

# Fine mapping

## Genômica Aplicada ao Melhoramento de Plantas

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# 1. Introdução ao Fine Mapping

## O que é Fine Mapping?

- Técnica utilizada para localizar com precisão genes ou QTLs responsáveis por determinada característica fenotípica.
- Etapa posterior ao mapeamento genético.
- Objetivo: reduzir a região candidata até alguns genes (ou até um gene único).

## Importância?

- Identificação de genes-alvo para programas de melhoramento.
- Facilita a clonagem de genes e estudos funcionais.
- Apoia estratégias de edição gênica e seleção assistida por marcadores (MAS).

## 2. Etapas do Fine Mapping

### a) Identificação prévia de um QTL

- Através de mapeamento por ligação (populações biparentais) ou GWAS (associação genômica ampla).

### b) Desenvolvimento da população

- Exemplos: RILs, backcross, ou  $F_2$ ,  $F_{2:3}$ .

### c) Genotipagem

- Uso de marcadores SNPs, InDels, SSRs.
- Beadchips, sequenciamento, plataformas como Genotyping By Sequencing (GBS).

### d) Fenotipagem

- Avaliação detalhada do fenótipo de interesse (ex: resistência à antracnose ou ferrugem).

### e) Refinamento da região

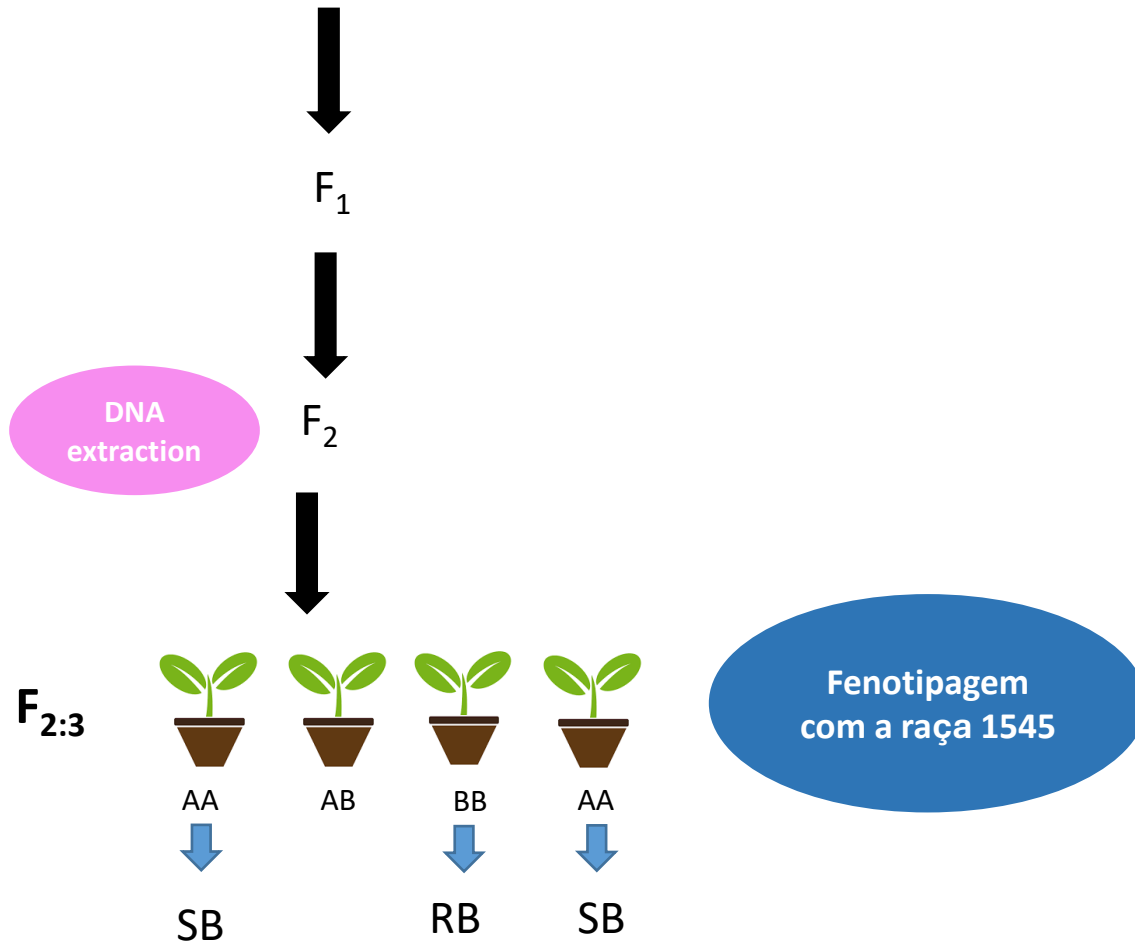
- Análise de associação entre marcadores e o fenótipo.
- Delimitação da região QTL em um intervalo genômico menor.

