Julius Kühn-Institut
Federal Research Centre for Cultivated Plants

Genebank Quality Manual


Julius Kühn-Institute

Institute for Breeding Research on Horticultural and Fruit Crops
Dresden-Pillnitz

Contact:

Julius Kühn-Institute - Federal Research Centre for Cultivated Plants,
Institute for Breeding Research on Horticultural and Fruit Crops,
Pillnitzer Platz 3a,
01326 Dresden,
Germany
Phone: 0049-351 261620
Fax: 0049- 351 2616212
Internet: www.jki.bund.de
Email: zgod@jki.bund.de

Head
Prof. Dr. Magda-Viola Hanke
Phone: 0049-351 2616214
Fax: 0049-351 2616213
Email: viola.hanke@jki.bund.de

Curator
Dr. Monika Höfer
Phone: 0049-351 2616222
Fax: 0049-351 2616213
Email: monika.hoefer@jki.bund.de

Operational Genebank Manual of the JKI Institute for Breeding Research on Horticultural and Fruit Crops Dresden

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1 Germplasm Acquisition and Accessioning

Genebanks can obtain the germplasm they want to conserve through a number of different ways. Conducting collecting missions is possibly the best way of acquiring germplasm material in the most reliable manner. Germplasm exchange with other genebanks is a third route to add genetic diversity to the collection. Obtaining and storing germplasm from researchers and plant breeders is another route to acquire genetic material. Such acquisitions should be guided by a formal mandate that the genebank concludes with its host organization or government and that provides the basis for a genebank acquisition policy. The actual accessioning of acquired germplasm samples, i.e. formally including it into the collection with its unique accession number, is a complex process during which the curator has to check a number of aspects such as the verification of the identity of the material, the health status, the availability of pertinent information, etc. It is further understood that also legal aspects form part of this activity, e.g. was the material collected/obtained in legal manner, are there any restrictions on its use, etc.

Box 1.1 Germplasm Acquisition and Accessioning

GA1 - Briefly describe any formal mandate that your genebank might have concluded with or received from your "mother organization" (e.g. institute, governmental body).

(*This description should include details on:*

a) *which species you conserve and make available;*
b) *who decides on what your mandate is and, if different,*
c) *from whom do you received the mandate;*
d) *the main aspects of the mandate; and*
e) *legal considerations on PGR as foreseen in national legislation).*

The Institute for Breeding Research on Horticultural and Fruit Crops is one of 15 research institutes of the Julius Kühn-Institute (JKI), Federal Research Centre for Cultivated Plants. The Institute has two locations – at Quedlinburg and at Dresden-Pillnitz.

Approximately 30 different fruit crops species are grown in commercial fruit production in Germany, whereas the range of cultivars presented is very limited. Thus the institute collects, preserves, characterizes and documents the diversity in fruit species and cultivars as well as in related wild species. Old German cultivars and cultivars with a socio-cultural, local and historical relation to Germany are in the focus of research.

The Fruit Genebank of the institute contributes to the implementation of the

'National Programme for Conservation and Sustainable Utilization of Genetic Resources of Agricultural and Horticultural Crops' and the realization of the international framework of European Cooperative Programme for Plant Genetic Resources', Working Groups *Malus/ Pyrus* and *Prunus*.

GA2 – Specific agreements. Does your genebank have any specific formal agreements with other genebanks regarding the conservation of specified germplasm?

(*This should include:*

a) *whether or not your genebank has any international agreements to conserve specified germplasm on behalf of other countries,*
b) *a specific region, and/or*
c) *the world*), and
d) *which crops or genepools fall under these agreements?*

The Institute for Breeding Research on Horticultural and Fruit Crops in Dresden is the National Coordination Centre of the German Fruit Genebank (www.Deutsche-Genbank-Obst.de).

GA3 -In case your genebank has a germplasm acquisition policy, what does the policy entail?.

a) please specify which crops or which geographic area, if applicable.

The genebank has no acquisition policy.

Crop specific expeditions have been performed for apple, pear and cherry. The goal is to capture and safeguard the genetic diversity on a region scale.

GA4 – How do you verify the identity of the germplasm material received (e.g. relying on the donor's information, comparing material with other accessions, involving (taxonomic) expertise, etc.)?

First, we rely on donor's information. Morphological verification for accessions of wild species and pomological verification of cultivars are conducted during field cultivation in the orchard starting from the fruiting period of the tree. Molecular analysis follow as a second part.

GA5 – Describe if and how you conduct an assessment of the various quality aspects of the seeds, tissue culture or plant material received.

(*This description includes:*

a) *quality aspects related to the correct identification of a given accession, but also*
b) *health*
c) *purity aspects of the sample/accession*), and
d) *use of a quality control system (e.g. ISO).*

Each accession of wild species and cultivars from unknown phytosanitary status that is supposed to be included into the collection is firstly grown in the greenhouse to determine its phytosanitary status. In case the check is positive the accession will be included into the active collection.

GA6 – Describe whether and how the SMTA is being implemented

a) Extent of materials covered by SMTA (crops, numbers of accessions)
b) Ways of SMTA implementation and documentation of transfers of PGR
c) *Other aspects (e.g. monitoring, supervision)*

SMTA has been implemented since 2008. *Fragaria* and *Malus* were chosen as these are genera on the Annex 1 list of the International Treaty on Plant Genetic Resources for Food and Agriculture and the access under the Multilateral System shall be provided pursuant to a Standard Material Transfer Agreement. The SMTA is used only for the delivery of vegetative propagation

material (SMTA article 2), scions in fruit trees of *Malus* and plants of *Fragaria*,. Special declarations are used for the delivery of material (1) to co-workers of other institutes belonging to the Julius Kühn-Institute - Federal Research Centre for Cultivated Plants, and (2) to private persons.
In addition and for all other deliveries (*Pyrus, Prunus* et al.) an Export list of the Institute for Breeding Research on Horticultural and Fruit Crops is used.

Box 1.2 Germplasm Collecting
GC1 – Describe here the details of the strategy that you follow in implementing germplasm collecting missions.
(*This description should include:*

a) *general aspects of planning and implementing a collecting mission,*
b) *the criteria you use for priority setting;*
c) *the actual strategy followed in sampling material from farmers' fields, from nature, etc.; and*
d) *how your germplasm acquisition policy underpins the mission*).

