Genomic and genetic variability of six chicken populations using single nucleotide polymorphism and copy number variants as markers

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Short Title: Genetic variability of six chicken breeds

Abstract

Genomic and genetic variation among six Italian chicken native breeds (Livornese, Mericanel della Brianza, Milanino, Bionda Piemontese, Bianca di Saluzzo and Siciliana) were studied using single nucleotide polymorphism (SNP) and copy number variants (CNV) as markers. A total of 94 DNA samples genotyped with

Axiom® Genome-Wide Chicken Genotyping Array (Affymetrix) were used in the analyses. The results showed the genetic and genomic variability occurring among the six Italian chicken breeds. The genetic relationship among animals was established with a principal component analysis. The genetic diversity within breeds was calculated using heterozygosity values (expected and observed) and with Wright's F-statistics. The individual-based CNV calling, based on log R ratio (LRR) and B allele frequency (BAF) values, was done by the Hidden Markov Model of PennCNV software on autosomes. A hierarchical agglomerative clustering was applied in each population according to the absence or presence of definite CNV regions (CNV were grouped by overlapping of at least 1 base pair). The CNV map was built on a total of 1003 CNV resulting in 564 unique CNV regions (344 gains, 213 losses and 7 complex), for a total of 9.43 Mb of sequence and 1.03% of the chicken assembly autosome. All the approaches using SNP data showed that the Siciliana breed clearly differentiate from other populations, the Livornese breed separates into two distinct groups according to the feather colour (i.e. white and black) and the Bionda Piemontese and Bianca di Saluzzo breeds resulted are closely related. The genetic variability found using SNP is comparable to that found by other authors in the same breeds using microsatellite markers. The CNV markers analysis clearly confirmed the SNP results.

Key words: SNP, Copy Number Variation, poultry, biodiversity, genetic variability

Implications

The aim of this study was to assess the genetic diversity of six Italian chicken breeds in order to define the status of in situ genetic collections and study their conservation potential. The genetic and genomic structure of the six Italian native chicken populations reported here will contribute to design coherent programs for in vivo and in vitro conservation, valorisation and utilization of the breeds. As these breeds represent a unique animal resource, these findings will impact the economic value and environmental sustainability of traditional food production..

Introduction

Genetic makeup of populations is the result of a long-term process of adaptation to specific environments and ecosystems and, of artificial selection. Local populations are usually well adapted to environment and capable to express optimal functionality of life cycle events, as reproduction and resistance to diseases despite environmental challenges and, at the same time, to exhibit a good food production (i.e. meat and eggs).

The Food and Agricultural Organization of United Nation (FAO) definition of animal genetic resources eligible for conservation includes animal populations with economic potential, scientific and cultural interest (FAO, 2009). In most of the World about 50% of documented breeds have been classified as extinct, at critical survival or endangered (Hammond, 1996); furthermore 31% of cattle breeds, 35% of pig breeds and 38% of chicken breeds are at risk of extinction. Additionally especially in poultry, local breeds have often been diluted by indiscriminate cross-breeding with imported stocks (FAO, 2009). As a consequence the conservation of domestic animal biodiversity has become a priority to develop sustainable, safe and diversified products and production systems. Considering that the 68% of the 53 Italian chicken breeds were classified as extinct (Zanon and Sabbioni, 2001), efforts for conservation of the remaining local populations are urgently required. Recently, national initiatives (Mosca *et al.*, 2015) have been undertaken in Italy to characterise local populations for resilience and for the nutritional properties of their primary production used as basis of regional food products often related to gastronomic traditions.

In the last decades, microsatellite markers have been used to perform phylogenetic analysis and studies on genetic variability in the chicken breeds (Strillacci *et al.,* 2009; Al-Qamashoui *et al*., 2014; Ceccobelli *et al.,* 2015). The availability of high-density Single Nucleotide Polymorphisms (SNP) arrays has opened the possibility to investigate the genetic structure of a population on a very large number of markers having uniform distribution on all chromosomes. Moreover, these arrays permit to identify and map copy number variants (CNV) on the genome. CNV are distributed over the whole genome in all species and are defined as large-scale genome mutations ranging from 50bp to several Mb (Mills *et al.,* 2011) compared with a reference genome (insertions, deletions and more complex changes). Involving large genomic regions, CNV may affect gene structure and determine expression and/or regulation gene changes (Redon *et al.,* 2006). Although CNV were recently mapped in several livestock species (Han *et al.,* 2014; Schiavo *et al.*, 2014; Bagnato *et al.,* 2015), their use as markers to explain intra-breeds genetic diversity has been explored only in few species (Gazave *et al.,* 2011; Xu *et al.,* 2016).

The aim of this study was to analyse the genomic and genetic variation in order to describe the existing variability among individuals of six Italian chicken breeds using both SNP and CNV as markers. We will then test the hypothesis that genetic variation exist among the six breeds considered in this study and highlighting that the new knowledge gained thanks to high throughput genotyping (SNP, CNV) strongly contribute to the characterization of genetic diversity among them.. The knowledge of the genetic structure of these breeds may be used to preserve the genetic variability and the phenotypic features peculiar of each population.

Material and methods

Sampling and genotyping

In this study, 6 Italian chicken breeds were used: Livornese (LI) from Tuscany, Milanino (MI) and Mericanel della Brianza (MB) from Lombardy, Bionda Piemontese (PI) and Bianca di Saluzzo (SA) from Piedmont, and Siciliana (SI) from Sicily (Supplementary Tables S1 and S2). All the populations are ancient Italian breeds except the composite MI. The MB is the only Italian bantam breed, with an official recognised standard.

Ninety-six blood samples (16 per breed) were randomly selected among blood bio-banks (stored in 0.5 M EDTA at −20°C) representative of flock nucleus conserved within the universities of Milano, Torino and Pisa. Genomic DNA was isolated using the NucleoSpin® Blood kit (Macherey-Nagel) according to the manufacturer’s instructions. DNA concentration was determined with the Qubit® dsDNA HS Assay kit (Life Technologies) using the proper Qubit® fluorometer; purity was assessed trough the evaluation of A260/280 and A260/230 ratios on the Infinite® 200 PRO NanoQuant spectrophotometer (Tecan) and integrity verified running samples E-Gel® 48 Agarose Gels, 1% (Invitrogen).

All DNA samples were genotyped using the Axiom® Genome-Wide Chicken Genotyping Array (Affymetrix) including 580961 SNP markers, distributed across the genome with an average spacing of 1.7 Kb (galGal4 assembly). Axiom™ Analysis Suite software (Affymetrix) was used to run raw intensity data Quality Control and

Genotyping Algorithms. Default quality control settings were applied to filter for low quality samples before running the genotyping analysis. Axiom CNV summary tool was used to generate input files for CNV prediction analysis software.

SNP analyses

SNP allele frequencies, expected (H_e) and observed (H_o) heterozygosity were computed separately for each breed using the PEAS software (Xu *et al.,* 2010). Genetic diversity within and among breeds was determined estimating the Wright’s F-statistics fixation index (F_{ST}) and inbreeding coefficient of an individual relative to a subpopulation (F_{IS}) on SVS Golden Helix software 8.3.1 (Golden Helix Inc.) (SVS).The genetic structure of Italian chicken populations was analysed using:

i) SVS: Principal Component Analysis (PCA) based on SNP allele frequencies.

ii) ADMIXTURE ver. 1.3.0 software (Alexander *et al.,* 2009): population structure analysis with a number of ancestral populations K that ranged from 2 to 8. To evaluate optimal number of ancestors, cross-validation error values (CVE) were computed for each K using a 5-fold cross-validation procedure, as reported by Nicoloso *et al.,* (2015). Each inferred chicken population structure was visualized using an R script.

iii) PEAS software: individual tree using Neighbor-Joining (NJ) algorithm. The NJ tree, constructed based on the allele sharing distance (DAs) as the genetic distance between individuals, was graphically represented using FigTree version 1.4.2 (Rambault, 2014).

CNV and CNVR Analyses

Both the Log R Ratio (LRR) and the B-Allele Frequency (BAF) values of each sample were obtained from the Axiom® CNV Summary Tool software. LRR and BAF were used in the individual-based CNV calling performed by PennCNV software (Wang *et al.*, 2007) on chromosomes 1–28, using the default parameters of the Hidden Markov Model (HMM): standard deviation of LRR <0.30, BAF drift as 0.01 and waviness factor at 0.05. The CNV regions (CNVR) were defined in each breed using the BedTools software, through merging overlapping CNV by at least 1bp, as described by Redon *et al.,* (2006).

Clustering analysis using CNVR. A clustering analysis for all samples was performed considering the identified CNVR as genetic makers (Tian *et al*., 2013). A scoring matrix of the CNVR data was built by encoding a value of “0” or “1” according to the absence or presence for each individual of any mapped CNV in the pertinent CNVR. A hierarchical agglomerative clustering was applied on the scoring matrix using the pvclust function from the pvclust R package (Suzuki and Shimodaira, 2006). Multiscale bootstrap resampling was applied to calculate the Approximately Unbiased P-value (AU) using 10000 bootstraps to assess the robustness of branches. Agglomerative method chosen was Unweighted Pair Group Method with Arithmetic mean (UPGMA).

Results and Discussion

SNP analyses

SNP analyses and the CNV detection were performed on 94 quality-filtered samples, as two samples belonging to MB and PI breeds were discarded for low raw signal intensity. SNP with Minor Allele Frequency (MAF) value ≤ 0.01, SNP with Hardy-

Weinberg equilibrium (HWE) ≤ 0.00001, SNP not on first 28 autosomal chromosomes and SNP having a call rate < 99% were excluded, reducing to 412336 SNP markers the number of loci used in the analysis. The number of polymorphic sites within breed ranged from 197099 (47.8%) to 383086 (92.8%) for SI and SA, respectively (Table 1).

For each breed, the effective number of heterozygous SNP (number of SNP in which at least one heterozygous individual was identified) represents more than 99% of polymorphic sites (Table 1). The H_o and H_e ranged from 0.210 and 0.170 (SI) to 0.345 and 0.320 (SA), whereas the F_{IS} values ranged from -0.192 (SI) to 0.094 (LI). The SI H_o and H_e values (0.210; 0.170) reflect the highest percentage of monomorphic SNP (52.2%) and the low variability within the breed. On the contrary, the SA breed has a low F_{IS} value (-0.045) and the highest H_o and H_e values confirming results previously obtained by Sartore *et al.,* (2014) using microsatellite markers.

In the LI breed, despite the high percentage of polymorphic SNP (75.9%), the H_o and H_e values are quite low (0.232 and 0.249), although the F_{IS} value (0.094) indicates a low level of inbreeding. Ceccobelli *et al.,* (2015) reported for the same breed similar F_{IS} value and higher H_o and H_e values obtained using microsatellites data. The low genetic variability measured in both LI and SI birds is suggested to be related to the small size of the population under conservation for many years, situation generally known to be associated with relevant value of inbreeding. The H_o and H_e values for the bantam breed MB (0.243 and 0.221) are lower than those obtained by Tadano *et al.,* (2008) on Japanese bantam breeds using a panel of 40 microsatellites. The F_{IS} value for MB (-0.060) is very low and quite similar to that identified in the Japanese Bantam breed Tosa-Jidori (Tadano *et al.*, 2008).

