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A novel high-density grapevine integrated linkage map using GBS in a multi-parental population: preliminary results

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► To cite this version:

Javier Tello Moro, Catherine Roux, Valerie Laucou, Gautier Sarah, Timothée Flutre, et al.. A novel high-density grapevine integrated linkage map using GBS in a multi-parental population: preliminary results. 12. International Conference on Grapevine Breeding and Genetics, Jul 2018, Bordeaux, France. hal-01857671

HAL Id: hal-01857671

<https://hal.science/hal-01857671>

Submitted on 5 Jun 2020

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INTRODUCTION

Understanding the genetic determinism of relevant grapevine traits like crop yield, phenology and fruit quality partially relies on the availability of sound genetic information. Likewise, the availability of reliable genetic maps are key elements to increase the knowledge about grapevine genome structural features [1]. The development of next-generation sequencing approaches and new sequencing platforms is boosting grapevine genetics research, allowing the generation of high-density linkage maps with relatively low-cost

and robust procedures for the subsequent identification of candidate loci and candidate genes for relevant traits [2]. Here, we report the preliminary results of the application of the genotyping-by-sequencing (GBS, Keygene patents) technology in a large grapevine population derived from a half-diallel crossing design for the generation of a novel high-density grapevine linkage map.

PLANT MATERIAL AND GENOTYPING PIPELINE

The half-diallel grapevine population (N=624) derives from 10 interconnected segregating progenies from the cross of 5 wine cultivars. The panel was planted in a randomized block design at the *Domaine du Chapitre* of Montpellier Supagro (Villeneuve-lès-Maguelone, France) in 2005.



♀ \ ♂	Pinot Noir	Syrah	Cabernet Sauvignon	Grenache	Terret Noir
Pinot Noir					
Syrah	58				
Cabernet Sauvignon	66	64			
Grenache	63	59	63		
Terret Noir	60	63	61	67	