Preferential planning and implementing collection missions for crops and their wild relatives are realized on the basis of available ecogeographical and ethnobotanical data in close cooperation with specialists in the host countries and consulting local experts in the collecting areas. Germplasm collecting activities are generally based on written contracts between JKI and host institution/ country.
During the expedition the original accessions are documented by photo, passport and first characterization data. Scion material and fruits (seeds) are collected. A total collection list with preliminary botanical characterization is summarized before including the material into the genebank.
SE2 – Provide any additional information on the germplasm collecting activities of your genebank, including the collaboration with others.

2 Ensuring Security

This chapter refers to the security of the genebank structure itself (i.e. its physical security), the safety of its germplasm (i.e. the maintenance of viability) as well as the institutional and personnel security, aspects which together will ensure the long-term conservation of the entire collection.

2.1 Physical Security

To ensure the physical security of the collections, the following aspects are regarded as essential elements for achieving the objective:

Box 2.1.1 Safety Duplication (of long-term conserved germplasm)
SD1 - Please describe how your genebank implements the safety duplication of your germplasm material.
(*This description should include the following aspects:*

a) The type of safety duplication (e.g. black-box; no specific arrangement; other);
b) The location(s) where you store your safety duplicates (country; genebank);
c) Whether or not you are using a formal agreement with the genebank(s) that store your duplicates?

d) *Whether the safety duplicates are stored under conditions comparable to your own? Please provide details;*
e) *Do you maintain safety duplicates from other genebanks at your genebank? If so, do you know any details of that material?*)

The cultivars of the fruit species are duplicated in the German Fruit Genbank. The German Fruit Genebank is a decentralized network, which is aimed on coordination of germplasm collections at different sites in Germany. The work is organized in species specific networks. (e.g. apple network, strawberry network etc.). Preservation guidelines for each fruit species have been developed within the species specific networks.
Wild species (e.g. *Malus, Fragaria, Pyrus, Prunus*) collections are duplicated in *ex situ* at the location Dresden-Pillnitz, in *in vitro* culture or in cryopreservation.

SD2 – Do you have a safety duplication policy? If so, please provide essential details.

Box 2.1.2 Structure

SS1 - Please provide details on how your genebank building has been designed to resist natural disasters (e.g. earthquakes; flood; storm).

No earthquake area; no high wind/storm exposure; standard construction practices were followed

SS2 - Please describe the security arrangements that you have in place to protect your genebank against burglars, fire and others.
(*Please include details on the following arrangements, as applicable:*
a) *Fences;*
b) *Security doors;*
c) *Alarm system;*
d) *Fire detectors;*
e) *Standby generator;*
f) *Others (please specify*).

Genebank is located in a fenced territory. The buildings have locked doors and fire detectors. At night they are supervised by a security service.

SS3 – Please provide information on any other structural security aspects that you might have in place.

Box 2.1.3 Security Equipment

SE1 - Provide details on the kind of emergency (back-up) equipment or arrangements that you have in place to ensure permanent electricity and cooling.
(*Aspects to consider are:*
a) *"back-up" compressors for your cold rooms;*
b) *generator;*
c) *regular maintenance and trial runs;*
d) *other*).

Based on a facility management system there is an alarm system for temperature deviations in cold stores. In case of blackouts standby generators will automatically kick in.

SE2 – Describe how you monitor temperature and relative humidity in your

cold stores and drying room? daily control by staff

Box 2.1.4 Institutional and Personnel Security
IPS1 – Provide details on the "institutional security", in particular with respect to the provision of financial means to operate the genebank
(*Aspects to consider are:*

a) *timely transfer of funds from the "mother" organization to the genebank;*
b) *do you have direct access to the "mother" organization that provides the budget?;*
c) *internal "security" of accessing these funds;*
d) *long-term security and stability of funding (compensation of inflation rates, avoiding variation in years)*
e) *any other observations that are relevant in this context)*.

Foundation under public law, funding provided annually by Federal Ministry of Food, Agriculture and Consumer Protection.
IPS2 – Describe how you secure adequate staffing of your genebank is? Staff secured by permanent work contracts.

Box 2.1.5 Contingency Plans:
CP1 - Describe the kind of emergency or contingency plan that your genebank has in place to cope with disaster situations.
The institute has a contingency plan to cover all conceivable risks.
CP2 - Provide information on the kind of training, security drills and other activities that your genebank gives to its staff to deal with emergency situations, if any.
Staff informed and trained regularly about emergency situations like fire and health hazards

3 Germplasm Maintenance

This chapter deals with key aspects of managing germplasm in a genebank, i.e. the maintenance of the viability, the genetic integrity, the availability of the conserved germplasm as well as the management of the corresponding information. Given the fact we are covering seed, in vitro cultures and entire plants it might well be that not all aspects are covered by one and the same genebank. In those cases it is suggested that only the applicable sections are completed. Accordingly, at the beginning of each section of this chapter you will find a "navigation box" (highlighted in yellow) that will help you as user of the template to complete the correct section(s).

3.1 Maintenance of Viability

This section refers to the maintenance of the longevity of the seeds or of tissue cultures or living plants in storage. A high initial viability is the most important pre-condition for achieving the longest lifespan of seed accessions in storage, hence maximum efforts need to be taken to ensure that seeds to be stored have the highest possible viability. Optimum growing conditions when multiplying/regenerating the accessions, efficient management of the

preparatory steps before storing the germplasm, adequate storage conditions as well as proper monitoring of the viability are critically important.

Navigation Box on Maintaining Viability section

Seed – If applicable, please complete the section on Maintaining Viability for the activities related to seed genebanks (i.e. boxes 3.1.1.A – 3.1.3.A)

In vitro cultures – If applicable, please complete the section on Maintaining Viability for the activities related to in vitro culture (i.e. boxes 3.1.1.B – 3.1.3.B

Cryopreservation – If applicable, please complete the section on Maintaining Viability for the activities related to cryopreserved collections (i.e. boxes 3.1.1.C – 3.1.3.C)

Field genebanks – If applicable, please complete the section on Maintaining Viability for the activities related to field genebanks (i.e. boxes 3.1.1.D – 3.1.3.D.
Maintaining the genetic diversity of vegetatively propagated crops, like fruit species, is more demanding than in the case of most seed-producing plants because the specific genotype must be maintained. Primary collections of clonal crops are in the field; however, backups for these materials are needed to provide security in case of a disease or environmental disaster.

