Biographical Sketch (Form B), and Space Flight Experiment Requirements Summary Form (Form F). In addition, proposals submitted to CSA should include Summary Budget (Form C) and Detailed Budget (Form D) and Other Support (Form E). Proposals submitted to an international solicitation which include Co-investigators from institutions in the U.S. or Canada should include Forms C, D and E, completed with the budgetary requirements of these Co-investigators.

The proposal must include the following material, in this order:

- (1) International Cover Page*
- (2) International Proposal Abstract
- (3) Proposal Title Page, with Notice on Restriction on Use and Disclosure of Proposal Information, if any
- (4) Project Description
- (5) Management Approach
- (6) Biographical Sketches (Form B)
- (7) Facilities and Equipment
- (8) Special Matters (specific information on human subjects protocol approval required, if applicable)*

- (9) Summary Budget/Budget Justification (Form C), if applicable
- (10) Detailed Budget, 12-Month (Form D), if applicable
- (11) Other Support (Form E), if applicable
- (12) Letters of Support from Collaborations and Letter of Assurance of Foreign Support (if applicable)
- (13) Appendices, if any (reviewers are not required to consider information presented in appendices)
- (14) Space Flight Experiment Information Summary (Form F)

* One signed original required

In addition, one copy of the Checklist for International Proposers Form should be submitted with the transmittal letter.

The Project Description section is limited to twenty (20) pages. Pages beyond the 20-page limit in this section will not be reviewed. There is no specific page limitation on other sections of submitted proposals. However, every effort should be made to keep proposals as brief as possible. The name of the Principal Investigator should appear in the upper right hand corner of each page of the proposal, except on the forms in this document where special places are provided for this information. Note that the proposal must specify the period of performance for the work described; periods of performance may be for any duration up to three years but should be suitable for the project proposed. This cost/budget information will be utilized by the review team to gauge a relative cost profile and magnitude of the overall budget. A more detailed budget assessment will be conducted during the definition period and presented as part of the formal selection for flight review.

The following paragraphs provide instructions for filling out the forms.

5.2 International Cover Page

All of the information requested on the International Cover Page Form must be provided, and one original signature version of this form should be submitted.

For Item (6) on this form, new means that a proposal for this project was not submitted to the last International Life Sciences Research Announcement (ILSRA) in 2001, renewal means that this proposal is for the continuation of an already funded task beyond the term of the funded proposal, and revised means that this proposal represents a revision of a proposal submitted to the 2001 ILSRA competition. A proposal previously submitted but not selected should be termed revised even if the original Principal Investigator has changed for 2004. Renewal and revised applications should contain special material described in the Project Description section below.

Note that Items (9) and (10) require assurance of compliance with human subject provisions of agency and governmental regulations. Applicants should refer to the agency solicitation for specific instructions in this area.

5.3 International Proposal Abstract Form

The information requested on this form is essential to the review of the proposal. It determines how the application will be evaluated and which agency manager(s) will receive the final review materials for possible inclusion in one of the research programs of the agency.

5.4 Proposal Title Page

The title page should contain the project title, name and address of the submitting institution, the name, address, and telephone number of the Principal Investigator, and the names and institutions of any Co-investigators. Principal Investigators should refer to agency-specific solicitations for instructions regarding additional information that should be included on the title page.

5.5 Project Description

The length of the Project Description section of the proposal should not exceed twenty (20) pages using regular (12 point) type. Any pages beyond the twenty-page limit will not be reviewed. The proposal should contain sufficient detail to enable a reviewer to make informed judgments about the overall merit of the proposed research and the probability that the investigators will be able to accomplish their stated objectives. The proposal should clearly indicate the relationship between the proposed work and the research emphases defined in the agency-specific solicitations. The development of a clear hypothesis, along with the available data evidence, should be emphasized in this section. In addition, the proposal should provide evidence of completed or planned ground research to justify the flight experiment.

