UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO DI RICERCA
Scienze e Biotecnologie
dei Sistemi Agrari e Forestali
e delle Produzioni Alimentari

Scienze e Tecnologie Zootecniche
Ciclo XXVI

Statistical Tools for Genome-Wide Studies

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Anno accademico 2012- 2013

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General Introduction

Selection in livestock is a technique that has been known for millenniums. In fact, Virgil, in the 3th book of the “Georgica” (36-29 B.C.), wrote about the procedures adopted in bovine selection in his era. Since then, the aim of animal selection has not changed substantially and is generally aimed to obtain animals with high resistance to diseases and high productive performance, both for milk yielded and meat produced. Many years later, Darwin (1869) proposed the use of selection in animal breeding and stated that “The key is man’s power of accumulative selection: nature gives successive variations; man adds them up in certain directions useful to him”.

In any selection procedure, animals have to be evaluated objectively. Therefore, after the traits of interest are individuated, they are studied by using numerical parameters. The first statistical evaluation of the genetic merit of a dairy sire was developed by Lush in 1931. In his work, Lush asserted that the evaluation of an animal was more accurate using a progeny test than a rating based on the pedigree. By using a path coefficient and assuming that genetic and environmental components of variance were known, Lush gave a formula for assessing the genetic merit of dairy sires for factors affecting milk production, using the correlation between the average record of the daughters and the genotype of the sire (Lush 1931).

Some years later, Hazel (1943) defined a selection index for measuring the net merit of individuals. To evaluate this index, multiple traits instead of a single trait were taken into account. Using traits of economic importance, an aggregate genotype value for each animal was obtained as a sum of its genotypes weighted by the relative economic value of that trait. Using this aggregate genotype, the selection index was obtained by maximizing the correlation between the aggregate genotype and the index itself, but to get a reliable index a well-estimated phenotype (measured on the animal itself and on its relatives) and a genetic variance-covariance matrix were used.

The introduction of the selection index was an important milestone in genetic selection because it was the first statistical method used to evaluate the genetic merit of an individual through its phenotype and the phenotypes of its relatives.

Pedigree and phenotype to compute EBV

The estimation of the breeding value (EBV) of animals involved in selection programs is the most important tool to obtain a high genetic improvement in livestock species.

The estimation of breeding value, evaluated by using both pedigree and phenotype recorded on the animals under study, depends on the knowledge of the relationships between the involved individuals. As a consequence, the estimation of the proportion of the phenotypic variance explained to the genotype is obtained by using the relationship matrix. The combination of pedigree and phenotype information with the estimated heritability allows to evaluate the breeding values of the animals. However, due to the enormous dimension of the relationship matrix, a huge amount of computer resources and long computational time are needed (Calus, 2009).

Henderson (1975) proposed a new computational method, named best linear unbiased prediction (BLUP), which is able to improve the accuracy of prediction of breeding values by using all relationships among animals. For many years, this technique has been largely applied and has led to positive results in genetic evaluation programs. However, to get a considerable genetic gain, lots of years are required, especially for traits that can be measured only in one sex (e.g. milk traits), after death (e.g. meat quality) or late in life (e.g. longevity) (Goddard and Hayes, 2009). Another negative aspect of the BLUP approach is that it contributes to an increase in the degree of inbreeding among animals, because it favors the close relatives. Finally, BLUP makes the assumption of the infinitesimal model (Fisher, 1918), where an infinite number of genes with very small effect contribute to the trait (Calus, 2009). This seems a practical but biologically unrealistic assumption because it is known that most of the infinitesimal model assumptions are not verified. Indeed, the number of loci is finite or, after repeated selection, the assumption of normality may not be reasonable (Fairfull et al. 2011)

EBV and quantitative trait loci

BLUP and similar statistical procedures, which belong to the so called “quantitative genetics” area, do not use any genetic information directly. The introduction of new molecular techniques able to map the DNA and produce a sparse map of genetic markers has given new momentum to genetic improvement. Fernando and Grossman (1989) applied the BLUP technique to a mixed linear model that also incorporated a marker factor containing information on the linked quantitative trait loci (QTL). Lande and Thompson (1990) showed how molecular genetics could integrate the traditional methods of genetic selection based on phenotypes and pedigree. These methods, where molecular genetics information is integrated in the selection procedures, are known as marker-assisted selection (MAS). This approach was able to increase the genetic gain by 9-38% (Meuwissen and Goddard 1995).

With this new approach a more realistic model, alternative to the infinitesimal model, was proposed. In this model, known as the finite locus model, most of phenotype expression is explained by a small number of loci with a large effect, i.e. the QTL, whereas the remaining part of phenotypic variance is explained by a great number of loci with an infinitesimal effect.

The initial expectations of a wide use of QTLs in MAS were not completely satisfied because of the presence of some undesirable aspects. Early marker maps were very sparse and, therefore, the QTL mapping was extremely difficult. Associations between chromosome regions and QTLs were studied by using the linkage analysis, which usually locates QTLs at intervals greater than 20 cM. In this scenario, the identification of underlying mutations and the use of marker information in MAS is very difficult (Goddard and Hayes 2009). Nevertheless, some important QTL regions that control milk production were detected in cattle populations (Georges et al. 1995; Weller et al. 1990). However, their use in animal breeding programs is not easy, because these models tend to overestimate the QTL effects (Beavis effect) (Xu, 2003b). Moreover, the estimated QTL effects should be validated in an independent population before this information could be used in genetic selection programs. More recent developments in QTL mapping methods have given more precise maps by using the linkage disequilibrium (LD) between markers and QTLs (Aulchenko et al. 2007). The advantage of using the LD for QTL mapping purposes is that the LD quickly decreases as the distance between markers and QTL increases. Consequently, a QTL can be located into a narrower region (Goddard and Hayes 2009). Recently, the availability of high density SNP platforms at reasonably low costs allows to map more and smaller QTLs. Nevertheless, the estimation of QTLs with small effects on the trait under study is difficult and decreases the precision with which the effects of total QTLs are estimated (Calus 2009). Another critical aspect of MAS is that, generally, few markers associated with a QTL are validated in an independent sample population. Using these validated markers, the ability to estimate the breeding value is limited because they explain only a small proportion of the genetic variance. This effect is also confirmed in complex traits studied in humans where only a proportion of the estimated trait hereditability, usually less than half, is explained by QTLs (Stranger et al. 2011).

Genomic Selection

Both accuracy and efficiency of breeding value estimation procedures increased by using the method of Meuwissen et al. (2001), who applied a multiple QTL approach known as genomic selection (GS). This method skipped the QTL-mapping step and estimated the effects of a high number of markers across the genome simultaneously. One of the main difference between the first type of MAS (QTL-MAS) and GS is that QTL-MAS uses the information of a few known QTLs in LD with some markers, whereas GS uses a huge number of markers available in a high density SNP platform. In this approach, all SNPs are considered in LD with a QTL and effects of known and unknown QTLs are accounted for. Furthermore, being all effects simultaneously estimated, the total genetic variance is not, on average, overestimated (Calus 2009; Goddard and Hayes 2009).

Genomic selection conceptually proceeds in two steps:

- Estimation of the effects of each marker in a reference population where genotypes and a reliable EBV are known;
- Prediction of the genomic estimated breeding values (GEBV) for animals not present in the reference population, such as young selection candidates, with known genotypes but without performance records.

In the second step, GEBVs of animals with genotype data but not phenotypes are estimated by summing the effect of each marker across the whole genome:

$$GEBV = X\hat{g}$$

where X is a design matrix allocating animals to genotypes, and $\hat{g}$ is the vector of marker effects.

There are, however, two main critical issues in the estimation of marker effects. The first is that the number of marker effects that have to be estimated is greater than the number of animals with known genotype and phenotype. The second regards the assumption related to the prior distribution of the variance of SNP effects. Some of the models proposed to solve these problems are the SNP-BLUP (Meuwissen et al. 2001; Moser et al. 2010), the GBLUP (Hayes et al. 2009, Van Raden et al. 2009) and the Bayesian approach termed as Bayes-alphabet (Meuwissen et al. 2001; Xu 2003a). Each model makes different assumptions about the prior distribution of marker effects.