Seed Collections

Box 3.1.1.A Initial seed viability
IV1 - Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your seed, in particular during regeneration and post-harvest (e.g. cultivation practices, pollination aspects, use of specific equipment as shelters, storage of harvested seeds, cleaning, etc.).
Seed lots from expedition missions are stored for a short time (maximum 1 year) at 4°C. Seedlings population of the material are established and after comprehensive evaluation, selected wild germplasm will be included into the Fruit Genebank of the Institute for Breeding Research on Horticultural and Fruit Crops Dresden, Germany
IV2 – Describe procedures how you deal with a) dormancy and b) hard seeds?
Protocols for seed pre-treatment (cold treatment) internally (only in German) available
IV3 – Please provide any other information on procedures that you follow to ensure highest possible initial viability.

Box 3.1.2.A Seed Viability Monitoring
VM1 - Describe the routine seed viability monitoring system that you use.
(*The monitoring system should include the following aspects:*
a) *frequency of testing;*
b) *sampling method applied;*

c) *any thresholds that you use;*
d) *whether you apply different procedures for crops/species with erratic initial viability or irregular viability lifespan;*
e) *etc*).

VM2 - Please describe the information "system" that you might have in place that allows you to make more species or even accession-specific decisions when the next monitoring should take place.

VM3 - Please provide information on non-specific thresholds that you might use for viability of seeds (i.e. percentage of germination) and for the amount of seeds left of an accession to initiate regeneration? *In case you differentiate between self- and outbreeding species, please answer for each category separately.*

Box 3.1.3.A Seed Storage Conditions (for the different types of collections, i.e. short/medium- or long-term storage)
SC1 - Please provide details on temperature and relative humidity conditions of your storage and drying rooms. In case they vary from room to room, please provide details for each.
Short term storage at 4°C; air drying
SC2 – Provide details on the type of containers and the packaging procedures (and the corresponding equipment, if any) that you use.

SC3 - What is the range of seed moisture contents (smc) of your stored seeds of different species; what measures do you apply to keep and/or monitor the (low) moisture level? Do you treat different species differently?

SC4- Provide data on the total storage capacity (number of containers, number of accessions) and an estimated percentage to which extent this capacity has been filled.

SC4 – Please include any other aspects regarding storage conditions at your genebank that you regard as important (e.g. anticipated lifespan of freezing and drying equipment and related prudent financial management).

A. In vitro Culture Collections

Box 3.1.1.B Initial viability
IV1 - Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your plant material, in particular during culture of donor plants (e.g. cultivation practices [field, greenhouse], phytosanitary pre-treatments, like use of pesticides).
Fragaria plantlets are aseptically initiated from new formed runners on potted plants forced in the greenhouse in March. For forcing the boxes are

transferred from the orchard (active collection) to the greenhouse in December.

IV2 – Describe procedures of explant isolation (organ source in the plant, manipulations) and sterilization (chemical and handling) of the explants.

Runners are surface-sterilized with 0.1% mercuric chloride and washed twice with sterile water. Explants are placed in a half strength modified Murashige & Skoog (1962)(MS) liquid medium with 256 mg/L peptone and 88 mg/L yeast extract (pH=6.9) to detect internal contamination,. If no contamination appeared after one week, meristems with the first primordia are isolated and placed on modified MS medium.

IV3 – Please provide any other information on procedures that you follow to ensure highest possible initial viability.

The trimmed bases of the runners are placed on 523 Viss detection medium for a second contamination check for three weeks (Viss et al., 1991). After 7 weeks MS medium with 0.1 mg/L BAP, 0.01 mg/L IBA and 30 g/L sucrose are used for further multiplication. The initial shoot induction is evaluated after 6 to 8 weeks. The method is described in detail in Höfer, Acta Hort. 908, 2011

Box 3.1.2 .B Viability Monitoring

VM1 - Describe the routine in vitro viability monitoring system that you use.
(*The monitoring system should include the following aspects:*

a) *regular control of contamination events,*
b) *control of hyper-hydricity,*
c) *control of health state (if different from a above),*
d) *etc*).

In vitro viability monitoring is performed regularly during transfer from one subculture to the other. Furthermore, control checks are conducted every second day. These checks cover visual controls on fungal contamination. Hyperhydricity is excluded during transfers between the subcultures.

VM2 - Describe the information “system” (i.e. an “expert system”) that you might have in place that allows you to make more species or even accession-specific decisions when the next monitoring should take place.

No specific system implemented. Experience and skill of the technical staff is crucial for special decision.

VM3 - Please provide information on non-specific thresholds that you might use for vigor of in vitro cultures (i. e. multiplication rates, loss by weak growth) and for the amount of culture vessels (tubes, jars) left of an accession to initiate additional multiplication measures?

Decisions on the multiplication media are taken according to the personal experience of the responsible staff members.

Decisions are made accession-specific and will be recorded in the running laboratory protocols.

Box 3.1.3.B Storage Conditions (for the different types of collections i.e. short/medium- or long-term storage)

SC1 - Please provide details on light, temperature and relative humidity conditions of your culture and storage rooms, as applicable. In case they vary from room to room, please provide details for each.

Growth room conditions are at 23°C with a 16h photoperiod under 60-65 µmol m^{-2} s^{-1} photon flux.
Cold storage is at 4°C in under low light**.**

SC2 – Provide details on the type of cultivation vessels (tubes, jars plastic vessels etc.) and the transfer procedures (including the corresponding equipment, if any) that you use.

Culture tubes are used for the initiation phase whereas glass jars are used for in vitro propagation.
For cold storage 5 chamber bags (PhytoTechnology Laboratories) are used.

SC3 – Please include any other aspects regarding in vitro culture and storage conditions at your genebank that you regard as important.

