Genetic Mapping of DNA Markers in Pineapple

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Abstract

Two genetic maps of DNA-markers (RAPD, AFLP and ISSR) of *Ananas comosus* var. *bracteatus* and *A. comosus* var. *comosus* have been previously published by our team. These maps were constructed at the Laboratory of Genetics and Plant Breeding of FERN, University of Algarve, using an F1 mapping population derived from a cross performed in Martinique between the two botanical varieties. However, the use of a F1 population, the small size of this population and the use of molecular markers with dominant inheritance allowed only markers that were heterozygous in the parents to be included in those maps and prevented the construction of an integrated high-density map. A new genetic map, intended to cover all the pineapple genome, is currently under construction using a mapping population of 142 F2 plants. This new map will integrate the already published maps and will include markers that had remained unlinked, as they were polymorphic but homozygous in parental genotypes, as well as newly identified markers. At the present time, the new map consists of seven linkage groups (> 40 cM) that integrate markers from both parents, 21 groups (> 40 cM) with markers mostly from one parent, six groups (< 40 cM and > 25 cM) with markers from one parent, and 12 smaller (< 25 cM) linkage groups, covering approximately 62% of the pineapple genome.

INTRODUCTION

The new pineapple taxonomy (Coppens d'Eeckenbrugge and Leal, 2003) recognizes five botanical varieties for *Ananas comosus* (L.) Merr.: var. *ananassoides*, var. *bracteatus*, var. *comosus*, var. *erectifolius*, and var. *parguazensis.* They are very similar in their floral structure and biology, chromosome number (2n=50), and they intercross successfully, producing fertile offspring (Coppens d'Eeckenbrugge et al., 1997). *Ananas comosus* var. *comosus* corresponds to the pineapple cultivated for fruit, characterized by large edible fruits, a trait resulting from human selection. *A. comosus* var. *bracteatus* (Lindl.) Coppens & Leal (grouping the former *A. bracteatus* (Lindley) Schultes f. and *A. fritzmuelleri* Camargo), characterized by a medium size inflorescence with long bracts, is cultivated as a living fence and as ornamental and is still found as a sub-spontaneous plant in southern South America. Two F1-based genetic maps have been published recently for these two varieties. The map of *A. comosus* var. *bracteatus* gathers 335 DNA markers assembled in 50 linkage groups covering 57.2% of the genome length. The map of *A. comosus* var. *comosus* gathers 156 markers in 30 linkage groups covering 31.6% of the genome. The locus *P*, whose dominant allele determines the morphological trait 'piping', a silvery streak and the absence of spines along the margin of the upper leaf (Cabral et al., 1997), was also included in the last map (Carlier et al., 2004).

The use of a F1 population, following the pseudo-test-cross methodology, allowed the rapid obtaining of these results. However, the small size of this population and the use

of molecular markers with dominant inheritance allowed only markers that were heterozygous in the parents to be included in those maps and prevented the construction of an integrated high-density map. In a second step, one of the F1 plants was selfed for the construction of a F2-based genetic map. This new genetic map, intended to cover all the pineapple genome, will integrate the already published maps and include newly identified markers, as well as markers that had remained unlinked, as they were polymorphic but homozygous in parental genotypes. We present here the first results of this work.

MATERIALS AND METHODS

Plant Materials

The mapping population consisted of 142 F2 plants obtained from a selfed F1 plant from a cross between *Ananas comosus* var. *comosus*, cv. Rondon, clone BR 50, and *Ananas comosus* var. *bracteatus*, cv. Branco do Mato, clone BR 20, performed at the CIRAD-FLHOR experimental station in Martinique. Leaves, collected from each F2 plant and progenitors, were sent to the University of Algarve for molecular marker analysis and map construction.

DNA Extraction and Molecular Markers Analyses

Procedures for DNA isolation and RAPD and AFLP analyses were as previously published (Carlier et al., 2004). ISSR analysis was performed as described in Farinhó et al. (2004).

