Quick Start Guide
DNA Tests for Rosaceae

DNA-informed breeding can enhance the efficiency, accuracy, pace, and creativity of breeding programs, enabling breeders to pursue new cross combinations and use their resources effectively.

There have been considerable advances in Rosaceae genetics through development of DNA tests for economically valuable traits and genetic characterization of promising germplasm. This 12-page resource provides an overview of how to use these DNA tests effectively for your breeding program.

The *Quick Start Guide* is intended for Rosaceae breeders who are aware of DNA-informed breeding and seeking additional information on implementation.

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The Process

Step 1: Set Goals

Step 2: Confirm DNA Predictions

Step 3: Create Workflow for Routine Use of DNA Tests

Step 4: Make DNA-Informed Breeding Decisions

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Background: Overview of the Process

2-Cycle Process

When implementing DNA tests for the first time in a breeding program or with new germplasm, **the genotyping step is done twice**.

First, only a subset of individuals from the target germplasm are evaluated along with appropriate trait data for the test. This preliminary cycle is recommended to confirm the utility of a DNA test for your program's situation – germplasm, environmental and cultural conditions, and evaluation methods. After the predictive power is confirmed, a larger group of germplasm can be genotyped.

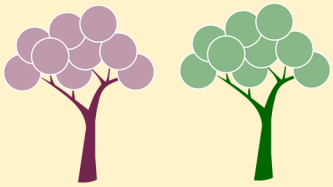
After confirming a test's predictiveness (as well as gaining a better understanding of its allele frequencies and distributions in your germplasm) it is important to reassess goals and logistics. Did you accurately estimate the cost and effort involved in implementing the genotyping steps? This is a good moment to make changes necessary for smoothly integrating DNA testing into your program. It is also the time to prepare for the potentially large amount of new DNA information by determining what your decisions will be once it arrives.

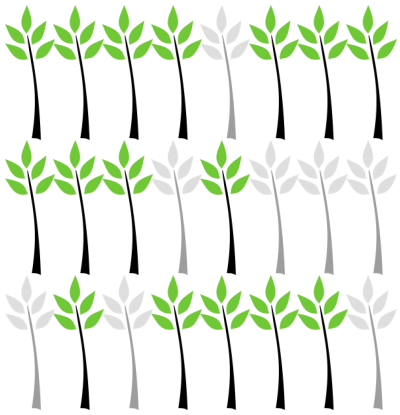
1	**Set Goals**	Determine what do you want to accomplish using DNA information.
2	**Confirm DNA Predictions**	Validate the utility of a DNA test in your breeding program using a representative sample of your germplasm.
3	**Create Workflow for Routine Use of DNA Tests**	Set a selection threshold for the predictions and decide how to manage the influx of data.
4	**Make DNA-Informed Breeding Decisions**	Genotype the full population and use those predicted phenotypes to make breeding decisions.

Background: Common Approaches to DNA-Informed Breeding

Parent Selection

Use DNA test results to choose the best parents for your breeding goals and maximize the effectiveness of cross combinations. Often, all current and possible parents are evaluated for the full panel of DNA tests available to maximize the information on hand for making crossing decisions with a breeding program's foundational germplasm.



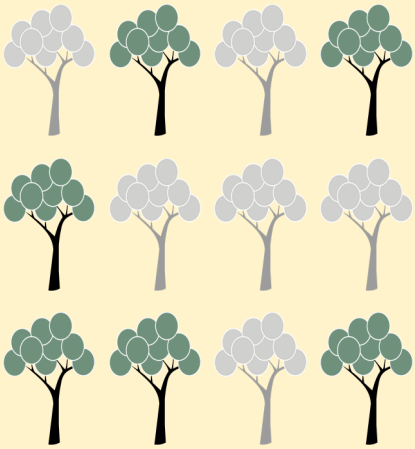


Seedling Selection

Evaluate a large number of seedlings and discard those predicted to be genetically inferior. Seedlings are typically evaluated with a small number of DNA tests for traits that are highly heritable or those essential for a successful new cultivar.

Elite Selection

Evaluate elite selections for all available DNA tests and advance only those with excellent commercial potential or as new parents. At this stage, it can be helpful to identify the presence of beneficial alleles, confirm selections have desired alleles for essential traits previously detected, and evaluate the overall combination of favorable alleles.