B. Cryopreserved Collections

Box 3.1.1.C Initial viability

IV1 - Describe the procedures or practices that you have in place to ensure the highest possible initial viability of your cryopreservation explant (source: in vitro pre-culture or directly from in situ explants), sterilization and explant isolation.

In *Fragaria*, we use a protocol optimized for a large scale of genotypes: three factors are most important for highest initial viability: 1) quality of explant preparation, 2) absence of endophytes 3) genotype.

In *Malus*, a modified winter vegetative bud method, according to the USDA-ARS National Centre for Genetic Resources Preservation, Fort Collins is used.

IV2 – Please provide any other information on procedures that you follow to ensure highest possible initial viability (e.g. elimination of virus diseases).

Box 3.1.2.C Viability Monitoring

VM1 – Please indicate whether (and if so when and how) you perform random viability tests after the initial viability test? [see also VM3 below]

In *Fragaria*, there is no policy for random viability tests. We monitor viability in all cases of request of material and renew the active collection.

The criterion for the suitability of the method used is the regeneration rate which did not significantly change in comparison to the initial rate.

VM2 - Please describe the information "system" that you might have in place that allows you to make more species or even accession-specific decisions.

No special system is present.

VM3 – Indicate for the initial regeneration control,

a. what is the percentage of regenerated control explants relative to the total number of explants per accession;
   In *Fragaria,* the regeneration control includes 5 shoot tips out of 20 cryopreserved shoot tips (20%).
   In *Malus,* the regeneration control includes 20 dormant buds out of 40 cryopreserved buds (33%).
b. any thresholds that you use [e.g. discard the material as not storable below a certain regeneration rate of the control],
   In *Fragaria*, a minimum recovery of 40% is a baseline for storage of a given accession. In *Malus*, a minimum recovery of 25% is a baseline for storage of a given accession.
c. whether you apply different procedures for accessions with erratic regeneration rates of the control [e.g. increase the amount of explants stored]; etc. and
d. what is the threshold number of remaining explants of a given accession under which you initiate regeneration for multiplication?
   Samples need regeneration when explant number falls below 50% of the cryopreserved samples.

Box 3.1.3.C Storage Conditions (for the different types of collections i.e. short/medium- or long-term storage)

SC1 - Please provide information on the general system used for cryopreservation (liquid nitrogen or vapor phase, automatic tank filling or filling by hand). In case they vary from tank to tank, please provide details for each.
Liquid nitrogen is conducted by supra-isolated pipe system from the bulk nitrogen tank to the storage containers. In *Fragaria,* liquid nitrogen and in *Malus* the vapor phase is used.
SC2 – Provide details on the type of cryopreservation tanks and storage system within the tank that you use.
We use cryotanks Biosafe 200 and Biosafe 100 (Cryotherm) provided with standard rack systems consisting 8 or 4 racks with each 11 boxes and 120 tubes (1.8ml) per box.
SC3 - Do you treat different species differently?
Different protocols are used for cryopreservation: *Fragaria* is cryopreserved by vitrification method and *Malus* by dormant budwood method. All these protocols are published (Höfer and Reed 2011 in Acta Hort.918 Vol.1; Höfer Höfer 2007 in Adv. Hort. Sci. 21).
SC4 – Please include any other aspects regarding storage conditions at your genebank that you regard as important.

C. Field Genebank Collections

Box 3.1.1.D Initial viability
IV1 - Describe the procedures or practices that you have in place to ensure the highest possible quality of your planting material, in particular during the growing from donor plants (e.g. cultivation practices in the field or greenhouse], phytosanitary pre-treatments, etc.).
Replantation of woody species is realized approx. after 15 – 20 years. Sampling of buds in winter or summer time is carried out after visual screening or necessary phytosanitary testing.
Replantation of the *Fragaria* collection is realized after two years. Sampling of runners is carried out after visual screening or necessary phytosanitary testing.
IV2 – Describe any particular procedures you use (e.g. which organ of the donor plant you use to reproduce the planting material).
Buds of woody species and runners of strawberry are used for replantation.
IV3 – Please provide any other information on procedures that you follow to ensure highest possible initial quality.

Box 3.1.2 .D Viability Monitoring
VM1 - Describe the routine field genebank monitoring system that you use.
(*The monitoring system could include the following aspects: regular control of disease or pest contamination, other types of damages to the plants, etc*).
Plant health is checked regularly and visually diseased plants are pruned or removed; pest control measures are taken according to good horticultural practice
VM2 - Describe the information "system" that you might have in place that allows you to make more species or even accession-specific decisions when the next monitoring should take place.
Monitoring takes place frequently during growing season by permanent staff;

once a year a phytosanitory control is realized by official office.
VM3 - Please provide information on non-specific thresholds that you might use for the quality of the individual plants (e.g. loss by weak growth) and for the amount of plants of an accession left in the field before additional initiating multiplication measures?

Box 3.1.3.D Maintenance Conditions
SC1 - Please provide details on your cultural practices (e.g. cultivation practices; pruning; irrigation; protection against animals etc.; pest and disease management; etc. applied to your field genebank material.
Cultivation and pest/disease management according to good horticultural practice (well trained staff); specialists for pest and disease control available; irrigation; fences against wild boars or deer
SC2 – In the case of annual or sub-perennial species that cannot over-winter in the field genebank, what measures do you take?
In *Fragaria*, cold sensitive accessions are held in the greenhouse during winter time.
SC3 – Please include any other aspects regarding field genebank maintenance conditions at your genebank that you regard as important.

3.2 Maintaining Genetic Integrity

Maintaining the genetic integrity of an accession can be achieved by minimizing genetic drift which may occur predominantly during the process of regeneration, due to too small numbers of individuals being planted, sub-optimal pollination and/or the introgression of alleles from other accessions or commercial crops or crop wild relatives. The following aspects are important and for achieving the objectives of maintaining genetic integrity and should be briefly described. Please note that a distinction should be made between seed numbers for an accession and seed numbers for sub-samples per accession. The latter only applies if the seeds of a given accession are being stored and distributed as sub-samples. As genetically modified materials get more widely distributed and as it might have specific (legal, technical, administrative) requirements a separate box on this type of material is included.

