

# Genotyping-by-sequencing to manage reference collections in DUS testing: a case-study on an ornamental species, Hydrangea

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# Introduction

**Hydrangeas, one of the world's most popular ornamental flowers, are also one of the most economically important ornamental species in France**, with Angers and the Maine-et-Loire accounting for 25% of European production of Hydrangeas. With 40-70 applications for plant breeder rights (PBR) each year, **Hydrangeas are on track to become one of the top 10 ornamental species in Europe in the next few years**. In France, the French Variety and Seed Study and Control Group (GEVES) is responsible for the Hydrangea national collection, an *in vivo* reference collection of over 900 taxa (species, sub-species and cultivars) maintained at the Gaston-Allard Arboretum in Angers. As the number of applications grows so does the size of the reference collection, with about 50 new varieties added to the reference collection every year, and new means are required to help manage the collection and secure Distinction, Uniformity, Stability (DUS) examinations.

## Objectives

In 2022, **GEVES (France)** and the **Bundessortenamt (Germany)** started an 18-month joint project co-funded by CPVO to develop a molecular toolset that associates neutral and gene-specific markers within a single framework to assist with the management of the **reference collection** and the selection of reference varieties to include in DUS trials.

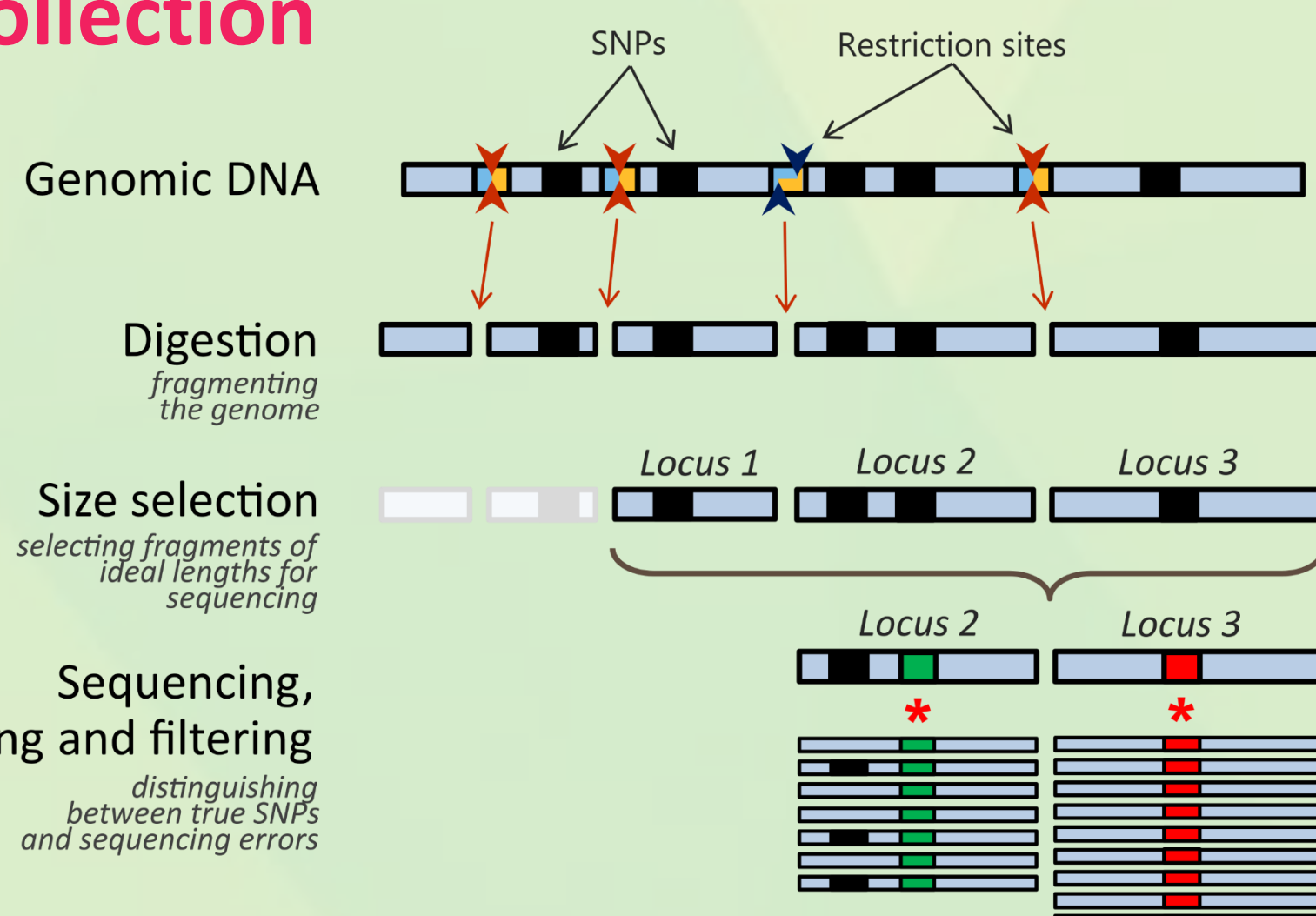
## Methods

Building upon the rapid development of high-throughput sequencing technologies (Next-Generation Sequencing) and the sharp decline of sequencing costs, **the project will use a targeted genotyping-by-sequencing (GbS) approach to characterize genetic diversity in the Hydrangea collection.** NGS allows thousands of individuals to be screened at 1000s–10,000s of loci simultaneously and building up comprehensive libraries of varietal molecular profiles for entire reference collections, all in one go.