Background: Standardized Phenotyping

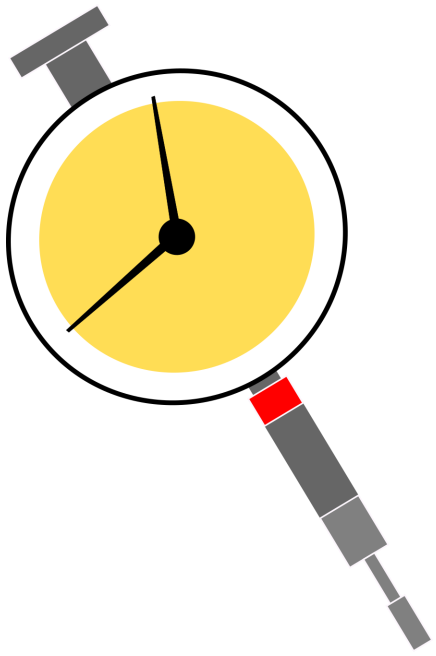
Reliable and consistent phenotyping protocols are essential for ensuring that DNA tests developed in research labs will work in an applied breeding programs across the U.S. The wide application of tests for traits such as soluble sugar content, texture and fruit size components rely on identical evaluation procedures. Standardized phenotyping protocols for major fruit quality traits have been established for **apple**, **tart cherry**, **sweet cherry**, **peach** and **strawberry**. These are available at www.rosbreed.org/breeding/tools.

Guidelines to Standardized Phenotyping

- **Relevance:** choose traits with high commercial impact to your industry.
- **Heritability:** choose traits which represent the genetic potential of your crop—those with the highest heritability.
- **Standardization:** measure traits using established protocols and check the repeatability of those traits in your program.
- **Use available expertise.** Seek out additional help when needed.
- **Replicate** as necessary across years, sites, and trees to achieve high precision.
- Use the most **cost efficient** approach—the cheapest per test and the most reliable method (resulting in the fewest evaluation rounds).
- **Avoid redundancy**. Avoid measuring the same trait in different ways.
- **Use instrumentation** whenever possible to replace repetitive labor that can fatigue personnel.

Synergy.

These protocols enable you to relate your breeding program to others around the world and leverage discoveries made at research institutions.

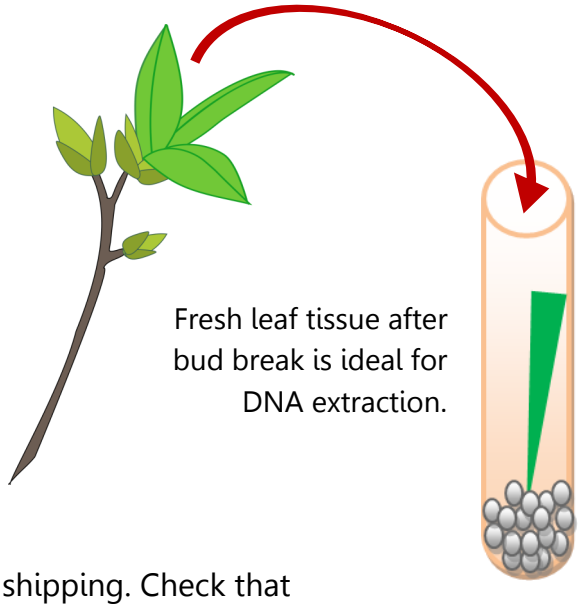


The penetrometer—a useful tool for evaluating fruit firmness.

Background: *Tissue Sampling & Genotyping*

Guidelines for leaf sample collection

1. Obtain a sampling protocol from the service lab you will be using. Often, labs prefer the youngest leaf possible (unfolded leaf tissue is best). Genotyping service labs will also specify how much tissue is needed.
2. Understand the how many tissue samples are needed per individual.
3. Understand how the service lab wants the samples arranged in the collection tubes and what documentation is needed to link tissue samples with your individuals (seedlings, parents, etc).
4. Decide what controls are needed—for instance, parents of seedlings or individuals with a known performance for the trait(s) of interest.
5. Establish practices to ensure accurate tracking of samples and individuals in your breeding program.
6. Determine the ideal shipping method and timing of shipping. Check that the service lab is expecting your samples.



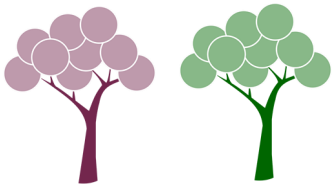
Fresh leaf tissue after bud break is ideal for DNA extraction.