SNP-BLUP (RR-BLUP)

The SNP-BLUP (RR-BLUP) model assumes that each of *m* SNP has a very small effect on the genetic variance of the trait. If n is the number of animals with known genotype and reliable EBV and m is the number of markers, the model is:

$$y = 1_n \mu + Xg + e$$

where y is the reliable EBV, 1_n is a vector of 1s, μ is the overall mean, X is a design matrix, allocating records to genotypes for markers (n rows and m columns), g is a vector of random effect of markers, and *e* is a vector of residuals that are assumed to be normally distributed with $e \sim N\left(0, I\sigma_e^2\right)$. In this model marker effects are assumed to be normally distributed with $g \sim N\left(0, I\sigma_g^2\right)$, where σ_g^2 is the variance of the marker effects. The solution of the previous model is given by:

$$\begin{bmatrix} \hat{\mu} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} 1_n' 1_n & 1_n' X \\ X' 1_n & X'X + I\lambda \end{bmatrix}^{-1} \begin{bmatrix} 1_n' y \\ X'y \end{bmatrix}$$

where $\lambda = \dfrac{\sigma_e^2}{\sigma_g^2}$ and I is the identical matrix. σ_g^2 is unknown but can be calculated from the total genetic additive variance σ_a^2, estimated, for instance, by REML (Gilmour et al. 2009). Therefore, assuming that all markers contribute equally to the total amount of the explained variance, the genetic variance can be estimated as $\sigma_g^2 = \dfrac{\sigma_a^2}{m}$. This assumption, however, seems unrealistic (Meuwissen et al. 2001). A more accurate estimation of σ_g^2 can be done by taking into account the differences in marker allele frequencies as follows: $\sigma_g^2 = \dfrac{\sigma_a^2}{2\sum_{j=1}^{m} p_j \left(1 - p_j\right)}$ where p_j is the allele frequency of marker *j*.

G-BLUP

An alternative and equivalent method to the SNP-BLUP, to estimate GEBV using marker information, is the G-BLUP, which uses a genomic relationship matrix G instead of the pedigree derived relationship matrix (Van Raden 2008, Hayes et al. 2009). Moreover, in the

G-BLUP, the genetic variance explained by each marker is not constant and changes according to marker allele frequencies. The G-BLUP model is:

$$y = 1_n \mu + Zg + e$$

where y is the reliable EBV, 1_n is a vector of 1s, μ is the overall mean, Z is a design matrix allocating records to breeding values, g is the vector of SNP effects, and *e* is a vector of random residuals, which are assumed to be normally distributed with $e \sim N\left(0, I\sigma_e^2\right)$. Let $g = Wu$ where u_i is the a vector of breeding values and $Var(g) = WW'\sigma_u^2$ where σ_u^2 is the variance breeding values. W is a design matrix allocating records to genotypes with $w_{i,j} = x_{i,j} - 2p_j$, where $x_{i,j}$ is the genotype *j*th SNP of the *i*th animal and p_j is the allele frequency of *j*th markers. If WW' is scaled, the genomic relationship matrix G is defined as $G = \frac{nWW'}{\sum_{i=1}^{n} w_{i,i}}$ and $Var(g) = G\sigma_g^2$. Using this model, the breeding value for both phenotype and non-phenotype individuals can be evaluated by the equations as follows:

$$\begin{bmatrix} \hat{\mu} \\ \hat{g} \end{bmatrix} = \begin{bmatrix} 1'_n 1_n & 1'_n Z \\ Z'1_n & Z'Z + G^{-1}\frac{\sigma_e^2}{\sigma_g^2} \end{bmatrix}^{-1} \begin{bmatrix} 1'_n y \\ Z'y \end{bmatrix}$$

This method is very attractive for populations without good pedigree records because the genomic relationship matrix will capture this information among the genotyped individuals. The accuracy of the estimation of GEBV in single breed populations of G-BLUP agrees reasonably well with the accuracy achieved with other methods such as BayesA. When the animals in the reference and validation sets are in a multi-breed population, the accuracy of G-BLUP is lower than that of BayesA (Hayes et al. 2009).

BAYESIAN METHODS

Both G-BLUP and SNP-BLUP approaches assume that all SNP effects are non-zero, small and normally distributed. Moreover, the two methods evaluate the genetic variance σ_g^2 from the additive variance σ_a^2. Under these assumptions, the vector of marker effects $\hat{g}$ can be easily estimated and consequently the GEBV of animals can be calculated. With different and more realistic assumptions about the variance explained by each locus or about the prior distribution of marker effects, the GEBV prediction could be more accurate. However, the evaluation of the genetic effects $\hat{g}$ is more complicated and requires complex statistical tools.

BayesA

The BayesA is an alternative method to BLUP to estimate the EBV. In this method data are modeled at two levels. The first model is developed at the level of the SNP and is similar to the SNP-BLUP model. The second model is developed at the level of variance across the SNPs.

The first model is:

$$y = 1_n \mu + Xg + e$$

where μ and g are calculated from the posterior distribution of mean and SNPs effects, given the data y. From the Bayes theorem

$$P(\mu, g \mid y) \propto P(y \mid \mu, g) P(\mu, g)$$

the posterior distribution of mean μ and effects g given the data y, $P(\mu, g \mid y)$ is proportional to the likelihood of the data given the parameters μ and g, $P(y \mid \mu, g)$, multiplied by the prior distributions of μ and g $P(\mu, g)$. In this method, as proposed by

Meuwissen et al. (2001), the prior distribution of μ is uniform, whereas the prior distribution of i^th SNP effect is $g_i \sim N\left(0,\sigma^2_{g_i}\right)$. The latter distribution highlights that the variance of each effect is not constant as in SNP-BLUP. This assumption seems to be more realistic. Indeed, if the variability of the variance that affects the effect $\hat{g}_i$, $\sigma^2_{g_i}$, is large then $\hat{g}_i$ can be large, whereas if $\sigma^2_{g_i}$ is small, then the effect $\hat{g}_i$ decreases towards zero. This model, termed as BayesA, can be solved as:

$$\begin{bmatrix} \hat{\mu} \\ \hat{g}_1 \\ . \\ \hat{g}_m \end{bmatrix} = \begin{bmatrix} 1'_n 1_n & 1'_n X_1 & . & 1'_n X_m \\ X'_1 1_n & X'X + I\dfrac{\sigma^2_e}{\sigma^2_{g_1}} & . & X'X \\ . & . & . & . \\ X'_m 1_n & X'X & . & X'X + I\dfrac{\sigma^2_e}{\sigma^2_{g_m}} \end{bmatrix}^{-1} \begin{bmatrix} 1'_n y \\ X'_1 y \\ . \\ X'_m y \end{bmatrix}.$$

The second model, considered at the level of variances of SNP effects, allows to evaluate the $\sigma^2_{g_i}$ for each SNP. The variance of effects is evaluated recursively. In the first step the prior distribution of σ^2_e and the prior distribution of $\sigma^2_{g_i}$ are fixed. After, the posterior distribution of effects across all the genome and the posterior distribution of the overall mean are evaluated. The prior distribution of error variance σ^2_e is chosen as $\chi^{-2}\left(2,0\right)$ because it gives an uninformative and uniform prior distribution. With these assumptions, the conditional posterior distribution of error variance is:

$$\Pr ior\left(\sigma^2_e\right) = \chi^{-2}\left(2,0\right) \qquad \rightarrow \qquad Post\left(\sigma^2_e \mid e_i\right) = \chi^{-2}\left(n-2, e'_i e_i\right)$$

where n is the number of markers. Finally, the prior distribution of $\sigma^2_{g_i}$ is obtained by using an inverted chi-squared distribution: $\Pr ior\left(\sigma^2_{g_i}\right) = \chi^{-2}\left(v,S\right)$ where v is the number of degrees of freedom (d.f.) and S is a scaled parameter. The chi-squared prior distribution is useful because, by combining it with the normal distribution of data, the posterior distribution of $\sigma^2_{g_i}$ also becomes a scaled inverted chi-squared:

$$\Pr ior\left(\sigma^2_{g_i}\right) = \chi^{-2}\left(v, S\right) \qquad \rightarrow \qquad Post\left(\sigma^2_{g_i} \mid g_i\right) = \chi^{-2}\left(v + n_i, S + g_i' g_i\right)$$