Map Construction

All segregating polymorphic markers were used for map construction, utilizing the JoinMap 3.0 program (Van Ooijen and Voorrips, 2001) set for the Kosambi's mapping function. Linkage groups were built with LOD thresholds varying between 3.0 and 7.0. The marker order within each linkage group was established using a LOD threshold of 1. Linkage groups established on the F2 population that exhibited at least two markers common to linkage groups of the F1-based maps were integrated with the latter. Linkage groups established on the F2 population that only shared one marker with linkage groups of the F1-based maps were placed side by side, connected through that specific marker. In the cases of such complex groups the larger group was used for map length estimation.

Markers and Linkage Group Identification

The linkage groups established on the F2 population are identified by the letter A followed by a number. Integrated groups are identified by the F2 group designation followed by the previous identification of the F1-based maps group(s). For example: A12+Ab4+Ac6 stands for group A12 (established using the F2 population) integrated with groups Ab4 and Ac6 of the F1-based pineapple genetic maps (Carlier et al., 2004).

RAPD and AFLP markers were designated as previously (Carlier et al., 2004). ISSR markers were identified by the capital letters ISSR followed by the two digit number ascribed to each ISSR primer (Table 1) and the estimated fragment size; e.g. ISSR221050 stands for a 1050 bp ISSR marker generated by primer 22.

Markers that segregate with a slight deviation from the expected ratio ($\chi^2_{\alpha=0.05} < \chi^2 \leq \chi^2_{\alpha=0.01}$) are identified with one asterisk and those having a more marked deviation ($\chi^2 > \chi^2_{\alpha=0.01}$) with two asterisks. The new markers, analysed among the F2 population, but not among the F1, are identified by the prefix "n". The suffix Ab and Ac at the end of marker designations refers to its origin, respectively *A. comosus* var. *comosus* or *A. comosus* var. *bracteatus*.

RESULTS AND DISCUSSION

The construction of the new genetic map using the F2 population was initiated using the RAPD primers and the AFLP primer combinations that, during the construction

of the F1-based genetic maps, had amplified either more polymorphic makers or markers strategically distributed along the linkage groups (Carlier et al., 2004). Twenty-one out of the tested 32 ISSR primers (Table 1) amplified 38 markers adequate for mapping. The type and number of molecular markers whose segregation was analysed among the F2 population are discriminated in Table 2.

The integration of the segregation data among the F1 and the F2 mapping populations resulted in an integrated map (Fig. 1) of 574 markers (454 AFLP, 79 RAPD and 41 ISSR) gathered in 46 linkage groups, spanning over 2421 cM (3.9 cM). Using the arithmetic average of the previously computed genome sizes of *A. comosus* var. *comosus* (4146 cM) and *A. comosus* var. *bracteatus* (3693 cM) as an estimate of the average genome size of *Ananas comosus* (3919.5 cM), we calculated that the new integrated map covers approximately 62% of the pineapple genome.

The integrated genetic map depicted in Fig. 1 is still incomplete. A very dense and integrated genetic map of molecular markers, complemented with microsatellite and SCAR markers, organised into 25 linkage groups covering almost all the pineapple genome will constitute a major scientific breakthrough. Such a map would allow the rapid location of markers flanking any gene of interest for use in marker assisted selection (MAS) or in gene isolation via map-based cloning and would constitute a reference network for physical mapping and genome sequencing programmes in pineapple.

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Tables

Table 1. Selected ISSR primers for the construction of a F2-based genetic map.