## A two-step, NGS-based approach to genotype the entire reference collection

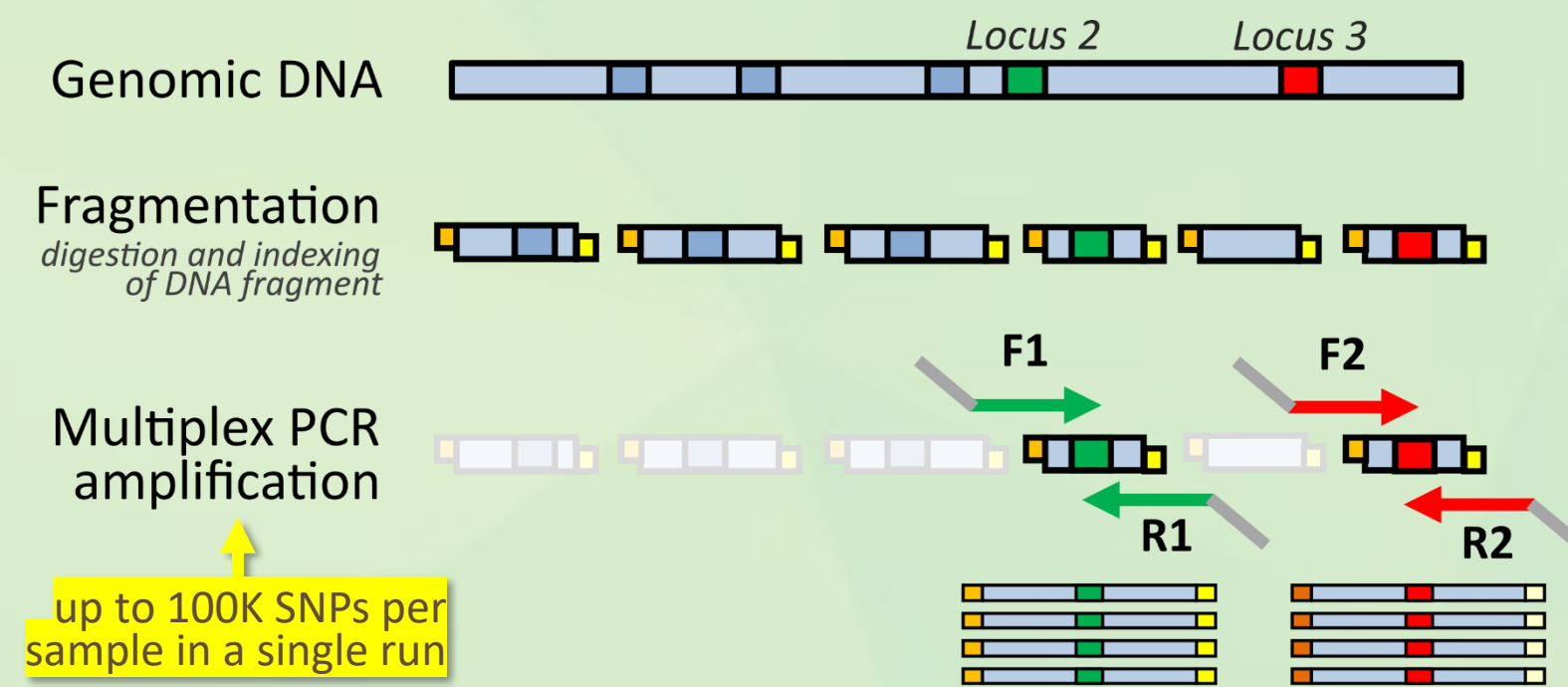
### Step 1. *de novo* SNP mining

First, GbS will be performed on a subset of 384 samples (core collection) to mine for new single nucleotide polymorphisms (SNP) representative of the specific and varietal diversity of the collection, using restriction enzymes to reduce genome complexity.



## Step 2. Targeted GbS

*Then, the rest of the collection will be genotyped using a targeted GbS approach with a subset of the newly identified SNPs (between 1000 and 5000 loci), selected in order to cover the whole genome. In addition, trait-specific markers will be tested.*