Except for LI and PI, the negative F_{IS} values detected in all other breeds reflect an excess (increasing) of heterozygosity, probably due to outbreeding (Tadano *et al.,* 2007). The heterozygous SNP were classified into three classes according to the number of individuals resulted heterozygous at the same locus: “01-05”, “06-10” and “11-16”. In fact, for MB, MI, SA and SI breeds (MB=4.7%, MI=6.6%, SA=6% and SI=7.4%) respect to LI and PI (LI=2.1%, PI=1.8%) a higher proportion of SNP were heterozygous in more than 10 samples (class “11-16”) (Figure 1). The same distribution applies for class of individuals “6-10”. On the contrary, if we consider the class of individuals “1-5” the two breeds LI and PI are those with the largest proportion of heterozygous SNP. This behaviour in SNP heterozygous loci agree well with the F_{IS} values found here.

The pairwise fixation indexes (F_{ST}) among the six Italian chicken breeds are presented in Figure 2. The F_{ST} values range from 0.082 (PI vs. SA) to 0.439 (SI vs. MB). The largest differences were between the SI breed and the other populations, with F_{ST} values ranging from 0.290 (SA) to 0.439 (MB). The F_{ST} values greater than zero can be related to the effect of genetic isolation respect to the other populations, which can lead to homozygous excess over time. As expected by their origin (i.e. same geographical region), the PI and SA breeds are closely related (F_{ST}=0.082) and their F_{ST} values against the other populations are very low. Sartore et al., (2016) considered PI as the ancestral population of the present day SA. These authors also report a similar F_{ST} value for the same breeds using a panel of 32 microsatellite markers.

The MI breed is relatively similar to PI and SA and differs from all other breeds in terms of genetic structure (Figure 2). The bantam breed MB differs from the MI and LI breeds (F_{ST}=0.356 and F_{ST}=0.324), but is relatively similar to the Piedmont PI and

SA breeds (F_{ST}=0.250 and F_{ST}=0.230). MB is very common breed in north-east of Milan and it is still not possible to determine the period in which this breed appeared. The breeds anyhow is reported to derived from dwarf rural chickens diffused in small rural farms at the beginning of last century (Ceppolina, 2015).

The overall F_{ST} value found across all breeds is 0.253, indicating that 25.3% of the genetic variation is explained by the breed differences, whereas the remaining 74.7% of the variance describes the differences among individuals. This value is higher than 0.15 considered by Frankham *et al.,* (2004) as an indicator of significant differentiation among populations. The genetic variability of local breeds here highlighted must be considered an important genetic resource as indicated by Muir *et al*., (2008). In fact, they reported in a recent analysis using SNP markers, that commercial pure line showed a substantial decrease of genetic diversity compared with non-commercial chicken populations.

The overall F_{ST} value identified here is similar to the previous reported using microsatellites markers in commercial chicken lines (Tadano *et al*., 2007), British (Wilkinson *et al.,* 2011) and Mediterranean chicken breeds (Ceccobelli *et al.,* 2015). In contrast, lower F_{ST} values were reported in Japanese, Italian and Swedish local populations (Tadano *et al.*, 2008, Zanetti *et al*., 2010; Abebe *et al.,* 2015). The higher chicken F_{ST} values, highlighted the larger genetic variability of chicken populations, respect to the one found in other livestock species. For instance, Wang *et al.,* (2015) reported a F_{ST} value of 0.149 in Chinese pig breeds and Makina *et al.,* (2014) a F_{ST} value of 0.149 in South Africa cattle breeds.

The PCA (Figure 3A) disclosed genetic differences among the six breeds and show that all individuals are well clustered by breed. The canonical variable plotted on the *y*-axis explained 1.93% of the overall SNP variance. On this axis, the LI breed is clearly separated in two different groups according to bird's feather colour (black upper group and white lower group) as well as the PI and SA breeds create two separated clusters closely related. The origin of LI breed is not so clear, probably from Central Italy, obtained from the selection of light chicken reared in Tuscany region. LI is worldwide spread with different colors of livery: black, white and brown (light and dark) (Ceppolina, 2015) and selected according to colour differences for decades.

The distinction among breeds was clearly displayed on the canonical variable plotted as *x-axis* representing 7.18% of the SNP variance. The SI breed is a distinct group, confirming results of F_{ST} values. In fact, this breed appears to derive from ancient inter-breeding of local Sicilian birds with North African sock (Ceppolina, 2015) The PCA plot shows the division of SI samples in three sub-groups. The major distance was identified between MI and SI breeds.

The results of the NJ analysis (Figure 3B), are consistent with those obtained by the PCA. The NJ dendrogram suggests the presence of three distinct clusters: cluster 1 includes the closely related PI and SA breeds (originating in Piedmont), cluster 2 includes the two varieties of LI breed and SI, and cluster 3 includes MI and MB breeds (originating in Lombardy).

An increasing number of assumed ancestors, from K=2 to 8 was used for global admixture analysis done by the ADMIXTURE software. The graphical representation of the estimated ancestor fractions in individual genomes is shown in Figure 3C. In fact, at K=2 two distinct ancestors are represented by SI and MB+MI, while LI, PI and SA genomes seem to include a major fraction of the MB+MI ancestor and a minor fraction the SI ancestor. K = 3 and 4 split MB from MI, and the above 3 composite breeds now had a major MI and minor MB and SI ancestor components. A similar albeit more complicate figure was kept by K=5. Based on agreement with the PCA and CNV analyses, the ADMIXTURE software identified K=6 as the most probable number of common ancestors of our samples. At K=6, MI, MB and SI breeds grouped again into independent ancestors, and the LI breed appears to be divided into two genetically distinguishable subgroups, confirming both PCA results and CNV cluster analysis.

Independently of the K number, individuals belonging to the PI and SA breeds seem to share the same ancestors composition, but when K increased to 7 they separated in two distinct groups, while retaining some common genetic features. At K=8 almost all breeds (except for MB) returned to show the same genetic features identified at smaller Ks. It is interesting to note that all the grouping strategies identify the MI breed as distinct from the other genetic groups: this is representative of the selection history of the breed initiated at the beginning of 20th century by crossing Valdarnese Bianca males to Horpington females (Mosca *et al.*, 2015).

CNV and CNVR analyses

In Table 2 the frequency of CNV identified, the mean and median values, as well as the CNV coverage per each breed compared to the chicken assembly autosomes are reported. In all breeds, the number of losses (state 0 and 1) is higher than the number of gains (state 3 and 4), except for the SA breed. This is indicated by the deletions/duplications ratios calculated as the total number of losses divided by number of gains: 1.56, 2.14, 1.11, 1.63, 1.12 and 0.45 for LI, MB, MI, PI, SI and SA, respectively. The majority of CNV (i.e. 91% among all breeds) identified in this study, have a length between 1 Kb and 100 Kb representing a proportion over the total number of CNV of 87.7% in the MI to 95.4% in the SI.

A total of 564 unique CNVR (344 gains, 213 losses and 7 complex) were found among all breeds. These CNVR covered a total of 9.43 Mb of sequence length corresponding to 1.03% of the chicken galGal4 assembly autosome. The total number of CNVR detected for each breed is 103 in LI, 57 in MB, 82 in MI, 174 in PI, 94 in SA and 123 in SI (Figure 4 and Supplementary Table S3). Table 3 shows the number of CNVR for each breed by chromosome. With the exception of chr21 and chr24, which contain CNVR identified only in two breeds (LI-PI and MI-PI, respectively), all other autosomes include CNVR from at least three breeds. CNVR on chromosomes 1, 2, 3, 4, 5, 8, 12, 14, 16 and 20 have been identified in all breeds. In the PI breed, the identified CNVR map on all chromosomes, with the exception of the chr26, while the CNVR identified in the LI breed are distributed on only 12 autosomes.

Among the identified CNVR, 426 (75%) were present in a single individual (singleton), 61 (10%) in two individuals, 23 (4%) in three individuals, 14 (2%) in four individuals, and 40 (7%) in more than five individuals. The high proportion of the singleton has been previously reported by Yi *et al.,* (2014) (68.8%) and by Han *et al.,* (2014) (76.5%), confirming that segregating CNV exist among individuals. The CNVR on chr16 at 215,410-330,020 bp was identified in 31 samples across all 6 chicken breeds (at least 2 samples/breed) as well as in chicken populations analysed by the latter above-cited authors.

Comparison of the CNVR in the six breeds (Figure 4) reveals that the number of CNVR shared among the breeds ranged from 15 (MI *vs* others) to 29 (PI *vs* others) whereas the number of intra-breed shared CNVR (mainly contributed by single sample variations) ranged from 41 (MB) to 145 (PI). Considering the CNVR identified by CNV common to individuals of different breeds, the most frequent combinations are: SI-PI (n=7) and SA-PI (n=6). Adding to these combinations those including other breeds, it gives a total of 11 and 10 CNVR common to SI-PI and SA-PI, respectively (Figure 4).

Despite recent studies on CNV in chicken have showed their role in metabolic pathways and their association with innate and adaptive immunity, morphological traits, developmental defects or disease susceptibility (Wang *et al.*, 2014; Yan *et al.*, 2015), the actual knowledge on CNV and their full role in the genomic expression is still limited and do not permit to understand the specific function of CNV here found.

Figure 5 shows the cluster-tree built for the six chicken breeds based on CNVR similarities. In the plot, the branch length is not directly proportional to the genetic distance estimated among samples. The Approximately Unbiased P-value (AU-P) and Bootstrap Probability value (BP-P) were shown for each node, as well as the Edge numbers. We focused on the AU-P because the BP-P is considered less accurate than AU-P and according to Suzuki and Shimodaira, (2006) the cluster (edges) with AU-P larger than 95% are the most plausible.

Edge numbers represent the order in which the clusters were built. More closely related samples have a smaller edge numbers, while higher edge numbers reflect clusters formed later in the breed evolutionary process. As shown in the plot, all samples of SI and almost all samples of MB were assigned to a single breed-cluster. The MI and LI samples are grouped in two distinct clusters each. Instead for PI and SA breeds, three and four clusters were identified respectively, two of which include samples belonging to both breeds.

Conclusion

This research represents a first approach to evaluate the genetic variability and diversity within and between six Italian chicken populations using SNP and CNV markers. The results highlight the existence of genetic variability and a low inbreeding coefficient in all Italian chicken breeds considered. Notably, the pairwise fixation indexes, the PCA and the NJ trees all show the clear separation of the SI breed from the others and in the LI, the presence of two distinct groups corresponding to the white and black varieties. In addition, PI and SA resulted closely related, highlighting the geographic common origin. The genetic variability found using SNP is comparable to the one reported by other authors in the same breeds, using microsatellite markers. In addition, the CNV markers analysis have well separated the breeds in terms of genetic identity, according to their breeding history. Some of the CNV interestingly maps in chromosomal regions where important functional genes are annotated (e.g. the MHC region on chromosome 16). A follow up analysis may further investigate functional association between CNV and genes.