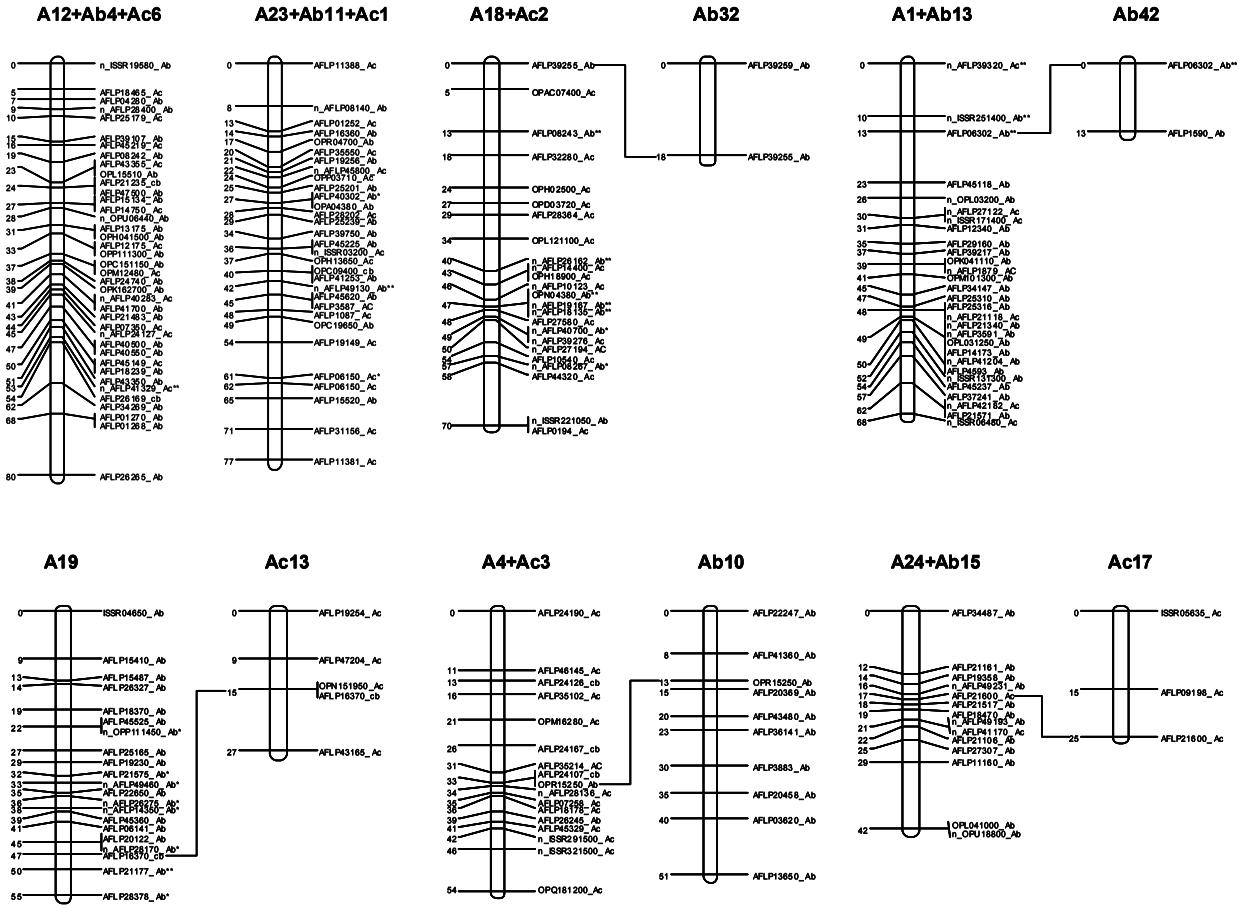
Code number	Primers	Code number	Primers	Code number	Primers
02	(CA)8ARY	13	(AC)8YT	22	(TC)8G
03	(GA)8YT	14	(AG)8T	23	(CT)8RC
04	(GA)8YC	15	(AG)8C	25	(TG)8RC
05	(GA)8AYC	17	(GA)8T	29	VHV(GT)7
06	(AG)8GYT	18	(GA)8C	30	HVH(TG)7
07	(AG)8YC	19	(GA)8A	31	(AG)8VC
09	(AC)8YG	21	(TC)8C	32	CCC(GT)7