Total population: 624 individuals

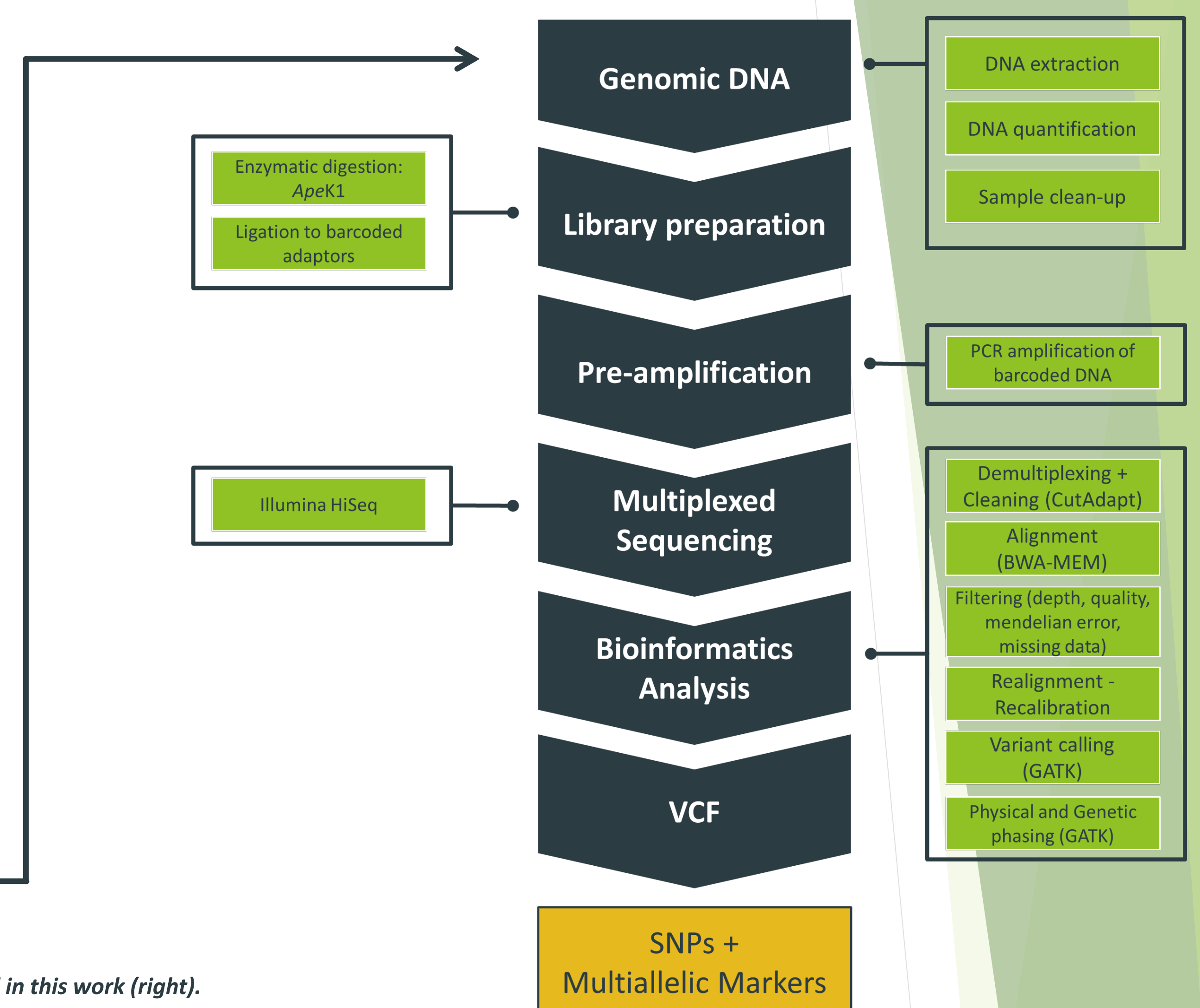


Figure 1. Origin and size of the ten interconnected grapevine progenies (left) and flow chart of the GBS genotyping pipeline used in this work (right).

PRELIMINARY RESULTS

GBS Results

The GBS approach and subsequent bioinformatics analysis was useful to identify 71720 genetic variants in the half-diallel population, including biallelic SNPs and multiallelic markers. We obtained an average of 17300 segregating markers per progeny, and a total of 9798 fully-informative ones (<abxac> or <abxcd>) (Table 1). Interestingly, 568 markers were found to be common to all (or 9) progenies.

Segregation type	SxPN	CSxPN	GxPN	TNxPN	CSxS	GxS	TNxS	GxCS	TNxCS	TNxG
<a0xab>	24	70	36	75	74	47	95	3	117	37
<aaxab>	6926	7064	4675	6047	7213	7406	5780	1945	5907	3731
<abxa0>	24	30	28	55	27	20	21	5	75	25
<abxaa>	8171	7315	4881	5240	5638	7095	4188	1952	4656	3512
<abxab>	7488	7095	3847	5757	7971	5288	5623	1607	5085	3376
<abxac>	792	1151	1301	1176	1834	1184	1209	572	1276	1000
<abxcd>	45	141	242	200	313	206	244	95	282	171
Total Markers	23470	22866	15010	18550	23070	21246	17160	6179	17398	11852

Table 1. Summary of the number of GBS-derived genetic variants in the 10 progenies of the half-diallel population

GBS-derived genetic variants' distribution was well balanced within the 19 grapevine chromosomes (Fig. 2A). A reduced set of 10306 genetic variants (Fig. 2B) were extracted for genetic grouping and mapping.