Results of this study represent a basis for the Italian chicken population’s valorisation as an important reservoir of genetic diversity. In Italy, Avian Research Units within Academic infrastructures are currently involved in in situ conservation programs of Italian poultry populations. Efforts to maintain genetic variability have been implemented and the small poultry flocks available need to be continuously monitored to avoid the loss of biodiversity.

As a conclusion, this manuscript confirm the existence of genetic and genomic variability in the Italian chicken populations suitable for their maintenance and genetic improvement. To enhance this process it is advisable that other researches on a larger population sample disclose the association between SNP and CNV markers with phenotype expression of quantitative traits.

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Table 1 *SNP statistics, observed heterozygosity (*H_o*), expected heterozygosity (*H_e*) and inbreeding coefficient (*F_{IS}*) values for the six Italian chicken populations (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda Piemontese, SA=Bianca di Saluzzo and SI=Siciliana)*

Breed	Size	No. of pol. SNP*	No. het SNP**	H_o	H_e	F_{IS}
LI	16	312823	310782	0.232	0.249	0.094
MB	15	263920	262346	0.243	0.221	-0.060
MI	16	270881	270039	0.258	0.237	-0.055
PI	15	366337	364921	0.312	0.304	0.008
SA	16	383086	382286	0.345	0.320	-0.045
SI	16	197099	196845	0.210	0.170	-0.192

*= number of polymorphic SNP; **= number of heterozygote SNP

Table 2 *Descriptive statistics of copy number variant (CNV) identified for each breed (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda Piemontese, SA=Bianca di Saluzzo and SI=Siciliana)*

Breed	No. CNV (min-max)*	No. losses State 0/1	No. gains State 3/4	CNV min length (bp)	CNV max length (bp)	CNV mean length (bp)	CNV median length (bp)	Coverage (bp)	Coverage (%)
LI	159 (3-17)	97	62	160	265647	17919.37	6535	2849180	0.31
MB	110 (5-10)	75	35	462	240256	17587.3	6381	1934603	0.21
MI	131 (4-29)	69	62	381	171360	15032.57	6133	1969267	0.21
PI	211 (6-28)	131	80	52	356281	19241.97	8497	4060057	0.44
SA	131 (5-11)	41	90	258	384766	35254.32	13306	4618316	0.50
SI	261 (7-46)	143	118	213	119253	16262.30	7910	4244461	0.46
Total	*1003*	*556*	*447*	*52*	*384766*	*19617.03*	*7380*	*19675884*	*2.14*

* min-max=minimum and maximum number of CNV for individual.

Table 3 *Descriptive statistics of copy number variant region (CNVR) identified for each breed (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda Piemontese, SA=Bianca di Saluzzo and SI=Siciliana) by chromosome (CHR)*

	Breeds					
CHR	LI	MB	MI	PI	SA	SI
1	24	15	24	40	21	24
2	15	9	9	20	14	20
3	5	9	11	14	5	15
4	6	4	4	15	6	9
5	8	3	6	11	8	12
6	3	0	1	9	3	4
7	3	0	1	3	7	5
8	3	1	2	3	1	2
9	3	2	4	4	0	4
10	1	0	2	5	4	0
11	1	0	2	3	4	3
12	2	2	1	5	2	2
13	5	2	2	5	2	0
14	3	2	3	5	3	2
15	3	0	2	3	0	0
16	1	1	1	1	1	2
17	3	0	0	3	1	0
18	0	2	1	3	1	3
19	1	0	1	2	3	4
20	1	1	1	3	1	1
21	4	0	0	1	0	0
22	0	1	0	2	1	1

23	2	0	1	2	1	2
24	0	0	1	2	0	0
25	1	0	0	3	0	5
26	1	1	1	0	2	3
27	2	2	1	3	2	0
28	2	0	0	4	1	1
Total	103	57	82	174	94	124

Figure captions

Figure 1 Proportion of heterozygous SNP classified into three classes according to the number of individuals resulted heterozygous at the same locus: “01-05”, “06-10” and “11-16” (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda Piemontese, SA=Bianca di Saluzzo and SI=Siciliana).

Figure 2 Matrix of pairwise fixation index F_{ST} among the six Italian chicken breeds. (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda Piemontese, SA=Bianca di Saluzzo and SI=Siciliana).

Figure 3 Population genetic analyses of the six Italian chicken breeds (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda Piemontese, SA=Bianca di Saluzzo and SI=Siciliana): A) Scatter plot (EV=Eigenvalues of canonical variables) from a PCA analysis based on SNP frequencies. B) Neighbour-Joining (NJ) dendrogram constructed using genetic sharing distances. C) Admixture plot for all Italian chicken breeds analysed based on different number of assumed ancestors (K).

Figure 4 Intra (Unique) and inter (Shared) breed variation of CNVR in the six Italian chicken populations (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda Piemontese, SA=Bianca di Saluzzo and SI=Siciliana).

Figure 5 Dendrogram generated by clustering all individuals of the Italian chicken breeds (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda

Piemontese, SA=Bianca di Saluzzo and SI=Siciliana) based on their CNV similarities. i) Approximate Unbiased (AU) p-value in dark grey colour, ii) Bootstrap Probability (BP) value in grey colour, iii) edge in light gray colour.