where n_i is either the number of haplotype effects at segment *i* or 1 when a single effect is estimated for each SNP. Meuwissen et al. (2001) fixed v and S as $v = 4.012$ and $S = 0.002$ to get a distribution similar to that of QTL effects obtained by Hayes and Goddard (2001) and to obtain the expected heterozygosity of QTL when the neutral model is considered (Hayes and Daetwyler 2013). Xu (2003a) proposed χ_1^{-2} (with 1 d.f.), whereas Ter Braak et al. (2005) proposed $\chi^{-2}_{0.998}$ (with $1-2\delta$ d.f.). As shown above, the posterior distribution of variance effects depends on the knowledge of the effect g_i and, therefore, $\sigma^2_{g_i}$ cannot be directly estimated. Likewise, g_i depends on $\sigma^2_{g_i}$. This problem can be solved using the Gibbs sampling to estimate effects and variances. The Gibbs sampler runs many times (more than 10,000 cycles) for each SNP and, once the first hundreds of evaluations of g_i are discarded, the final effect of the ith SNP, $\hat{g}_i$, is obtained as the average of the remaining evaluations of g_i. The combination of the assumptions of normality distribution of marker effects and inverted chi-squared distribution of variance effects results in a *t-distribution* of the posterior conditional distribution of marker effects, where the probability of getting SNPs with moderate or large effects is greater than in a normal distribution.

Bayesian Lasso

Bayesian Lasso (BayesL) (Xu 2003a; Yi and Xu 2008) is similar to the BayesA approach. BayesL uses the same model and the same procedure of BayesA to evaluate marker effects, but it makes a different assumption about the distribution of markers variance. In BayesL, $\Pr ior\left(\sigma^2_{g_i}\right)$ is assumed to have an exponential distribution and, after integration, the posterior distribution of SNP effects $\hat{g}$ results in a double-exponential expression. Double-exponential distribution has a larger peak at zero and heavier tails than the normal distribution. As a consequence, the effects of a large number of markers will be very close to zero.

BayesB

Another possible assumption about the distribution of marker effects is a situation where a lot of SNPs are located in regions with no QTL and, consequently, have zero, whereas some SNPs have a moderate or large effect because they are in linkage disequilibrium with QTLs. Meuwissen et al. (2001) called this method BayesB and proposed a prior distribution of marker effects where many SNPs have zero effects whereas the remaining markers have a normal distribution. In BayesB, the prior distribution is fixed with a high density, π, at $\sigma_{g_i}^2 = 0$ and with an inverse chi-square distribution at $\sigma_{g_i}^2 > 0$:

$$\sigma_{g_i}^2 = 0 \qquad \text{with probability } \pi$$

$$\sigma_{g_i}^2 = \chi^{-2}\left(v, S\right) \qquad \text{with probability } (1\text{- }\pi),$$

where v = 4.234 and S = 0.0429 (Meuwissen et al. 2001). The Gibbs sampler described in BayesA cannot be used in the BayesB method, because it moves only where $\sigma_{g_i}^2 > 0$. Indeed, if $g_i \neq 0$, it is not possible to sample from a distribution with $\sigma_{g_i}^2 = 0$, whereas the probability of finding $g_i = 0$ is infinitesimal when $\sigma_{g_i}^2 > 0$. This problem was solved by sampling $\sigma_{g_i}^2$ and g_i simultaneously using a Metropolis-Hastings algorithm (Meuwissen et al. 2001).

Even if there are many works where Bayesian methods yield a more accurate prediction of GEBV than SNP-BLUP, these results are often obtained using simulated published data (Meuwissen et al. 2001; Habier et al. 2007). However, when using real data, the best performances of Bayesian methods are not consistently verified. One reason for the disagreement observed between real and simulated data could be differences between the genetic architecture of the real population and that of simulated data. It is well known that accuracy is proportional to hereditability (h^2) and to the number of individuals in train population (N_p). Daetwyler et al. (2010) demonstrated that the accuracy of SNP-BLUP, for a given N_p and h^2, was not dependent on the number of QTL (N_{QTL}), whereas the accuracy of BayesB was high when N_{QTL} was low but it decreased when N_{QTL} increased. In addition, sometimes, the accuracy of SNP-BLUP was higher than the accuracy of BayesB when N_{QTL} was high.

Another problem that affects both BayesA and BayesB is their sensitivity to the prior distribution and the parameter specification. In a simulated dataset, Lehermeier et al. (2013) tested the sensitivity of four Bayesian methods frequently used in genome-based prediction: Bayesian Ridge, BayesL, BayesA and BayesB. The authors found that the predictive abilities of the tested Bayesian methods were similar, but the performances of BayesA and BayesB depended substantially on the choice of parameters. However, all Bayesian approaches require huge computer resources and are time expensive (Shepherd et al. 2010). The reason is that Markov Chain Monte Carlo techniques, such as Gibbs sampling and Metropolis-Hasting algorithm, require thousands of samplings to detect the effect of each SNP. If the data dimension is small, these techniques are feasible. However, in genomic selection, animals are genotyped by using high density SNP platforms and, in this case, a huge computational time is needed.

Several other methods have been proposed to predict the genomic breeding values of animals in selection programs. Apart from few approaches which assume an equal contribution of all loci to the genetic variance, a common challenge of the most part of these methods is to reduce the dimensionality of the SNP data (Calus 2009). The reduction of the number of SNP involved in genomic evaluations brings down the genotyping costs and might reduce the bias due to SNP that are not in LD with any QTL.

Genome-wide association studies

Genome-wide association studies (GWAS) is a way to detect associations between markers and production or functional traits or diseases. Associations are studied by examining many common genetic variants in different individuals and then verifying if any variant is associated with a trait of interest. In animal breeding programs, knowledge of the genes that affect a particular trait can be used to select animals carrying desirable alleles (Goddard and Hayes, 2009; Ron and Weller, 2007; Wiener et al. 2011). There are many approaches to implement GWAS for quantitative traits, and the simplest one is the use of a linear regression for each marker.

Single marker regression

Under the assumption of random mating among animals with no population structure, the association between SNPs and traits can be tested by using the following model:

$$y = Wb + Xg + e$$

where y is the trait, W is a design matrix for fixed effects (e.g. mean, age and season of birth), b is the vector of fixed effects, X is the vector of the SNP genotypes, g is the effect of the markers, and e is the vector of residuals, assumed to be normally distributed with mean zero and variance σ_e^2: $e \sim N\left(0, \sigma_e^2\right)$. In this model the effect of each marker is additive and is considered as a fixed effect. The null hypothesis H_0 is that the marker has no effect on the trait, whereas the alternative hypothesis H_1 is that the marker is in LD with a QTL that affects the trait. The statistical test used to test the H_0 is a F-test and H_0 is rejected if $F > F_{\alpha,n,m}$ where α is the level of significance and *n* and *m* are the degrees of freedom. The choice of the level α of significance is a crucial point in GWAS. In genomic data analyses, tens of thousands of markers are tested and, therefore, the α value of 0.05 normally used leads to a very high number of false positive associations. For example, the 50K Illumina's chip contains around 50,000 SNP. If a threshold is fixed, the expected false positive associations are $50,000 \times 0.05 = 2,500$. To overcome this problem, a correction for the multiple test error can be applied. Usually, the Bonferroni correction is adopted, but it is extremely conservative and discards most of possible true associations. In fact, referring to the previous example, the threshold that should be fixed with the Bonferroni correction is $\alpha = \frac{0.05}{50,000} = 10^{-6}$ and this value would probably cut off most associations. An alternative empirical procedure is the permutation test (Churchill and Doerge, 1994), which is an excellent method for setting significance thresholds in a random mating population. On the other hand, the permutation test takes a lot of time because it fixes the α threshold by randomly shuffling, for each marker, the phenotypes across individuals thousands of times.

Another source of spurious associations is the stratification of the population due to the genetic drift or to the artificial selection that exists in some livestock populations (Ma et al., 2012). These effects can be removed by using a mixed model with the population structure as random effect.