For in vitro cultured and cryopreserved material, which are normally maintained as clones, genetic stability is as important as genetic integrity of the seed-stored material.

Navigation Box on Maintaining Genetic Integrity section

Seed – If applicable, please complete the section on Genetic Integrity for the activities related to seed genebanks (i.e. boxes 3.2.1.A – 3.2.5.A)
See above
In vitro cultures – If applicable, please complete the section on Genetic Integrity for the activities related to in vitro culture (i.e. boxes 3.2.1.B – 3.2.3.B

Cryopreservation – If applicable, please complete the section on Genetic

Integrity for the activities related to cryopreserved collections (i.e. boxes 3.2.1.C – 3.2.3.C)

Field genebanks – If applicable, please complete the section on Genetic Integrity for the activities related to field genebanks (i.e. boxes 3.2.1.D – 3.2.3.D

A. Seed Collections

Box 3.2.1.A Seed Containers and Sample Size

SCSS1 – Do you document the initial number of seeds of individual accessions (either as received from collecting missions or through exchange)?

Yes, number of seeds.

SCSS2 – Please describe what kind of containers (and equipment) you use, the procedure you follow with respect to sub-sampling, seed numbers per container, etc.

Woody species – paper bags; *Fragaria* – glass tubes

SCSS3 - What is the number of seeds that you use as the minimum threshold per accession? Are these seed numbers of a given accession based on genetic parameters (such as reproduction biology; heterogeneous samples)? Please provide URL of your protocols if these are on-line available

No minimum threshold

SCSS4 – Please provide details on other aspects that are important in this context.

Box 3.2.2.A Pollination Control

PC1 - Please describe the regeneration procedures that you follow for self- and outbreeding species.

(*Please include in your description the following aspects:*

a. *Any control measures to minimize or avoid cross pollination between accessions;*
b. *The use of pollination cages for insect pollinated species;*
c. *The use of specific pollinators for insect pollinated species;*
d. *Strategies to ensure that males and females participate equally in the reproduction*).
e. *Strategies to avoid any genetic drift (minimum number of plants, minimum number of plants at flowering stage before pollinators introduction, similar quantity of seeds harvested from each plant, etc.)*

PC2 – Provide any other relevant information on procedures that you apply to control pollination of your germplasm.

Box 3.2.3.A Regeneration Environment and Procedures

RE1 – Describe the regeneration environment and conditions that you apply. If applicable, you might want to distinguish between different types of germplasm (e.g. wild relatives, landraces, modern varieties, breeding material, genetic stocks, etc.).

(*Consider the following aspects:*

a) *In how far are the environmental conditions of the current*

regeneration of individual germplasm accessions comparable to the environmental conditions that existed at the original collecting or breeding site?;

b) *Do you use controlled environments?;*

c) *Do you collaborate with other genebanks in Europe?;*

d) *others*).

RE2 – Please include any other relevant points on regeneration environment.

Box 3.2.4.A Seed Processing Procedures

SPP1 – Describe the protocol(s) that you use for threshing and seed cleaning.

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SPP2 – Describe the protocol(s) that you use for seed drying, including whether you use different drying procedures for different types of species.

SPP3 – Please describe how you keep the time between harvesting and the actual (long-term) storage of seeds as short as possible.

SPP4 – Please describe how and where you store (in a temporary manner) newly harvested seeds.
(Please provide details on the temperature and relative humidity of the storage room/space; what type of containers do you use, if any).

SPP5 – Describe the criteria you use to decide on seed quantity per accession for the long-term storage.

Box 3.2.5.A Genetically Modified Material

GMM1 – In case you treat GMO material differently from "normal germplasm", please provide here the details for each of the deviating procedures (and equipment).

No GMOs in the genebank

GMM2 – Describe the policy and procedures (if any) in your genebank, related to ensuring that distributed samples are not containing GMOs.

B. In vitro Culture Collections

Box 3.2.1.B In vitro Culture Vessels and Sample Size

SCSS1 – Indicate if you document the initial number of explants of individual accessions when culture is initiated (from one or from more clonal donor plants)?

When it is clear, that the accessions are true clones, culture is initiated from more clonal donor plants.**SCSS2** – Please describe in general terms the type of culture vessels (as far not already done in section SC2 in Box 3.1.3.B), media and phytohormones you use as well as the procedures you follow with respect to cutting technique, callus exclusion, etc.

Glass jars are used for in vitro propagation. For in vitro cold storage 5 chamber bags (PhytoTechnology Laboratories) are used. Medium is usually based on Murashige and Skoog's (1962) formula. As a rule, phytohormones are not used for cold storage.

SCSS3 – Please indicate whether or not you use a minimum number of in

vitro plantlets per accession?
In vitro culture is the base to start an in vitro cold storage and the cryopreseravation. For cold storage we have 10 plants in 5 chamber bags (PhytoTechnology Laboratories).
SCSS4 – Please provide details on other aspects that are important in this context.

Box 3.2.2.B In vitro Culture Procedures
SPP1 – Describe the numbers of sub-clones you may cultivate per accession (assuming that this is not crop specific)
SPP2 – Describe the sub-culture duration (if not crop specific)
In vitro culture – 4 weeks; In cold storage inventory will be done every two months.
SPP3 – Describe the criteria you use to decide on in vitro plant quality (if not crop specific). No bacteria/fungi/callus formation, healthy looking. Criteria are good vigor and no hyperhydricity.

Box 3.2.3.B Genetically Modified Material
GMM1 – In case you treat GMO material differently from "normal germplasm", please provide here the details for each of the deviating procedures (and equipment).
We do not store genetically modified material.

C. Cryopreserved Collections

Box 3.2.1.C Cryopreservation Containers and Sample Size
SCSS1 – Indicate if you document the initial number of explants of individual accessions?
The initial number of explants and the regeneration control explants are documented in our system.
SCSS2 – Please describe what kind of cryopreservation vessels (and equipment) you use (only if they differ from the corresponding answers in previous boxes), the procedure you follow with respect to separate material containing viruses or bacteria from healthy material
See above
SCSS3 - What is the number of explants that you use as the minimum threshold per accession?
See above
SCSS4 – Please provide details on other aspects that are important in this context.