### **3. Exemplo Aplicado: Fine Mapping para Resistência à Antracnose**

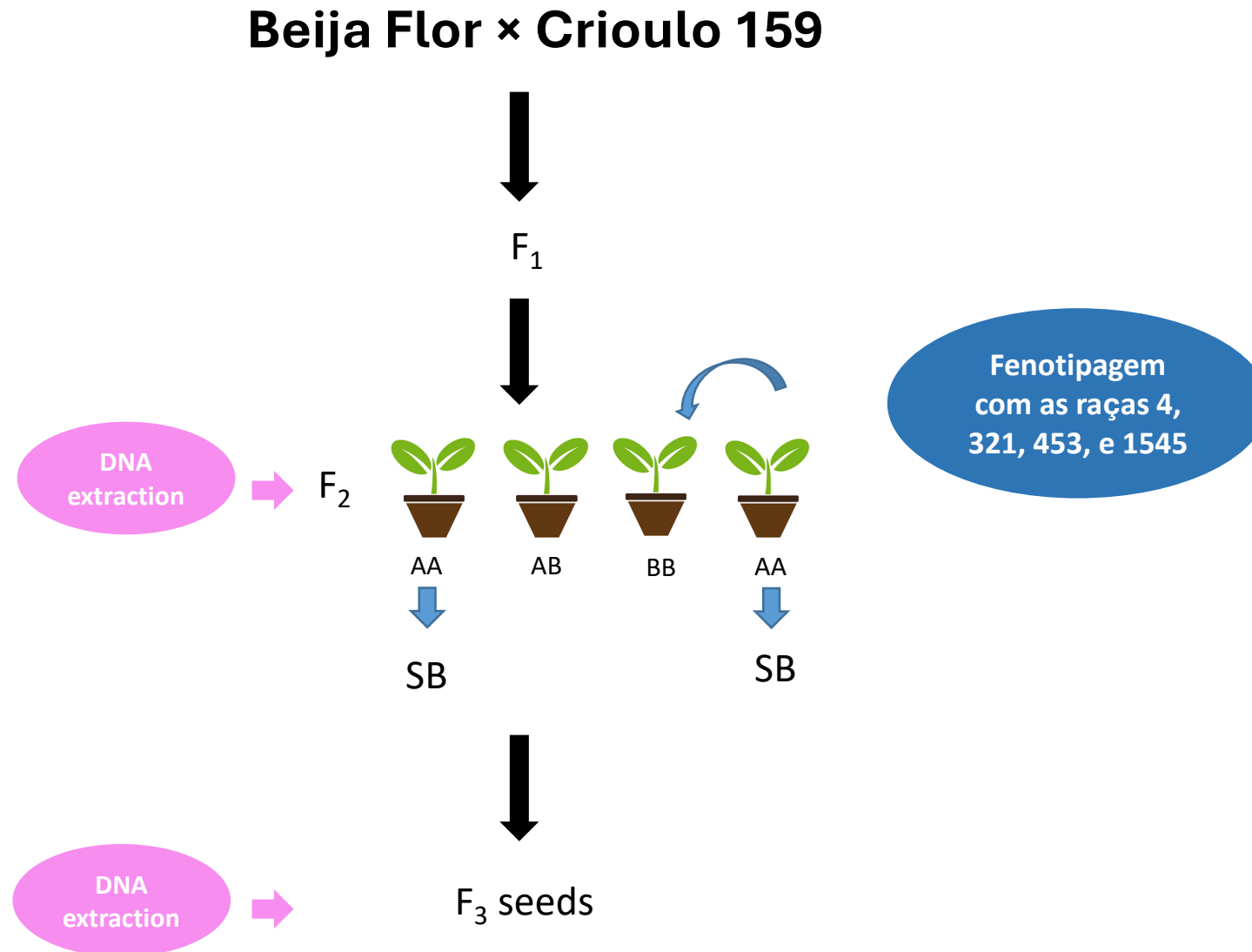
- 1- Desenvolvimento da população
- 2- Fenotipagem da população (plantas  $F_2$ )
- 3- Teste de herança
- 4- Genotipagem com Beadchip
- 5- Bulk segregant analysis (Genome Studio)
- 6- Encontrar a região e o cromossomo
- 7- Desenhar marcadores SNPs
- 8- Genotipar a população  $F_2$
- 9- Linkage map (JoinMap and MapChart)
- 10- Fenotipar as plantas  $F_3$
- 11- Genotipagem com marcadores SNPs
- 12- Encontrar recombinantes
- 13- Repetir as etapas 7, 10, 11 e 12 de acordo com a quantidade de plantas e marcadores
- 14- identificar genes candidatos

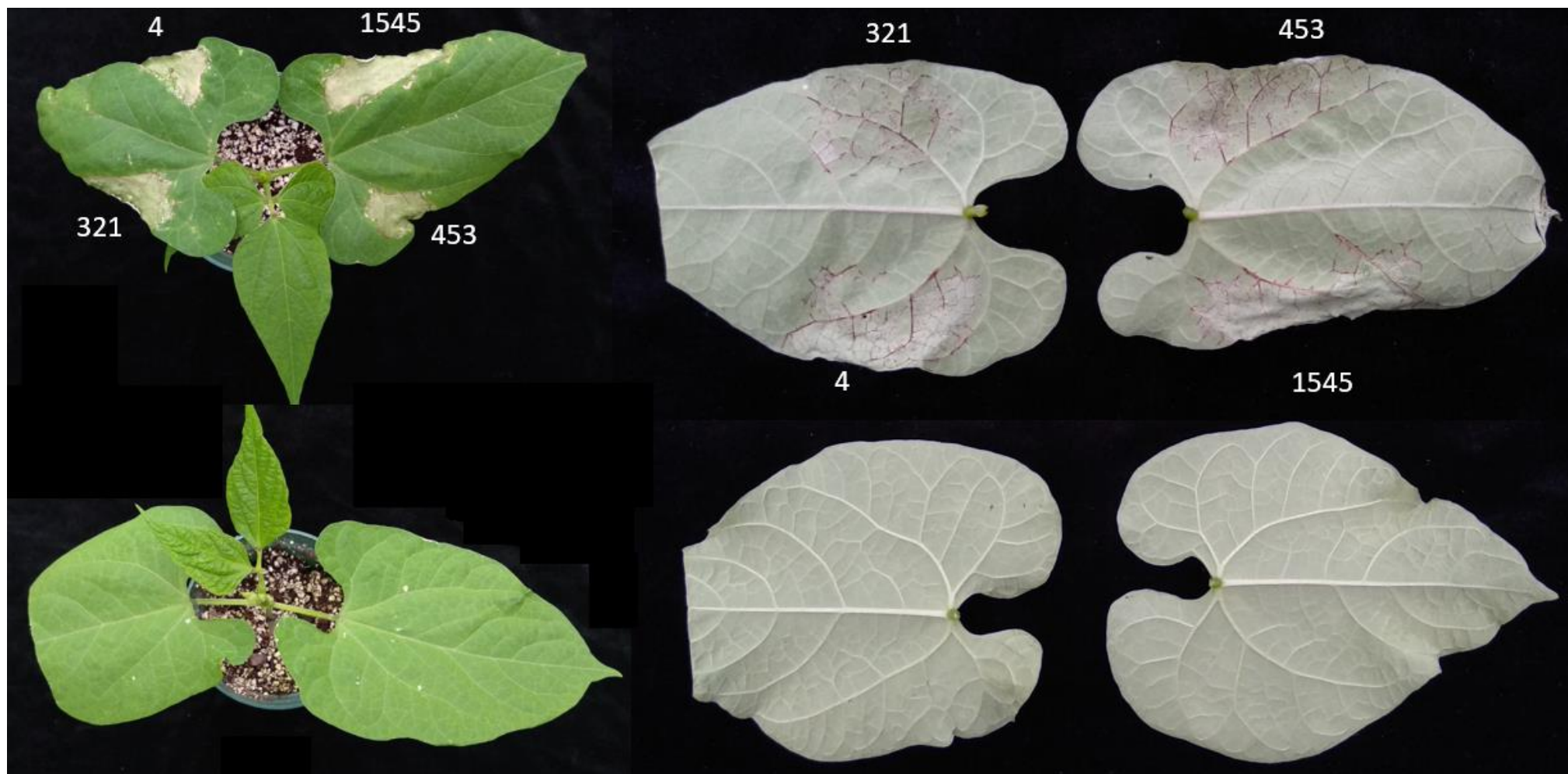
1- Desenvolvimento da população; 2- Fenotipagem da população (plantas  $F_{2:3}$ )