The mixed model

In mixed models, the expectation of the outcome y is modeled using both fixed and random effects. Fixed effects are the same as those of the single marker regression, whereas random effects are the polygenic effect due to population structure. In cattle breeds, the assumption of independence between traits does not hold because relatives in the sample population share genomes and the traits are controlled by genome. The heritability *h*^{2} characterizes the strength of control of the trait by genome, whereas the coefficient of relationship $\phi_{i,j}$, which characterizes the relationship between a couple of relatives *i* and *j*, is roughly proportional to the genome shared identical-by-descent. Correlations among phenotypes of the relatives *i* and *j* depend on the degree of relatedness $\phi_{i,j}$ and on the heritability *h*^{2} of the trait, and are evaluated by the relation $\rho_{i,j} = h^2\phi_{i,j}$. The model which takes into account the correlation structure is the following:

$$y = 1_n \mu + bX + Za + e$$

where y is the vector of reliable EBV, 1_n is a vector of 1s, μ is the overall mean, X is the vector of the considered SNP genotype, b is the regression coefficient, Z is a design matrix for animal effects, a is the vector of the random additive polygenic effects with $a \sim N(0, \Phi\sigma_a^2)$, where $\Phi = \left\{\rho_{i,h}\right\}$ is the additive genetic relationship matrix, and e random residual effect with $e \sim N(0, I\sigma_e^2)$ (Yu et al. 2006, Aulchenko et al. 2007). The structure of the mixed model is like that of BLUP and, therefore, its solutions are obtained as previously described for the BLUP model. The significance of the regression coefficient b and consequently the associations between SNPs and traits are assessed by using a t-test or Wald chi-squared. Even if the mixed model solves the problem of the population stratification, it still has the shortcomings of multiple testing. When a single-marker linear regression is used to test associations for complex traits, the model might lead to inconsistent estimation of marker effects because markers are in linkage disequilibrium with many QTL (de Los Campos et al. 2010). In animal breeding, most of the productive traits are affected by a large number of genes with possible interactions among them. As a consequence, in genetic studies of complex traits, the single-locus analysis does not produce reliable results (Cordell, 2009). Another disadvantage of the single SNP approach is that LD could extend to a wide genome region. In this case, the detection of the region containing the true mutation and the significant associated SNPs could be difficult (Pryce et al. 2010). A possible solution to this problem could be to fit all SNPs simultaneously by using the Bayesian-alphabet model.

Whatever the method used for GWAS, SNPs declared associated with a trait have to be validated, even if a stringent threshold is used to detect the statistical associations. The best way to validate the detected SNPs is to verify the associations in an independent population. In livestock, where the degree of inbreeding is high and the pedigree structure could affect independent samples, the most convincing validation method is across breeds. However, if a SNP does not segregate in the breeds considered in the validation procedure, the validation of the SNP across breeds might fail.

Imputation

Genotype imputation indicates the process of predicting genotypes that are not directly assayed from a SNP chip panel. These "*in silico*" genotypes can be used to boost the number of SNPs across the whole genome as part of a GWAS or a GS program. The imputed markers can be also used in a more focused region as part of a fine-mapping study (Marchini and Howie 2010). In GWAS and GS, high-density marker panels of different SNP densities (50K and 777K) are currently used to genotype bulls and elite cows under study (Hayes et al. 2009, Schopen et al. 2011, Chamberlain et al. 2012). In animal science, genotyping costs are one of the major constraints which limit a large-scale implementation of GS. However, the commercial availability of low-density SNP panels has offered new opportunities to increase the number of animals involved in association studies and, above all, in selection programs. Genotypes obtained from a low-density panel are currently imputed to a high-density chip and used in addition to genotypes obtained with a high density panel.

Imputation is very useful when genotypes coming from different chips panel have to be joined (Druet et al 2010). In this case, imputation can increase the sample size of the population under study. In GWAS this implies an increase in the power of a given study and can also facilitate meta-analyses in studies that combine genotypes obtained from different sets of variants (Howie et al. 2011).

The Hidden Markov Model (HMM) is the most useful approach to perform imputation. It is used in many of the available software suite programs, such as Beagle (Browning and Browning 2009), IMPUTE2 (Howie et al. 2009) and FastPHASE (Scheet and Stephens 2006).

Hidden Markov model

HMM are probabilistic models where the resulting sequences are generated by two concurrent stochastic processes. The first is a one-state Markov model where the probability of transition from state *j-1* to state *j* depends only on state *j-1*. In the second process, there is the emission of a value (the haplotypes or the genotypes) which is regulated by an emission probability depending on the state. The result is a sample of sequences conditioned by the transition between states (i.e. ACCGTC). Because only the final sequence can be observed, with no understanding of the Markov process, the model is termed *hidden*.

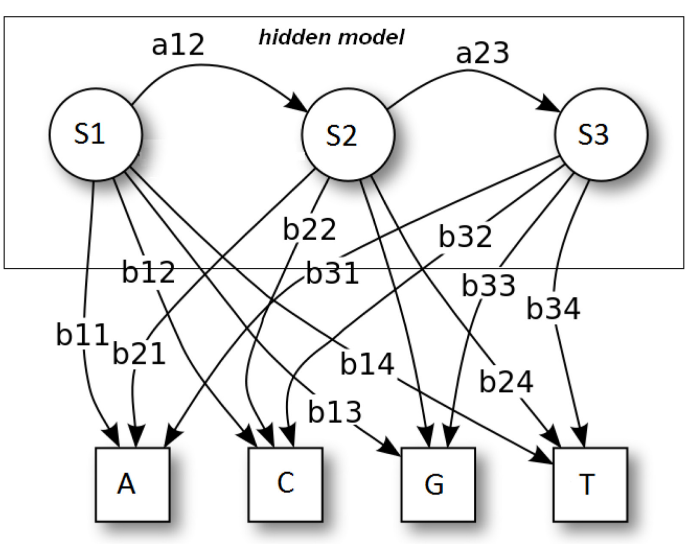

Figure 1 A Hidden Markov model for DNA sequences. The circled *Si* are the hidden states and the arrows between the states indicate the state-transition probabilities. Letters inside squares indicate the symbols of emission and the arrows between a state and a symbol are the emission probabilities.

Using Rabiner's notation (Rabiner 1989), the five components of a HMM are as follows:

- N hidden states: $S_1, S_2, \ldots, S_N$;
- M different symbols (the haplotypes A C G T): $v_1, v_2, \ldots, v_M$;
- State-transition probabilities $A = \{a_{i,j}\}$: $a_{i,j} = P\left(x_t = S_j \mid x_{t-1} = S_i\right)$ that is the probability to transit from the state S_i to the state S_j ;

- Emission probabilities $B = \{b_{j,k}\}$: the probability of observing the symbol v_k in the state S_j;
- Initial-state probabilities distribution $\pi = \{\pi_i\}$: $\pi_i = P(x_1 = S_i)$ that is the probability that the HMM process starts at state S_i.

In Figure 1 there is a HMM for DNA sequences with the Rabiner's notation.

Once parameters N and M are fixed, the model is described by means of $\lambda = \{A, B, \pi\}$, which is obtained fixing suitable values for *A, B* and π. Several problems arise with a HMM inferring the probability of an observed sequence or detecting which could be the most likely sequence. If the entire sequence *s* of length L generated by the HMM is known and if *w* is the path of the starting state till the final state, the joint probability to observe *s* is: $P(s, w \mid \lambda) = a_{0,1} \prod_{t=1}^{L} a_{t,t+1}\ b_{t,k}$. Being *w* unknown, all possible paths should be considered and, consequently, the probability to observe the sequence *s* is $P(s \mid \lambda) = \sum_{w} P(s, w \mid \lambda)$. The procedure to evaluate *s* is computationally expensive, even for simple applications. To solve this problem, the forward-backward algorithm was proposed (Baum and Egon 1967; Baum 1972). This algorithm reduces the number of paths to be considered and, consequently, the probability of sequence *s* can be determined. Once the sequence is fixed, the next step is to detect the most probable state sequence that generated it. This issue can be efficiently solved by using the Viterbi algorithm (Viterbi 1967).

In conclusion, an important shortcoming of the methods based on HMMC is that all of them require a very long computation time.

Outline of the thesis

The overall aim of this thesis is to propose some alternative approaches to evaluate the genomic breeding value of animals involved in genomic selection programs. Moreover, a new method to develop genome wide association studies is proposed. This new method was also used to reduce the dimensionality of the SNP data. These selected SNPs were then used to estimate the breeding values.

