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## **BSA-Seq: an efficient method to decipher a complex trait on Poplar, a highly heterozygous diploid genome**

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### ► To cite this version:

Aurelie Canaguier, Véronique Jorge, Vanina Guérin, Odile Rogier, Vincent Segura, et al.. BSA-Seq: an efficient method to decipher a complex trait on Poplar, a highly heterozygous diploid genome. PAG XXVI - Plant and Animal Genome Conference, Jan 2018, San Diego, United States. , 1 p., 2018. hal-02736419

**HAL Id: hal-02736419**

**<https://hal.inrae.fr/hal-02736419v1>**

Submitted on 2 Jun 2020

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## INTRODUCTION

The efficiency of the Bulk Segregant Analysis (BSA) has clearly been demonstrated to detect genomic regions and genes involved in various traits. It allows large experiments reducing the cost and time and preserving the power of full individual's population analysis. Over the past few years the combination of BSA and Next Generation Sequence (NGS) data (BSA-Seq) has given a new accuracy and robustness to the discovery of genes and genomic regions underlying traits of interest, mainly on crop and model species (1).

In our study, we applied the BSA-Seq on Poplar, a heterozygous and diploid genome, to decipher the genetic determinism of leaf rust resistance. As a proof of concept, we focused on  $R_{US}$ , a major gene previously fine-mapped on Chromosome 19 and controlling the uredinia size during the rust-Poplar interaction (2,3).

## MATERIAL AND METHODS

### MATERIAL

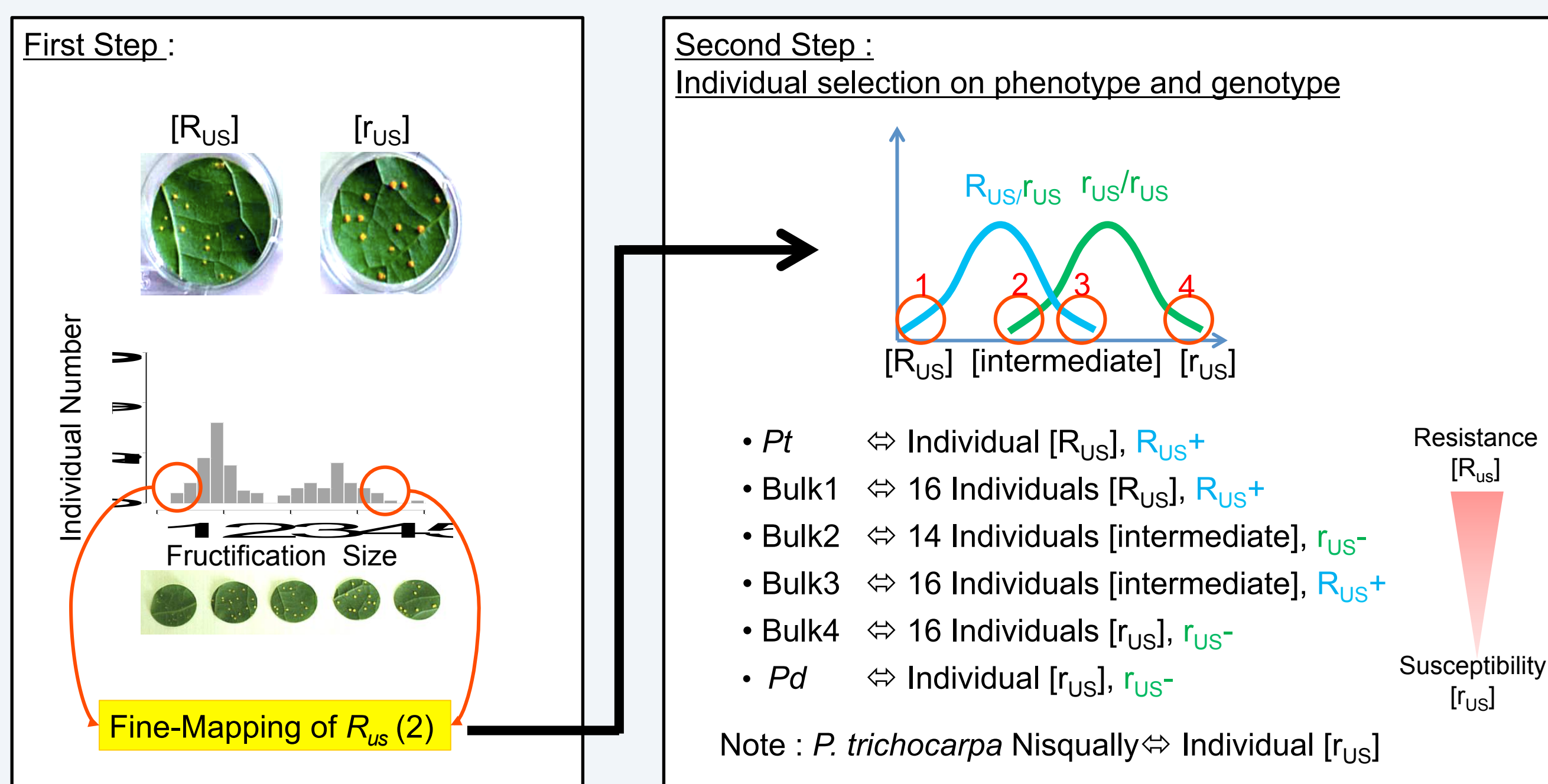
- **Phenotyping** for traits associated to the resistance to *Melampsora larici-populina* (*Mlp*) leaf rust of parents and **1414 progenies** from an interspecific cross :

***Populus deltoides* clone 73 028-62 (Pd) x *Populus trichocarpa* clone 101-74 (Pt)**

- Independently DNA extractions with Qiagen Kit and **genotyping** (2,3).