Example Collection Map of Tissue Samples

	1	2	3	4	5	6	columns 7 - 12
A	*control 1*	*control 1*	sample 7	sample 7	sample 15	sample 15	...
B	*control 2*	*control 2*	sample 8	sample 8	sample 16	sample 16	
C	sample 1	sample 1	sample 9	sample 9	sample 17	sample 17	
D	sample 2	sample 2	sample 10	sample 10	sample 18	sample 18	
E	sample 3	sample 3	sample 11	sample 11	sample 19	sample 19	
F	sample 4	sample 4	sample 12	sample 12	sample 20	sample 20	
G	sample 5	sample 5	sample 13	sample 13	sample 21	sample 21	
H	sample 6	sample 6	sample 14	sample 14	sample 22	sample 22	

Step 1: Set Goals

Before investing any resources in DNA-informed breeding, establishing objectives for what you want to do with DNA predictions is important.



- What are my specific objectives?
- What resources can I commit to this endeavor?
- What goals can I reach given my resource limitations?

DNA tests are available for Rosaceae crops (see www.rosbreed.org/breeding/dna-tests for a current list) that address common breeding objectives via specific trait loci:

- ✓ Disease resistance
- ✓ High quality fruit
- ✓ Good productivity

Major strategies of DNA-informed breeding

	Advantages	Disadvantages
Parent Selection	• Easiest to implement • Best value per dollar spent • Can characterize entire parental set	• Only indicates parental phenotypes, not progeny
Seedling Selection	• Cost savings realized by not field planting and evaluating inferior seedlings	• Can be costly to evaluate a large number of progeny
Elite Selection	• More accurate information for making advancement decisions • Can be less expensive than seedling selection because a smaller number	• Will not avoid costly field planting and evaluation for potentially inferior individuals

Step 1: Goal Setting Worksheet

Use the following worksheet to determine the optimal conditions for using DNA tests in your breeding program.

1. **Write down your breeding goals ranked by importance.**

2. **For your crop, which of the currently available DNA tests align with your breeding goals?**

3. **Do you know the phenotypes of all individuals used as parents in your program for the DNA tests that align with your breeding goals?** Knowing the predicted values for key traits in your parent pool is a good place to get started with DNA-informed breeding.

4. **When are those traits evaluated during cultivar development (for example, as seedlings, early generation material, in advanced trials)?** When could you use DNA tests to substitute for or supplement trait evaluation?

5. **For an individual trait that has a DNA test for it, how many breeding lines, clones, individuals, etc would you normally evaluate phenotypically in a year?**

6. **What resources will you commit to this endeavor?**

Consider monetary cost for testing, additional labor needed, time of existing staff to sample tissue, interpret results and implement decisions.

Financial Resources for Genotyping_____

Staff Time (number of staff and hours each will dedicate to this)_____

Your Time _____

Other _____

7. **Can you accomplish your goals with these resources?**

Step 2: Confirm DNA Predictions

When introducing DNA tests for previously untested germplasm, the predictiveness of the DNA test should be confirmed. The general stability of DNA test predictions over diverse germplasm, years, time of the year, organ maturity, storage conditions, or shelf life durations – or their genetic insights in particular situations – depend on alleles present and their frequencies, trait heritability, and method of phenotyping. Your breeding program will benefit from understanding the reliability and accuracy of each DNA-based assay under your program's conditions and germplasm.

Choose a representative sample of germplasm

The first time, only a subset of individuals from the target germplasm needs to be evaluated. An effective subset represents the phenotypic range of the trait across which you hope the DNA test will distinguish and the genetic diversity of the breeding germplasm to which you hope to routinely apply the DNA test. This set could include the parents of target progenies, some related selections or cultivars expected (or known) to be above and below your target trait threshold, and a handful of segregating seedlings.