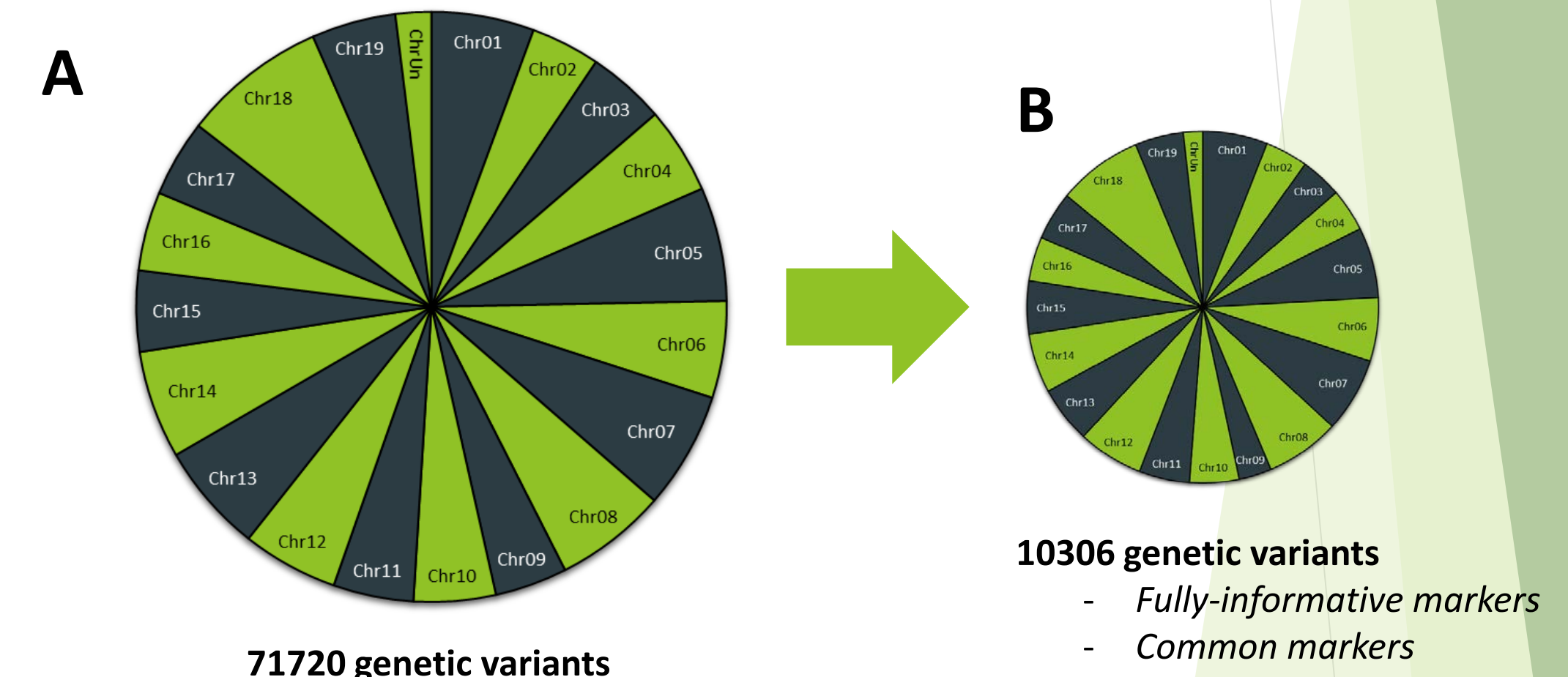


Figure 2. Proportions of GBS-derived genetic markers anchored to the 19 grapevine chromosomes in the full (A) and the reduced (B) sets of detected markers

Consensus and integrated maps construction

CONSENSUS MAPS FOR EACH PROGENY (LG02)