Figure_1

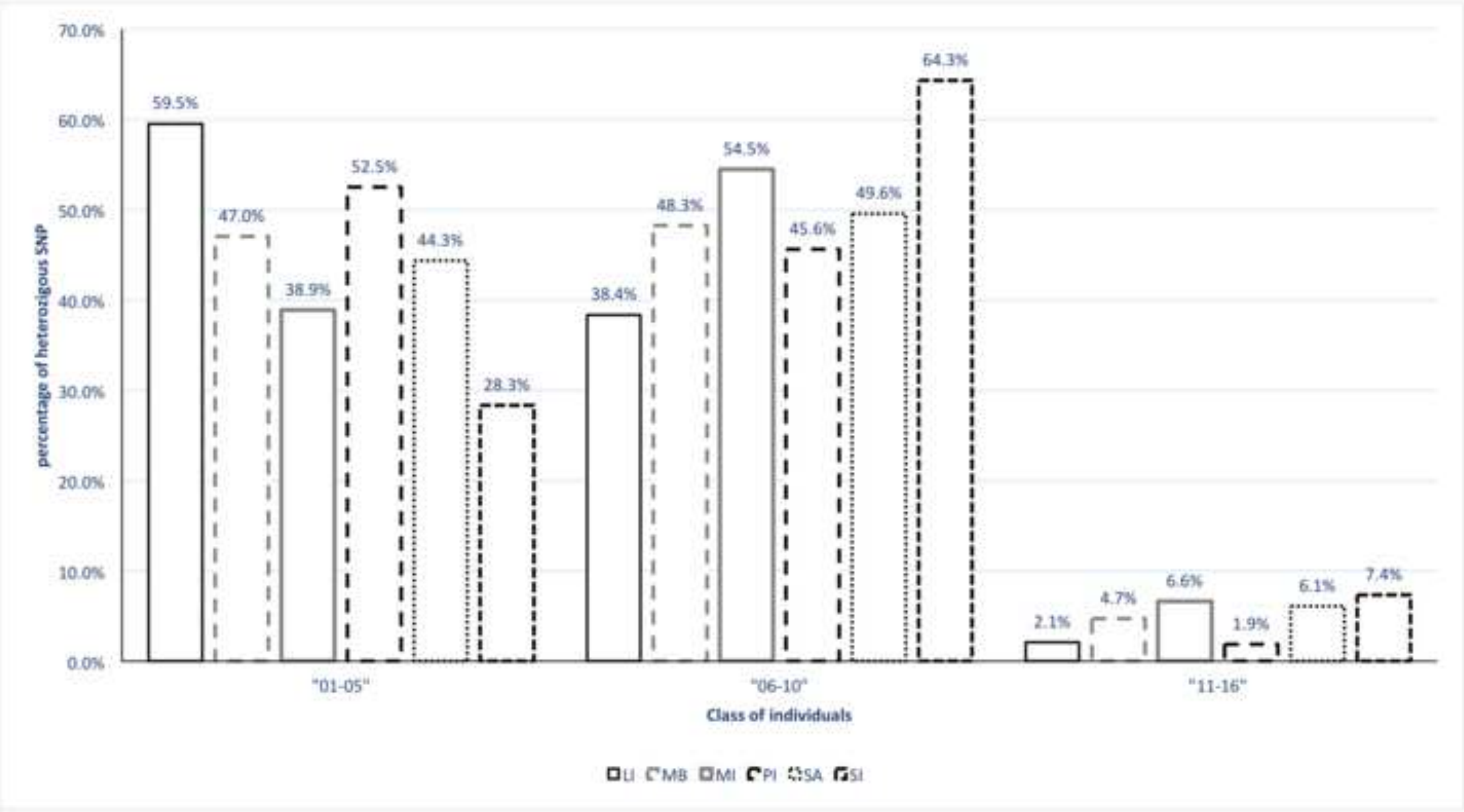
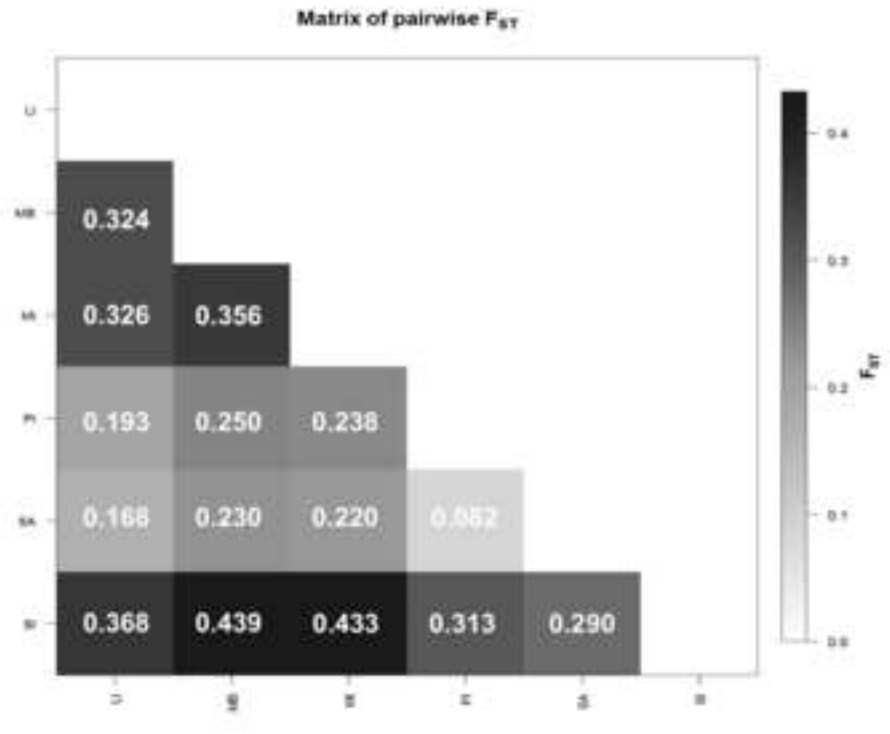
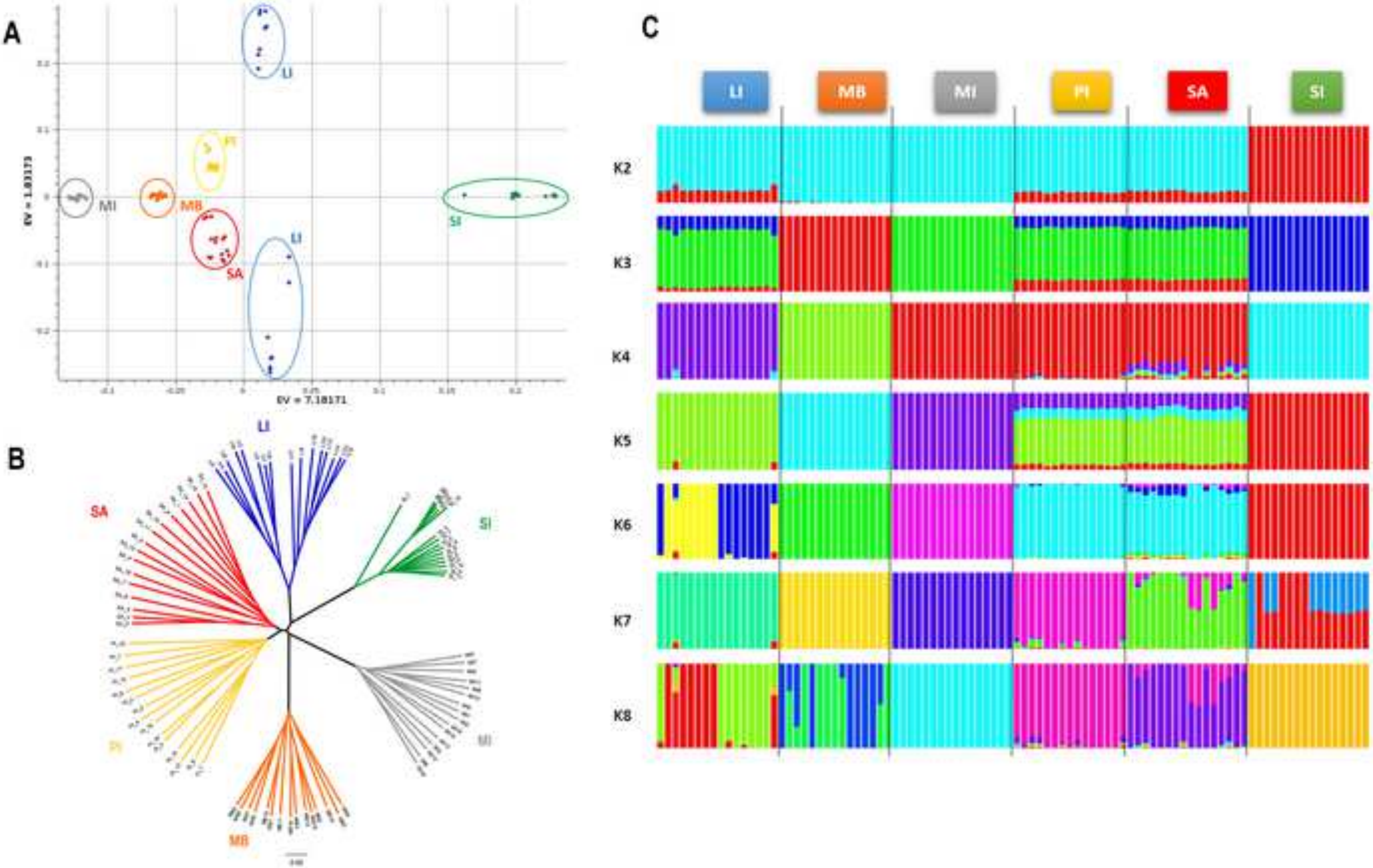
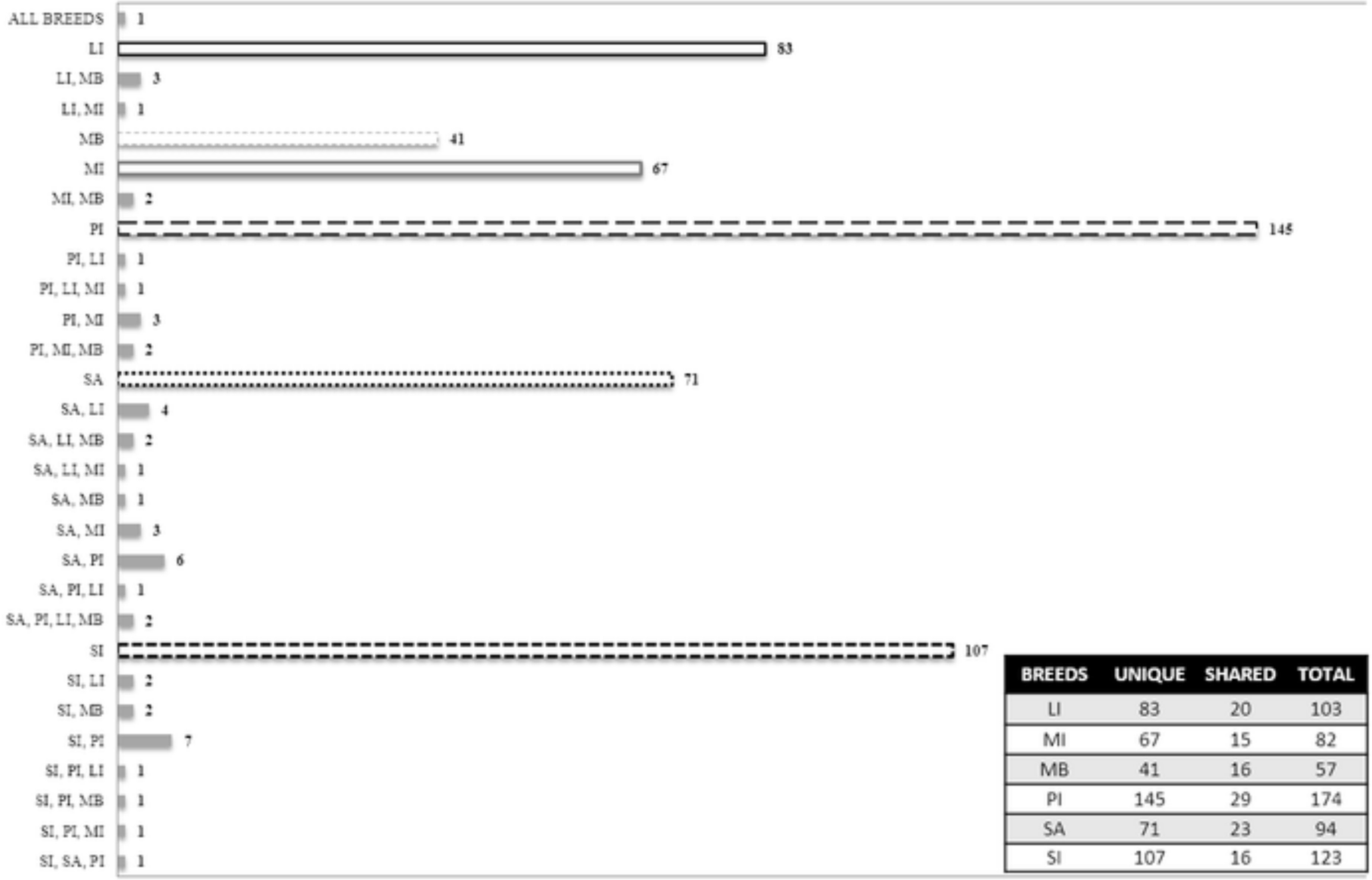

percentage of heterozigous SNP
70.0%
60.0%
50.0%
40.0%
30.0%
20.0%
10.0%
0.0%
59.5%
47.0%
38.9%
52.5%
44.3%
28.3%
38.4%
48.3%
54.5%
45.6%
49.6%
64.3%
2.1%
4.7%
6.6%
1.9%
6.1%
7.4%
"01-05"
"06-10"
"11-16"
Class of individuals
LI
MB
MI
PI
SA
SI

Figure 2

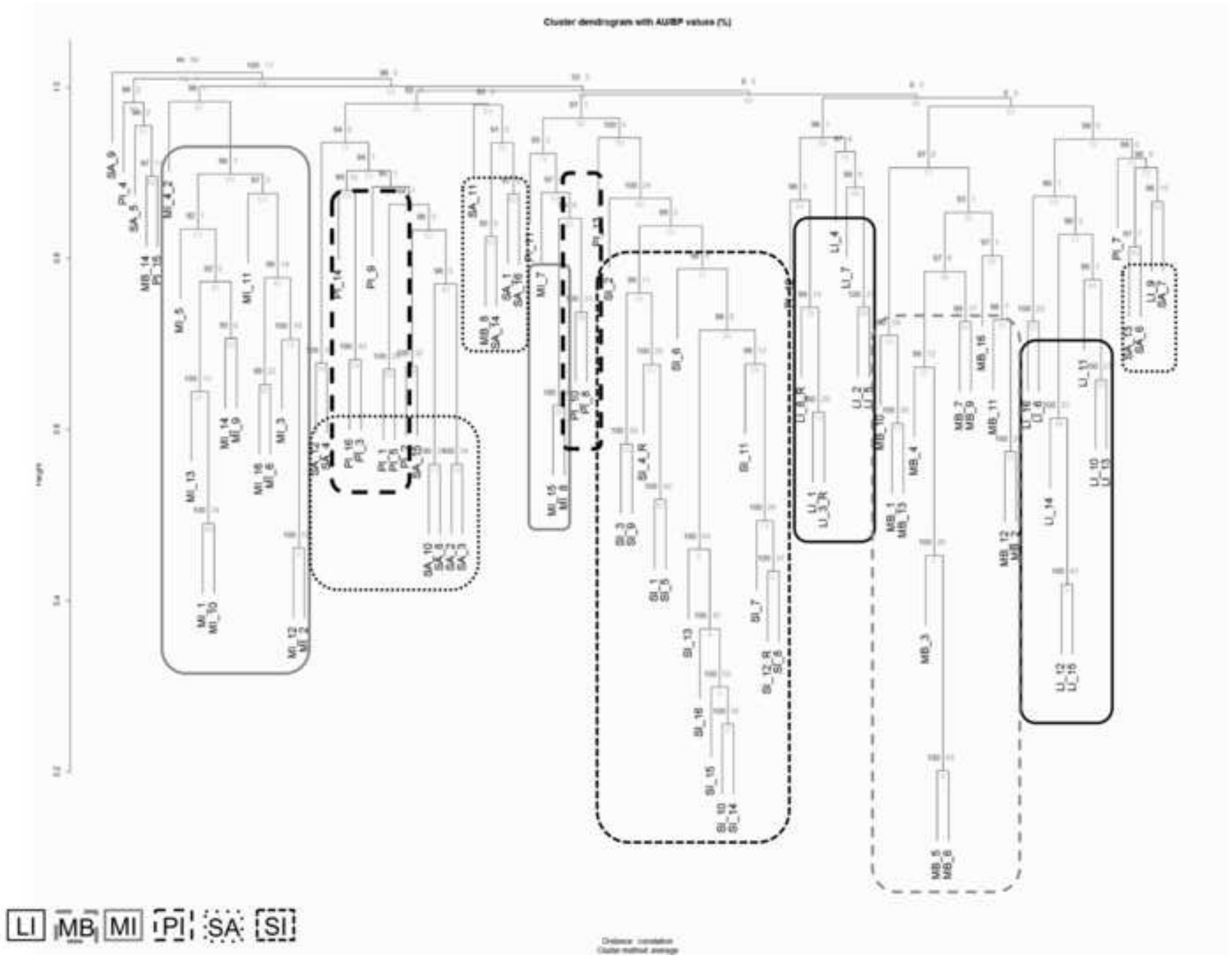

Matrix of pairwise F_{ST}
LI
MB
MI
PI
SA
SI
0.324
0.326
0.356
0.193
0.250
0.238
0.168
0.230
0.220
0.062
0.368
0.439
0.433
0.313
0.290
0.4
0.3
0.2
0.1
0.0
F_{ST}