5.6 Management Approach

Each proposal must specify a single Principal Investigator who is responsible for carrying out the proposed project and coordinating the work of other personnel involved in the project. In proposals that designate several senior professionals as key participants in the research project, the management approach section should define the roles and responsibilities of each participant and note the proportion of each individual's time to be devoted to the proposed research activity. The proposal must clearly and unambiguously state whether these key personnel have reviewed the proposal and endorsed their participation.

5.7 Personnel/Biographical Sketches (Form B)

The Principal Investigator is responsible for direct supervision of the work and must participate in the conduct of the research regardless of whether or not compensation is received under the award. A short biographical sketch of the Principal Investigator, including his or her current position title, educational background, a list of major publications, and a description of any exceptional qualifications, must be included. In chronological order (concluding with present

position), list previous employment, experience, and honors. Include present membership on any government public advisory committees. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. Do not exceed two pages. Omit personal information that does not merit consideration in evaluation of the proposal. Complete Form B for other senior professional personnel who will be directly associated with the project. Provide the names and titles of any other scientists and technical personnel associated substantially with the project in an advisory capacity. Universities should list the approximate number of students or other assistants, together with information as to their level of academic attainment. Any special industry-university cooperative arrangements should be described.

5.8 Facilities and Equipment

Describe the available facilities and major items of equipment specially adapted or suited to the proposed project, and any additional major equipment that will be required. Identify any government-owned facilities, industrial plant equipment, or special tooling proposed for use on the project. Provide evidence that such facilities or equipment will be made available if the applicant is successful in obtaining funding. Before requesting a major item of capital equipment, investigators should determine if sharing or loan of equipment already within the organization is a feasible alternative to purchase. Where such arrangements cannot be made, the proposal should so state. The need for items that can be typically used for research and non-research purposes should be explained.

5.9 Special Matters

The Special Matters section must contain appropriate statements regarding human subject provisions. Investigators should refer to agency-specific solicitations for instructions on this section.

5.10 Detailed Budget and Supporting Budgetary Information

Applicants responding to the CSA solicitation are required to submit Budget Forms C and D. In addition, applications to the ESA or JAXA solicitations which include Co-investigators from institutions in the U.S. or Canada should provide these forms for those investigators. Proposals from organizations outside the U.S. and Canada which do not have a U.S. or Canadian Co-investigator should not submit these forms.

Principal Investigators in Japan should complete form JP-4, provided in the JAXA Announcement. This form should also be completed by Co-investigators in Japanese institutions named in proposals to other agencies' research announcements.

This section must include information that supports the costs submitted in Forms C and D. In this solicitation, the terms "cost" and "budget" are used synonymously. Sufficient proposal cost detail and supporting information are required; funding amounts proposed with no explanation (e.g., Equipment: \$1,000, or Labor: \$6,000) may cause delays in evaluation and award.

Generally, costs will be evaluated for reasonableness, allowability, and allocation. The budgetary forms define the desired detail, but each category should be explained in this section. Investigators should exercise prudent judgment in determining what to include in the proposal, as the amount of detail necessarily varies with the complexity of the proposal.

The following examples indicate the suggested method of preparing a cost breakdown:

Direct Labor

Labor costs should be segregated by titles or disciplines with estimated hours and rates for each. Estimates should include justification, such as currently paid rates or outstanding offers to prospective employees. This format allows the agency to assess cost reasonableness by various means, including comparison to similar skills at other organizations.

Other Direct Costs

Please detail, explain, and substantiate other significant cost categories as described below:

- a) Subcontracts: Describe the work to be contracted, estimated amount, recipient (if known), and the reason for subcontracting.
- b) Consultants: Identify consultants to be used, why they are necessary, the time they will spend on the project, and the rates of pay (not to exceed the equivalent of the daily rate for Level IV of the Executive Schedule, exclusive of expenses and indirect costs).
- c) Equipment: List separately. Explain the need for items costing more than \$5,000 USD. Describe the basis for the estimated cost. For proposals including U.S. Co-investigators, general purpose equipment is not allowable as a direct cost unless specifically approved by the NASA Grant Officer. Any equipment purchase requested to be made as a direct charge under this award must include the equipment description, how it will be used in conducting the basic research proposed, and why it cannot be purchased with indirect funds.
- d) Supplies: Provide general categories of needed supplies, the method of acquisition, and estimated cost.
- e) Travel: Describe the purpose of the proposed travel in relation to the grant and provide justification, including information on the destination and the number of travelers, where known.
- f) Other: Enter the total of direct costs not covered by a) through e). Attach an itemized list explaining the need for each item and the basis for the estimate.