**Beija Flor × Cornell 49242**



1- Desenvolvimento da população; 2- Fenotipagem da população (plantas F<sub>2</sub>)





### 3- Teste de heranca

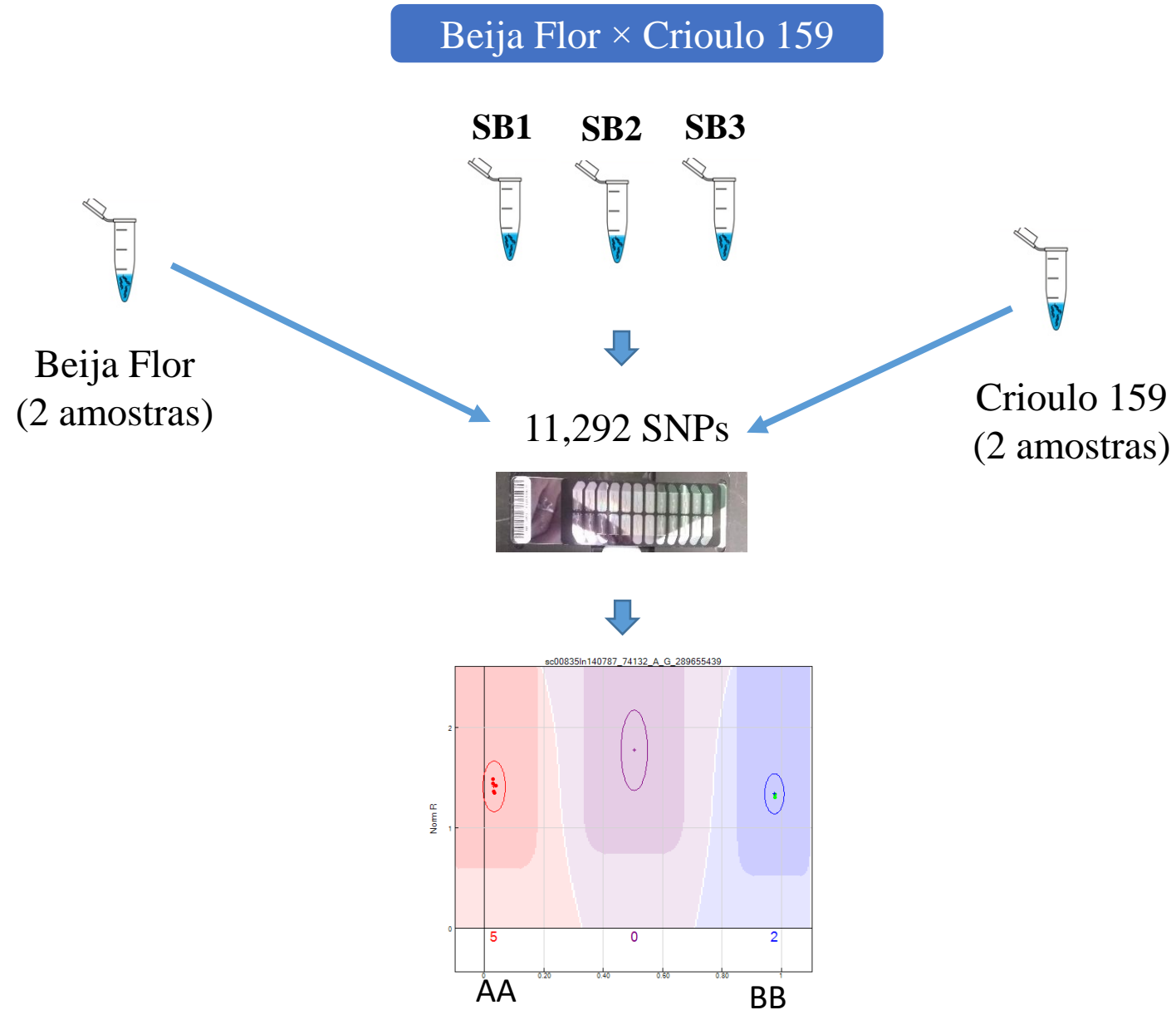
Population	Observed	Expected	Chi square	P value	Race
F <sub>2</sub> Beija Flor × Crioulo 159	286: 104	292: 98	0.491	0.4836	321 e 1545

