##### **PRT-CGN-PG-117 PROTOCOL mulitiplication clover**

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

### **Introduction**

Multiplications have to fulfil minimum quality requirements to ensure maintenance of genetic identity and integrity (avoiding seed mixing among accessions and minimizing loss of diversity through genetic drift) and high seed quality (absence of diseases and sufficient germination ability).

Contamination with Genetically Modified Organisms (GMO) should be prevented.

*Any deviation from this protocol should be reported to CGN, after which it will be recorded by CGN in the Multiplication logbook (FOR-CGN-PG-002).*

**Multiplication**

Maintiaining genetic integrity

* Isolation
* Clover is a cross-pollinator. The material is therefore propagated per accession in isolation fields, spaced at least 40 m apart. The isolation crop is rye or Triticale.
* Population size
* Multiplication is done on about 70 plants, the minimum number of plants is 25 to a maximum of 150.
* When pricking out and transplanting, the required number of plants is chosen without applying selection in the population. However, plants that lag much behind in growth may be omitted if the lag would result in these plants not eventually contributing to seed multiplication of the accession.
* It is recorded how many plants per accession participated in multiplication. These data are recorded in the Multiplication logbook.
* Sowing
* Any seed hardiness is taken into account. Germination recommendations or local germination methods are followed.
* The number of seeds to be sown will be determined by the CGN.
* If material germinates poorly or very slowly, these findings are recorded and copied into the Multiplication logbook.
* Cultivation
* No selection takes place. If the accession is a mixture of different species, the CGN will be notified. The CGN decides whether and how selection may take place. This is recorded and copied into the Multiplication logbook.
* Red clover, if there is irregular flowering, can be mowed in June to encourage synchronous flowering.
* Pollination
* There is pollination by on-site insects.
* Harvest
* All inflorescences with the mature seed are harvested and bagged by field number.
* All seed produced is returned to the CGN.

Maintaining identity

* Characteristics
* During sowing, cultivation and harvesting, accessions should be clearly marked with a label indicating the field number. All field numbers assigned prior to seed sowing should remain unchanged up to and including harvesting and seed cleaning.

Maintaining seed quality:

* Seed decontamination before sowing
* Not relevant.
* Pruning
* Not relevant.
* Inspection
* Control against diseases and pests takes place.
* Seed treatment after harvest
* Bags with the harvested plant parts are pre-dried.
* In consultation with the CGN, it will be decided how the seed will be cleaned after harvest.

**Concluding actions**

* All deviations during cultivation are noted and sent with the seed. These notes are copied into the Multiplication logbook.
* The harvested seed is sent to the CGN as soon as possible, but no later than 6 months after harvest, with the seed bags bearing the CGN number and field number.