ESA-ISLWG *Arabidopsis* Workshop

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Workshop Report

Final

Prepared by C. Brillouet
ESA-ESTEC

List of Participants:

Name	Affiliation
BRIARTY, Greg	Univ. of Nottingham (ret.)
BRILLOUET, Claude	ESA-ESTEC, MSM-GF
BRINCKMANN, Enno	ESA-ESTEC, MSM-GM
BUCKLEY, Nicole	Canadian Space Agency
EODICE, Michael	NASA-Ames Research Center
EPPLEY, Zoe	NASA-HQ, Washington
FERL, Robert	Univ. of Florida, Gainesville
GRUENDEL, Douglas	NASA, Kennedy Space Center
ISHIOKA, Noriaki	NASDA, Tsukuba
KERN, Volker	NASA-Ames Research Center
KISS, John	Miami Univ., Oxford, Ohio
LOMAX, Terri	NASA-HQ, Washington
MANIERI, Pierfilippo	ESA-ESTEC, MSM-GF
MUSGRAVE, Mary	Univ. of Connecticut, Storrs
NISHITANI, Kazuhiko	Tohoku Univ., Sendai
PALME, Klaus	Universität Freiburg
PERBAL, Gérald	Univ. P.& M. Curie, Paris
REED, David	NASA, Kennedy Space Center
SCHMITT, Didier	ESA-ESTEC, MSM-GA
SHIMAZU, Toru	Japan Space Forum, Tokyo
STEELE, Marianne	NASA-Ames Research Center
VISO, Michel	CNES-HQ, Paris

Goals of the Workshop

- To present *Arabidopsis* scientists with a description of available facilities, additional supporting resources (*e.g*. cold stowage), and logistics operations, which may be suitable to conduct *Arabidopsis* experiments in Space.

- For scientists to expose their needs and define typical *Arabidopsis* experiments scenarios

- To combine this information in a synthetic Matrix to define a generic "*Arabidopsis* Growth Facility" (AGF) which will summarize all needs and available facilities.

- From the AGF Matrix, elaborate (post-workshop) an ISLSWG Workshop Report summarizing the discussions and providing recommendations to generate the greatest science yield from available resources.

Summary of Space Agencies Presentations

Didier Schmitt (ESA) welcomed the participants and recalled that the "model organisms" approach and focused research using *Caenorhabidtis elegans* and *Arabidopsis thaliana* was recommended, by the ISLSWG, primarily to speed-up the space experiments selection and implementation process. Therefore, the main goal of this Workshop should be to provide recommendations and help to define a typical *Arabidopsis thaliana* growth scenario (or scenarios) in Space. Those recommendations should be included in the Companion Document for the next International Life Sciences Research Announcement (ILSRA), which is to be released by 15th October 2003.

Claude Brillouet (ESA) presented the organization and goals of the workshop, as listed above.

Pierfilippo Manieri (ESA) presented the BIOLAB Facility, which is suitable only for seedlings of *Arabidopis*. Fully-grown plants (>60mm) and seed-to-seed experiments are not possible using Biolab. The Biolab standard Experiment Containers (EC) are significantly shorter than the EMCS-ECs and, furthermore, the illumination system provides only $10W/m^2$ (approx. 20 µmol photons/m^2/sec) at the substrate level; video observation is possible on each rotor. The life support system controls oxygen, CO_2 and humidity and removes ethylene.

Biolab is equipped with a Bio-GloveBox, Temperature Controlled Units (-20°C to +10°C), an incubator (+18°C to +40°C) with 2 centrifuges (0.001g to 2.0g), a microscope and a spectrophotometer. A liquid handling mechanism allows fully automated fluid transfer operations (*e.g*. fixation); liquid samples may also be automatically transferred to the microscope or to the spectrophotometer.

Launch of Biolab to the ISS is now expected for the second half of 2005.

David Reed (NASA-KSC), presented the KSC developed facilities for plant growth experiments in Space, and associated equipments:

- the Plant Growth Facility (PGF)
- the Controlled Environment Research System (CERES, *formerly PGF Split-Plenum*)
- the Biological Research In Canisters (BRIC) hardware
- the KSC Gaseous Nitrogen Freezers (KSC-GN2)

The PGF includes six 76 cm^2 x 17.5 cm growth chambers and occupies 1 Middeck Locker Equivalent (MLE), plus additional stowage. The illumination system provides a PAR level of 50 to 300 $\mu mol.m^2.s^{-1}$. The PGF is equipped with a CO_2 and ethylene removal system. The temperature is ambient +/- 1°C.