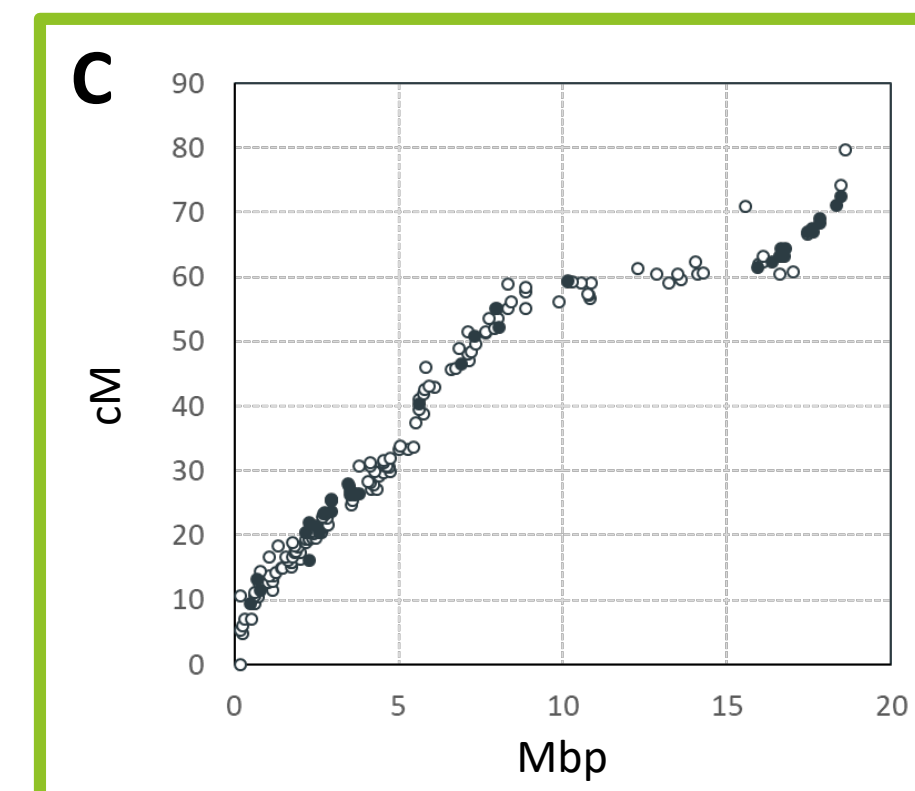
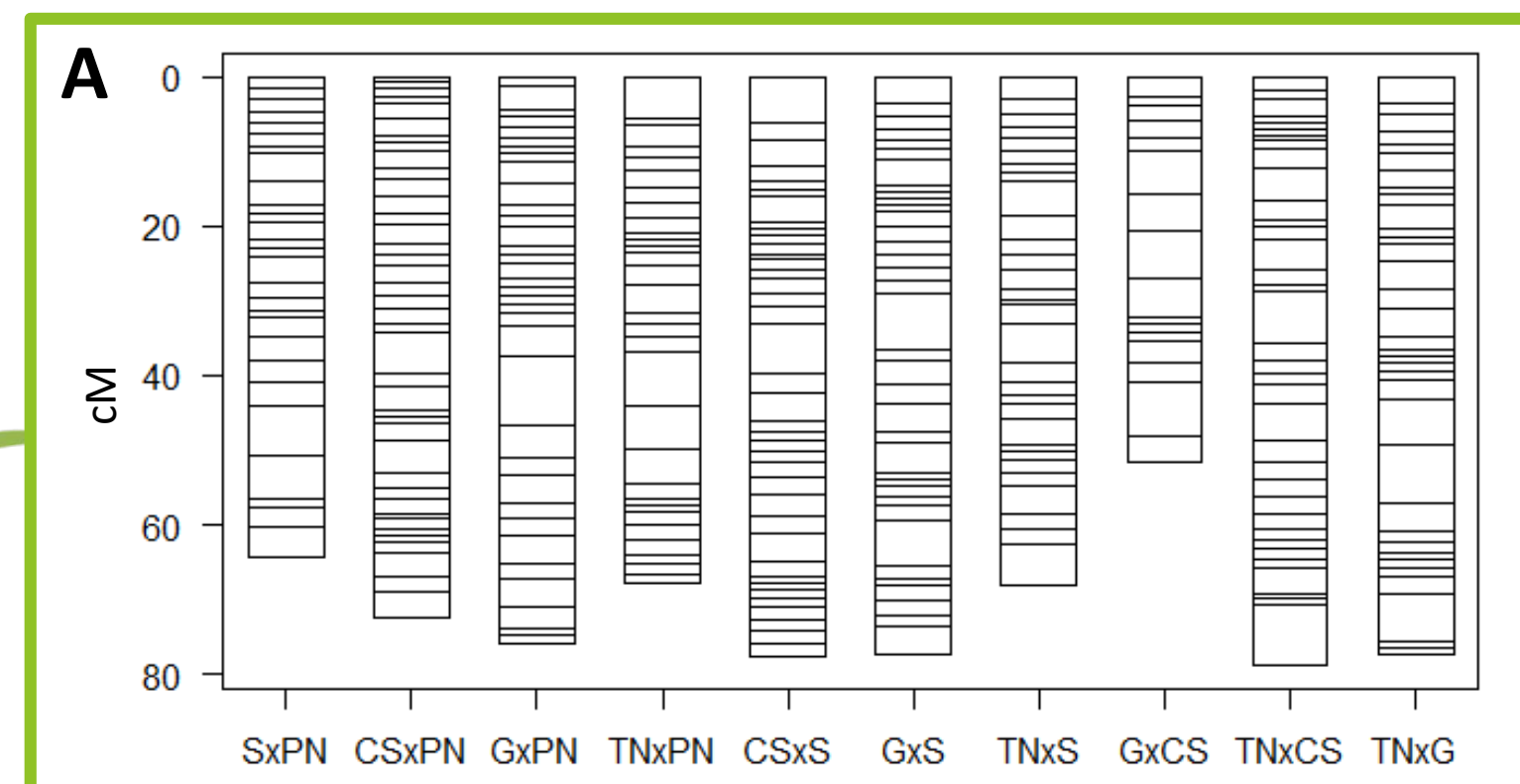