Niver de significância de 1% = 0.01

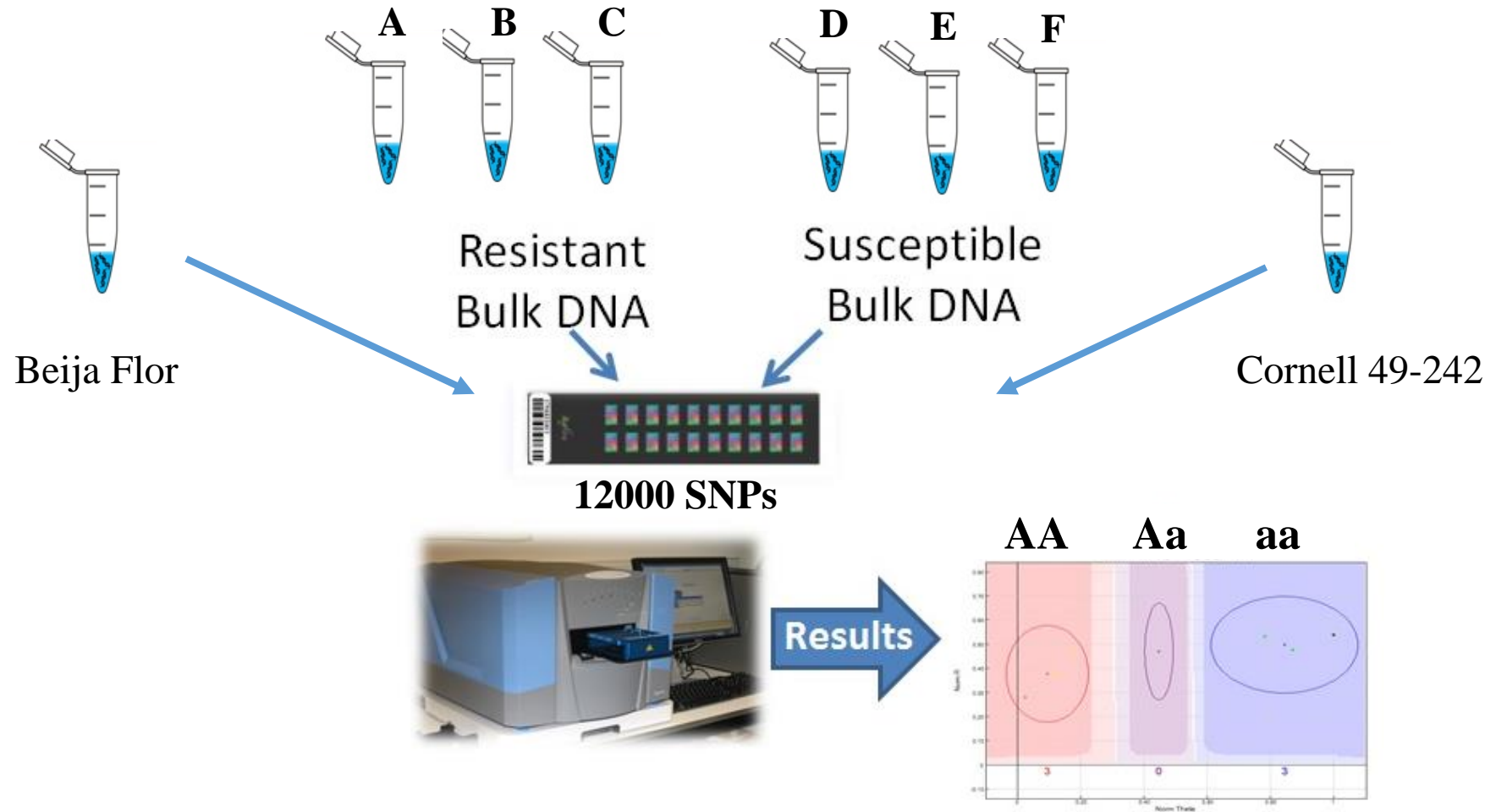
P-valor > 0.01, não rejeita a hipótese nula.



## 4- Genotipagem com Beadchip



#### 4- Genotipagem com Beadchip



## 5- Bulk segregant analysis (Genome Studio); 6- Encontrar a região e o cromossomo

id	SNP_position_Bean_v2.0	chr	Crioulo159_1	Crioulo159_2	BeijaFlor_1	BeijaFlor_2	BFxC159_SB1	BFxC159_SB2	BFxC159_SB3
sc00518ln214431_146536_C_T_234672203	120,328.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00518ln214431_147554_A_G_234673221	121,346.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00518ln214431_55133_G_A_234580800	275,483.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00518ln214431_27922_T_C_234553589	302,160.00	Chr04	AA	AA	BB	BB	AA	AA	AA
Chr04_443035_C_A	443,035.00	Chr04	AA	AA	BB	BB	AA	AA	AA
Chr04_459430_G_T	459,430.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00835ln140787_51913_C_A_289633220	473,538.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00835ln140787_67166_T_C_289648473	488,793.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00835ln140787_74132_A_G_289655439	495,761.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00835ln140787_101089_C_A_289682396	521,957.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00835ln140787_114250_G_A_289695557	535,120.00	Chr04	AA	AA	BB	BB	AA	AA	AA
Chr04_547025_A_G	547,025.00	Chr04	AA	AA	BB	BB	AA	AA	AA
Chr04_620164_A_C	620,164.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00112ln569344_292205_A_G_98011205	1,381,835.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00112ln569344_243297_C_A_97962297	2,040,423.00	Chr04	AA	AA	BB	BB	AA	AA	AA
sc00283ln338214_159814_C_T_171567968	2,110,777.00	Chr04	AA	AA	BB	BB	AA	AA	AA

## 7- Desenhar marcadores SNPs

### KASP Genotyping Chemistry

#### 1) Assay components:

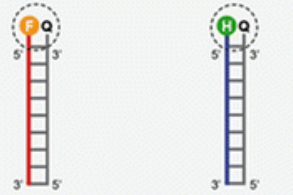
##### A) Primer mix

Allele specific forward primers:

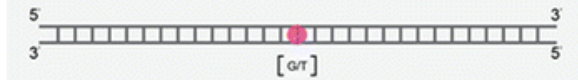
allele-1  
5' — 3' C  
allele-2  
5' — 3' A

Reverse primer:  
3' — 5'

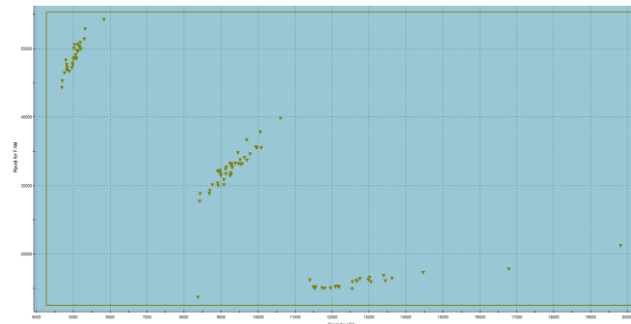
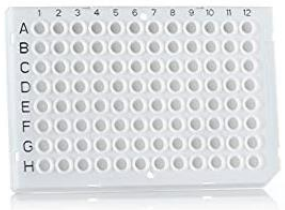
##### B) Master mix



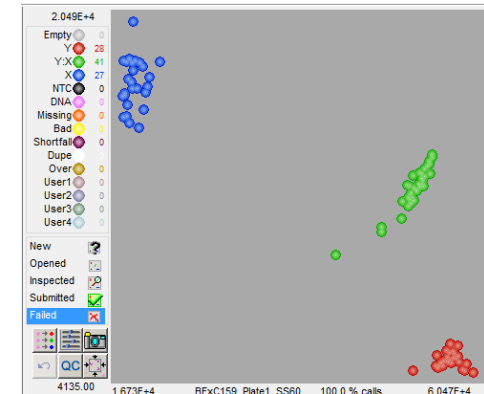
##### C) DNA template (sample)



Mx3000P qPCR machine



MxPro software



Klustercaller software.