It is foreseen that, as from 2004, after the Shuttle fleet has been returned to flight, 20 MLEs will be available for research equipment on each "Sortie" flight to the ISS.

CERES offers a temperature range from 23 to 26°C and an illumination level identical to the PGF, it has a Green Fluorescence Protein (GFP) imaging capability. The two growth chambers measure 284 cm^2 x 19 cm and independently control atmosperic composition and temperature.

Both the PGF and CERES exchange air with the cabin. Filters need to be changed every five days to maintain ethylene removal capability. This will impack stowage to support these hardware. Neither the PGF or CERES have centrifugation capability.

The BRIC hardware, in its different versions, has been flown many times on the Shuttle and on Mir: BRIC-60 (vented) accommodates 60 mm Petri dishes and BRIC-100 (vented or sealed) accommodates 100mm Petri dishes. The BRIC-100VC is a gas purgable version, which can accommodate up to four 100 mm Petri plates. BRICs are passive hardware, do not have centrifugation capability, and when sealed, result in elevated ethylene levels in the hardware.

The KSC Gaseous Nitrogen Freezers (KSC-GN2, -196°C) have been presented in the 1 MLE, 1.5 MLE and MPLM versions, as well as the BRIC Passive Cooler (BRIC-PC) for 4-6°C samples preservation and the KSC Fixation Tube (KFT), which provides 3 levels of containment and allows the use of Tox. Level 2 fixatives without the need for a Glovebox.

Enno Brinckmann (ESA) presented the European Modular Cultivation System (EMCS), which is a multi-purpose facility suitable for experiments with small plants (<160mm), small animals, micro-organisms and cell cultures, dependent upon the Experiment Unique Equipment (EUE) developed. The EMCS Experiment Containers (ECs) have power, data, gas and water interfaces with the facility, on two centrifuge platters, each platter holds 4ECs. An illumination system (using white, red and infrared LEDs) provides 50 or 75 W/m^2. Infra-Red LEDs are used for "dark" observation. EMCS features both CO_2 and ethylene removal systems. Relative Humidity control is provided individually at the EC level. The EMCS provides an imaging system for digital stills and video, temperature control, and a controlled atmosphere independent of air cabin.

EMCS launch to the ISS, on the ULF-2 Shuttle Mission, is now tentatively scheduled for September 2005.

Noriaki Ishioka (NASDA) and Toru Shimazu (Japan Space Forum) presented the Space Experiments Planning in Japan and the available, or under development, equipment and, in particular, the Plant Experiment Unit (PEU) which is a specialized experiment container, with a growth chamber of approx. 5 x 5 x 5 cm, which provides LED illumination (Red + Blue, 7:3, 110 $\mu mol.m^{-2}.s^{-1}$), watering, humidity control and video observation (NTSC), data and power interface.

The PEUs are designed for use inside the NASDA Cell Biology Experiment Facility (CBEF), which can accommodate 6 PEUs under microgravity and 4 PEUs on a centrifuge, with variable speed (g-levels) capability. However, CBEF does not include an ethylene removal system.

Michael Eodice (NASA-ARC) presented the ISS Cold Stowage capabilities and equipment existing or under development.

- the Minus Eighty degree Laboratory Freezer for the ISS (MELFI), built by ESA, which includes four 44 liters Dewars, each independently settable to -80°C, -26°C and +4°C.
- the ARCTIC (MERLIN2) active incubator/refrigerator/freezer (-20°C to +48°C), single Middeck locker equivalent unit. Both flight units failed in orbit, repair/upgrade option is currently on hold.
- the Passive Nitrogen Dewars, for -180°C transportation (KSC-GN2 freezers).
- the Cryofreezer: active -180°C with Snap Freezing capability (availability: 2007).
- the General Laboratory Active Cryogenic ISS experiment Refrigerator (GLACIER): active, -180°C , with 30 liters usable cold stowage volume (availability: 2006)
- the Low Temperature, Low Energy Carrier (LoTEC): passive transportation transport canister single MLE, 22.4 liters internal volume. Phase Change Material (PCMs) are available for 4° to 6°C, up to 2 weeks temperature holding capability. Other PCMs and temperature ranges are under analysis (expected flight certification: July 2003).

Some standard, ambient, Transport & Stowage equipment was also presented: the Resupply Stowage Rack (RSR), the Resupply Stowage Platform (RSP) and the Zero-G Stowage Rack (ZSR), as per NASA document SSP-50467.