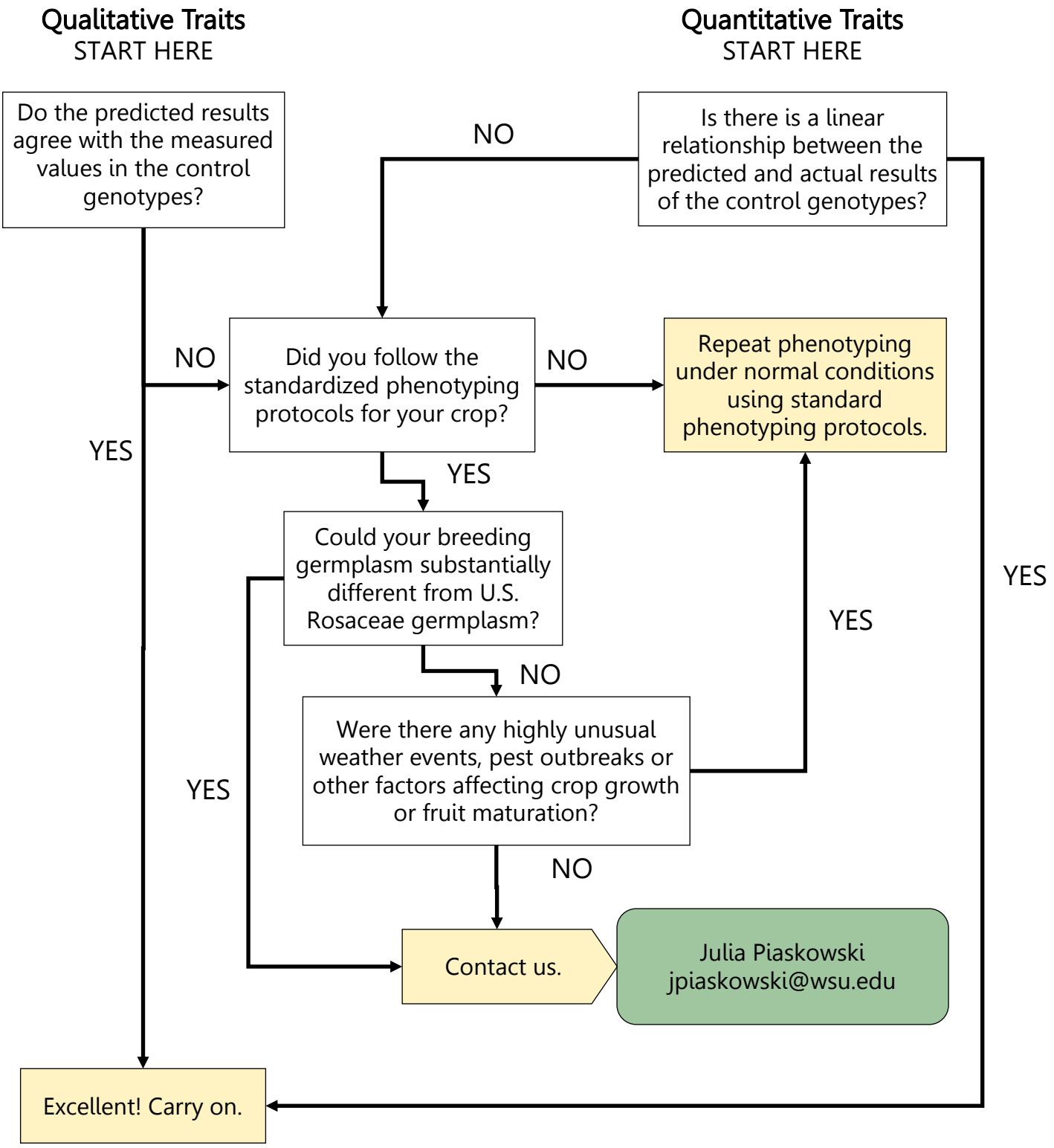
Phenotype the germplasm

Obtain or compile performance information for the traits of interest for each member of the representative subset. Accurate phenotypic data is important for comparing the DNA predictions with the actual values.

Genotype the germplasm

Obtain genotypic outcomes for the DNA test for each phenotyped member of the germplasm subset. Such genotypic data can be obtained in-house or via one of numerous competitively priced and customer-oriented service providers in the U.S. or beyond.

Step 2: DNA Test Validation Flow Chart



Step 3: Create Workflow for Routine Use of DNA Tests

Use the following worksheet to determine the optimal conditions for using DNA tests in your breeding program.

1. **Determine the number of individual samples that will be tested.** How many individuals would you normally evaluate for the target trait?

2. **Designate personnel for implementation tasks.** Who—specifically—will be responsible for collecting leaf samples? For sending them to the DNA testing service provider? For checking the summary of genotypic results? For implementing DNA-informed decisions? Make sure your technical personnel have the training/experience needed and time available to manage this work.

3. **Ascertain when certain tasks need to be done.** Determine what time of the year you or your team can complete these activities and when you will need the results to inform timely decisions:

   Sample tissue for routine DNA testing_____

   Process and use results_____

4. **Decide in advance what your selection decisions will be.** Understand what results to expect for a particular test and your intended action for those test results (for example, the selection threshold).