## 8- Genotipar a população F<sub>2</sub>

F <sub>2</sub> ID	Reaction to race1545	Genotype with KASP markers <sup>2</sup>							Reference genome
		33,578	164,385	521,957	552,696	1,299,082	1,301,156	1,842,115	<i>Pv2.1</i>
		90,666	218,204	580,673	611,412	1,036,037	1,038,110	1,728,453	<i>Pv1.0</i>
		SS129	SS120	SS58	SS284	SS260	SS60	SS149	
Crioulo 159	8	AA	AA	AA	AA	AA	AA	AA	
Beija Flor	1	BB	BB	BB	BB	BB	BB	BB	
BxC_1	1	AB	AB	AB	AB	AB	AB	AB	
BxC_2	1	AB	AB	AB	AB	AB	AB	AB	
BxC_3	8	AA	AA	AA	AA	AA	AA	AA	
BxC_4	1	AB	AB	AB	AB	AB	AB	AB	
BxC_5	1	AB	AB	AB	AB	AB	AB	AB	
BxC_6	1	AB	AB	AB	AB	AB	AB	AB	
BxC_7	1	AB	AB	AB	AB	AB	AB	AB	
BxC_8	1	AB	AB	AB	AB	AB	AB	AB	
BxC_9	1	AB	AB	AB	AB	AB	AB	AB	
BxC_10	1	BB	BB	BB	BB	BB	BB	BB	
BxC_11	1	AB	AB	AB	AB	AB	AB	AA	
BxC_12	1	BB	BB	BB	BB	BB	BB	BB	
BxC_13	1	AB	AB	AB	AB	AB	AB	AB	
BxC_14	1	AB	AB	AB	AB	AB	AB	AB	
BxC_15	3	AB	AB	AB	AB	AB	AB	AB	

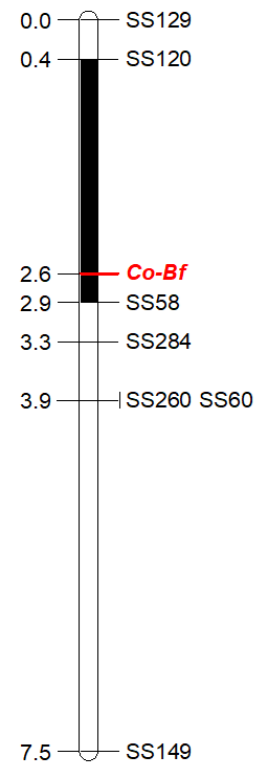
## 9- Linkage map (JoinMap and MapChart)

### MapChart

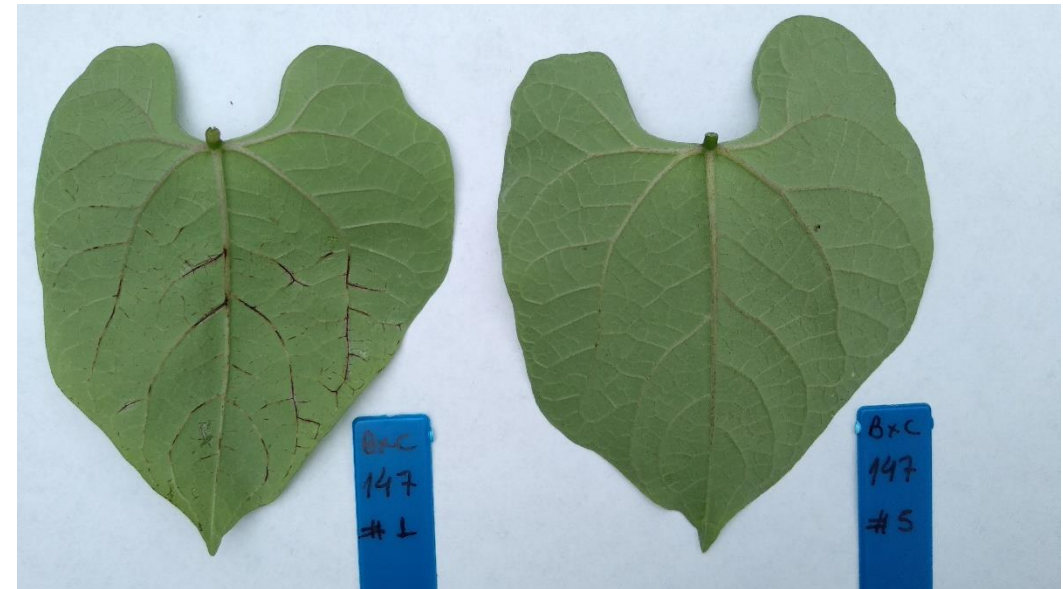
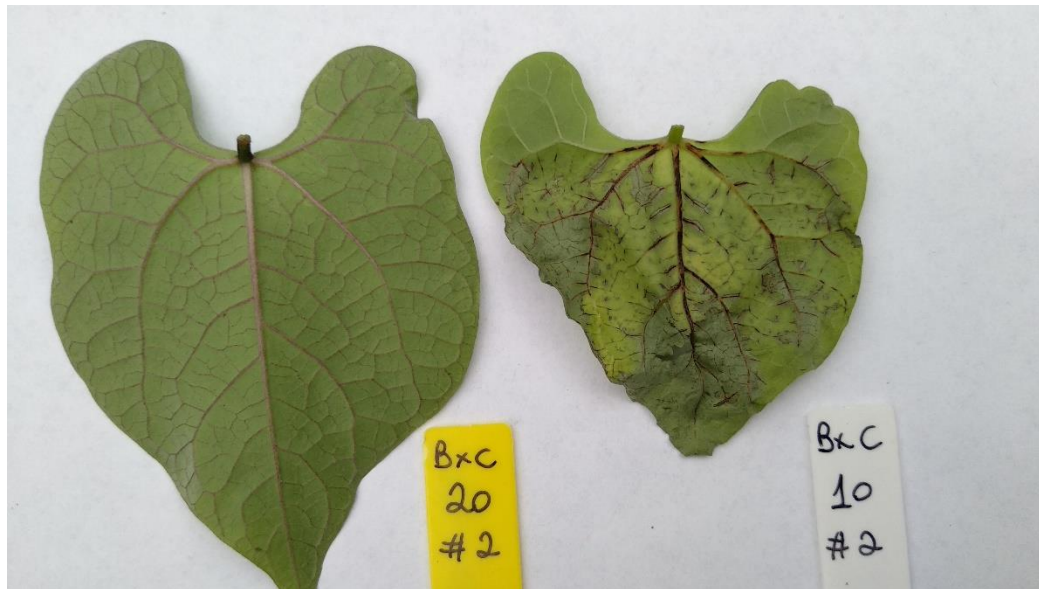
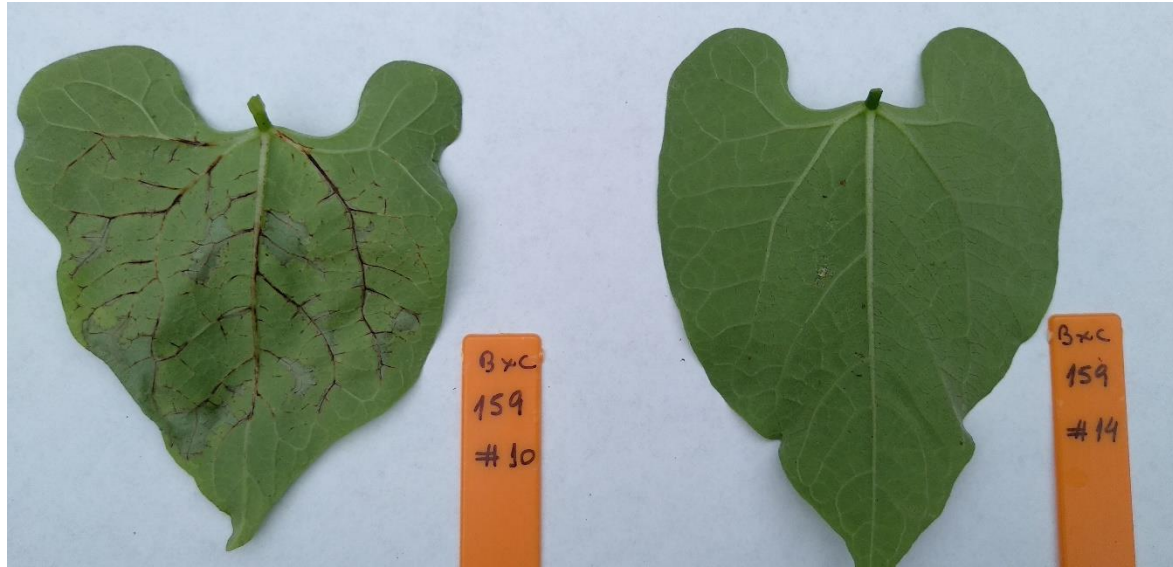
GROUP Pv04