A
B
C
LI
PI
MI
MB
SA
SI
EV = 1.83173
EV = 7.18171
LI
MB
MI
PI
SA
SI
K2
K3
K4
K5
K6
K7
K8



BREEDS	UNIQUE	SHARED	TOTAL
LI	83	20	103
MI	67	15	82
MB	41	16	57
PI	145	29	174
SA	71	23	94
SI	107	16	123

Figure_5


Cluster dendrogram with AU/BP values (%)
Height
Distance: correlation
Cluster method: average
LI
MB
MI
PI
SA
SI

Genomic and genetic variability of six chicken populations using single nucleotide polymorphism and copy number variants as markers

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Supplementary Table S1 *Origin and phenotypic characteristics in the six Italian chicken breeds*

Breed*	Origin	Region of origin	Use	Colour				Comb type	Bird size
				Plumage	Beak	Shank	Ear-lobe		
LI-B	Ancient	Tuscany	eggs	white	yellow	yellow	white	simple	full
LI-N	Ancient	Tuscany	eggs	black	black	yellow	white	simple	full
MB	Last century	Lombardy	broodiness		yellow	yellow	red&white	simple	bantam
MI	Composite reconstituted	Lombardy	meat	white	yellow	white	white	simple	full
PI	Ancient	Piedmont	eggs/meat	golden/red	yellow	pink/yellow	white	simple	full
SA	Ancient	Piedmont	eggs/meat	white	yellow	pink/yellow	white	simple	full
SI	Ancient	Sicily	eggs	Golden	black	green	red&white	crown	full

*LI-B= Livornese, white variety; LI-N=Livornese, black variety; MB=Mericanel della Brianza; MI=Milanino; PI=Bionda Piemontese; SA=Bianca di Saluzzo; SI=Siciliana

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Supplementary Table S2 *Means of phenotipic quantitative traits recorded in females and males chickens of the Italian breeds: body weight (BW), body length (BL), shank length (SL), chest circumference (CC) and wing span (WS)*

Breed*	Females					Males				
	BW (g)	BL (cm)	SL (cm)	CC (cm)	WS (cm)	BW (g)	BL (cm)	SL (cm)	CC (cm)	WS (cm)
LI-B	1995	36.8	8.5	35.4	37.6	2524	42.3	11.0	40.8	45.0
LI-N	1877	37.3	8.7	34.3	38.2	2209	40.0	10.0	37.0	44.7
MB	761	28.1	5.5	24.9	27.4	1056	30.8	6.0	26.0	31.8
MI	2705	41.5	8.2	36.7	37.3	3199	47.8	9.0	37.0	43.7
PI	2140	40.6	7.9	32.6	36.2	2534	46.0	9.0	34.3	41.6
SA	2035	36.8	7.5	31.0	35.7	2747	45.0	8.9	37.0	44.4
SI	1672	37.5	8.5	32.1	37.2	1909	40.0	10.0	35.5	40.0

*LI-B= Livornese, variety white; LI-N=Livornese, variety black; MB=Mericanel della Brianza; MI=Milanino; PI=Bionda Piemontese; SA=Bianca di Saluzzo; SI=Siciliana

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Supplementary Table S3 *Copy Number Variant Regions (CNVR) mapped (chromosome (Chr) region start, end and length and CNVR state) for each of the six Italian chicken breed (LI=Livornese, MB=Mericanel della Brianza, MI=Milanino, PI=Bionda Piemontese, SA=Bianca di Saluzzo and SI=Siciliana). Individuals are reported in Breed_SampleID column.*

Chr	CNVR Start (bp)	CNVR End (bp)	CNVR Length (bp)	Breed_SampleID	CNVR state
1	9126940	9143601	16661	LI_7	GAIN
1	23812864	23817003	4139	LI_10	LOSS
1	25835971	25839970	3999	LI_6	LOSS
1	28444831	28448719	3888	LI_13	LOSS
1	28460553	28469033	8480	LI_13	LOSS
1	29649120	29656052	6932	LI_16	LOSS
1	32261418	32264785	3367	LI_4	GAIN
1	43445087	43450974	5887	LI_10	LOSS
1	73474060	73480039	5979	LI_9	GAIN
1	77610657	77617319	6662	LI_4	GAIN
1	88082730	88092227	9497	LI_4	GAIN
1	102931495	102966351	34856	LI_9	GAIN
1	105454801	105459538	4737	LI_7	LOSS
1	124424407	124425025	618	LI_16	GAIN
1	137262485	137266365	3880	LI_2	GAIN
1	139290303	139417687	127384	LI_7	GAIN
1	141493034	141501579	8545	LI_9	GAIN
1	151077811	151103187	25376	LI_11,LI_5	GAIN
1	151120104	151126098	5994	LI_14,LI_15	LOSS
1	160947041	160965926	18885	LI_2	LOSS
1	161230334	161231780	1446	LI_7	LOSS
1	165174530	165183916	9386	LI_3_R	GAIN
1	184911407	184915744	4337	LI_11,LI_13	LOSS
1	188915548	188920734	5186	LI_11	LOSS
2	2584064	2595497	11433	LI_4	GAIN
2	22957325	22958518	1193	LI_7	GAIN
2	25859462	25952423	92961	LI_3_R,LI_8_R,LI_2,LI_1	LOSS
2	57663103	57669441	6338	LI_15	GAIN
2	60096217	60109363	13146	LI_4	GAIN
2	61558638	61574562	15924	LI_9	GAIN
2	61831467	61848698	17231	LI_9	GAIN
2	63628602	63630632	2030	LI_10	LOSS
2	75782432	75789532	7100	LI_9	GAIN
2	77522436	77527196	4760	LI_12,LI_13	LOSS
2	87680240	87694251	14011	LI_10,LI_12,LI_13,LI_15,LI_16	LOSS
2	93132478	93157538	25060	LI_10,LI_12,LI_15	LOSS
2	93671386	93699066	27680	LI_3_R	GAIN
2	121130610	121142245	11635	LI_3_R	GAIN
2	141127635	141134337	6702	LI_16,LI_6,LI_7	LOSS

3	11654769	11661304	6535	LI_14,LI_5	GAIN
3	37240711	37258225	17514	LI_5	GAIN
3	58097854	58102975	5121	LI_9	LOSS
3	75203576	75206622	3046	LI_11	LOSS
3	97775486	97778052	2566	LI_2,LI_4,LI_5,LI_6,LI_7	LOSS
4	5111388	5114996	3608	LI_7	LOSS
4	13316392	13319428	3036	LI_15	GAIN
4	42076031	42080664	4633	LI_10,LI_13	LOSS
4	47202618	47205335	2717	LI_1,LI_16,LI_9	LOSS
4	63010731	63024133	13402	LI_4	GAIN
4	76259738	76278990	19252	LI_11	GAIN
5	18270982	18273279	2297	LI_5	GAIN
5	19630190	19707342	77152	LI_11,LI_16,LI_10,LI_13	LOSS
5	21668927	21676808	7881	LI_3_R,LI_7	LOSS
5	22407746	22410680	2934	LI_2	LOSS
5	29070715	29073718	3003	LI_9	GAIN
5	40024565	40039414	14849	LI_9	GAIN
5	48851655	48858827	7172	LI_11	LOSS
5	49067692	49072565	4873	LI_2,LI_7	LOSS
6	7298525	7303004	4479	LI_12,LI_13,LI_14,LI_15, LI_3_R	LOSS
6	12304431	12307723	3292	LI_9	GAIN
6	34190817	34195158	4341	LI_13	LOSS
7	8119314	8120216	902	LI_10,LI_13	LOSS
7	29697191	29699187	1996	LI_7	LOSS
7	31266237	31278978	12741	LI_2,LI_3_R,LI_5	LOSS
8	157378	202883	45505	LI_10,LI_11,LI_12,LI_4,LI_8_R	LOSS
8	15437515	15466850	29335	LI_7	GAIN
8	16570088	16580656	10568	LI_9	GAIN
9	5602283	5604846	2563	LI_9	GAIN
9	9156044	9160537	4493	LI_10,LI_15	LOSS
9	21327153	21327678	525	LI_8_R	LOSS
10	2053074	2056292	3218	LI_4	GAIN
11	13391667	13412283	20616	LI_12,LI_14,LI_15	LOSS
12	1407088	1672735	265647	LI_4	GAIN
12	2158256	2158611	355	LI_9	GAIN
13	1136171	1143178	7007	LI_16	LOSS
13	3647002	3647419	417	LI_4	LOSS
13	5377387	5381011	3624	LI_11	GAIN
13	7021881	7025909	4028	LI_4	LOSS
13	7202531	7206406	3875	LI_10,LI_13	LOSS
14	777214	798132	20918	LI_10	GAIN
14	8270701	8288943	18242	LI_5	GAIN
14	14169357	14183169	13812	LI_9	GAIN
15	5531575	5541371	9796	LI_13	GAIN
15	8554518	8555460	942	LI_10,LI_11	LOSS
15	10788530	10811194	22664	LI_4	GAIN
16	219031	306007	86976	LI_9,LI_3_R,LI_14,LI_6,LI_11, LI_13,LI_15,LI_16	GAIN
17	1863910	1864637	727	LI_1,LI_3_R	LOSS
17	1973371	1975564	2193	LI_6	LOSS
17	10386087	10407714	21627	LI_7	GAIN
19	9967973	9973808	5835	LI_16	GAIN
20	1946946	1950706	3760	LI_12,LI_15	LOSS
21	474196	477570	3374	LI_14	GAIN

