

## NEW CONVENTIONS FOR DNA FINGERPRINTING: METHODOLOGICAL WORKFLOWS AND FUTURE PERSPECTIVES.

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DNA fingerprinting of fruit trees has its historical utilization in the identification and classification of genetic resources, often conserved in *ex situ* and *in situ* collections. These can greatly vary in size, nature of the plant material, management and networking capabilities, and are organized in the form of living germplasm repositories, botanical gardens or even private collections. An emerging field of application is the genetic identification of varieties for the protection of plant breeders' right and for the management of plant material in nurseries. This latter field of application is destined to further expansion with the growing release of essentially derived varieties produced by the new breeding techniques (NBT), which differ from the original variety by one or a few point mutations in the genome. The need to create networks between international groups responsible for the collections of living germplasm and varietal identification is leading to the building of panels of shared Single Nucleotide Polymorphisms (SNPs) to be used as "barcodes". Selected panels have already been proposed, consisting of 45, 21, and 37 SNPs, respectively for pear (*Pyrus spp* L.), apple (*Malus domestica* (Suckow) Borkh.), and sweet chestnut (*Castanea sativa* Mill.). Particularly, in sweet chestnut, the availability of fast, easy, scalable, and cost-effective workflow for genotyping of a small to large number of unknown new

accessions is aiding the diffusion of the barcoding approach. Indeed, the re-ordering of the Italian chestnut germplasm has been entrusted to CREA by the Italian Ministry of Agriculture, Food Sovereignty and Forests (MASAF) and it is ongoing using a panel of 37 SNPs, which will be expanded to 120 in a core group of reference genotypes. Furthermore, the innovation project KASTRACK funded by Campania Region is going to transfer this method for chestnut DNA fingerprinting to small private laboratories. This important step involves the release of an interactive public database for reference fingerprints, as well as the implementation of specific protocols for the collection of samples in the field and their investigation by direct Polymerase Chain Reaction (dPCR). At the same time, some of the 45 SNPs proposed for the identification of pear varieties are being tested for dPCR applications and to ascertain their discriminating power within the Italian pear germplasm. The experiences gained up to now encourage us to consider the conventional identification of these SNP panels as a keystone for an effective and rapid recovery of the germplasm in large and small public/private collections. To this aim, an adequate subset of SNPs should be selected by mutual agreement for species not yet explored.