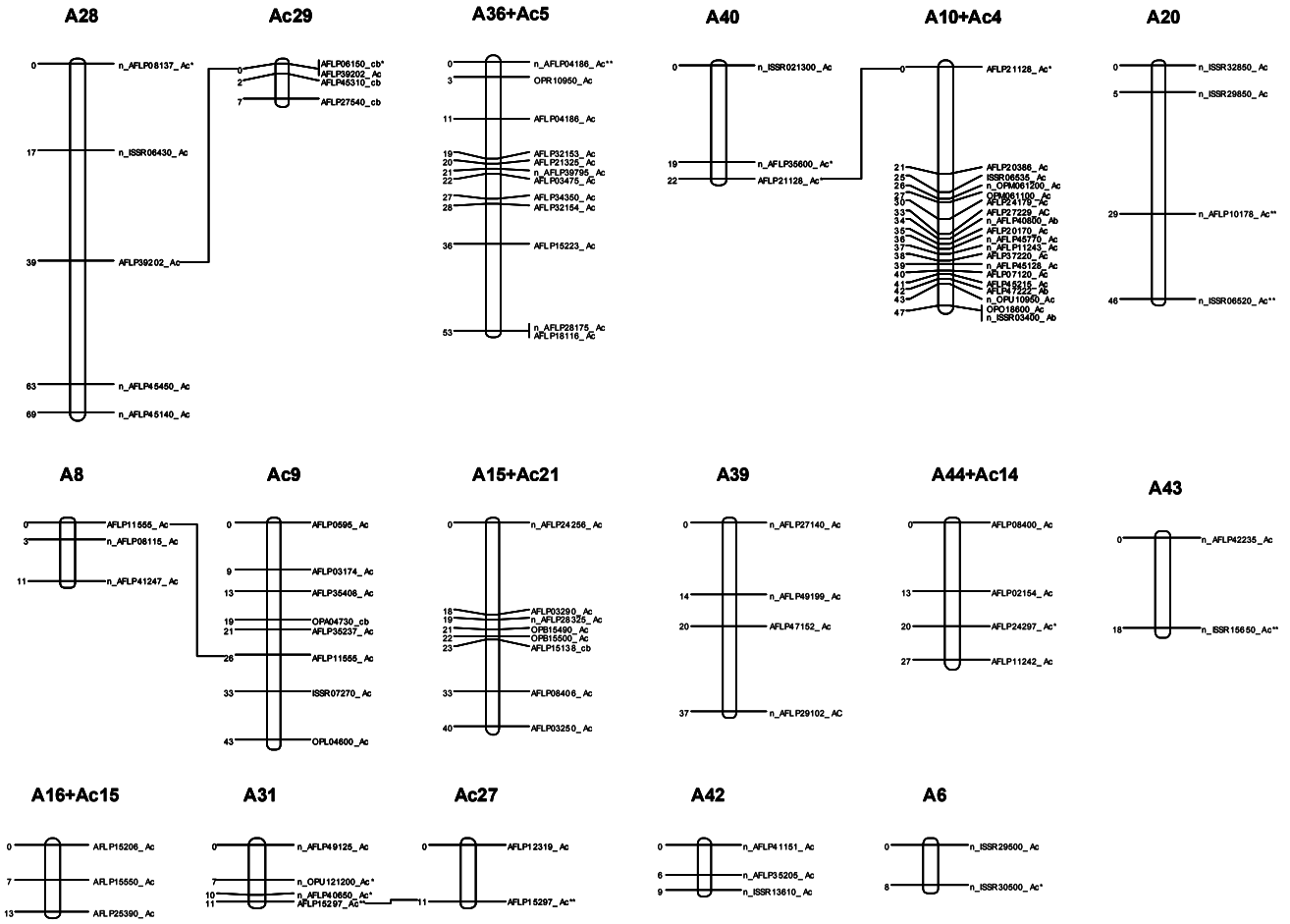
Table 2. Number of primers and number of markers analysed among the F2 population.