Indirect Costs

Indirect costs should be explained to an extent that will allow the agencies to understand the basis for the estimate.

5.11 Other Support (Form E)

This form is required for investigators from Canadian and U.S. institutions only.

Use Form E to list other sources of research support for the Principal Investigator and each of the Co-investigators. Please list all active support as well as any pending support.

5.12 Letters of Collaboration/Support

Include letters of support from collaborators. Please refer to the individual agency's Space Life Sciences Research Announcement about including a **Letter of Assurance of Foreign Support**.

5.13 Appendices

Appendices may be included, but investigators should be aware that reviewers are not required to consider information presented in appendices.

5.14 Space Flight Experiment Requirements Summary (Form F)

All applicants proposing space flight research must provide the information requested on Form F. The information on this form is essential for the technical evaluation of the feasibility of the proposed study. In addition, Form F should be used by the investigator to determine all required components of the flight experiment, from preflight preparation and data collection to tests and data/specimen processing. Before filling out this form, applicants should read Sections 1 and 2 of this document carefully to make certain that they understand the constraints that are associated with flight experiments. This form is used primarily by a team of technical experts which does not necessarily have expertise in every area of science. Be sure to clearly and succinctly explain all experiment requirements, from trivial to grand, in terms that an intelligent non-scientist can understand. The Principal Investigator should contact the appropriate Agency Point of Contact for questions or clarification before submitting a proposal.

5.15 Checklist for International Proposers Form

One copy of a completed version of this checklist should be attached to the submittal letter.

Proposals must be submitted to NASA Peer Review Services (NPRS) by May 5, 2004. Proposals must be received by 4:30 PM Eastern Time. Proposals and NOIs mailed through the U.S. Postal Service by express, first class, registered, or certified mail are to be sent to the following address:

NASA Peer Review Services
SUBJECT: ILSRA Flight Experiments in Space Life Sciences
500 E Street, SW - Suite 200
Washington, DC 20024-0001

INTERNATIONAL COVER PAGE

SOLICITED PROPOSAL APPLICATION FOR SPACE LIFE SCIENCES IN RESPONSE TO ANNOUNCEMENT ILSRA-2004	NUMBER
PLEASE FOLLOW INSTRUCTIONS CAREFULLY	REVIEW GROUP
	DATE RECEIVED

1. COMPLETE TITLE OF PROJECT

2. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR (First, middle, and last name; degrees; position)

3. COMPLETE MAILING ADDRESS

Internal Mail Code or Location
Office or Organization Division
Agency/Center, Company, or Institution
Street or P.O. Box

City, Province Country

4. TELEPHONE NUMBER (country code, city code, number, extension)	
FAX NUMBER E-MAIL ADDRESS	

5. THIS PROPOSAL IS: NEW RENEWAL REVISED

6. HAS THIS PROPOSAL (OR SIMILAR REQUEST) BEEN SUBMITTED TO ANY OTHER AGENCY?
 No Yes IF YES, SPECIFY AGENCY AND YEAR SUBMITTED:

7. HUMAN SUBJECTS: No Yes

7b. Exemption # or IRB Approval Date:
7c. Assurance of Compliance #:

8. CO-INVESTIGATORS (First, middle, and last name; degrees)	9. CO-INVESTIGATOR'S ORGANIZATION
---	-----------------------------------

10. DATES OF ENTIRE PROPOSED PROJECT PERIOD From:	11. COSTS REQUESTED FOR FIRST 12-MONTH BUDGET PERIOD 11a. Direct Costs 11b. Total Costs	12. COSTS REQUESTED FOR ENTIRE PROPOSED PROJECT PERIOD 12a. Direct Costs 12b. Total Costs
Through:	<i>Canadian and US only</i>	<i>Canadian and US only</i>