21	2752552	2757496	4944	LI_7	GAIN
21	3518438	3522020	3582	LI_10	LOSS
21	3659215	3661071	1856	LI_15	LOSS
23	1667735	1683487	15752	LI_6	GAIN
23	3178691	3189074	10383	LI_7	GAIN
25	1367825	1368519	694	LI_7	GAIN
26	628372	635367	6995	LI_9	GAIN
27	544009	730872	186863	LI_5	GAIN
27	3136238	3136779	541	LI_14,LI_16	LOSS
28	1514399	1517816	3417	LI_7	GAIN
28	3060312	3062116	1804	LI_10,LI_12,LI_15	LOSS
1	9259807	9263237	3430	MB_14	GAIN
1	34232188	34242449	10261	MB_11,MB_13,MB_14,MB_8	LOSS
1	38278789	38289573	10784	MB_14	LOSS
1	42816757	42823179	6422	MB_9	LOSS
1	54569850	54571368	1518	MB_10	LOSS
1	77596019	77619334	23315	MB_13	GAIN
1	78851811	78931472	79661	MB_16	GAIN
1	78999223	79091079	91856	MB_16	GAIN
1	137179950	137183097	3147	MB_10	GAIN
1	139290303	139376850	86547	MB_1,MB_10	GAIN
1	177529984	177537827	7843	MB_13	LOSS
1	179187409	179193848	6439	MB_14	LOSS
1	180949032	180957360	8328	MB_13	LOSS
1	181735053	181742346	7293	MB_13	GAIN
1	194701436	194715342	13906	MB_1	LOSS
2	7704086	7705442	1356	MB_11,MB_4	LOSS
2	11622978	11624639	1661	MB_14,MB_16,MB_2	LOSS
2	63303760	63326303	22543	MB_1	GAIN
2	71929233	71953128	23895	MB_7	GAIN
2	72023967	72033615	9648	MB_12	GAIN
2	86814986	86821326	6340	MB_11	LOSS
2	117216281	117223458	7177	MB_16	GAIN
2	129267623	129269192	1569	MB_8	LOSS
2	139983056	139987280	4224	MB_11	LOSS
3	5336469	5365139	28670	MB_16,MB_6,MB_11,MB_3, MB_2	GAIN
3	34095935	34101091	5156	MB_3,MB_4,MB_5,MB_6, MB_7, MB_8,MB_9	LOSS
3	36114091	36119498	5407	MB_3,MB_4,MB_5,MB_9	LOSS
3	36491792	36499933	8141	MB_8	LOSS
3	36593046	36597882	4836	MB_5,MB_6	LOSS
3	36639251	36645136	5885	MB_5,MB_6	LOSS
3	40256767	40259368	2601	MB_14	LOSS
3	62353496	62354043	547	MB_6	LOSS
3	107454355	107455846	1491	MB_11	LOSS
4	51219942	51231546	11604	MB_2	GAIN
4	56026210	56043376	17166	MB_11,MB_9	GAIN
4	61027539	61080022	52483	MB_12,MB_9,MB_7	GAIN
4	73092714	73104673	11959	MB_14	GAIN
5	1954724	1961355	6631	MB_9	LOSS
5	11921964	11925669	3705	MB_4	GAIN
5	23910847	23918123	7276	MB_11,MB_12,MB_2,MB_3, MB_5,MB_6,MB_1	LOSS

8	157378	202883	45505	MB_11,MB_13,MB_3,MB_4, MB_14	LOSS
9	8133230	8138012	4782	MB_1,MB_2,MB_3,MB_5, MB_13, MB_16,MB_6	LOSS
9	21327153	21328896	1743	MB_9	LOSS
12	10922550	10923116	566	MB_7	GAIN
12	14395236	14395698	462	MB_14,MB_9	LOSS
13	4310530	4312716	2186	MB_1,MB_10,MB_13	LOSS
13	7202082	7203839	1757	MB_13,MB_1,MB_10,MB_7	LOSS
14	8271822	8276372	4550	MB_11,MB_8	GAIN
14	15124958	15132783	7825	MB_2	GAIN
16	245855	330020	84165	MB_12,MB_2,MB_11	GAIN
18	693906	695111	1205	MB_11	LOSS
18	699667	706189	6522	MB_11	LOSS
20	6408986	6410289	1303	MB_10	LOSS
22	3527384	3534897	7513	MB_12,MB_16,MB_3	LOSS
26	1823618	1829006	5388	MB_9	LOSS
27	463174	483459	20285	MB_14	GAIN
27	533750	774006	240256	MB_12,MB_2	GAIN
1	1317654	1324805	7151	MI_10	LOSS
1	3224832	3246090	21258	MI_15,MI_7,MI_8,MI_8	LOSS
1	4013951	4044109	30158	MI_13,MI_11,MI_1	GAIN
1	11634775	11639454	4679	MI_2	LOSS
1	20473440	20474632	1192	MI_9	GAIN
1	23812864	23817003	4139	MI_1,MI_10,MI_13,MI_14, MI_6, MI_9	LOSS
1	32372412	32376897	4485	MI_12,MI_2	LOSS
1	32463008	32546752	83744	MI_4_2	GAIN
1	43001844	43066722	64878	MI_5,MI_6,MI_7,MI_14,MI_9	GAIN
1	55013914	55020128	6214	MI_2	GAIN
1	62279659	62285139	5480	MI_14	LOSS
1	72345535	72351290	5755	MI_5	GAIN
1	75709646	75719210	9564	MI_15	GAIN
1	79024615	79030474	5859	MI_4_2	GAIN
1	94564270	94566171	1901	MI_4_2	GAIN
1	95963827	95965305	1478	MI_3	LOSS
1	109100362	109108759	8397	MI_15	LOSS
1	134253652	134257407	3755	MI_4_2	GAIN
1	146282800	146286641	3841	MI_14	GAIN
1	147717421	147773690	56269	MI_4_2	GAIN
1	149877721	149880263	2542	MI_7	LOSS
1	150669350	150840710	171360	MI_4_2	GAIN
1	160947041	161061487	114446	MI_7	LOSS
1	165869717	165871075	1358	MI_3	GAIN
2	2680117	2681152	1035	MI_6	GAIN
2	11209864	11275503	65639	MI_4_2	GAIN
2	28849148	28857742	8594	MI_15,MI_16	GAIN
2	61903581	61906355	2774	MI_4_2,MI_5	LOSS
2	79145360	79168177	22817	MI_14	GAIN
2	79680088	79703232	23144	MI_4_2	GAIN
2	131836214	131845973	9759	MI_10	GAIN
2	132550262	132555172	4910	MI_4_2,MI_12,MI_2,MI_5	LOSS
2	143156984	143202642	45658	MI_4_2	GAIN

3	6261877	6263795	1918	MI_10,MI_15	LOSS
3	22817122	22824497	7375	MI_11	GAIN
3	30251485	30260622	9137	MI_4_2	GAIN
3	34352233	34363532	11299	MI_4_2	GAIN
3	36801293	36822942	21649	MI_4_2	GAIN
3	56773444	56810263	36819	MI_4_2	GAIN
3	59406354	59411276	4922	MI_4_2	GAIN
3	64211482	64214927	3445	MI_4_2	GAIN
3	83221366	83265188	43822	MI_4_2	GAIN
3	102224929	102246295	21366	MI_5,MI_6,MI_9	LOSS
3	104628962	104649974	21012	MI_1	GAIN
4	10452517	10476672	24155	MI_4_2	GAIN
4	18972227	18975272	3045	MI_2	LOSS
4	39852446	39853779	1333	MI_7	GAIN
4	82566185	82575845	9660	MI_4_2	GAIN
5	6980375	6982401	2026	MI_4_2	GAIN
5	9160978	9164588	3610	MI_4_2	LOSS
5	30836594	30904211	67617	MI_4_2	GAIN
5	34488308	34617188	128880	MI_4_2	GAIN
5	42318001	42353346	35345	MI_4_2	GAIN
5	55701139	55705607	4468	MI_7	GAIN
6	1361	42885	41524	MI_4_2,MI_1	GAIN
7	35030551	35036989	6438	MI_8	LOSS
8	16510295	16517358	7063	MI_7	GAIN
8	26060670	26063448	2778	MI_13	GAIN
9	753605	763648	10043	MI_2	GAIN
9	1891690	1895268	3578	MI_11	GAIN
9	6114018	6121595	7577	MI_16,MI_6	LOSS
9	8133230	8138012	4782	MI_12	LOSS
10	9223993	9242432	18439	MI_4_2	GAIN
10	17944255	17945851	1596	MI_7	LOSS
11	4962435	4965379	2944	MI_14	LOSS
11	13051825	13066669	14844	MI_12,MI_16,MI_2,MI_3,MI_6	LOSS
12	2081163	2090078	8915	MI_12,MI_15,MI_8	GAIN
13	1126898	1143178	16280	MI_10,MI_5	GAIN
13	4312184	4312716	532	MI_1,MI_10,MI_14,MI_5	LOSS
14	1514372	1515896	1524	MI_5	LOSS
14	8195675	8198667	2992	MI_2	LOSS
14	15145902	15148673	2771	MI_14	GAIN
15	4390371	4398451	8080	MI_4_2	LOSS
15	8711904	8720968	9064	MI_10,MI_13	GAIN
16	251597	298221	46624	MI_8,MI_9	GAIN
18	888250	888631	381	MI_1,MI_10,MI_11,MI_12, MI_14,MI_2,MI_3,MI_6	LOSS
19	1999676	2004067	4391	MI_10,MI_4_2,MI_6,MI_7	LOSS
20	8646822	8655741	8919	MI_11,MI_10,MI_3	LOSS
23	1592366	1593082	716	MI_11	GAIN
24	3438826	3440881	2055	MI_3	LOSS
26	4891341	4898142	6801	MI_16	LOSS
27	467647	472507	4860	MI_6	LOSS
1	3218895	3246090	27195	PI_10,PI_11,PI_5,PI_8, PI_9,PI_5	LOSS
1	11441978	11447557	5579	PI_12	LOSS
1	11724225	11728415	4190	PI_14	LOSS
1	13205120	13206656	1536	PI_14	LOSS

1	15896361	15926518	30157	PI_9	GAIN
1	18180690	18183579	2889	PI_13	GAIN
1	20037753	20042920	5167	PI_14	LOSS
1	23247889	23252232	4343	PI_11	GAIN
1	32503622	32511387	7765	PI_14	GAIN
1	36840282	36889950	49668	PI_4	GAIN
1	39726847	39729063	2216	PI_13	LOSS
1	40234626	40249288	14662	PI_11	GAIN
1	41682591	41698727	16136	PI_4	GAIN
1	57964239	57980899	16660	PI_15	GAIN
1	64361722	64367347	5625	PI_9	GAIN
1	64876486	64884121	7635	PI_4	LOSS
1	68551124	68552422	1298	PI_13	LOSS
1	71486860	71522594	35734	PI_15	GAIN
1	75516142	75520450	4308	PI_14	GAIN
1	76098060	76113281	15221	PI_4	LOSS
1	77596019	77617319	21300	PI_3	GAIN
1	82035758	82049063	13305	PI_11	GAIN
1	90915258	90925139	9881	PI_2	LOSS
1	93609076	93618668	9592	PI_8	GAIN
1	94209271	94214144	4873	PI_14	LOSS
1	101250973	101269092	18119	PI_4	GAIN
1	130884485	130908797	24312	PI_4	GAIN
1	139290303	139417687	127384	PI_1,PI_10	GAIN
1	139935853	139939921	4068	PI_2	LOSS
1	153533922	153537110	3188	PI_13	LOSS
1	154369977	154380331	10354	PI_11	GAIN
1	155623091	155629031	5940	PI_14	GAIN
1	163164808	163521089	356281	PI_16	LOSS
1	178962865	178964753	1888	PI_1,PI_13	LOSS
1	181720987	181754708	33721	PI_14,PI_3	GAIN
1	181864092	181870278	6186	PI_14	GAIN
1	184907096	184914376	7280	PI_10	LOSS
1	187648204	187657075	8871	PI_9	LOSS
1	189464714	189465405	691	PI_12	GAIN
1	194347634	194467211	119577	PI_16,PI_3	GAIN
2	15674407	15680332	5925	PI_10	LOSS
2	20679230	20682669	3439	PI_13	LOSS
2	25868266	25876407	8141	PI_12	GAIN
2	29089897	29096904	7007	PI_1	LOSS
2	46854428	46861745	7317	PI_7	LOSS
2	47107428	47113922	6494	PI_11	GAIN
2	54188096	54216108	28012	PI_14	GAIN
2	57565703	57570258	4555	PI_13	GAIN
2	61223815	61234190	10375	PI_13	GAIN
2	63596395	63606171	9776	PI_16,PI_3	LOSS
2	70070949	70080701	9752	PI_12	GAIN
2	108035821	108043134	7313	PI_11	LOSS
2	108420471	108433824	13353	PI_4	GAIN
2	116777703	116781914	4211	PI_3	LOSS
2	118393587	118442146	48559	PI_4	GAIN
2	122097779	122104510	6731	PI_4	LOSS
2	122333875	122338519	4644	PI_11	GAIN
2	129104580	129169307	64727	PI_2,PI_3	GAIN
2	138451360	138463990	12630	PI_11	GAIN