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Chapter 2

The impact of the rank of marker variance-covariance matrix in principal component evaluation for genomic selection applications

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Published in Journal of Animal Breeding and Genetics (2011) 128: 440-445.

Summary

In genomic selection (GS) programs, direct genomic values (DGV) are evaluated by using information provided by high-density SNP chip. Being DGV accuracy strictly dependent on SNP density, it is likely that an increase of the number of markers per chip will result in severe computational consequences. Aim of present work was to test the effectiveness of principal component analysis (PCA) carried out by chromosome in reducing the marker dimensionality for GS purposes. A simulated data set of 5,700 individuals with an equal number of SNP distributed over 6 chromosomes was used. PCs were extracted both genome-wide (ALL) and separately by chromosome (CHR) and used to predict DGVs. In the ALL scenario, the SNP variance-covariance matrix (S) was singular, positive semi-definite and contained null information which introduces 'spuriousness' in the derived results. On the contrary, the S matrix for each chromosome (CHR scenario) had a full rank. Obtained DGV accuracies were always better for CHR than ALL. Moreover, in the latter scenario DGV accuracies became soon unsettled as the number of animals decreases whereas, in CHR, they remain stable till 900-1,000 individuals. In real applications where a 54K SNP chip is used, the largest number of markers per chromosome is about 2,500. Thus a number of around 3,000 genotyped animals could lead to reliable results when the original SNP-variables are replaced by a reduced number of PCs.

Introduction

In the last decade, several countries have developed breeding programs based on genomic selection (GS). In this approach, the genetic merit of an animal is assessed by using marker information provided by dense SNP platforms (Fernando et al. 2007). The BovineSNP50 BeadChip (Illumina Inc., San Diego, CA), which contains 54K SNP-markers, has been the most used platform in bovine genomic studies. It is likely that SNP chip density will be further enlarged in the very next future, being direct genomic value (DGV) accuracy strictly dependent on SNP density (Solberg et al. 2008). Recently, a 777K SNP platform has been made available (Illumina Inc., San Diego, CA) for bovine genotyping. In human genetics, for example, over one million SNPs are usually typed per individual (Hinds et al. 2005; The International Hapmap Consortium 2005). However, expertise is hardly transferable to animals being genomic information, in human genetics, mainly used for association studies. In genomic selection, the primary aim of animal genotyping is the estimation of DGV which is highly computational demanding. Moreover, being DGV accuracy strictly dependent on the number of animals with genotypes and phenotypes available (i.e. size of the reference population), a large number of individuals has to be genotyped, thus increasing the amount of data to be processed. As an example, a data matrix (X) of nearly 4 billion columns is generated if 5,000 animals are genotyped with the 777K chip. Such amount of records is very difficult to handle and the use of complex algorithms such as BLUP, Bayes A (Meuwissen et al. 2001) or LASSO (Park & Casella 2008) requires a huge computational capacity. Therefore, the search for methods able to reduce the dimension of the X matrix represents a priority. With this aim, Vazquez et al. (2011) proposed to select relevant SNP by single marker regression on phenotypes. However, results on actual data highlight a reduction of DGV accuracy when a number of SNP are deleted. Moreover, being SNP selection based on their relevance on the analyzed phenotype, specific sets of SNP should be needed for different traits (Habier et al. 2009).

Actually, the deletion of some columns in the data matrix X should be avoided, considering the great economic effort for genotyping a large number of animals with the highest marker density available. A more rational approach should summarize information contained on the whole SNP panel in a smaller set of new variables. This is the case of the principal component analysis (PCA) (Hotelling 1933). This technique removes any redundancy in the original data by searching for a new set of mutually orthogonal variables (the principal components, PC), each accounting for decreasing amount of variance in the data. PCA has been used to analyze human genetic patterns (Cavalli Sforza & Feldman 2003; Paschou et al. 2007). Recently, Lewis et al. (2011) applied PCA to a genomic dataset (30,000 SNP) generated in a study involving 19 breeds (13 taurine, three zebu, and three hybrid breeds). Authors demonstrated that 250-500 carefully selected SNP are sufficient to trace the breed of unknown cattle samples. In GS simulated experiments, PCA has been used to reduce the dimension of the SNP data matrix for DGV prediction (Macciotta et al., 2010; Solberg et al., 2009), obtaining similar accuracies when either SNPs or PCs were used as predictors. These results indicate that PCA can be considered a suitable tool to reduce the number of SNP variables in GS programs.

Aim of this work was to demonstrate, both in theory and in practice, that a proper use of PCA may be effective in reducing the marker dimensionality for GS purposes.

The Principal Component Analysis

PCA is a statistical procedure that transforms a number of (possibly) correlated variables into an equal number of uncorrelated variables called PCs. The objective of PCA is to redistribute the original variability of data. Thus, the first principal component accounts for as much as possible of original variability in the data, and all components are extracted in order to maximize successively the amount of variance explained (Morrison 1976; Krzanowsky 2003). In other words, to summarize information contained in the starting m-dimensional space (the m SNP-variables), original directions are rotated into a new m-dimensional space. The new m-directions are the principal components where the jth PC is represented by a linear combination of the observed variables X_m:

$$PC_j = v_{1j}X_1 + \ldots + v_{mj}X_m$$

with j=1,……,m. The v_{mj} weights are the components of the eigenvectors extracted from the variance-covariance (correlation) matrix (S) in a so called "eigenvalue problem". The S matrix is symmetric and positively semi-definite. It has on the diagonal the variances of each m-variable and off diagonal the covariance between variables. The trace of S (trS) represents the total variance of the multivariate system. The eigenvalue problem applied to S gives the following results:

i) m eigenvalues, $\lambda_1 > \lambda_2 > \ldots\ldots\ldots > \lambda_m \geq 0$, such as $\sum_i^m \lambda_i = trS$.

ii) a set of m vectors (eigenvectors), one for each eigenvalue. These vectors are mutually orthogonal and their components are the weights v_{mj} used to compose the PCs. These vectors constitute the matrix V of the eigenvectors.

The first eigenvalue is greater than the second, the second is greater of the third and so on. The proportion of the total variance accounted by the *i*th component (var_{expl}) can be empirically evaluated as:

$$\text{var}_{\exp 1} = \frac{\lambda_i}{trS}$$

Finally, the matrix P whose columns are the new variables, can be calculated as:

$$P = X \cdot V$$

whose dimension is (nxm).

One crucial step of PCA concerns the choice of the number of PCs to be retained. Several methods have been proposed (see Jolliffe, 2002, for a review of the most frequently used criteria). The simplest is to retain a number of p components (p<m) until the cumulative variance explained reach a fixed value. Generally this value is fixed at around 80 – 85% of the total variance.

The rank of the genomic variance-covariance S matrix and its effect on PC extraction

The rank (ρ) of a matrix is defined as the maximum number of independent rows (or columns). For a rectangular matrix A_{nxm}, ρ is minor or equal to the minimum value between n and m, i.e. $\rho \leq \min(n; m)$ (Bumb 1982; Patterson et al. 2006). In the case of the data matrix

X_{nxm}, being n<<m, $\rho_x \leq n$. Therefore, its variance-covariance square matrix S has dimension mxm but not full rank ($\rho_S \leq n\text{-}1$). As a consequence, it has one or more eigenvalues equal to zero.

Let we consider a real situation where X has n=4k rows and m=50k columns. The extraction of principal components starts from a S matrix with dimension $50k \times 50k$ and rank $\rho_S \leq 4k-1$. In the best situation, only 4k-1 eigenvalues are greater than zero, and therefore, the maximum number of non-redundant PCs is 4k-1. The remaining PCs are directions along which the observations do not have components. The total variability, originally distributed over 50k variables, has been compressed in 4k-1 directions, being $\sum_{1}^{4k-1} \lambda_i = trS$. This result is a non-sense because, being the PCs new axes obtained by rotation, their number should be equal to the original axes. Moreover, the number of PCs is further reduced if a threshold of 85% of the total variance explained is considered.

The same problem has been raised by Bumb (1982) for factor analysis, another dimension-reduction multivariate technique. The author observed “spurious” results, i.e. characterized by a random variability, when the number of variables exceeds the number of observations.