During this presentation, it was pointed out that the NASA-JSC Cold Stowage Working Group needs experiments/PIs inputs one year before required use !

Dr. Nicole Buckley (CSA) sent, post-workshop for insertion in the report, the following information about the Aquatic Research Facility (ARF):

The ARF currently has a limited fixation capability that is designed to function with liquid media only inside each aquaria. This mechanism could be used to hydrate seeds on a substrate surface, however it is inadequate to fix grown plants.

Fixation and/or freezing of *Arabidopsis* plant material could be accomplished with a minor redesign of the Sample Container Unit (SCU). Uncaptured screws used to close the SCU during

preflight processing would need to be replaced by captured screws. With captured screws, the SCUs could be disassembled on orbit, a simple crew operation, and the plants could be removed for preservation using other available hardware such as the KFT or any of several onboard freezers. A more complex redesign could replace one of the two aquaria in each SCU with a larger fixation device thereby providing completely autonomous operation of the ARF.

The ARF provides two carousels each of which is capable of containing six SCUs. The carousels can independently provide 0 - 1 g, thermal control, and video imaging (white light and infrared). Each SCU provides a maximum usable volume of 25mm x 101mm x 66mm and passive gas exchange with the ambient environment.

Summary of *Arabidopis* Scientists Presentations

Dr. John Kiss (Dept. of Botany, Miami Univ., Oxford OH)) talked about "*Arabidopsis in Space: Past, Present and Future*", recalling his past experiment, especially on the ESA Biorack Facility, emphasizing the importance of an onboard 1-g control centrifuge and of an efficient ethylene removal system: a typical "ethylene effect" was observed in the Biorack Shuttle-to-Mir experiments, due to a high ethylene level in Mir's atmosphere. He also stressed the importance, for newcomers in the space experiments field, to get contact and help, mostly informal, from experienced PIs. Other lessons learned from his flight experience included: getting the crew involved with the experiment and having hardware engineers interact with scientists. He also recommended that the space agencies put an accurate list of hardware actually available for flight experiments in their AO's.

Dr. Kiss also described how microgravity allows performing pure phototropism experiments, without gravitropism perturbation. This is especially true for roots phototropism experiments and the investigation of the phytochrome family of photoreceptors. Pure phototropic response can be studied only in Space.

Currently, his experiments on phototropism in microgravity are in development for the EMCS facility, and he presented information on Experiment Unique Equipment (EUE) for this project.

Dr. Kiss also expressed his concerns that, although high-resolution digital video cameras are available, it is not currently possible to have digital image recording on the ISS (and especially for EMCS). The current standard is NTSC video tapes… when it should be digital HDTV !

Prof. Mary Musgrave (Dept. of Plant Science, Univ. of Connecticut) spoke about "*Physiological challenges to seed production during Spaceflight*". Prof. Musgrave pointed-out that *Arabidopsis thaliana* may not always be the material of choice for plant experiments in space. Especially for plant embryogenesis studies: due to the fact that *A. thaliana* is a self-fertilizing (auto-pollination) plant, it is not possible to know the exact age of the embryos and it is very difficult to accurately study the early phases of embryo development. In this respect, *Brassica rapa*, which needs manual pollination by the crew, is much more appropriate.

The fact that the use of *Arabidopsis* has been recommended as a "model organism" should not impair the selection of experiments with other plants, when they are more appropriate for a specific type of investigation.

Dr. Musgrave described how "roots oxytropism" has been evidenced during some spaceflight experiments, using the Plant Growth Unit (PGU) and *Arabidopis* grown on agar-filled tubes. Under microgravity, water distribution within micropores of the substrate (agar) creates hypoxia at a lower water content than in 1-g. This root-zone hypoxia may have been implicated in many observed effects on *Arabidopsis* somatic embryos grown on agar.

Brassica seed production has been achieved on the ISS during a hardware test of the Biomass Production Chamber (BPC), seeds were in-flight frozen using the ARTIC freezer. Higher chlorophyll, soluble carbohydrates and starch levels have been observed on the flight samples, compared to ground controls.

Dr. Musgrave reported that a critical issue is to evaluate the influence of microgravity on the internal atmosphere composition (and gas-mixing) around the seeds, which may, in turn, affect the ripening of the fruit.

Dr. Musgrave also recommended the use of other intermediate g-levels (apart from 0-g and 1-g), to simulate *e.g.*, Mars gravity (0.38g).