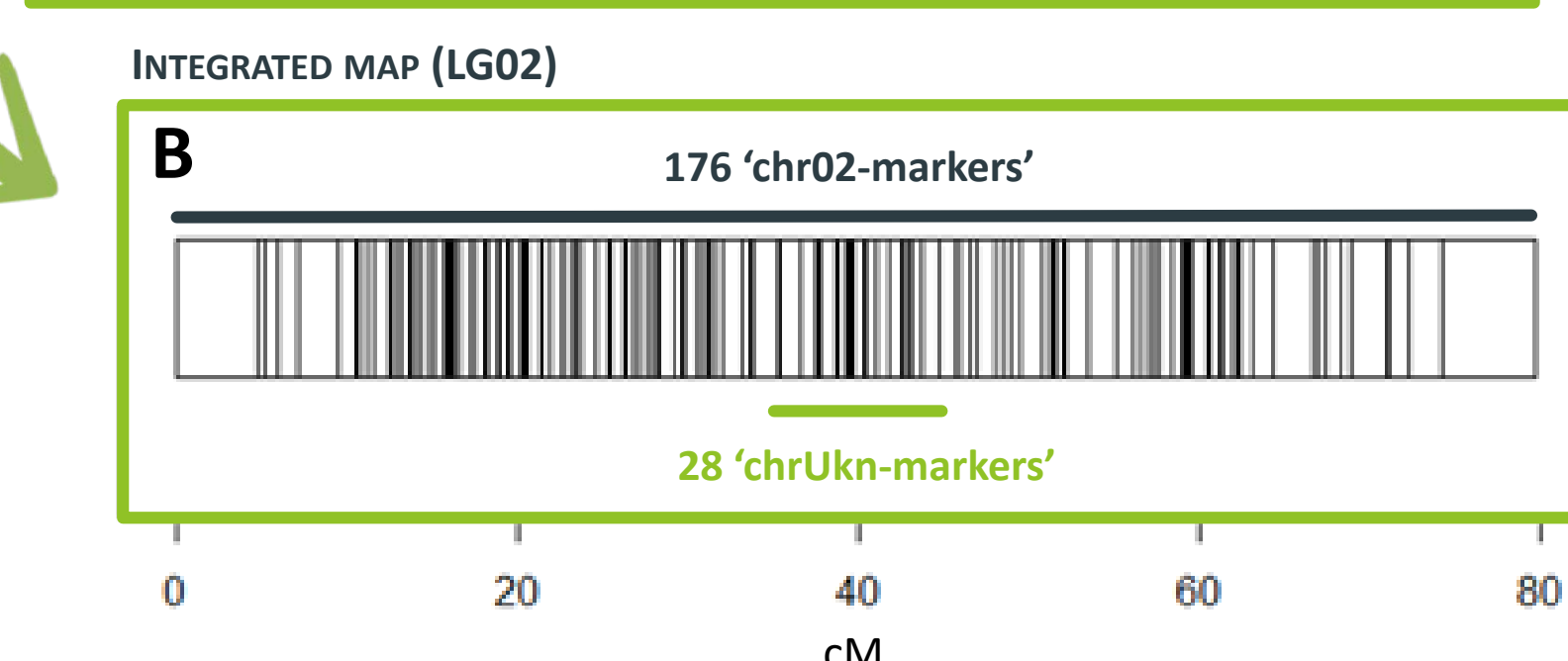