Box 3.2.2.C Cryopreservation Procedures (as long as not crop specific)
SPP1 – Describe the protocol(s) that you use for preculture and pretreatment such as cold acclimation and dehydration.
Fragaria - 14 d alternating-temperature cold acclimation (16 h at -1°C and 8 h at 22°C) + shoot tips are dissected and cultured on MS medium with 5% DMSO for two days under cold acclimation conditions
Malus - sections are desiccated to 30 % moisture by placing them into a -5 °C cold chamber.

SPP2 – Describe the protocol(s) that you use for cryopreservation proper (such as slow freezing, droplet freezing, vitrification, encapsulation etc.)
See above
SPP3 – Describe the protocols that you use for regeneration (slow or fast rewarming, washing, dark periods etc.)
In *Fragaria* - the vials are rewarmed by plunging into sterile water (40°C) for 2 min. The PVS2 solution is removed from the vials and the unloading solution (1.2 M sucrose) is added and maintained for 20 min. Finally, the shoot tips are transferred to normal proliferation medium in Petri dishes.
In *Malus* - after cryopreservation the scion pieces are transferred into moist peat moss for a 15 d rehydration period at 4 °C. For chip-budding each rewarmed single bud is grafted onto a rootstock 15 – 20 cm above soil level.
SPP4 – Describe the time span and method(s) of survival and regeneration controls
Recovery is assessed in *Fragaria* after 6-8 weeks and in *Malus* after 3-4 months.
SPP5 – Describe the criteria you use to decide on explant quantity per accession for the long-term storage.
See above

Box 3.2.3.C Genetically Modified Material
GMM1 – In case you treat GMO material differently from "normal germplasm", please provide here the details for each of the deviating procedures (and equipment).
Not relevant

D. Field Genebank Collections

Box 3.2.1.D Accession Sample Size
SCSS1 – Indicate if you document the initial number of plants of individual accessions (either as received from collecting missions or through exchange)?
Initial number of plants is documented.
SCSS2 – Please describe what kind of procedures you follow, if any, with respect to sub-sampling and subsequent place/container/etc. of maintenance?
none
SCSS3 - What is the number of plants that you use as the minimum threshold per accession? Are these plant numbers of a given accession based on genetic parameters (such as reproduction biology; heterogeneous samples)?
In woody species we have two trees per accession. In *Fragaria* we have 3 - 6 plants depending on the existing duplicate collection.
SCSS4 – Please provide details on other aspects that are important in this context.

Box 3.2.2.D Multiplication not relevant
PC1 - Please describe the multiplication procedures that you follow for your field genebank material (both, annual as well as perennial species)?
(*Please include in your description the following aspects if they would apply to your field genebank management procedures): :*

a. *Any control measures to minimize or avoid cross pollination between accessions (if applicable/relevant);*
b. *The use of pollination cages for insect pollinated species;*
c. *The use of specific pollinators for insect pollinated species;*
d. *Strategies to ensure that males and females participate equally in the reproduction*).
e. *Strategies to avoid any genetic drift (minimum number of plants, minimum number of plants at flowering stage before pollinators introduction, similar quantity of seeds harvested from each plant, etc.)*

PC2 – Provide any other relevant information on procedures that you apply to control pollination of your germplasm in case of harvesting planting material from your field genebank material?

Box 3.2.3.D Planting Material Processing Procedures not relevant

SPP1 – Describe the protocol(s) that you use for threshing and seed cleaning, if used as an intermediate step for the management/multiplication of your field genebank accessions
.

SPP2 – Please describe how and where you store (in a temporary manner) newly harvested planting material.
(Please provide details on the temperature and relative humidity of the storage room/space; what type of containers do you use, if any, etc.).

SPP3 – Describe the criteria you use to decide on the number of plants per accession intended for the long-term conservation.

3.3 Ensuring Availability

An important objective of conservation efforts is to facilitate the effective utilization of germplasm accessions by researchers, breeders and farmers. Thus, ensuring the ready availability of stored germplasm is an important principle. It refers to the ability of genebanks to supply and distribute the stored germplasm, together with any associated information, in an adequate way to users. Aspects that can affect the availability include: (a) policies, (b) seed stock, (c) health status of accessions, and (d) distribution quantity. Although most of the questions are not relevant in the ECPGR/AEGIS context, it was decided to keep the questions and to allow for a comprehensive genebank manual that can be used “globally”.

Navigation Box on Ensuring Availability

Seed – If applicable, please complete the section on Ensuring Availability for the activities related to seed genebanks (i.e. boxes 3.3.1.A – 3.3.4.A)

In vitro cultures – If applicable, please complete the section on Ensuring Availability for the activities related to in vitro culture (i.e. boxes 3.3.1.B – 3.3.4.B

Cryopreservation – If applicable, please complete the section on Ensuring

Availability for the activities related to cryopreserved collections (i.e. boxes 3.3.1.C – 3.3.4.C)

Field genebanks – If applicable, please complete the section on Ensuring Availability for the activities related to field genebanks (i.e.boxes 3.3.1.D – 3.3.4.D

A. Seed Collections

Box 3.3.1.A Ensuring Availability of Germplasm – Policy Aspects
AGP1 – Describe the germplasm distribution policy that you follow at your genebank.
(*You might want to consider in your response the following aspects:*
a) *crop/species specificity;*
b) *whether or not sufficient seed stock is available; who the requestor is;*
c) *what the purpose of the germplasm request is;*
d) *any restrictive conditions and/or*
e) *the total amount of accessions sent per request for distribution of germplasm;*
f) *use of a formal agreement to distribute the germplasm*).

AGP2 - Do you have as part of your service rendering policy aspects such as a "maximum time" between receiving a germplasm request and distribution of the germplasm?

AGP3 – Describe how you treat "related information" about the requested accessions that you make available to the requestor, i.e. provide details on the typical information you send out with the germplasm.

Box 3.3.2.A Ensuring Availability of Germplasm – Seed/Germplasm Stock Aspects
AGSS1 - Please provide details on the minimum/maximum amount of seed, plant, in vitro samples that you distribute (where relevant, differentiated by species groups, i.e. self-pollinating, cross-pollinating and/or whether an accession is homo- or heterogeneous).