### METHOD – BULK CONSTITUTION

The selection of 62 progenies, based on genotyping of markers physically linked to  $R_{US}$  and the phenotypic information, was realized as described in **Figure 1**. Then, the corresponding DNA were pooled equimolarly into 4 bulks.



**Figure 1.** Population phenotyping and genotyping to select progenies for extreme and intermediate Bulks.

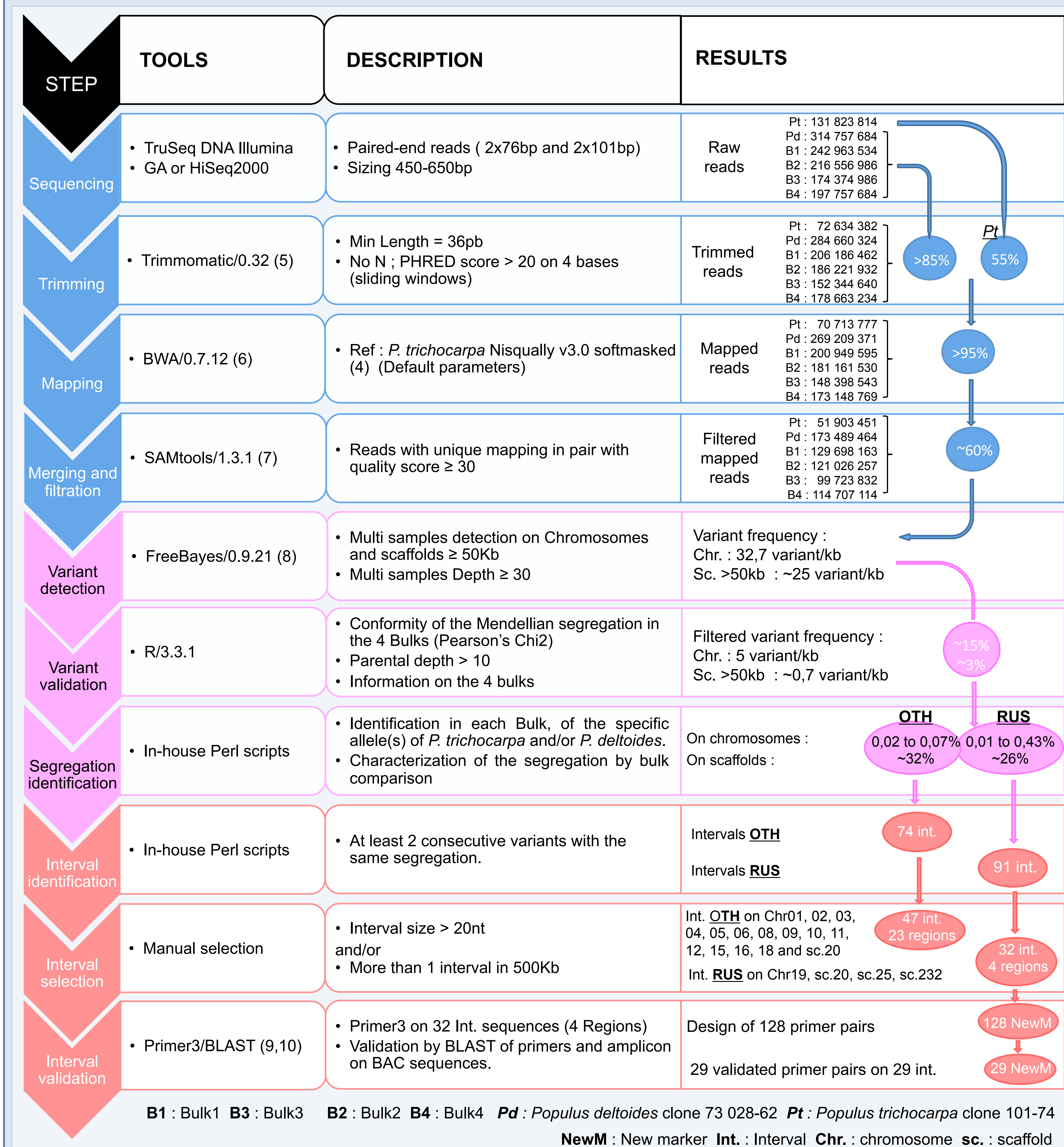
### METHOD – BULK COMPARISON

The **RUS** markers are expected to be polymorphic between the 2 parents and to cosegregate with [R<sub>US</sub>] and R<sub>US</sub><sup>+</sup>. More precisely, a variant was considered as **RUS** whenever it fulfilled the following two conditions: (i) its alleles differed between the 2 parents; and (ii) its *P. trichocarpa* allele was present in bulks 1 and 3 and absent in bulks 2 and 4.