13. APPLICANT ORGANIZATION (Organization Name)

14. ORGANIZATION OFFICIAL TO BE NOTIFIED IF AN AWARD IS MADE (Name, title, address, and telephone number)	15. OFFICIAL SIGNING FOR APPLICANT ORGANIZATION (Name, title, and telephone number)
---	---

16. PRINCIPAL INVESTIGATOR/PROGRAM DIRECTOR ASSURANCE: I agree to accept responsibility for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application. Willful provision of false information is a criminal offense (U.S. Code, Title 18, Section 1001).	SIGNATURE OF PERSON NAMED IN 2 (In ink; "Per" signature not acceptable.)
	<div style="border: 1px solid black; width: 100px; height: 20px; margin-left: auto;"></div> DATE

17. CERTIFICATION AND ACCEPTANCE: By submitting the proposal identified in this Cover Sheet/Proposal Summary in response to ILSRA-2004, the Authorizing Official of the proposing institution (or the individual proposer if there is no proposing institution): 1) certifies that the statements made in this proposal are true and complete to the best of his/her knowledge; 2) agrees to accept the obligations to comply with the sponsoring agency award terms and conditions if an award is made as a result of this proposal; and 3) if the applicant organization is an entity of the United States of America, confirms compliance with all provisions, rules, and stipulations set forth in the three Certifications contained in this ILSRA [namely, i) Certification Regarding Debarment, Suspension, and Other Responsibility Matters Primary Cover Transactions, ii) Certification Regarding Lobbying, and iii) Certification of Compliance with the NASA Regulations Pursuant to Nondiscrimination in Federally Assisted Programs]. Willful provision of false information in this proposal and/or its supporting documents, or in reports required under an ensuing award, is a criminal offense (U.S. Code, Title 18, Section 1001).	SIGNATURE OF PERSON NAMED IN 15 (or person named in 2, if there is no proposing institution) (In ink; "Per" signature not acceptable.)
	<div style="border: 1px solid black; width: 100px; height: 20px; margin-left: auto;"></div> DATE

INTERNATIONAL PROPOSAL ABSTRACT

Principal Investigator: _____

Co-Investigators: _____

Proposal Title: _____

Abstract

Prepare a brief description of the application stating the broad, long-term objectives and specific aims of the proposed work. Describe concisely the research design and methods for achieving these objectives and aims. This abstract is meant to serve as a succinct and accurate description of the proposed work when separated from this application. Limit abstract to 300 words or fewer.

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
------	----------------

EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).

INSTITUTION(S) AND LOCATION	DEGREE(S) (if applicable)	YEAR(S)	FIELD(S) OF STUDY

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years, and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. **DO NOT EXCEED TWO PAGES.**

BUDGET FOR ENTIRE PROJECT PERIOD

DIRECT COSTS ONLY

<i>BUDGET CATEGORY TOTALS</i>		<i>1st BUDGET PERIOD</i>	<i>ADDITIONAL YEARS OF SUPPORT REQUESTED</i>		
			<i>2nd</i>	<i>3rd</i>	<i>4th</i>
PERSONNEL (Salary and Fringe Benefits) (Applicant organization only)					
SUBCONTRACTS					
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES					
TRAVEL	DOMESTIC				
	NON-DOMESTIC				
OTHER EXPENSES					
TOTAL DIRECT COSTS FOR EACH PERIOD					
TOTAL INDIRECT COSTS FOR EACH PERIOD					
TOTAL DIRECT + INDIRECT COSTS FOR EACH PERIOD					
TOTAL DIRECT + INDIRECT COSTS FOR ENTIRE PROJECT					

JUSTIFICATION FOR UNUSUAL EXPENSES :