Figure 3. Consensus (A) and integrated (B) maps obtained for LG02. In C, the relationship between genetic (in cM) and physical (in Mbp) distances are shown (each marker is represented as a dot, with common markers highlighted as filled dots).



Consensus maps were obtained for each progeny with CarthaGene as previously detailed [3], considering the reduced subset of 10306 GBS-derived markers. Consensus maps obtained for LG02 in each progeny are graphically shown in Fig 3A as an example.

Although slight differences in map length were observed, we obtained a high degree of agreement in marker order between the 10 consensus maps. Thereafter, consensus maps were merged by the linear programming approach implemented in LPmerge [4] to obtain an integrated genetic map.

The integrated map for LG02 contained 204 markers spanning 79.7 cM (Fig. 3B), which corresponded to 162 unique marker positions with an average gap of 0.5 cM between them. This length is similar to the one reported in previous *Vitis vinifera* L. integrated maps [1, 5], but with a considerable higher marker density. Interestingly, 28 markers not assigned to any of the 19 predicted grapevine chromosomes ('chrUkn-markers') grouped in the central part of chr02 (in consensus and integrated maps), suggesting it as its actual placement. Additionally, a good agreement between genetic and physical distances of the grouped markers was observed (Fig. 3C), supporting the confidence in the maps obtained.

Altogether, our results confirm the usefulness of the GBS technology for the high-throughput discovery of novel genetic variants in grapevine, and suggest their efficiency for obtaining dense genetics map with high informative content for both basic and applied research.

FUTURE WORK

Consensus and integrated maps will be used in further genetic research aimed to unravel the genetic determinism of grapevine traits like crop yield components, phenology and berry and must quality traits, and to test genomic prediction in specific configurations.

REFERENCES

[1] Doligez *et al.* (2006) TAG 113, 369–382. [2] Teh *et al.* (2017) Mol. Breed. 37, 1. [3] Houel *et al.* (2015) BMC Plant Biol. 15:205. [4] Endelman and Plomion (2014) Bioinformatics 30, 1623-1624. [5] Vezzulli *et al.* (2008) TAG 117, 499-511

ACKNOWLEDGEMENTS

This work has received the support of the AgreenSkills+ fellowship programme, funded by the EU's Seventh Framework Programme under grant agreement N° FP7-609398, the *Géno-Vigne*® Technological Unit, and the *Agropolis Fondation* under the ARCAD project No 0900-001.