	Number of primers	Number of markers			
		A. comosus var. *bracteatus*		*A. comosus* var. *comosus*	
		Previously mapped[2]	New	Previously mapped[2]	New
RAPDs	25	18	8	5	3
ISSRs	21	0	16	0	22
AFLPs	30[1]	116	82	41	61
Sub-total		134	106	46	86
Total		240		132	

[1] Primer combinations
[2] Mapped in the F1-based genetic maps (Carlier et al., 2004)

Figures


A12+Ab4+Ac6
A23+Ab11+Ac1
A18+Ac2
Ab32
A1+Ab13
Ab42
A19
Ac13
A4+Ac3
Ab10
A24+Ab15
Ac17


A28
0 n_AFLP08137_Ac*
17 n_ISSR06430_Ac
39 AFLP39202_Ac
63 n_AFLP45450_Ac
69 n_AFLP45140_Ac
Ac29
0 AFLP06150_cb*
0 AFLP39202_Ac
2 AFLP45310_cb
7 AFLP27540_cb
A36+Ac5
0 n_AFLP04186_Ac**
3 OPR10950_Ac
11 AFLP04186_Ac
19 AFLP32153_Ac
20 AFLP21325_Ac
21 n_AFLP39795_Ac
22 AFLP03475_Ac
27 AFLP34350_Ac
28 AFLP32154_Ac
36 AFLP15223_Ac
53 n_AFLP28175_Ac
AFLP18116_Ac
A40
0 n_ISSR021300_Ac
19 n_AFLP35600_Ac*
22 AFLP21128_Ac
A10+Ac4
0 AFLP21128_Ac*
21 AFLP20386_Ac
25 ISSR06535_Ac
26 n_OPM061200_Ac
27 OPM061100_Ac
30 AFLP24178_Ac
33 AFLP27229_AC
34 n_AFLP40880_Ab
35 AFLP20170_Ac
36 n_AFLP45770_Ac
37 n_AFLP11243_Ac