Prof. Kazuhiko Nishitani (Tohoku Univ.) talked about "*Cell Wall Construction as Affected by Gravity Signal*" emphasizing the role of the Xyloglucan endo-transglucosylase/hydrolase (XTH) enzymes which are encoded by a multi-gene family, often with many copies (762 genes). Individual XTH genes exhibit cell-type specific expression profiles and are regulated individually by different sets of plant hormones. Distinct sets of cell wall proteins are forming different cell types by a control at the transcriptional level.

Oligo-DNA microarray capable of measuring expression of 762 cell-wall genes has been established.

Less rigid cell walls have been observed under microgravity and the causal mechanism of this effect is not known.

Protocols for space experimentation to study XTH gene family expression under microgravity are currently under development.

Prof. Klaus Palme (Univ. of Freiburg) talked on *"Arabidopsis thaliana: a tool for exploring the molecular mechanisms underlying gravity perception and transduction*", emphasized more on the methods and techniques which are actually required for an adequate investigation of plant gravitropism at the cellular and molecular level, on Earth and in Space.

With respect to the preceding talk, Prof. Palme stressed that micro-arrays are not suitable for single-cell measurements, however single-cell data (proteins contents, not only DNA-RNA) are needed. This is an extremely complex field which requires both an extended range of single-cell quantitative measurement methods, coupled to advanced robotics and computerized analysis tools, but also a world-wide network of institutions.

Powerful, fast, advanced computers with advanced image processing software and pattern-searching algorithms will also be needed. Automated microscopes, with low-light fluorescence capability, time-space visualization (to follow transportation of a protein within a single-cell) and 3-D optical sectioning/reconstruction should be made available.

Dr. Palme illustrated those needs with the study of polar auxin transport and distribution in the root cap, as affected by gravity/microgravity. This process involves a complex set of genes

and proteins (AUX-1/LAX and the PIN family), plant hormone (IAA), intra- and extra-cellular molecules transport.

Comments and Recommendations from Presentations and AGF Matrix (see Annex. 1)

Concerning the **STS flight schedule**, Dr. Terri Lomax (NAS-HQ) confirmed that a "Return-to-Flight" announcement is not expected until mid- or late August 2003, after analysis of the Columbia Accident Investigation Board (CAIB) report, which is expected to be released by end of July. The return-to-flight target timeframe is December 2003 to April 2004.

A generic slip of approximately 12 months is expected for all flights.

Note: since this workshop, the release of the CAIB report has been delayed until end of August 2003.

The next LSRA should be released by 15th October 2003 and will cover the utilization period 2005-2007. For this period, some Middeck Lockers will be made available for experiments hardware on the Shuttle, but very limited experiment volume (if any) will be available on the ISS.

Launch of experiments hardware and samples using the Multi-Purpose Logistics Module (MPLM) may be a problem for time-critical samples, since "late-access" for MPLM loading is L-88 hours.

***Arabidopsis thaliana* as a "model organism**":

It is clear that the "Models Organisms (*C. elegans and A. thaliana*) approach" has non-questionable merits and will streamline the selection and accommodation process. However, as exemplified by Dr. Musgrave's presentation, it must not prevent, or impair, the selection of experiments using other plant organisms when their use are fully justified for specific scientific investigations (*e.g*. the use of *Brassica rapa* for plant embryo studies). Little enthusiasm was expressed for the idea of pre-flying *Arabidopsis* and then offering the flight materials as a resource for proposal from the NRA process. It was generally felt that the level of sophistication in plant space research demands that the materials to be flown and the manner in which they are flown play a major role in the science. The use of specific strains, mutants and developmental stages, together with the use of specific environmental parameters, essentially precludes the potential for selecting a standard set of plants, environmental parameters and procedures.

Dr. Nishitani recommended that the *Arabidopsis* wild type "Columbia" should be preferably used since it is, by far, the most well known from a genetic point of view.

The use of transgenic *Arabidopsis* strains has been discussed; concerns were expressed whether the Experiment Containers and Facilities, or a Glovebox, will provide the required biohazard containment levels. Dr. Terri Lomax said that this issue is under consideration but is not considered critical. Dr. Ferl commented that the use of transgenic Arabidopsis in his experiments

on STS-93 were reviewed by his institutional review board and found to be exempt from NIH concerns.

Owing to the extensive spaceflight history and publications on *Arabidopsis*, the basic research questions which could be addressed using the "model organism" concept have already been answered. Past experience shows that the more detailed research questions that might now be explored using *Arabidopsis* require far more sophisticated ecosystems, such as the EMCS and BIOLAB. With this in mind, the participants in the workshop agreed that *Arabidopsis* is beyond the "model organinism" stage, and their preference in the upcoming solicitation was to clearly define the realistic constraints (e.g. organism, preflight, on orbit, recovery operations, cold / ambient stowage, crew time, etc.) and then allow the PIs to propose "clever" experiments that would fit within the envelope and make maximum use of the available resources.