2	143235268	143238971	3703	PI_7	GAIN
3	4643013	4662485	19472	PI_13	GAIN
3	19091474	19120795	29321	PI_16	GAIN
3	34694086	34695062	976	PI_9	LOSS
3	35493517	35501706	8189	PI_10	GAIN
3	36667591	36668879	1288	PI_10	GAIN
3	38249302	38253923	4621	PI_7	GAIN
3	46991968	46995596	3628	PI_1	GAIN
3	54087820	54093710	5890	PI_4	GAIN
3	60637923	60641744	3821	PI_16	GAIN
3	61112549	61114138	1589	PI_16	GAIN
3	61688227	61704997	16770	PI_15	GAIN
3	66272850	66279076	6226	PI_15	LOSS
3	98290080	98291559	1479	PI_10	GAIN
3	106305069	106306023	954	PI_16	GAIN
4	8607594	8629823	22229	PI_11	GAIN
4	30843777	30888475	44698	PI_11	GAIN
4	32038130	32045970	7840	PI_1	LOSS
4	37876329	37886316	9987	PI_13	GAIN
4	60584245	60604379	20134	PI_11	GAIN
4	61823268	61834915	11647	PI_3,PI_4,PI_1,PI_14, PI_16, PI_5	LOSS
4	65420193	65438719	18526	PI_10	LOSS
4	66546653	66561413	14760	PI_16	GAIN
4	69780004	69783759	3755	PI_14	GAIN
4	77000679	77001964	1285	PI_9	GAIN
4	77667355	77681253	13898	PI_9	GAIN
4	79270341	79281903	11562	PI_8	GAIN
4	81409594	81425814	16220	PI_8	GAIN
4	85254962	85395894	140932	PI_13,PI_5,PI_1	GAIN
4	86222920	86258708	35788	PI_4	GAIN
5	240948	310523	69575	PI_4	GAIN
5	5183559	5190380	6821	PI_3	GAIN
5	11815839	11822303	6464	PI_1	LOSS
5	19718770	19721817	3047	PI_4	GAIN
5	20474613	20478312	3699	PI_10	GAIN
5	36018763	36030838	12075	PI_1	GAIN
5	48921509	48936803	15294	PI_10,PI_13,PI_8	LOSS
5	49072565	49084257	11692	PI_5,PI_9	LOSS
5	55934915	55988044	53129	PI_4	GAIN
5	56614989	56766967	151978	PI_4,PI_15,PI_12	GAIN
5	58823251	58823303	52	PI_7	GAIN
6	1715628	1722140	6512	PI_13	GAIN
6	3089155	3102715	13560	PI_1	GAIN
6	3245000	3248341	3341	PI_2	LOSS
6	4588275	4608888	20613	PI_12	LOSS
6	15410672	15418016	7344	PI_12	LOSS
6	16571491	16578846	7355	PI_12	LOSS
6	17141315	17144773	3458	PI_9	LOSS
6	25614355	25623899	9544	PI_16	GAIN
6	27241761	27244509	2748	PI_9	LOSS
7	23994881	23998014	3133	PI_1	LOSS
7	24023402	24035680	12278	PI_11	GAIN
7	34266357	34285445	19088	PI_13	GAIN
8	4472273	4479576	7303	PI_14	GAIN

8	8734809	8910898	176089	PI_9	LOSS
8	11941233	11955539	14306	PI_4	GAIN
9	8133230	8138012	4782	PI_11,PI_2	LOSS
9	17383018	17384534	1516	PI_13,PI_4	COMPLEX
9	19132182	19134581	2399	PI_4	GAIN
9	22082371	22084901	2530	PI_16	GAIN
10	589629	608449	18820	PI_9	GAIN
10	8345612	8354399	8787	PI_7	GAIN
10	16460511	16464740	4229	PI_3	LOSS
10	18235411	18238416	3005	PI_8	LOSS
10	19862709	19866067	3358	PI_15	GAIN
11	5478524	5481519	2995	PI_1	GAIN
11	9223312	9232374	9062	PI_16	GAIN
11	18005900	18013411	7511	PI_4	GAIN
12	1122474	1223234	100760	PI_16,PI_3,PI_1,PI_14,PI_5,PI_9,PI_2,PI_12	GAIN
12	4214092	4215737	1645	PI_16,PI_3	LOSS
12	4360784	4371186	10402	PI_9	LOSS
12	5138363	5152260	13897	PI_12	GAIN
12	15102030	15106137	4107	PI_5	GAIN
13	1120443	1128603	8160	PI_13	GAIN
13	7306384	7307816	1432	PI_14	LOSS
13	8564174	8569610	5436	PI_13	GAIN
13	9282142	9282445	303	PI_1	GAIN
13	9497347	9497640	293	PI_15	LOSS
14	6295255	6300266	5011	PI_14	GAIN
14	6455192	6463527	8335	PI_4	GAIN
14	7308112	7312732	4620	PI_4	GAIN
14	8195675	8198667	2992	PI_3	LOSS
14	9755259	9759074	3815	PI_4	LOSS
15	910061	913281	3220	PI_11	GAIN
15	2354124	2362299	8175	PI_12	GAIN
15	2804884	2809037	4153	PI_4	GAIN
16	215410	315114	99704	PI_13,PI_7,PI_5,PI_1	COMPLEX
17	4509860	4512922	3062	PI_14	GAIN
17	5611710	5614167	2457	PI_13	GAIN
17	8822007	8826934	4927	PI_3	LOSS
18	2560868	2564292	3424	PI_11	GAIN
18	3518846	3519650	804	PI_7	GAIN
18	5960569	5963938	3369	PI_4	GAIN
19	2482316	2489696	7380	PI_9	GAIN
19	8440799	8450059	9260	PI_14	GAIN
20	7179441	7211282	31841	PI_4	GAIN
20	7452654	7454307	1653	PI_14	GAIN
20	8648685	8655741	7056	PI_9	LOSS
21	2854020	2854666	646	PI_1	GAIN
22	3898	9154	5256	PI_1	LOSS
22	486545	489696	3151	PI_7	LOSS
23	3537025	3552310	15285	PI_4	GAIN
23	5256953	5257940	987	PI_4	GAIN
24	446179	459648	13469	PI_11	GAIN
24	5460205	5476146	15941	PI_12	GAIN
25	75666	76674	1008	PI_13	GAIN

25	800162	801901	1739	PI_14	LOSS
25	988739	989777	1038	PI_9	LOSS
27	467647	472507	4860	PI_15	LOSS
27	912632	913923	1291	PI_13	LOSS
27	1926235	1929503	3268	PI_16	GAIN
28	425658	428045	2387	PI_16,PI_7	LOSS
28	1184191	1196218	12027	PI_16	GAIN
28	2618390	2618786	396	PI_15	LOSS
28	3100523	3108956	8433	PI_13	GAIN
1	712002	738339	26337	SA_2,SA_3,SA_5	GAIN
1	3927484	3931561	4077	SA_9	GAIN
1	4006738	4044109	37371	SA_16,SA_3,SA_13	GAIN
1	35792995	35796117	3122	SA_11	LOSS
1	42054978	42064075	9097	SA_9	GAIN
1	44015316	44024068	8752	SA_11	LOSS
1	77596019	77621395	25376	SA_13	GAIN
1	86103785	86111630	7845	SA_1	GAIN
1	88087545	88092227	4682	SA_4	GAIN
1	91897368	91909088	11720	SA_3	LOSS
1	98042613	98046884	4271	SA_4	GAIN
1	98616097	98621166	5069	SA_9	LOSS
1	133550038	133566314	16276	SA_9	GAIN
1	135000914	135002598	1684	SA_5	LOSS
1	139290303	139384505	94202	SA_13,SA_2,SA_3,SA_6	GAIN
1	160947041	161110014	162973	SA_16	GAIN
1	161374003	161376417	2414	SA_5	GAIN
1	163136323	163521089	384766	SA_1,SA_14,SA_10,SA_16	LOSS
1	175263746	175284778	21032	SA_2	GAIN
1	184911407	184915122	3715	SA_12,SA_4	LOSS
1	188586941	188592484	5543	SA_12	LOSS
2	27799754	27801335	1581	SA_14	LOSS
2	54737872	54749833	11961	SA_7,SA_12,SA_4	LOSS
2	61903581	61906355	2774	SA_8	LOSS
2	71929233	71954714	25481	SA_10	GAIN
2	73831264	73847626	16362	SA_10,SA_6	GAIN
2	76880818	76893143	12325	SA_6	GAIN
2	78844264	78854658	10394	SA_10	LOSS
2	82274348	82283458	9110	SA_4	GAIN
2	82326757	82331888	5131	SA_5	GAIN
2	97088838	97099282	10444	SA_7	GAIN
2	102386064	102396900	10836	SA_11	GAIN
2	113623482	113643659	20177	SA_5	GAIN
2	122101503	122103098	1595	SA_6	LOSS
2	129085437	129172949	87512	SA_1,SA_14,SA_4,SA_13	GAIN
3	4305248	4321508	16260	SA_9	GAIN
3	78020691	78026532	5841	SA_16	LOSS
3	78513742	78517962	4220	SA_11	GAIN
3	89541217	89547453	6236	SA_7	GAIN
3	95499094	95517006	17912	SA_11	GAIN
4	6459515	6464278	4763	SA_14	GAIN
4	24898015	24916382	18367	SA_15	GAIN
4	30102774	30163522	60748	SA_14,SA_9	LOSS
4	33502139	33509354	7215	SA_16	GAIN
4	59237251	59238335	1084	SA_15	LOSS
4	81412294	81431796	19502	SA_7	GAIN