The S rank issue is particularly relevant in genomic selection due to the huge number of columns in the SNP data matrix. The extraction of PCs by chromosome instead of genome-wide could represent a possible strategy to deal with this problem. The approach is supported by the substantial biological orthogonality between chromosomes. Moreover, as stated in the previous section, the number of markers per chromosome is lower than 2,500 in the commercial 54K SNP platform. The current size of reference populations in genomic projects often exceeds 3,000 individuals. Therefore, both X and S matrices evaluated by chromosome (X_{CHR} and S_{CHR}) could have a full rank and the related PCs would not lead to spurious results.

A simulation study

Materials

Data were extracted from an archive generated for the XII QTLs – MAS workshop, freely available at: http://www.computationalgenetics.se/QTLMAS08/QTLMAS/DATA.html. Briefly, a genome of six chromosomes with 6,000 biallelic evenly spaced SNP was generated. A total of 300 SNP were deleted: 75 monomorphic, and 225 with MAF lower than 10%. A number of animals (5,700) equal to the retained SNP was considered: 5,600 of reference (REF), and the remaining 100 younger individuals as prediction population (PRED). All animals had phenotypes available. For complete details on the data generation see Lund et al. (2009).

Methods

Effects of SNP markers on phenotypes in the REF population were estimated by using a BLUP mixed linear model that included either the fixed effects of mean, sex and generation, and the random effect of principal component scores (Meuwissen et al. 2001). The overall mean and the estimated effects of PC scores were then used to predict DGV in PRED population (for more details on DGV evaluation see Macciotta et al. 2010). Accuracy of DGV prediction was evaluated by calculating Pearson correlations between DGV and true breeding value (TBV) in PRED animals.

Two scenarios were simulated. PCs were extracted on all SNP simultaneously (ALL) or separately by chromosome (CHR). Different sizes of REF population and number of extracted PCs (corresponding to different percentages of the total variance explained) were tested for each scenario. In particular, the size of REF was fixed at 5,700, 3,000, 1,000, 900, 800, 500, 400, and 300 animals. Variance retained by PCs ranged from 60% to 95% by a step of 5%.

Results and discussion

The ability of PCA in reducing the space of the 5,700 SNP-variables can be seen in Figure 1, where the first 2,000 PCs are displayed. In particular, the percentage of explained variance is around 85% and 95% when 300 or 700 PCs are retained, respectively. Thus the information contained in around 6k markers can be summarized in a small number of PCs (5 or 12% of the total PCs).

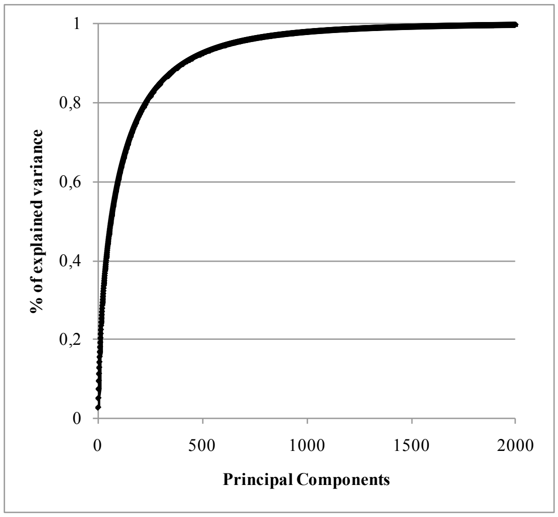

Figure 1 Pattern of the cumulative variance explained as the number of retained principal components increases

Table 1 displays the number of retained PCs for increasing amounts of explained variance and for different sizes of the REF population, both for CHR and ALL approaches. As expected, the number of extracted PCs decreases together with the population size in each scenario. For example, when the REF size reduces from 5,700 to 1,000 individuals and 85% of variance explained is considered, the reduction in predictor dimensionality obtained by PC extraction is equal to 37% and 13% for ALL and CHR scenarios, respectively. These results highlight that in PCA the total variance is compressed in a smaller space when the number of observations is lower than the number of variables (as in ALL). On the other hand, in the CHR scenario the correct number of PCs is retained till the number of individuals exceeds the maximum number of SNP per chromosome (i.e. 1,000). Therefore, in a real situation where animals are genotyped with the 54K chip the number of retained PCs, for 85% of variance accounted, is likely to be around 3,000-3,500. Such a number of variables can be easily managed with any personal computer and the computational time for DGV evaluation reduces to few minutes.

Table 1 Number of retained principal components in genome-wide (ALL) and by chromosome (CHR) scenarios both for original variance explained and the number of involved animals' reduction

	Number of animals									
Variance	**5700**		**1000**		**800**		**500**		**300**	
explained (%)	CHR	ALL	CHR	ALL	CHR	ALL	CHR	ALL	CHR	ALL
50	95	64	89	42	89	41	84	34	84	34
55	116	80	107	52	107	51	101	43	98	41
60	140	99	130	65	128	62	121	53	117	50
65	169	123	155	80	153	77	144	64	140	61
70	205	152	186	98	184	95	174	79	170	74
75	251	190	225	122	222	118	210	97	205	90
80	312	240	277	153	272	148	257	120	250	109
85	400	313	350	196	344	190	323	150	313	135
90	542	430	466	263	455	253	429	193	409	170
95	831	670	696	383	677	366	630	261	589	224

Figure 2 displays DGV accuracies for decreasing sizes of REF population and for different amounts of accounted variance. Values are in agreement with reports on simulated and real data (Van Raden et al., 2009). The starting point of simulation is when both S_{ALL} and S_{CHR} have full rank (figure 2a), i.e. when the number of animals is approximately equal to the number of SNP. In particular, DGV accuracies show a regular rising pattern both for ALL and CHR, with higher values for the latter scenario. This result is probably due both to mathematical and "biological" reasons. For a fixed amount of explained variance, the number of components extracted by chromosome is greater than those obtained genome-wide. This result seems to indicate a redundant PC calculation in CHR, because PCA is more efficient when the same amount of variance is accounted by a smaller number of new variables. As a consequence, higher DGV accuracies for ALL compared to CHR should be expected. However, results reported on figures 1 highlight a similar behavior of the two methods. Thus the substantial chromosome orthogonality allows, in the CHR approach, for a correct assessment of PCs number. Moreover, it can be seen that CHR outperforms ALL for low percentages of retained variance. The gap between the two scenarios reduces when variance is > 95% or more, i.e. when almost all the total variance is accounted for.