AGSS2 – Describe how you store the seeds/etc. of a given accession with respect to the use of single or multiple bags or containers per accession.

AGSS3 – Describe how you manage the availability of adequate seed/etc. stock per accession, including the use of an absolute lower minimum of seeds per accession as the threshold to decide to regenerate.

AGSS4 – Provide here information on any other aspects that are relevant to manage seed/etc. stocks.

Box 3.3.3.A Ensuring Availability of Germplasm – Health Aspects
AGHA1 – Describe how you store seed/other germplasm with respect to germplasm health considerations, including whether you have a "policy" of storing only "disease free" (as far as you can see or determine) accessions, at

least for the quarantine pests and diseases.

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).

AGHA3 – Describe if and how you distribute germplasm accompanied by a phytosanitary certificate or a "plant passport".

AGHA4 – Provide any other relevant information on procedures that you follow with respect to germplasm health aspects.

Box 3.3.4.A Germplasm Supply

GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes, including whether you differentiate between germplasm from self- or outbreeding species, heterogeneous accessions, and possibly other aspects.

GS2 – As GS1 above, but in case your germplasm samples do not possess the minimum viability, would you increase the number of seeds?

GS3 – Please provide information on any other aspects related to seed supply.

B. In vitro Culture Collections

Box 3.3.1.B Ensuring Availability of Germplasm – Policy Aspects

AGP1 – Describe the germplasm distribution policy that you follow at your genebank.
(*You might want to consider in your response the following aspects: is the user informed about the option to get provided with in vitro cultures and whether they are available all the time of the year, are in vitro samples an option or the only way to get material; who the requestor is; what the purpose of the germplasm request is; any restrictive conditions and/or the total amount of accessions sent per request for distribution of germplasm; use of a formal agreement to distribute the germplasm)*
In *Fragaria in vitro* cultures are distributed if there is *in vitro* material available. *in vitro* samples are one of two options, not distributed to private individuals. Restrictive conditions/limitations in total amount of accessions; SMTA as formal agreement to distribute the germplasm

AGP2 – Indicate if you have as part of your service rendering policy aspects such as a "regular or a maximum time" between receiving a germplasm request and distribution of the germplasm?
due to maintenance cycle requirements no time period

AGP3 – Describe how you treat "related information" about the requested accessions that you make available to the requestor, i.e. provide details on the typical information you send out with the germplasm.
typical information: acc. name and number

Box 3.3.2.B Ensuring Availability of Germplasm – Germplasm Stock

Aspects

AGSS1 - Please provide details on the maximum amount of in vitro samples that you distribute.

no distinct limits fixed, but usually 5 plantlets per accession

AGSS2 – Describe how you store the samples of a given accession with respect to the use of vessels for culture and vessels for distributions (glasses of plastic bags).

Distributed in 5 chamber bags (PhytoTechnology Laboratories).

AGSS3 – Describe how you manage the availability of adequate plants per accession, including the use of an absolute lowest minimum of plants per accession as the threshold to decide to regenerate.

The request has to be sent early to have time for propagation.

AGSS4 – Provide here information on any other aspects that are relevant to manage stocks (e.g. transfer of material through greenhouse transfer phases in case a user cannot handle in vitro cultures).

Box 3.3.3.B Ensuring Availability of Germplasm – Health Aspects

AGHA1 – Describe how you store germplasm with respect to germplasm health considerations, including whether you have a "policy" of storing only "disease free" (as far as you can see or determine) accessions, at least for the quarantine pests and diseases.

After culture initiation all accessions of *Fragaria* are tested for *Xanthomonas fragariae.*

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).

within EU provision of plant passports, phytosanitary certificate for outside EU provision (valid health tests and mostly import permits required)

AGHA3 – Describe if and how you distribute germplasm accompanied by a phytosanitary certificate or a "plant passport".

See AGHA2

AGHA4 – Provide any other relevant information on procedures that you follow with respect to germplasm health aspects.

Box 3.3.4.B Germplasm Supply

GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes.

usually 5 plantlets per accession

GS2 – Please provide details of your routine methodology of containers etc. that you use to distribute in vitro cultures.

Distributed in 5 chamber bags (PhytoTechnology Laboratories).

GS3 – Please provide information on any other aspects related to in vitro plant supply.

not shipped in periods of frost; express delivery for remote countries, even within Europe (periods without light as short as possible)

C. Cryopreserved Collections

Box 3.3.1.C Ensuring Availability of Germplasm – Policy Aspects

AGP1 – Describe the germplasm distribution policy that you follow at your

genebank. No distribution of cryopreserved material
(*Cryopreserved material is for distribution in exclusive cases only – e.g. for special research, please describe your policy*; *who the requestor is; what the purpose of the germplasm request is; any restrictive conditions and/or the total amount of accessions sent per request for distribution of germplasm; use of a formal agreement to distribute the germplasm*).

AGP2 – Indicate if you have as part of your service rendering policy aspects such as a "regular or maximum time" between receiving a germplasm request and distribution of the germplasm?

AGP3 – Describe how you treat "related information" about the requested accessions that you make available to the requestor, i.e. provide details on the typical information you send out with the germplasm.

Box 3.3.2.C Ensuring Availability of Germplasm – Germplasm Stock Aspects
AGSS1 - Please provide details on samples that you distribute (where relevant).

AGSS2 – Describe how you store, for distribution, the cryopreserved material of a given accession with respect to the use special equipment such as dry-shippers etc.

AGSS3 – Describe how you manage the availability of adequate cryopreserved material.

AGSS4 – Provide here information on any other aspects that are relevant to manage seed/etc. stocks.

Box 3.3.3.C Ensuring Availability of Germplasm – Health Aspects
AGHA1 – Describe how you store seed/other germplasm with respect to germplasm health considerations, including whether you have a "policy" of storing only "disease free" (as far as you can see or determine) accessions, at least for the quarantine pests and diseases. You could also add data on separation of differently infested material in separate cryotanks etc.

AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).

AGHA3 – Describe if and how you distribute germplasm accompanied by a phytosanitary certificate or a "plant passport".