BUDGET FOR 12 MONTH PERIOD

DIRECT COSTS ONLY

DETAILED BUDGET FOR 12-MONTH BUDGET PERIOD DIRECT COSTS ONLY		FROM		THROUGH	
Duplicate this form for each year of grant support requested		FUNDING AMOUNT REQUESTED			
PERSONNEL (Applicant Organization Only)					
NAME	ROLE IN PROJECT	EFFORT ON PROJECT	SALARY	FRINGE BENEFITS	TOTALS
	Principal Investigator				
SUBTOTALS →					
SUBCONTRACTS					
CONSULTANT COSTS					
EQUIPMENT (Itemize; use additional sheet if needed)					
SUPPLIES (Itemize by category; use additional sheet if needed)					
TRAVEL	DOMESTIC				
	NON-DOMESTIC				
OTHER EXPENSES (Itemize by category; use additional sheet if needed)					
TOTAL DIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD					
INDIRECT COSTS FOR FIRST 12-MONTH BUDGET PERIOD					
TOTAL COST FOR FIRST 12-MONTH BUDGET PERIOD					

OTHER SUPPORT

Please provide information regarding specific sources of other support for the PI and each Co-I (not consultants). This information should be provided separately for each individual in the format shown below. List all active support for an individual before listing pending support. Include the investigator's name at the top of each page and number pages consecutively.

NAME OF INDIVIDUAL		
ACTIVE/PENDING		
Project Number (Principal Investigator)	Dates of Approved/ Proposed Project	Percent Effort
Source	Annual Direct Costs	
Title of Project (or Subproject)		
One-sentence description of project goals. (The major goals of this project are...)		
Brief description of potential scientific or commitment overlap with respect to this individual between this application and projects described above (summarized for each individual).		

Form F

SPACE FLIGHT EXPERIMENT REQUIREMENTS SUMMARY

In addition to the actual proposal, Form F is required for the Flight Feasibility Review. This form has been designed for a description of all preflight, inflight and post-flight components of the flight experiment. Form F consists of three sections:

- A general section to be completed for all flight proposals,
- A section to be completed only for experiments that require human subjects, and
- A section to be completed only for experiments that require non-human specimens.

If an experiment requires both human and non-human specimens, the entire form must be completed. If no specimens are required (e.g., radiation dosimetry), complete Part 1 and other applicable hardware and procedures questions. If the proposal consists of distinct segments with different requirements, fill out multiple forms to fully describe all segments. **Form F is mandatory for flight experiments.** Flight experiment proposals submitted without Form F completed will not be evaluated.

Please read the questions carefully and keep answers brief but thorough, ensuring that all requested information has been provided. Expand tables/response space as needed. Downloading the RTF file is the most effective way to complete this form.

Part I: General Information

1. Principal Investigator name: _____
2. Proposal title: _____

3. Duration of flight experiment
 - a. Minimum number of days in flight:
 - b. Desired number of days in flight:
4. Describe the types of procedures required for the inflight portion of the experiment. List each type of procedure separately (e.g., blood sample, record EKG, fix culture, etc.).

5. Storage of equipment and supplies other than animal/plant/specimen habitats (for all flight experiments)

Is temperature control of equipment/supplies needed:	Yes	No	Not Applicable	Not Known	Temperature (°C)	Estimated Volume (cm3)
-- for launch?						
-- in flight?						
-- for return?						

6. Hazardous materials and controlled/radioactive substances (for all flight experiments)
Add more lines if necessary.

Material	Estimated Volume (cm3)	Usage Time Period (e.g., Preflight, Inflight, Post-flight)
----------	---------------------------	---

- 1.
- 2.
- 3.

Part II: Research Involving Crewmembers as Subjects

7. Subjects
 - a. Number of subjects required for statistical significance:
 - b. Special requirements (e.g., gender, age, etc.):
 - c. Are inflight procedures needed?
 - d. Are pre- and post-flight procedures needed?
8. List all human subject restrictions (*e.g., specific dietary regimens, fluid intake regulation, work/rest cycles, exercise, etc.*). *Indicate the impact on scientific outcome if restrictions cannot be met.*
9. Is loading of experiment supplies or equipment less than 90 hours before launch required? If so, explain why.
10. Is removal of the experiment samples, data, or equipment less than 24 hours after landing required? If so, explain why.
11. What procedures will the crew need to learn in order to perform their role as subjects for the experiment?
List and briefly describe each procedure separately. Be sure to rate the difficulty of learning each procedure (1= easy; 10= difficult) and indicate when each procedure will be used (e.g., preflight, inflight, post-flight). Assume that the crewmembers do not have a medical background or prior experience with these kinds of experiments.