5	6791975	6807441	15466	SA_9	GAIN
5	14852222	14865343	13121	SA_11	LOSS
5	19630190	19707342	77152	SA_8,SA_10,SA_12	COMPLEX
5	29069437	29080034	10597	SA_7	GAIN
5	41923441	42121531	198090	SA_14,SA_2,SA_8, SA_10, SA_11	GAIN
5	43482910	43497003	14093	SA_8	GAIN
5	45230370	45232473	2103	SA_11	LOSS
5	56625359	56766967	141608	SA_4	GAIN
6	10654524	10680799	26275	SA_6	GAIN
6	10704786	10731614	26828	SA_5	GAIN
6	12476598	12534084	57486	SA_16	LOSS
7	2638088	2651394	13306	SA_6	LOSS
7	8164811	8176551	11740	SA_12	LOSS
7	17668992	17678986	9994	SA_9	GAIN
7	18624373	18631083	6710	SA_11	LOSS
7	20060536	20064374	3838	SA_10	LOSS
7	23432696	23438586	5890	SA_7	LOSS
7	34736224	34747585	11361	SA_13	GAIN
8	25577287	25582258	4971	SA_16	LOSS
10	7162636	7165946	3310	SA_1	GAIN
10	15613783	15644242	30459	SA_12	LOSS
10	16768901	16771909	3008	SA_6	GAIN
10	18103633	18106871	3238	SA_3	LOSS
11	9226448	9235695	9247	SA_16	GAIN
11	12666424	12670482	4058	SA_16	LOSS
11	13050902	13072246	21344	SA_3	LOSS
11	17771921	17780560	8639	SA_7	GAIN
12	1122474	1223234	100760	SA_2,SA_3,SA_12,SA_15, SA_8,SA_10	GAIN
12	8979651	9006779	27128	SA_7,SA_6	GAIN
13	14577833	14581514	3681	SA_13	LOSS
13	16995864	16998627	2763	SA_9	GAIN
14	777214	798132	20918	SA_12,SA_4	GAIN
14	1661995	1664367	2372	SA_3	LOSS
14	8271822	8287465	15643	SA_14	GAIN
16	216711	330020	113309	SA_6,SA_13,SA_10,SA_7	GAIN
17	1891058	1898872	7814	SA_13	LOSS
18	10228101	10229839	1738	SA_1	LOSS
19	2484083	2493424	9341	SA_3,SA_15	GAIN
19	3414324	3415644	1320	SA_7	GAIN
19	6175580	6198717	23137	SA_9,SA_12	GAIN
20	9380018	9382079	2061	SA_4	GAIN
22	3824813	3825071	258	SA_15	GAIN
23	19365	41759	22394	SA_5	GAIN
26	3549636	3555452	5816	SA_9	GAIN
26	4939026	4945581	6555	SA_13	GAIN
27	655362	730872	75510	SA_4,SA_6	GAIN
27	2763442	2767149	3707	SA_9	GAIN
28	900944	916233	15289	SA_6	GAIN
1	3220733	3251128	30395	SI_10,SI_13,SI_14,SI_15, SI_14	LOSS
1	17974844	17983725	8881	SI_6	GAIN
1	21065469	21099951	34482	SI_2	GAIN

1	28379369	28382637	3268	SI_1	LOSS
1	30456065	30464380	8315	SI_11	GAIN
1	32824810	32826891	2081	SI_13	GAIN
1	36236367	36252075	15708	SI_2	GAIN
1	43337259	43343246	5987	SI_1	LOSS
1	46954348	46977988	23640	SI_2	GAIN
1	71481449	71522594	41145	SI_9,SI_1	GAIN
1	90706897	90710453	3556	SI_2,SI_6	LOSS
1	93479822	93516055	36233	SI_2,SI_13	GAIN
1	93775729	93803110	27381	SI_2	GAIN
1	99088395	99094316	5921	SI_11	GAIN
1	105233539	105238985	5446	SI_5	GAIN
1	112231619	112236512	4893	SI_15	GAIN
1	113433478	113477304	43826	SI_1,SI_3,SI_5,SI_9	GAIN
1	113527039	113659727	132688	SI_3,SI_5,SI_9,SI_1	GAIN
1	114412511	114435091	22580	SI_2	GAIN
1	116545042	116592957	47915	SI_2	GAIN
1	133202223	133207506	5283	SI_2	GAIN
1	148324090	148328215	4125	SI_3	LOSS
1	158145819	158158411	12592	SI_11	GAIN
1	181731621	181752254	20633	SI_10,SI_6,SI_12_R,SI_13,SI_14,SI_15,SI_7,SI_8	GAIN
2	928384	942023	13639	SI_13,SI_14	LOSS
2	25859462	25952423	92961	SI_1,SI_11,SI_12_R,SI_5,SI_8,SI_9,SI_2	LOSS
2	27335793	27360988	25195	SI_1,SI_14,SI_5	GAIN
2	27471883	27478356	6473	SI_12_R,SI_9	GAIN
2	28266857	28321381	54524	SI_2,SI_13	GAIN
2	32190085	32255964	65879	SI_2	GAIN
2	51709054	51749553	40499	SI_2	GAIN
2	55063690	55095520	31830	SI_9	GAIN
2	66013851	66065937	52086	SI_2	GAIN
2	81952642	81962048	9406	SI_1,SI_4_R,SI_5	LOSS
2	83674703	83695919	21216	SI_2,SI_10	GAIN
2	85115029	85119510	4481	SI_11	GAIN
2	86849998	86856411	6413	SI_1,SI_5	GAIN
2	90305163	90314923	9760	SI_16,SI_5	GAIN
2	94041568	94135075	93507	SI_2	GAIN
2	99727585	99734823	7238	SI_13	GAIN
2	122417706	122433674	15968	SI_15,SI_16	LOSS
2	125351754	125354703	2949	SI_11	GAIN
2	129477998	129478452	454	SI_13,SI_15,SI_16	LOSS
2	138731319	138759199	27880	SI_6	GAIN
3	2437089	2441423	4334	SI_2	GAIN
3	9015436	9021140	5704	SI_1	GAIN
3	11556397	11570561	14164	SI_13	GAIN
3	19456437	19474613	18176	SI_2	GAIN
3	25213329	25249979	36650	SI_11	GAIN
3	38030440	38042818	12378	SI_11	GAIN
3	39426685	39431324	4639	SI_1	GAIN
3	40446754	40471392	24638	SI_11,SI_2,SI_12_R,SI_13,SI_6,SI_7	LOSS
3	63568510	63579364	10854	SI_13,SI_10,SI_14,SI_15,SI_16	LOSS

3	66522713	66529906	7193	SI_6	GAIN
3	73712844	73719632	6788	SI_11	LOSS
3	79572513	79583555	11042	SI_10,SI_11,SI_13, SI_14,SI_15, SI_16,SI_2,SI_3,SI_4_R, SI_5,SI_7,SI_8,SI_9	LOSS
3	92316897	92317634	737	SI_1	LOSS
3	103364349	103366668	2319	SI_11,SI_12_R,SI_16, SI_7,SI_9	LOSS
3	104851927	104866866	14939	SI_11	GAIN
4	1169607	1216538	46931	SI_11	GAIN
4	9088302	9096261	7959	SI_4_R	LOSS
4	39804638	39808060	3422	SI_11	GAIN
4	39975564	39992341	16777	SI_2	GAIN
4	48309951	48341896	31945	SI_2,SI_13	GAIN
4	58771139	58787472	16333	SI_11,SI_2	GAIN
4	63435279	63442498	7219	SI_14,SI_16	GAIN
4	69976217	69979968	3751	SI_3	GAIN
4	85226873	85292322	65449	SI_6	GAIN
5	3091754	3109737	17983	SI_1	LOSS
5	23910847	23918123	7276	SI_13	LOSS
5	29592886	29594502	1616	SI_7	LOSS
5	40021762	40029943	8181	SI_11	GAIN
5	45658625	45665805	7180	SI_11,SI_1,SI_13,SI_2, SI_3,SI_8	LOSS
5	47420680	47433235	12555	SI_2	GAIN
5	48921509	48936803	15294	SI_1,SI_10,SI_11,SI_12_R, SI_13, SI_3,SI_6,SI_7,SI_8,SI_9	LOSS
5	50151867	50159015	7148	SI_1	GAIN
5	54073626	54075872	2246	SI_16	GAIN
5	54998413	55022936	24523	SI_2	GAIN
5	58790805	58795489	4684	SI_15,SI_10,SI_12_R,SI_13, SI_14,SI_16,SI_2,SI_6, SI_7,SI_8,SI_9	LOSS
5	59253017	59256313	3296	SI_10,SI_13,SI_15,SI_16, SI_2,SI_6,SI_8,SI_12_R, SI_14,SI_7,SI_9,SI_9	LOSS
6	949346	963033	13687	SI_10,SI_13,SI_14,SI_15,SI_16	LOSS
6	3447947	3486942	38995	SI_13	GAIN
6	4395786	4399822	4036	SI_15	LOSS
6	17141315	17144773	3458	SI_10,SI_13,SI_14,SI_15, SI_16,SI_4_R,SI_7	LOSS
7	3990089	3996538	6449	SI_6	GAIN
7	5661560	5726624	65064	SI_5	GAIN
7	24558429	24562846	4417	SI_2	GAIN
7	27756301	27769436	13135	SI_12_R	GAIN
7	34262022	34285445	23423	SI_2	GAIN
8	3426059	3430208	4149	SI_6	LOSS
8	8449020	8489515	40495	SI_2	GAIN
9	3429115	3463492	34377	SI_8	GAIN
9	3694268	3704433	10165	SI_6	GAIN
9	14851112	14866888	15776	SI_11	GAIN
9	17380217	17384534	4317	SI_10,SI_15,SI_13,	LOSS

				SI_14,SI_16	
11	1565290	1569986	4696	SI_11	GAIN
11	6355091	6362007	6916	SI_11,SI_13	LOSS
11	17216551	17230323	13772	SI_13	GAIN
12	1181561	1212051	30490	SI_1	GAIN
12	2422961	2425174	2213	SI_2	GAIN
14	8418295	8422378	4083	SI_11	GAIN
14	14406520	14419809	13289	SI_2	GAIN
16	60835	101809	40974	SI_2	GAIN
16	233569	330020	96451	SI_11,SI_2,SI_10,SI_12_R, SI_13, SI_14,SI_15,SI_16,SI_5,SI_8	COMPLEX
18	537037	557577	20540	SI_11,SI_2	GAIN
18	699667	706189	6522	SI_11,SI_2	LOSS
18	735315	742868	7553	SI_11,SI_2	LOSS
19	3679079	3686041	6962	SI_2	GAIN
19	5028683	5033359	4676	SI_4_R	GAIN
19	7457093	7474834	17741	SI_2	GAIN
19	8159262	8165868	6606	SI_1	GAIN
20	8693844	8696998	3154	SI_11	GAIN
22	3757673	3764333	6660	SI_1,SI_11,SI_12_R,SI_15, SI_4_R,SI_5,SI_8	LOSS
23	2947239	2954827	7588	SI_2	GAIN
23	3710926	3726715	15789	SI_4_R,SI_5,SI_7,SI_1	GAIN
25	319961	329270	9309	SI_2	GAIN
25	743116	744762	1646	SI_13	LOSS
25	1365792	1369882	4090	SI_2	GAIN
25	1471515	1471746	231	SI_3	LOSS
26	2115014	2120628	5614	SI_8	GAIN
26	2285168	2289168	4000	SI_2	GAIN
26	4585159	4610871	25712	SI_2	GAIN
28	1113623	1194121	80498	SI_2	GAIN