5. **Determine how routine DNA testing will affect recordkeeping in the breeding program?** Where will the results be stored? Consider how to incorporate DNA predictions into your information management system.

6. **Determine how routine DNA testing will affect planting decisions, the tracking of seedlings, selections and other individuals and other aspects of the field program?** Changes in organization and labeling of seedlings or other material might be needed. Reduced field plantings offer new opportunities in resource allocation.

Step 4: Make DNA-Informed Breeding Decisions

Genotype the Target Germplasm

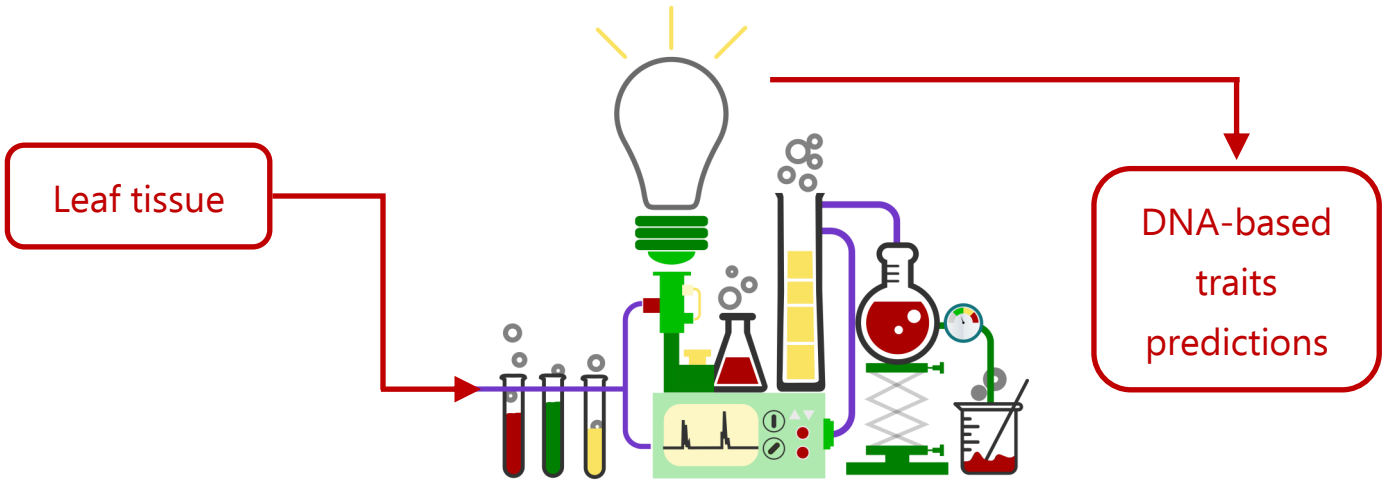
The entire panel of lines you would like to use for DNA-informed breeding can be genotyped for the tests confirmed in your germplasm. While other DNA tests can also be included, until they are validated with phenotypic data from your program, their predictive capacity in your breeding program is not known.

Interpret DNA Test Results

Use information from the DNA test validation step to understand how DNA trait predictions will manifest themselves in your breeding program and target environments. For some traits such as peach fruit type (nectarine versus peach), there should be no difference between predictions and actual values. Other traits, such as days to maturity, will differ slightly from program to program.

Use DNA Information in Your Breeding Program to:

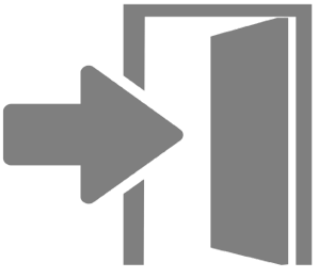
- Choose the best parents and cross combinations that target your breeding objectives.
- Predict segregation ratios in newly generated families for trait loci of interest.
- Discard seedlings or selections that do not meet minimum expectations.
- Choose suitable elite selections for comprehensive evaluation towards commercial release and discard those lacking desired attributes.
- Pyramid alleles that might otherwise be difficult to detect individually based on phenotypic information alone.



Visit the RosBREED DNA-Informed Breeding Web Portal

www.rosbreed.org/breeding/dna-tests

Explore Training Resources on DNA-Informed Breeding



Explore DNA Tests and Order Plant Sampling Kits

Download Phenotyping Protocols and Other Breeding Tools

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