38 AFLP37220_Ac
39 n_AFLP45128_Ac
40 AFLP07120_Ac
41 AFLP49215_Ac
42 AFLP47222_Ab
43 n_OPU10950_Ac
47 OPO16600_Ac
n_ISSR03400_Ab
A20
0 n_ISSR32850_Ac
5 n_ISSR29850_Ac
29 n_AFLP10178_Ac**
46 n_ISSR06520_Ac**
A8
0 AFLP11555_Ac
3 n_AFLP08115_Ac
11 n_AFLP41247_Ac
Ac9
0 AFLP0595_Ac
9 AFLP03174_Ac
13 AFLP35406_Ac
19 OPA04730_cb
21 AFLP35237_Ac
26 AFLP11555_Ac
33 ISSR07270_Ac
43 OPL04600_Ac
A15+Ac21
0 n_AFLP24256_Ac
18 AFLP03290_Ac
19 n_AFLP28325_Ac
21 OPB15490_Ac
22 OPB15500_Ac
23 AFLP15138_cb
33 AFLP08406_Ac
40 AFLP03250_Ac
A39
0 n_AFLP27140_Ac
14 n_AFLP49199_Ac
20 AFLP47152_Ac
37 n_AFLP29102_AC
A44+Ac14
0 AFLP08400_Ac
13 AFLP02154_Ac
20 AFLP24297_Ac*
27 AFLP11242_Ac
A43
0 n_AFLP42235_Ac
16 n_ISSR15650_Ac**
A16+Ac15
0 ARLP15206_Ac
7 ARLP15550_Ac
13 ARLP25390_Ac
A31
0 n_AFLP49125_Ac
7 n_OPU121200_Ac*
10 n_AFLP40650_Ac*
11 AFLP15297_Ac**
Ac27
0 AFLP12319_Ac
11 AFLP15297_Ac**
A42
0 n_AFLP41151_Ac
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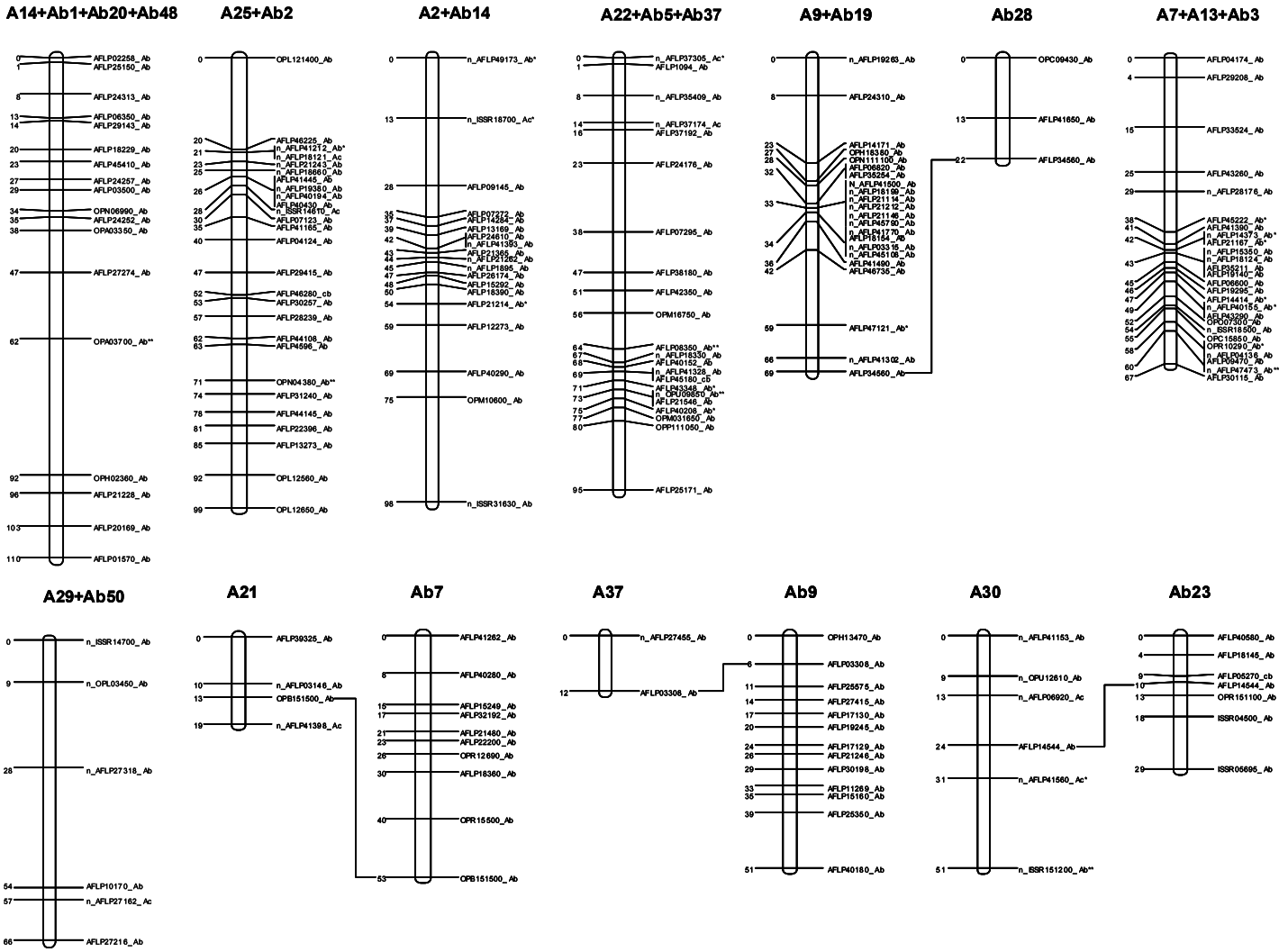
9 n_ISSR13610_Ac
A6
0 n_ISSR29500_Ac
8 n_ISSR30500_Ac*