AGHA4 – Provide any other relevant information on procedures that you follow with respect to germplasm health aspects.

Box 3.3..C4 Germplasm Supply
GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes.

GS2 – Please provide details of your routine methodology of containers etc. that you use to distribute cryopreserved material.

GS3 – Please provide information on any other aspects related to cryopreserved material supply.

D. Field Genebank Collections

Box 3.3.1.D Ensuring Availability of Germplasm – Policy Aspects

AGP1 – (*You might want to consider in your response the following aspects: crop/species specificity; whether or not sufficient seed stock is available; who the requestor is; what the purpose of the germplasm request is; any restrictive conditions and/or the total amount of accessions sent per request for distribution of germplasm; use of a formal agreement to distribute the germplasm*).

For all species according the availability. Material requests for *Fragaria* have to be sent until the end of May; plantlets will be distributed in August. For woody species the requests have to be sent until October; dormant scions will be distributed the following year until March.

AGP2 – Indicate if you have as part of your service rendering policy aspects such as a "maximum time" between receiving a germplasm request and distribution of the germplasm?

See above

AGP3 – Describe how you treat "related information" about the requested accessions that you make available to the requestor, i.e. provide details on the typical information you send out with the germplasm.

typical information: acc. name and number

Box 3.3.2.D Ensuring Availability of Germplasm – Seed/Germplasm Stock Aspects

AGSS1 - Please provide details on the minimum/maximum amount of plants or organs (cuttings, bulbs, tubers, etc.) per plant that you distribute per accession (where relevant, differentiated by species groups, i.e. annual or perennial; woody or herbaceous; other) and/or whether an accession is clonally or sexually propagated).

In *Fragaria* 2-3 plants per accession; in woody species (2-3 scions per accession).

AGSS2 – Describe how you manage the availability of adequate organs per accession, including the use of an absolute lower minimum of plants per accession as the threshold to decide to multiply.

See above. In woody species we have two trees per accession. In *Fragaria* we have 3 - 6 plants depending of the existing duplicate collection

AGSS3 – Provide here information on any other aspects that are relevant to manage plant material stocks.

Box 3.3.3.D Ensuring Availability of Germplasm – Health Aspects

AGHA1 – Describe how you maintain field genebank (and any intermediate storage step) accessions with respect to health considerations, including

whether you have a “policy” on accepting/planting only “disease free” planting material (as far as you can see or determine) accessions, at least for the quarantine pests and diseases.
See above. No further policy is followed on disease-free material
AGHA2 – Describe how you follow plant quarantine rules and regulations when exporting germplasm abroad (especially to countries at another continent).
within EU provision of plant passports, phytosanitary certificate for outside EU provision (valid health tests and mostly import permits required)
AGHA3 – Describe if and how you distribute germplasm accompanied by a phytosanitary certificate or a “plant passport”.
See above
AGHA4 – Provide any other relevant information on procedures that you follow with respect to germplasm health aspects.

Box 3.3.4.D Germplasm Supply
GS1 – Describe the policy of your genebank with respect to the sample size that you use for distribution purposes, including whether you differentiate between germplasm from annual or perennial species, clonally or sexually propagated accessions, and possibly other aspects.
Only depending on availability
GS2 – Please provide information on any other aspects related to seed supply.

4 Providing Information

The lack of adequate information on a given accession may well decrease the value of that accession to the user. The information on individual accessions should be as complete as possible in order to facilitate the identification of duplicates and/or to select accessions with desirable characteristics. A genebank should have a documentation system in place that allows to optimize management of the collections as well as to provide access to information about the collection to users.

Box 4.1 Genebank Documentation System
GD1 - Please provide details on the technical aspects of the genebank information management system(s) that you use.

a) On which software is the system based (i.e. Oracle, Fox Pro, MS Access, MS excel, MS Word, other?).
b) In case you use a manual information management system, please provide details.
c) In case your “internal” database(s) is/are different from the publicly available database(s), please provide details on both,
d) Describe which activities of the genebank are covered by the system.

Genebank information system (My SQL database) consisting of passport data, botanical determination, handling of botanical names, etc. will be developed. In addition, agronomical and characterization data are recorded using paper sheets.
GD2 - Provide details on which types of data you handle in your documentation system, e.g. passport data, characterization & evaluation data,

cultivar data, material distribution etc.
passport data, botanical determination, handling of botanical names (taxonomy), characterization and evaluation data etc.

GD3 - In case your internal database(s) is/are different from the publicly available database(s), please provide details on both.
In the first step only passport data will be available in the public data base.
GD4 – Describe in which form you send accession specific data (e.g. as hard copy, electronically – if the latter, please specify (in plain text) which file format, i.e. Excel, Access, others is used).
with short excerpt of passport data; if requested and available additional data can be provided as Excel file
GD5 - Provide information on how technical support for development and maintenance of the documentation system is arranged

GD6 – Describe your genebank policy with respect to backing-up of the database contents, including with which frequency?
In the future a permanent back up is the aim.
GD7 – Provide any other information on your information management system that is not covered in one of the above questions.

Box 4.2 Information Exchange
IE1 – Please describe how you make your passport data available to users (i.e. as hard copy; via the internet; other?).
internet, Excel file if requested
IE2 - Please indicate if your data is available as machine to machine web-services. In case it is, describe

a. what types of data (passport data, characterization & evaluation data etc) and
b. which web-service interfaces are available (i.e. GBIF IPT, BioCase, TapirLink).

See above
IE3 - Please indicate if your data is published to EURISCO. Describe which data is published to EURISCO and at which intervals.
Our data are published to EURISCO and updated regularly.
IE4 – Please provide any other information on information exchange that is important for others to know.

IE5 - Describe the kind of information you distribute together with the germplasm to persons that request germplasm?
(*Please consider the following data types: Passport, Characterization; Evaluation, and/or Germplasm management data (e.g. viability percentage; protocols followed for routine operations; etc.*).
typical information: acc. name and number

Thank you for the efforts you have made to answer all the questions. This information will be important to you and your colleagues at the genebank as well as to the Working Groups and other bodies in ECPGR for the establishment of a quality genebank management system!

The ECPGR Secretariat