SS129 0.000  
SS120 0.387  
Co-Bf 2.600 i b c2  
SS58 2.905  
SS284 3.292  
SS260 3.940  
SS60 3.940  
SS149 7.450

### Pv04



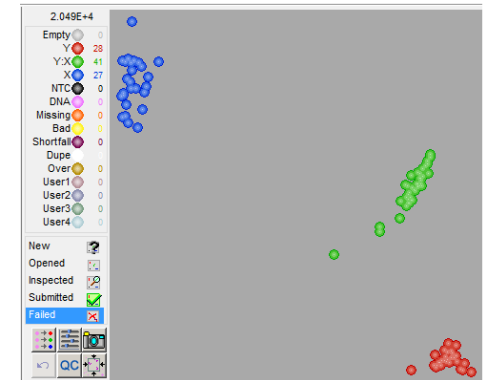
## 10- Fenotipar as plants F<sub>3</sub>





## 11- Genotipagem com marcadores SNPs

Number of Plants	F <sub>2</sub> family ID	Reaction to race 1545	Markers		Recombinant?
			SS129 33,578	SS260 1,299,082	
1 ...	Crioulo 159	8	AA	AA	-
	Beija Flor	1	BB	BB	-
	BxC_1#1	1	AB	AB	No
	BxC_1#2	8	AA	AA	No
	BxC_2#2	2	BB	BB	No
	BxC_2#3	1	AB	AB	No
	BxC_14#1	1	BB	AB	Yes
	BxC_18#10	8	AA	AB	Yes
	BxC_20#9	8	AA	AA	No
	BxC_21#1	1	AB	AB	No
	BxC_21#2	1	BB	BB	No
	BxC_26#4	8	AA	BB	Yes
	BxC_41#9	1	AB	AA	Yes
	BxC_61#3	8	AB	AA	Yes
	BxC_61#4	1	BB	BB	No
780	BxC_62#1	8	AA	AA	No
	BxC_62#2	2	AB	AB	No



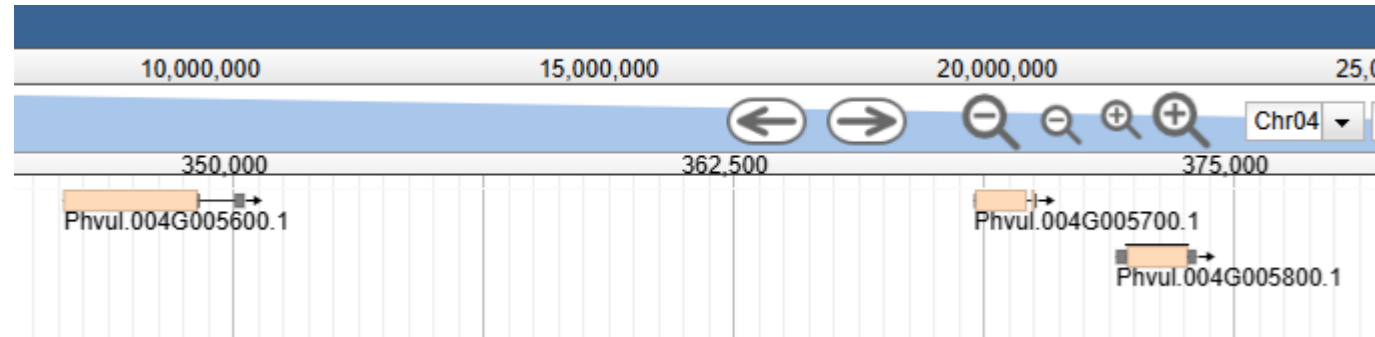


## 12- Encontrar recombinantes

F <sub>3</sub> family ID	Reaction to race 1545	Genotype with KASP Markers								Reference genome
		33,578	164,385	302,160	448,849	495,761	521,957	552,696	1,299,082	
		90,666	218,204	381,360	507,568	554,477	580,673	611,412	1,036,037	
		SS129	SS120	SS130	SS142	SS123	SS58	SS284	SS260	
Crioulo 159	8	AA	AA	AA	AA	AA	AA	AA	AA	
Beija Flor	1	BB	BB	BB	BB	BB	BB	BB	BB	
BxC_14#1	1	BB	BB	BB	AB	AB	AB	AB	AB	
BxC_18#10	8	AA	AA	AA	AB	AB	AB	AB	AB	
BxC_41#10	8	AA	AA	AA	AB	AB	AB	AB	AB	
BxC_98#1	8	BB	BB	BB	AA	AA	AA	AA	AA	
BxC_120#15	1	AA	AA	AA	AB	AB	AB	AB	AB	
BxC_134#2	1	BB	BB	BB	AA	AA	AA	AA	AB	
BxC_155#5	1	AB	AB	AB	AA	AA	AA	AA	AA	
BxC_179#8	1	AA	AA	AA	AB	AB	AB	AB	AB	
BxC_98#6	8	BB	BB	BB	AA	AA	AA	AA	AA	
BxC_131#10	1	AB	AB	AB	AA	AA	AA	AA	AA	