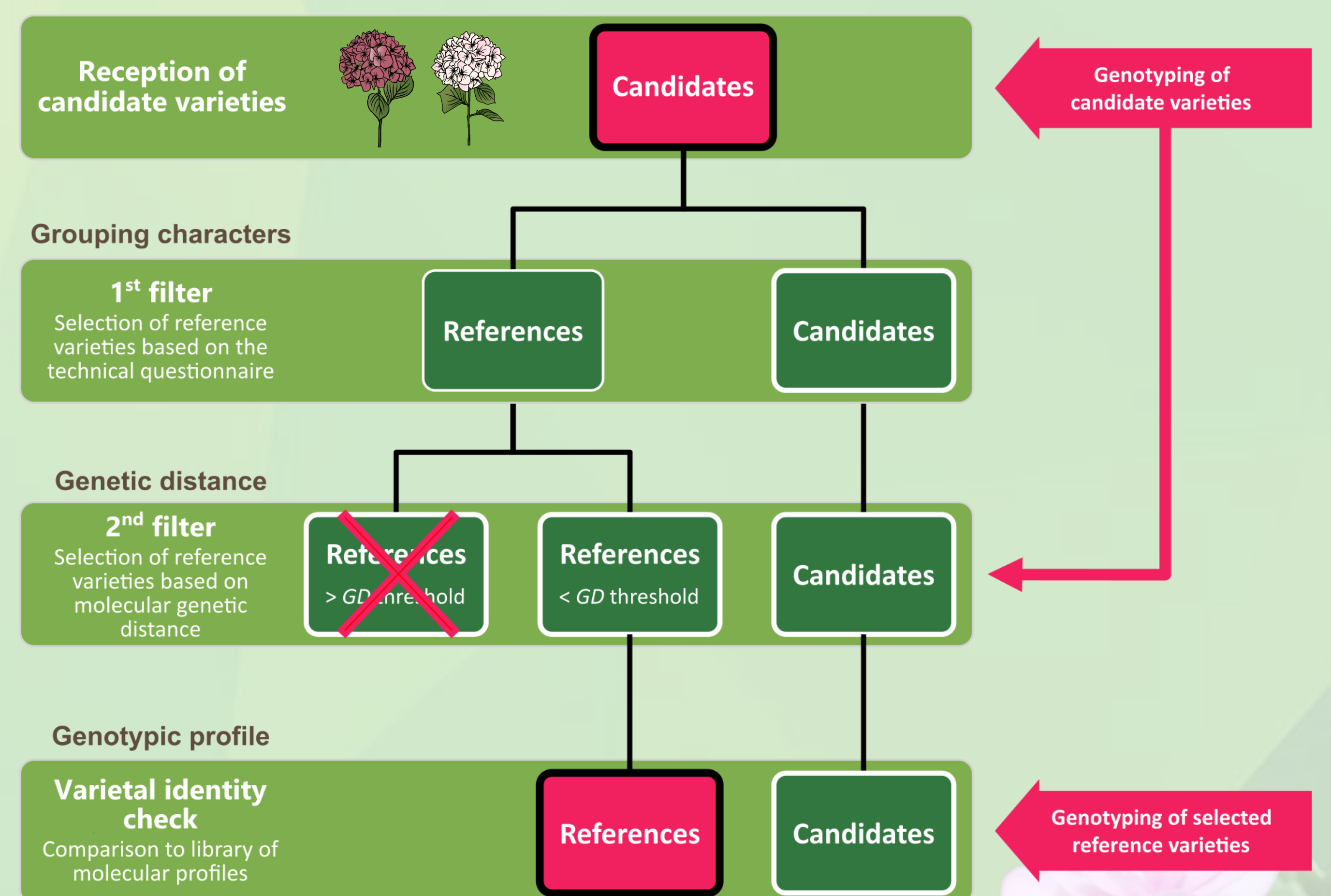


over 3000 samples in a single sequencing lane → Sequencing, demultiplexing and variant calling

[illegible]

## Proposed model

**This new molecular toolset will be used to design a SNP-informed DUS model for Hydrangeas** derived from UPOV model 2 (*“Combining phenotypic and molecular distances in the management of variety collections”*, TGP/15/3), in which reference varieties will be selected first based on grouping characteristics according to the technical questionnaire, then based on molecular data (genetic similarity and presence/absence of trait-specific genotypes). This new set of markers will not only help structure the collection (identification of duplicates and mutants) but also confirm variety identity and resolve potential cases of Intellectual Property Rights (IPR) infringement.



**A schematic view of the proposed model for integrating molecular data in DUS examinations of Hydrangeas.** Candidates are genotyped upon reception while reference varieties are chosen based on the information provided in the technical questionnaire (1<sup>st</sup> filter). A matrix of pairwise genetic distance is then calculated between candidates and reference varieties; all reference varieties above a given genetic threshold are excluded from pairwise comparisons (2<sup>nd</sup> filter). After plant cuttings were prepared, the varietal identity of the reference material is confirmed by comparing the genotype of the plant material collected against a reference library of molecular profiles.

This is a joint project between GEVES (France) and the Bundessortenamt (Germany). The project is co-funded by the Community Plant Variety Office (CPVO Grant Agreement N° 7519780) and by GEVES.



GEVES is a public interest group (GIP) founded by the French National Institute for Agricultural and Environmental Research (INRAE), the French Ministry of Agriculture, and the French Interprofessional Organisation for Seeds and Plants (SEMAE). **The official missions of GEVES are:**

- to carry out DUS and VCUS testing for the **registration of new varieties** in the Official Catalogue (national listing)
- to carry out DUS testing for the **legal protection of varieties** (Plant Breeders' Rights)
- to evaluate the quality and varietal identity of seed lots for **seed certification** according to international standards

GEVES is also responsible for the **national coordination of plant genetic resources** for the Ministry of Agriculture.