Facilities recommended for *Arabidopsis* experiments:

CNES (Dr. Michel Viso) suggested the use of the, under-development, KUBIK facility which is planned to first fly of the Dutch Soyuz Mission in Spring 2004. The main concern here is the absence of any CO_2 control and ethylene removal system in a small and densely pack incubator. Furthermore, KUBIK uses the Biorack type I/E containers, which have a very small internal volume and would only allow for seed and seedlings experiments. No illumination, video-observation and data transmission systems are provided.

Therefore, use of KUBIK for plant experiments should be restricted to scientific investigations for which the ethylene level is not critical.

Three facilities clearly emerge as the equipments of choice for *Arabidopsis* (and other plants) experiments:

EMCS (ESA) - Note that EUE to support seedling growth in ECs for the EMCS has been developed and successfully tested by NASA-ARC. Cultivation Chambers for whole plants are being developed by ESA.

PGF & **CERES** (NASA) - Disadvantages include no centrifugation capability or controlled atmosphere. Filter exchanges and stowage also limit usefullness on the ISS.

The NASDA **CBEF/PEU** also offers interesting capabilities for very small plants (growth chamber 5x5x5 cm). However, the absence of a dedicated ethylene removing system may be a limiting factor for many experiments.

BIOLAB could be suitable for some experiments with *Arabidopis* seedlings, but not for seed-to-seed or whole plant experiments due to the smaller volume of the standard Biolab EC, compared to the EMCS-EC. In addition the Biolab illumination system does not provide a level that would allow full growth. On the other hand, its sophisticated liquid handling mechanism, built-in microscope and spectrophotometer allow complex, fully automated operations to be performed. The BioGlovebox allows manual crew operations when automation is not possible.

Biolab may be recommended for *Arabidopsis* experiments at the cellular level (biochemistry, molecular biology) or with seedlings and not requiring full-size plants.

The CSA Aquatic Research Facility (**ARF**) may be suitable for seeds and seedlings experiments. However, in its current configuration, it allows fixation for only samples/organisms in liquid media inside each aquaria. According to Dr. Buckley, a minor re-design of the ARF's Sample Container Unit (SCU) would allow fixing and/or freezing *Arabidopsis* samples from ARF (in conjunction with other on-board fixation devices and/or freezers

Experiment Preparation:

Although some investigators indicated that they may prepare their experiment either at their home lab or at the launch site, a majority of them clearly stressed that preparation at the launch site is preferred. This is particularly critical for launch delays and scrubs.

Shared use of the newly built Space Experiments Preparation and Research Laboratory (SERPL) by the international ISS partners will then be a critical issue.

Experiment Termination, Post-Experiment Storage & Return:

A majority of investigators indicated the criticality of -80°C (or lower) deep-freezing for an adequate preservation of the samples, especially for molecular biology analysis purposes. Availability of onboard deep-freezers (MELFI, KSC-GN2 freezers) is then mandatory for this category of experiments, for termination, post-experiment stowage and return to ground.

Additional cold stowage equipments may be available for transportation from (and to) the ISS: the NASA BRIC-PC (Passive Cooler), but it has a temperature hold time limited to 48 hours only at 4-6°C, and the ESA Passive Thermal Conditioning Units (PTCUs) which have been extensively flown, in particular in connection with the Biorack facility.

Depending on the PCM used, the PTCUs can hold +5°C for 22 days and an upgraded version of the -10°C PTCU is able to maintain this temperature for also 3 weeks, further potential upgrades of the design may even increase the temperature hold time.

Re-conditioning of the PCMs, using an ISS onboard freezer may also be considered.

