#### PROTOCOL FOR MULTIPLICATION OF CUCUMBER

This protocol applies to all parties involved in multiplication of CGN material.

#### Introduction

Multiplications have to fulfill minimum quality requirements to ensure maintenance of genetic identity and integrity (no unwanted mixing among accessions, no genetic narrowing through drift) and a high quality of seed (no diseases, good germination capacity). Contamination through Genetic Modified Organisms (GMO) must be prevented to the best ability.

Any deviation from this protocol must be reported to CGN and will be recorded by CGN in the Multiplication logbook (FOR-CGN-PG-002).

#### Multiplication

##### Maintaining genetic integrity

* isolation
* All accessions are multiplied in pollinating-insects free greenhouses.
* population size
* Ten plants are multiplied per accession. In the case of heterogeneity, material that is hard to multiply or other considerations a minimum of fifteen plants must be used. In consultation with CGN, population sizes can be adjusted when modification is considered necessary.
* In planting out, the required number of plants is chosen non-selectively from the population, although plants that lay considerably behind in growth may be left out because their poor performance could mean that these plants eventually do not contribute to seed multiplication of the accession.
* The number of plants that have been included in a multiplication is recorded for every accession. These data are copied into the Multiplication logbook.
* sowing
* The possibility of dormancy or poor germination capacity of the seed is taken into account. Germination advice sent with the seeds from CGN or own methods of breeders are followed.
* If material germinates poorly or very slowly, these findings are recorded and copied into the Multiplication logbook.
* pollination
* Normally the Chain Pollination method is used in order to maintain the genetic diversity within an accession. Self-Pollination is used, when indicated by CGN in the excel logbook.
* Companies determine themselves which methods they use to induce male or female flowering. In case there is no information whether an accessions produces only male of female flowers, it needs to be checked during the growing of young plants. This gives enough time to take measures for inducing male or female flowering. CGN informs when an accession is hermaphrodite.
* harvest
* From each plant approximately the same amount of seeds is harvested, to get a representative seed mixture. This is realized by harvesting the same amount of fruits per plant. If this is not realistic, CGN is consulted.
* If very little seed is produced, it should also be returned.

##### Maintaining identity

* characteristics
* During sowing, cultivation and harvest, accessions must be clearly marked with a label giving the field number. All field numbers assigned before sowing remain unchanged up to and including harvest.

##### Maintaining seed quality

* seed disinfection before sowing
* Not all seed lots from CGN are heat treated. It is recommended to give a heat treatment before sowing.
* inspection
* The crop is monitored closely. Attention should be paid to seed born viruses and viroids. A report is required of this visual inspection. Where applicable the material will be tested according the instructions, mentioned in the actual EU guidelines/regulations. Heat treatment does not guarantee that the material is free of viruses. Diseases or plagues are combated; this is recorded and copied into the Multiplication logbook. When a disease is identified that might jeopardize proper multiplication of the seed, CGN is notified.
* seed handling after harvest
* In consultation with CGN, a decision is made how the seeds will be cleaned after harvest. The cleaning method is registered in the logbook.
* Harvested seeds are NOT given a heat treatment because this can influence the germination rate and storability of the seeds.
* Cleaned seed samples must be dried well and stored under circumstances with low temperature and Relative Humidity.

#### Concluding actions

* Any deviations during cultivation or cleaning are noted in the appropriate records, which are sent with the seed. These notes are copied into the Multiplication logbook.
* Harvested seed is sent to CGN as soon as possible, in any case no later than six months after harvest. The seed bags are identified by their field number and their CGN number.