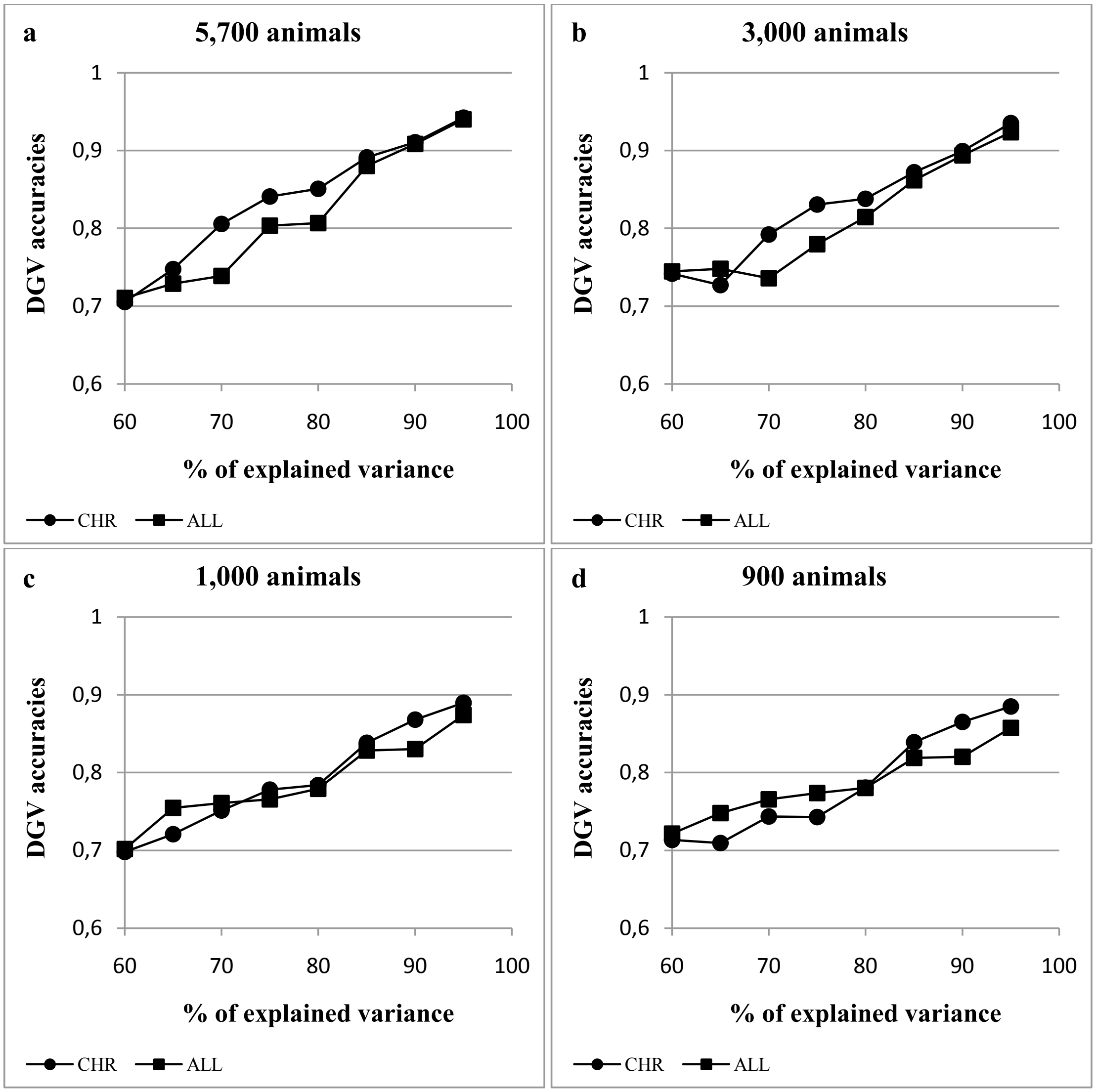

Figure 2 Accuracies of direct genomic value (DGV) for increasing values of variance explained and decreasing number of animals in training population

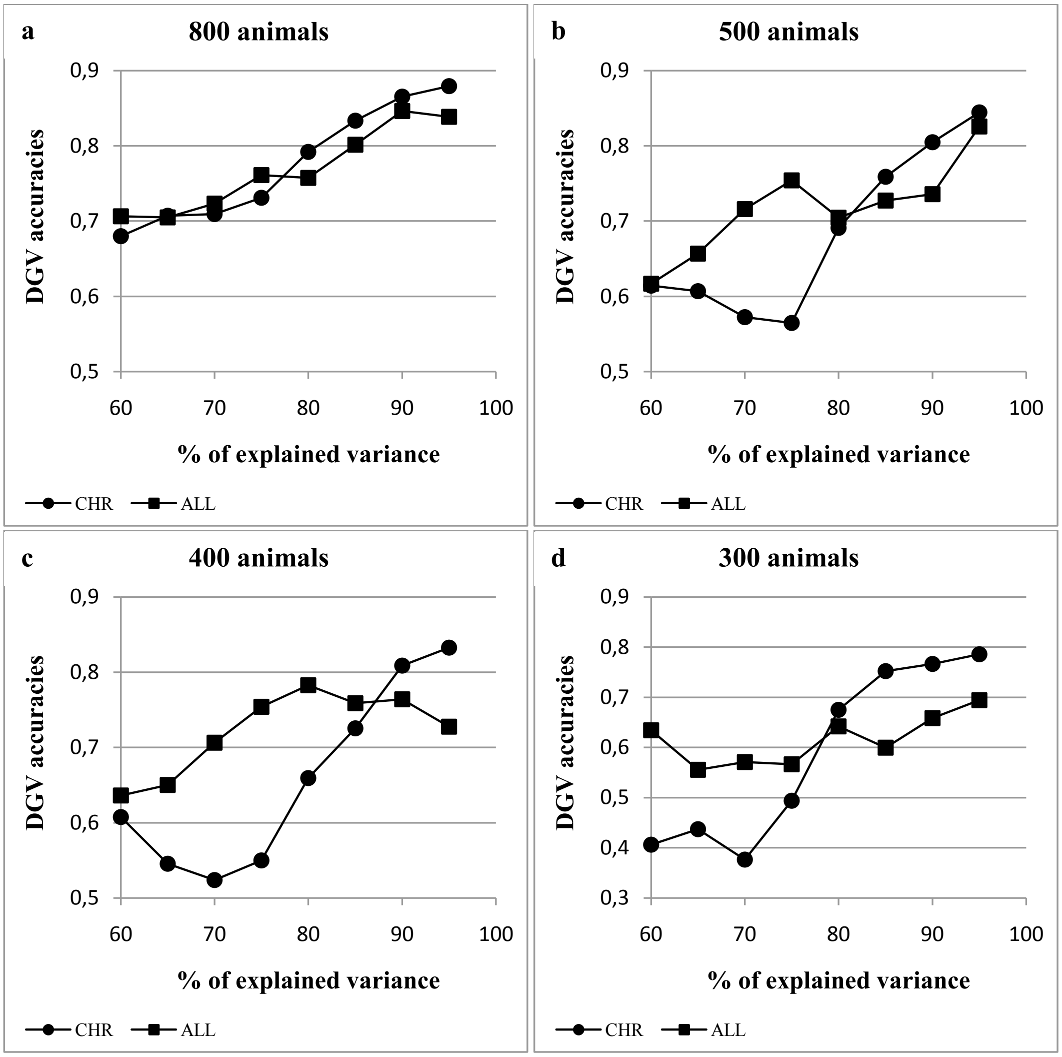

Figure 3 Accuracies of direct genomic value (DGV) for increasing values of variance explained and decreasing number of animals in training population

Differences between accuracies obtained in the two approaches tend to increase as the number of animals decreases (figure 2). Moreover, the pattern becomes more irregular for ALL. These figures are in agreement with other studies that observed spurious results when the rank of S is markedly smaller than its dimension (Bumb 1982). Figure 3 clearly displays this effect highlighting that also CHR pattern tends to be irregular for a REF size lower than 1,000 animals. Starting from figure 3a, the behavior became more unpredictable with a random loss of accuracy (Figure 3b) when 75% of variance is explained by PCs. However, both figure 2 and 3 shows that DGV accuracies in CHR are always higher than ALL for an accounted variance greater than 80-85%. Such a value could be used as a criterion for retaining PC extracted chromosome-wide in an implementation of the PC approach on real genomic data. Moreover, for these values of variance, DGV accuracies range from 90 to 80% until the S_{CHR} has a full rank (1,000 animals, in our simulation). On the other hand, they decrease till around 70% (figure 2d) for a REF size of 300. Thus a number of animals greater than the number of SNP per chromosome should be used to obtain good accuracies.

Conclusions

With the recent development of high-density marker chips that are routinely used in genomic selection programs, the need for reducing predictor dimensionality is of primary importance. The principal component analysis can represent a useful tool for summarizing and reallocating the overall information contained in the SNP data. A proper use of the technique requires a full rank S matrix to produce reliable results. This is a relevant issue in genomic analysis where the number of variables always exceeds the number of genotyped animals. According to the results of the present work, such an issue can be addressed by extracting PCs separately by chromosome, i.e. by using this technique on a series of full rank S_{CHR} matrices. Better accuracies of DGVs have been obtained when PCs are extracted by chromosome instead of genome-wide, even with both S_{ALL} and S_{CHR} at full rank. In the Illumina 54K chip the largest number of markers per chromosoma, about 2,500, is located on BTA1. Thus a number around 3,000 genotyped animals could lead to reliable results when the original SNP-variables are replaced by a reduced number of PCs. Results of the present work, although obtained with a genome size and number of markers different from the conditions found on field data, seems to be rather realistic. The recently released Bovine3k genotyping BeadChip is finding a large use in genomic selection programs. Thus in the very next future several animals will have genotypes available with this marker density.