Appendix 1 - Facilities availability & expected launch date, time-frame:

ARF	available, already flown on STS-77
BIOLAB	late 2005 (ISS-1E, Columbus launch)
BRIC (all)	available, already flown on STS & Mir
CBEF-PEU	under review (JEM launch)
CERES	February 2005 (*tbc*)
EMCS	September 2005 (ULF-2)
KFT	available, already flown on STS/ISS
KSC-GN2	available, already flown on STS/ISS (1.5 MLE version)
KUBIK	April 2004 (Dutch Soyuz Mission)
MELFI	2004 (ULF-1)
PGF	available, already flown on STS

Annex.1

Generic "*Arabidopsis* Growth Facility" (AGF) Requirements Matrix

	Scientists Input								Suitable Facilities				
Experiments Type/Requirements	**G. Briarty**	**R. Ferl**	**N. Ishioka**	**J. Kiss**	**M. Musgrave**	**K. Nishitani**	**K. Palme**	**G. Perbal**	**NASA**	**NASDA**	**ESA**	**CSA**	**CNES**
1- Seedlings:Roots (**SR**), Shoots (**SS**)	SR, SS, *A.Thaliana* & legume	SR, SS	SR	SR, SS	n/a	n/a	SR, SS	SR	PGF, CERES	CBEF PEU	EMCS. BLB	ARF	KUBIK
2- Whole plants: from seed to seed (**StS**), Seed harvesting	n/a	n/a	n/a	n/a	*Brassica*	n/a	(n/a)	n/a	PGF, CERES	CBEF PEU	EMCS	n/a	n/a
3- Whole plants: **organ harvesting**: roots (**R**), shoots (**S**), leaves **(L**), fruits (**F**)	n/a	R, SL, F	n/a	n/a	F	S	all	R, L	PGF, CERES	CBEF PEU	EMCS	n/a	n/a
4- Plant material: wild type (**WT**), mutants (**M**), transgenic (**Tr**)	WT	WT, M	WT	WT, M	WT	WT Columbia	WT, M	WT, Tr	W, M, Tr	CBEF PEU	W, M, Tr	W, M, Tr	n/a
5- Growth environment: light intensity (**L**), gas composition (**G**), nutrient supply (**N**)	L, G, N	Standard	Standard	L: 70 µmole G, N	Standard	L, N	Standard	L, G, N	PGF, CERES	CBEF PEU	EMCS	ARF	n/a
6- Observation possibility: white (**W**) or Infra Red (**IR**) light source, resolution, picture frequency	n/a	W	W	W, IR (root)	W	n/a	W, IR	W, (IR)	PGF, CERES	CBEF PEU	W, IR 0.1 mm	W, IR ARF 2 mm	n/a
7- Experiment start: from seeds (**SE**), from seedlings (**SL**), from plants (**PL**)	SE	SE, SL, PL	SE	SE	SE, SL, PL	SE or SL	SE	SE	SE, SL, PL	CBEF PEU	SE, SL, PL	SE, SL	KUBIK
8- Experiment preparation: home lab (**HL**) or launch site (**LS**)	HL / LS	HL / LS	LS	HL / LS	HL / LS	HL	HL / LS	LS	n/a	n/a	n/a	n/a	n/a
9- Experiment duration: one run (**1R**), several runs (**xR**)	1R	xR	1R	3R	1R	1R	xR	xR	1R, Xr	1R PEU	1R, xR	1R, xR	1R KUBIK
10- Experimental treatment: *e.g* . staining (**St**), g-vector change **(gV**), g-level change (**gL**)	gL	St	n/a	µg, 0.1 - 0.7g, 1-g	gas	0-g, 1-g	ALL	St, 0-g, 1-g		CBEF PEU	EUE: St, gV 0.001 - 2g	0.1 - 1-g	St ? gL ?
11- Sample replicas (**n=**)	30	30	> 5	30	30	6 3 fixed 3 frozen	> 3	40	6 PGCs	24, PEU	2 x 6 EC BLB 2X4 EC EMCS	12 SEUs	tbd EC # ?
12- Experiment termination: fixative (**FIX**), which one ? , freezing -20°, -80°C (**FR - x°C**)	FIX: GA / PF FR -80°C	FIX RNA later	FR -80°C	FIX (GA) FR -20°C	FR FIX DRY	FIX FR -80°C	FIX FR -80°C	FIX FR -80°C	KFT ARCTIC LSG (PGB)	MELFI	BLB BGB (PGB)	FIX for Aquatics only	FIX
13- Post-experiment storage: duration (**x days, weeks**), thermal condition (**x °C**)	FIX: +4°C FR at -80°C	FIX at +4°C	FR at -80°C up to 90 days	FR at -20°C, FR at -80°C	FR at -20°C, FR at -80°C	FIX: +4°C FR < -20°C	FIX: +4°C FR at -80°C	FIX: +4°C FR at -80°C	(MELFI) ARCTIC KFT	MELFI	BLB -20°C, +10°C	n/a	+20°C +6°C
14- Transport to / from ISS									GN2, BRIC PGF CERES		PTCUs		