A14+Ab1+Ab20+Ab48
A25+Ab2
A2+Ab14
A22+Ab5+Ab37
A9+Ab19
Ab28
A7+A13+Ab3
A29+Ab50
A21
Ab7
A37
Ab9
A30
Ab23

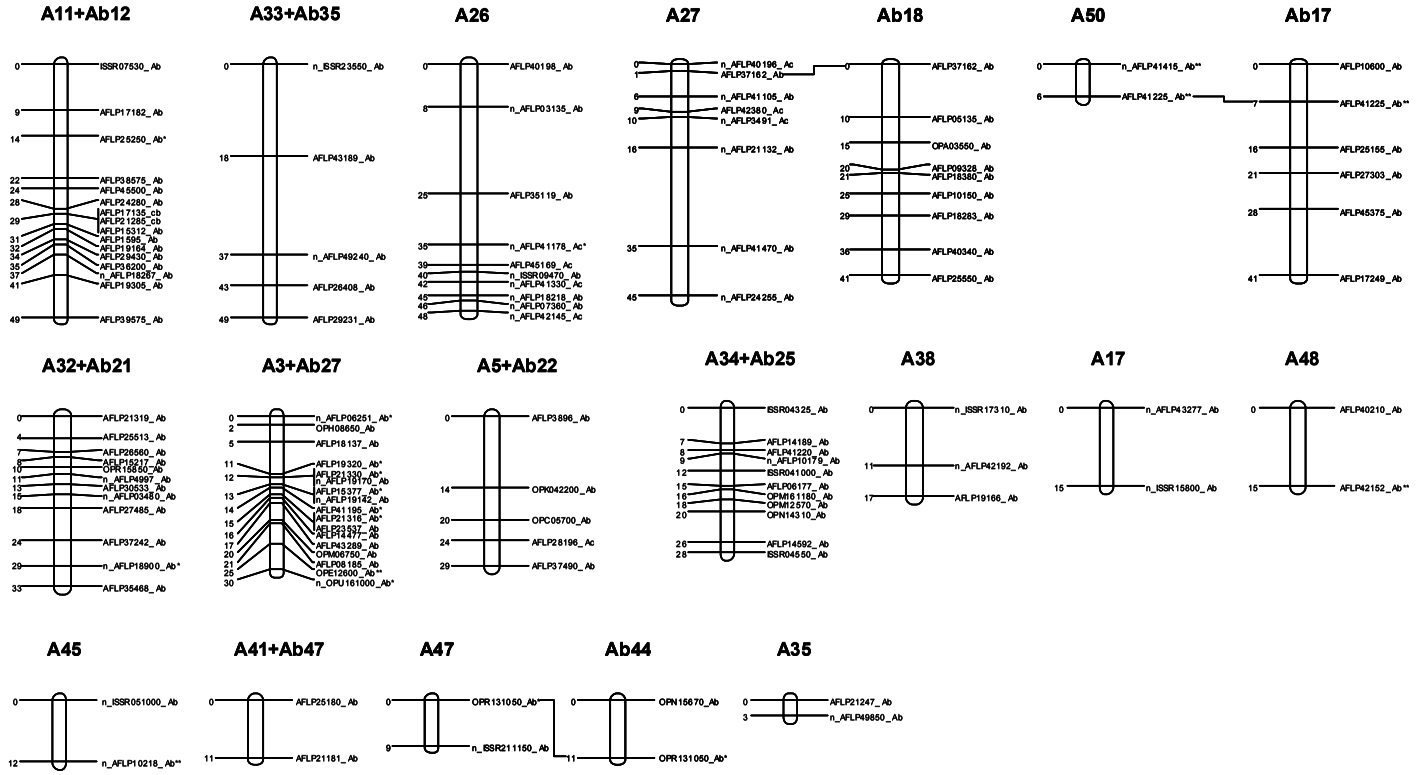


Fig. 1. Integrated genetic map of *Ananas comosus*.