## 14- Identificar genes candidatos

Phytozome v2.1



Phytozome v2.1

Gene Report: **Phvul.004G005600**  
Genome: *Phaseolus vulgaris* v2.1 (Phytozome genome ID: 442 - NCBI taxonomy ID: 3885)  
Gene Identifier: Phvul.004G005600 (PAC:37162633)  
Transcript: **Phvul.004G005600.1**  
Location: Chr04:345783..350309 forward  
Alias: Phvul.004G005600.v1.0  
Auto Define: (1 of 124) PTHR23155/PTHR23155:SF414 - LEUCINE-RICH REPEAT-CONTAINING PROTEIN // SUBFAMILY NOT NAMED  
View: **8**

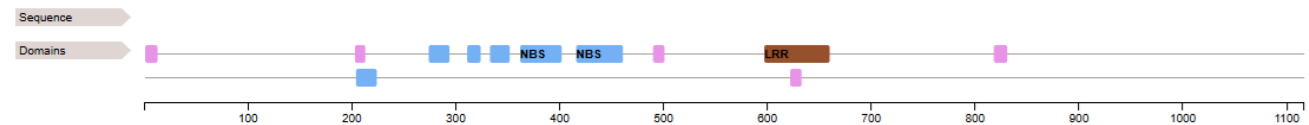
Functional Annotations [Export](#)



<https://phytozome-next.jgi.doe.gov/>

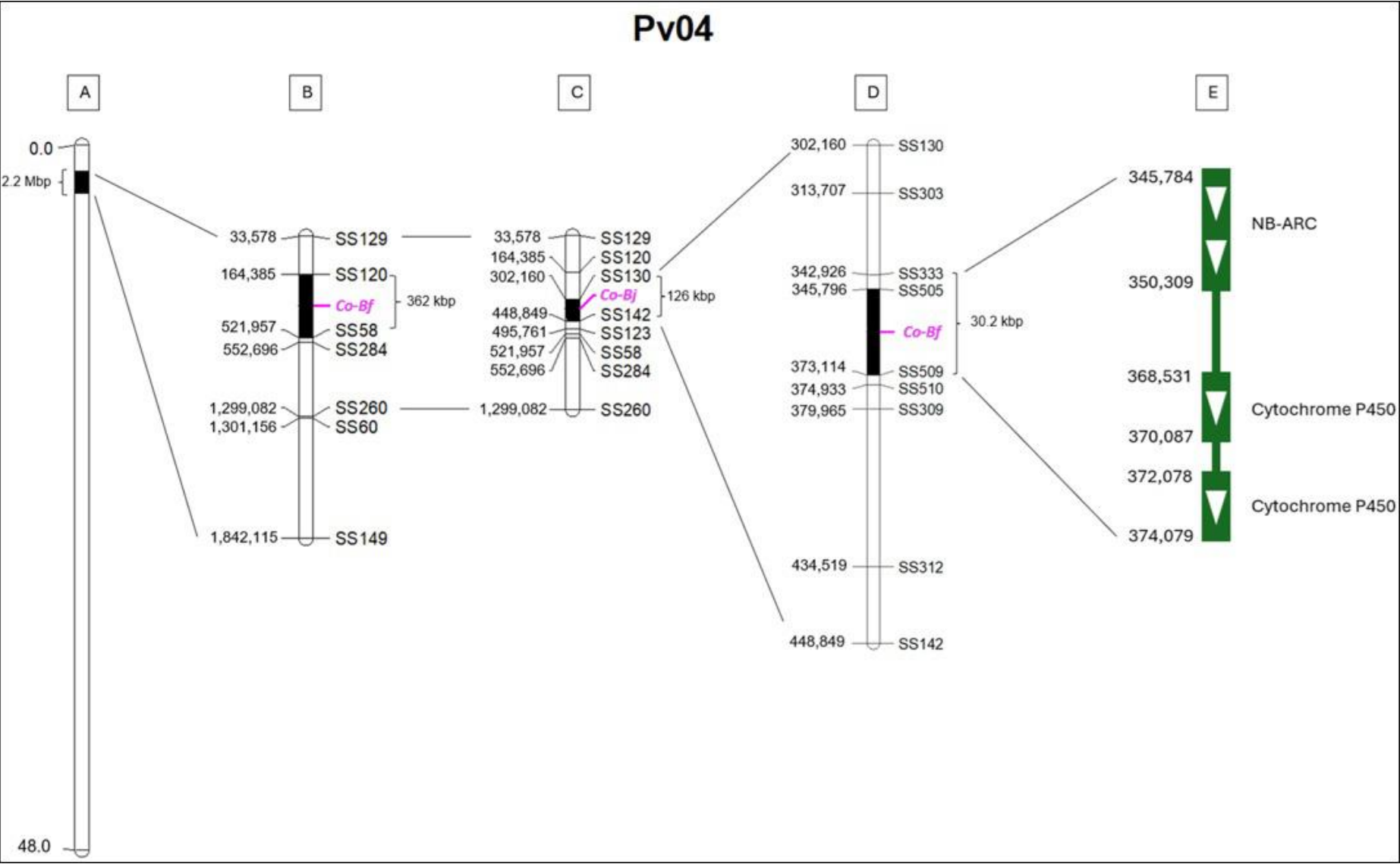
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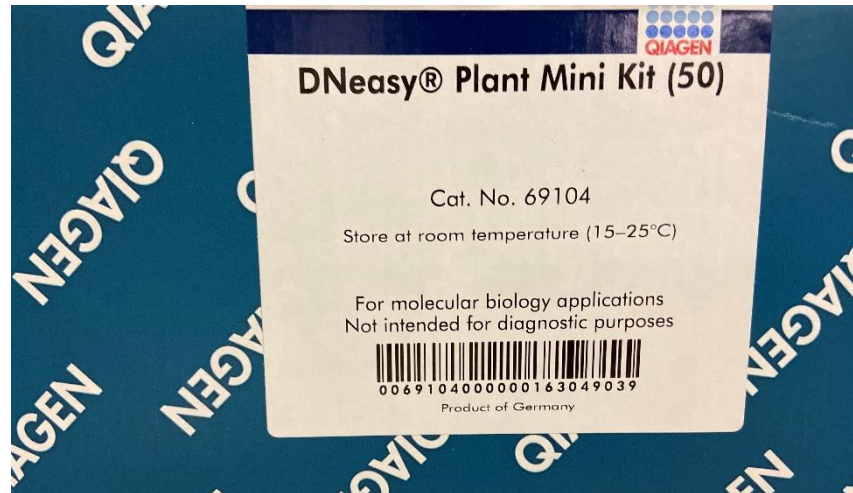
PRGbd