12. Does the experiment require a person to assist (operator) with data collection? If so, what procedures will be performed by this person?

List and briefly describe each procedure separately. Be sure to rate the difficulty of learning each procedure (1= easy; 10= difficult) and indicate when each procedure will be used (e.g., preflight, inflight, post-flight). Assume that the crewmembers do not have a medical background or prior experience with these kinds of experiments.

13. Equipment for human subject measurements

Add more lines if necessary.

a. Pre- and Postflight

What Variable will be Measured?	Equipment Needed for Measurement	Equipment Provider (Agency or PI)
1.		
2.		
3.		
4.		

b. Inflight (*List ALL needed inflight equipment for measurement, sample collection, or storage.*)

What Variable will be Measured?	Equipment Needed for Measurement	Equipment Provider (Agency or PI)
1.		
2.		
3.		
4.		

Part III: Research Involving Non-Human Subjects

18. Use the table below to list the requirements for non-human specimens. *Add more rows if necessary.*

Specimen Type (e.g., species, strain, gender, weight, age)	Drugs, Tracers, Tags, etc.	Number of Specimens Required for Flight Experiment	Number of Specimens Required for Ground Control of Flight Experiment
1.			
2.			
3.			
4.			
5.			

19. Use the table below to list the required inflight experimental conditions for all non-human specimens and samples. *Be sure to completely describe, for each specimen or sample, the environmental parameters (e.g., temperature, humidity, CO₂, light level, atmospheric pressure) and allowable range for each parameter. Also indicate when the environmental conditions will be needed (e.g., Flight Day 3-10, mission duration, pre-injection, after fixation).*

Requirement	Tolerance (e.g. $\pm 1^{\circ}\text{C}$)	When needed?	Specimen/Sample
1.			
2.			
3.			
4.			
5.			

25. List the procedures from Item 23 in the table below. *Indicate the frequency and an acceptable time range for each procedure (e.g., change media every 5 days \pm 1 day, fix sample on day 10 \pm 6 hours).*

Procedure	Flight Day and Time (if necessary)	Frequency	Acceptable Range
1.			
2.			
3.			
4.			

26. For each specimen, list preferred habitat or indicate NO PREFERENCE.

27. List equipment, tools, supplies needed for inflight experiment procedures.

28. List any special requirements for specimen and/or sample accommodation or manipulation.

CHECKLIST FOR INTERNATIONAL PROPOSERS

This checklist should be annotated to indicate that the stated items have been included in the proposal package.

Principal Investigator/Program Director:

- | | |
|---|---|
| <input type="checkbox"/> International Cover Page* | <input type="checkbox"/> Summary Budget Form (Form C) |
| <input type="checkbox"/> International Proposal Abstract | <input type="checkbox"/> Detailed 12-Month Budget (Form D) |
| <input type="checkbox"/> Title Page | <input type="checkbox"/> Supporting Budgetary Information |
| <input type="checkbox"/> Project Description | <input type="checkbox"/> Other Support (Form E) |
| <input type="checkbox"/> Management Approach | <input type="checkbox"/> Letters of Support from Collaborators and Letter of Assurance of Foreign Support (if applicable) |
| <input type="checkbox"/> Biographical Sketches (Form B) | <input type="checkbox"/> Appendices, if any |
| <input type="checkbox"/> Facilities and Equipment Description | <input type="checkbox"/> Space Flight Experiment Requirements Summary (Form F) |
| <input type="checkbox"/> IRB letter/form (if applicable)* | <input type="checkbox"/> 25 copies of all materials listed above |

* One signed original required

Only one copy of the following needs to be submitted:

- This checklist indicates all applicable items have been enclosed.