Positions which segregates in conformity with the resistance leaf rust and not with R<sub>US</sub><sup>+</sup> are called « other » (**OTH**).

## RESULT 1

### ANALYSIS WORKFLOW



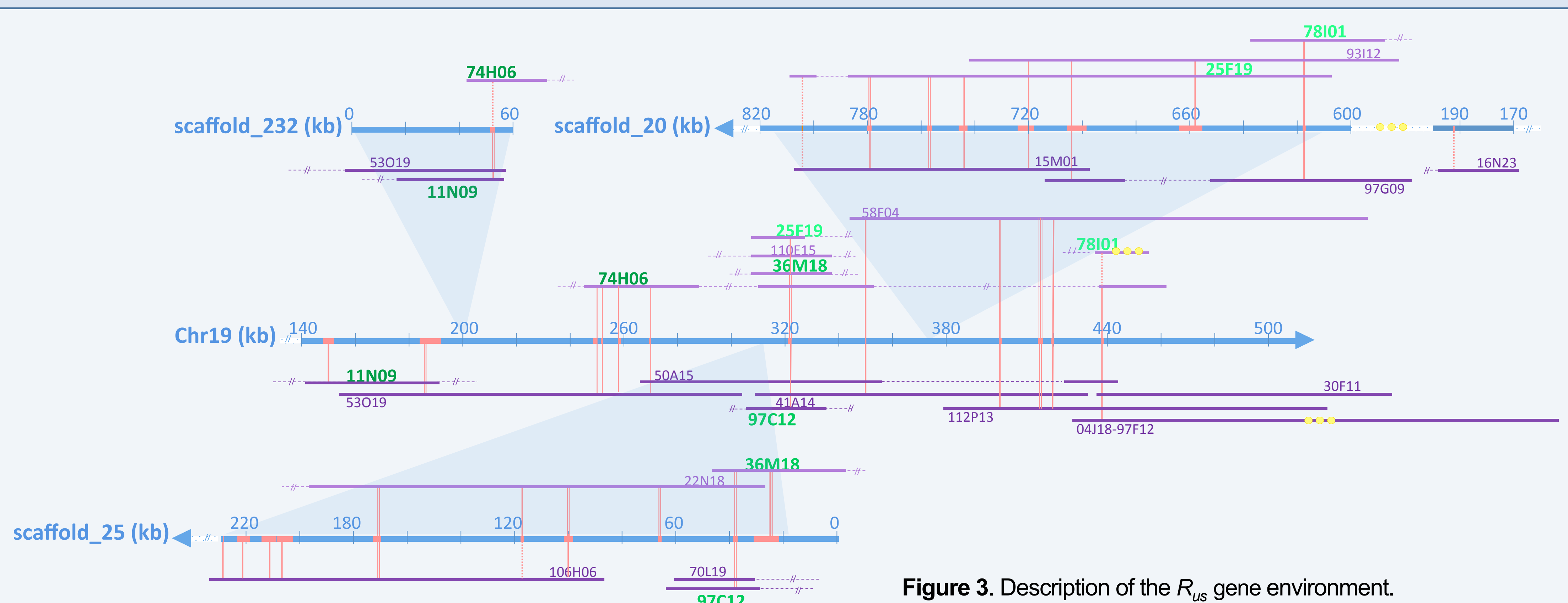
**Figure 2.** Analysis workflow and general results.

## RESULT 2

### DESIGN OF NEW MARKERS ON A SPECIFIC REGION

#### Legend

- Chromosome 19 or scaffolds of *P. trichocarpa* Nisqually v3.0
- Interval on *P. trichocarpa* Nisqually v3.0
- 1 partial new marker : one primer and partial amplicon
- 1, 2 or 3 new marker(s) : 2 primers and amplicon
- Pt BACs related to  $R_{US}$  allele
- Pt BACs related to  $r_{US}$  allele
- 74H06, 97C12 BACs anchoring by new markers on one scaffold and on the Chromosome 19
- 25F19... Previous genetic and physical markers linked to  $R_{US}$  (2).



**Figure 3.** Description of the  $R_{US}$  gene environment.

## CONCLUSION & PERSPECTIVES

BSA-seq method allows identification of *P. trichocarpa* and/or *P. deltoides* specific variants for complex trait in a diploid and heterozygous context and this, even if the mapping reference doesn't carry the searched region of interest.

Next steps are first to proceed the PCR experiments with the new markers on the parents and progenies to enrich the  $R_{US}$  fine-map ; second to characterize the 23 other regions in segregation with the resistance to leaf rust.

Moreover this pipeline, usable on any heterozygous species, releases to the scientific community a high-confidence set of variant positions based on the conformity of the allele frequencies within the bulks.

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THANKS TO CEA-CNG : A. Boland-Augé group for performing DNA samples QC, M.T Bihoreau and D. Lechner for providing INRA-EPGV group with access to their Illumina Sequencing Platform.