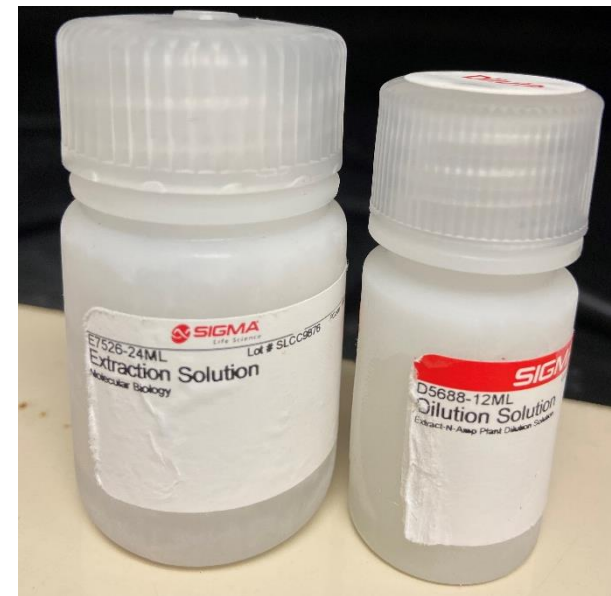
<http://prgdb.org/prgdb4/>

Fine mapping

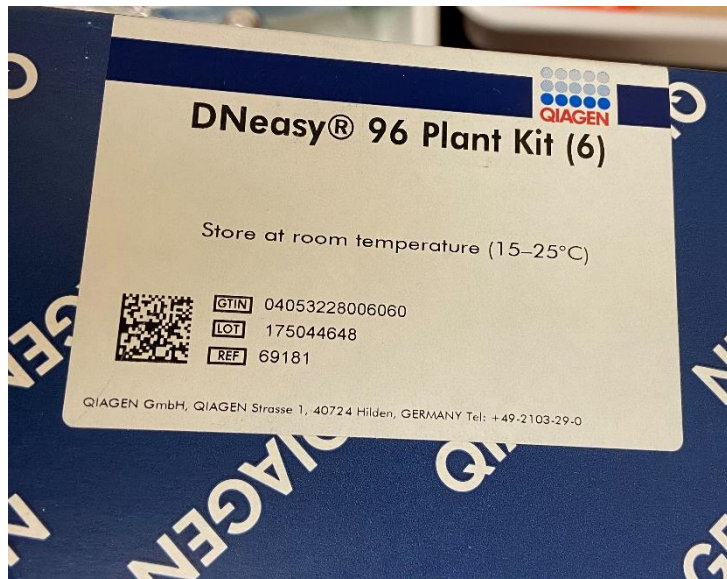




Kit de extracao de DNA – Eppendorf - QIAGEN



Kit de extracao de DNA rapido - SIGMA



Kit de extracao de DNA – Placa de 96 células - QIAGEN



Kit de extracao de RNA - QIAGEN

## Referências

- de Lima Castro SA, Gonçalves-Vidigal MC, Gilio TAS, Lacanallo GF, Valentini G, Martins VSR, Song Q, Galván MZ, Hurtado-Gonzales OP, Pastor-Corrales MA (2017) Genetics and mapping of a new anthracnose resistance locus in Andean common bean Paloma. BMC Genom 18:306. <https://doi.org/10.1186/s12864-017-3685-7>
- Gilio TAS, Hurtado-Gonzales OP, Gonçalves-Vidigal MC, Valentini G, Elias JCF, Song Q, Pastor-Corrales MA (2020) Fine mapping of an anthracnose-resistance locus in Andean common bean cultivar Amendoim Cavalo. PLOS ONE 15:1-17. <https://doi.org/10.1371/journal.pone.0239763>
- He C, Holme J, Anthony J (2014). SNP Genotyping: The KASP Assay. In: Fleury, D., Whitford, R. (eds) Crop Breeding. Methods in Molecular Biology, vol 1145. Humana Press, New York, NY. [https://doi.org/10.1007/978-1-4939-0446-4\\_7](https://doi.org/10.1007/978-1-4939-0446-4_7)
- Hurtado-Gonzales OP, Valentini G, Gilio TAS, Martins AM, Song Q, Pastor-Corrales MA (2017) Fine mapping of *Ur-3*, a historically important rust resistance locus in common bean. G3 7(2):557–569. <https://doi.org/10.1534/g3.116.036061>.
- ILLUMINA. GenomeStudio® genotyping module v2.0: software guide. Illumina, inc, 2018.
- Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci USA 88:9828-9832. <https://doi.org/10.1073/pnas.88.21.9828>
- Song Q, Jia G, Hyten DL, Jenkins J, Hwang EY, Schroeder SG, Osorno JM, Schmutz J, Jackson SA, McClean PE, Cregan PB (2015) SNP assay development for linkage map construction, anchoring whole-genome sequence, and other genetic and genomic applications in common bean. G3: Genes, Genomes, Genet 5(11):2285-2290. <https://doi.org/10.1534/g3.115.020594>
- Valentini, G, Hurtado-Gonzales, OP, Xavier, LFS et al. Fine mapping of the unique *Ur-11* gene conferring broad resistance to the rust pathogen of common bean. Theor Appl Genet 138, 64 (2025). <https://doi.org/10.1007/s00122-025-04856-5>
- Van Ooijen JW (2006) JoinMap 4, Software for the Calculation of Genetic Linkage Maps in Experimental Populations. Kyazma B.V., Wageningen, Netherlands
- Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78
- Xavier, LFS. Tese de doutorado: Mapeamento do gene de resistência à antracnose na cultivar Andina de feijão comum Beija Flor. Programa de Pós graduação em genética e melhoramento. Universidade Estadual de Maringá. 2021.

# Obrigada!

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