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Chapter 3

Use of partial least squares regression to impute SNP genotypes in Italian Cattle breeds

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Published in Genetics Selection Evolution (2013), 45: 15.

Abstract

Background

The objective of the present study was to test the ability of the partial least squares regression technique to impute genotypes from low density single nucleotide polymorphisms (SNP) panels i.e. 3K or 7K to a high density panel with 50K SNP. No pedigree information was used.

Methods

Data consisted of 2,093 Holstein, 749 Brown Swiss and 479 Simmental bulls genotyped with the Illumina 50K Beadchip. First, a single-breed approach was applied by using only data from Holstein animals. Then, to enlarge the training population, data from the three breeds were combined and a multi-breed analysis was performed. Accuracies of genotypes imputed using the partial least squares regression method were compared with those obtained by using the Beagle software. The impact of genotype imputation on breeding value prediction was evaluated for milk yield, fat content and protein content.

Results

In the single-breed approach, the accuracy of imputation using partial least squares regression was around 90 and 94% for the 3K and 7K platforms, respectively; corresponding accuracies obtained with Beagle were around 85% and 90%. Moreover, computing time required by the partial least squares regression method was on average around 10 times lower than computing time required by Beagle. Using the partial least squares regression method in the multi-breed resulted in lower imputation accuracies than using single-breed data. The impact of the SNP-genotype imputation on the accuracy of direct genomic breeding values was small. The correlation between estimates of genetic merit obtained by using imputed versus actual genotypes was around 0.96 for the 7K chip.

Conclusions

Results of the present work suggested that the partial least squares regression imputation method could be useful to impute SNP genotypes when pedigree information is not available.

Background

In genomic selection programs, the breeding value (GEBV) of an individual is assessed by combining both genomic and traditional pedigree-based predictions. High-density marker platforms (HDP) of different SNP (single nucleotide polymorphism) densities (50K and 777K) are currently used to genotype bulls under selection (Hayes et al. 2009) and elite cows and to test for marker-phenotype associations (Schopen et al. 2011, Chamberlain et al. 2012).

Genotyping costs are among the major constraints for large-scale implementation of genomic selection in many breeds. However, the commercial availability of low density SNP panels (LDP), such as the Illumina Bovine3K Genotyping BeadChip or the Illumina BovineLD BeadChip, which contains around 7K markers (Boichard et al. 2012), has offered new opportunities to increase the number of animals involved in selection programs. Genotypes obtained from an LDP must be imputed to the 50K platform by using suitable algorithms. Genotype imputation can also be useful when combining data sets that were generated using different SNP chips (Druet et al. 2010).

Genotype imputation refers to in silico reconstruction of missing genotypes. Several techniques have been proposed to routinely impute SNP genotypes. The following three steps are common to all procedures: (1) a training population (TP) genotyped with an HDP is created; (2) a prediction population (PP) is generated by using an LDP; and (3) a suitable algorithm is used to impute missing SNPs in the PP.

On the basis of the information considered to infer missing marker genotypes, imputation methods can be classified into three groups. The first relies on linkage and family information

(Daetwyler et al. 2011, Hickey et al. 2011)), the second uses linkage disequilibrium based on population information (Scheet et al. 2006, Browning et al. 2009), and the third combines the two former sources of information (Druet et al. 2010, Van Raden et al. 2011). Several factors affect imputation accuracy. In particular, imputation accuracy strongly depends on the number of individuals in the training population and on the marker density of the LDP (Druet et al. 2010, Weigel et al. 2010a, Weigel et al. 2010a, Zhang et al. 2010b).

The impact of imputed genotypes on GEBV accuracies has been investigated. Results are sometimes discordant or expressed in different ways. For example, Chen et al. (2011) compared GEBV values obtained with actual and imputed data. Two computer programs, Findhap (Van Raden et al. 2011) and Beagle (Browning et al. 2009), were used to impute SNP genotypes from a 3K panel to a 50K panel. The loss of reliability in GEBV prediction by using imputed data was around 6.5% and 2.6% with Findhap and Beagle, respectively. Recently, Segelke et al. (2012) reported a reduction in reliability of genomic predictions, averaged over 12 traits, ranging from 5.3% to 1% for the 3K and 7K chips, respectively. Moser et al. (2010) proposed the use of an LDP that included the highest ranked SNPs for a trait under study. However, the gain in accuracy of GEBV obtained with the highest ranked SNP was only slightly higher (5-6%) than the accuracy obtained with an equal number of evenly spaced markers. Nevertheless, with this strategy, considering that a specific pool of markers is required for each trait, the use of evenly spaced SNP seems to be preferable over choosing a specific SNP set for each trait.

Several imputation algorithms have been proposed and implemented in freely available software such as Beagle (Browning et al. 2009), DAGPHASE (Druet et al. 2010) and Findhap (Van Raden et al. 2011). Chen et al. (2011) found Beagle to be the most accurate but at the expense of longer computation time.

A method that uses the Partial Least Squares Regression (PLSR) technique to impute SNP genotypes was proposed recently (Dimauro et al. 2011). It was tested on a simulated genome consisting of 6000 SNPs equally distributed on six chromosomes and a data set of 5865 individuals (TP = 4665 and PP = 1200). The PLSR method yielded accuracies in marker imputation ranging from 0.99 to 0.86 when 10% or 90% genotypes were imputed, respectively. In the latter case, the accuracy of direct genomic values (DGV) dropped from 0.77 to 0.74. Furthermore, Dimauro et al. (2011) highlighted that, with a fixed percentage (50%) of SNPs to be predicted, imputation accuracies slowly decreased from 98% with TP = 5000, to 87% with TP = 1000 and to 69% with TP = 600. PLSR requires only genotype data, and other data, such as pedigree relationships, is not needed. Therefore, this approach could be useful when the population structure is not known.

The aim of the present work was to test the PLSR imputation method on real data. In particular, a scenario with a 50K genotyped TP and a PP genotyped using either the 3K or 7K panel was simulated. Moreover, the ability of the PLSR method to predict SNP genotypes for different bovine breeds and in a multi-breed approach was tested.

Methods

Data

Data consisted of SNP genotypes belonging to 2179 Italian Holstein bulls genotyped with the Illumina 50K Beadchip (single-breed dataset). Only markers located on the 29 autosomes were considered. Monomorphic SNPs and SNPs with more than 2.5% missing values were discarded. No editing for minor allele frequency (MAF) was applied. A total of 43 427 SNPs were retained and any missing genotypes for these SNPs were replaced by the most frequent genotype at that locus. Data on a total of 86 bulls were discarded, of which 48 were replicates or had inconsistent Mendelian inheritance information, and 38 had a low overall call rate (lower than 95%).

To study the performance in a multi-breed sample, 749 Brown Swiss and 470 Simmental bulls were also available. For the multi-breed data set, data from the three breeds were edited together to obtain the same SNPs in all data sets. At the end of the editing procedure, 30 055 markers were retained.

Genotypes were coded according to the number of copies of a given SNP allele they carried, i.e. 0 (homozygous for allele B), 1 (heterozygous) or 2 (homozygous for allele A). The phenotypes available for all animals were polygenic estimated breeding values for milk yield, protein and fat content. Animals were ranked according to their age: the oldest were designed as TP with all genotypes considered known, whereas the youngest represented the PP. For both the single and multi-breed approach, SNPs belonging to 3K and 7K LDP were identified in the PP animals and all other genotypes were masked, thus mimicking the two Illumina LDP.

The partial least squares regression imputation method

PLSR is a multivariate statistical covariance-based technique that is able to predict a response matrix $Y_{(n \times p)}$ from a predictor matrix $X_{(n \times m)}$ and to describe the common structure of the two matrices (Dimauro et al. 2011). In both X and Y, n represents the number of animals involved, m is the number of SNPs in the LDP and p is the number of SNPs to be imputed. PLSR allows for the identification of underlying variables (known as latent factors) which are linear combinations of the explanatory variables X, that best model Y. Dimauro et al. (2011) demonstrated that the accuracy of PLSR prediction increases with the number of latent factors approaching the number of SNPs to be predicted (the columns of Y). The maximum number of latent factors depends on the size of X, which has a lower number of columns than Y. For this reason, in each run, the number of extracted latent factors was fixed to be equal to the number of predictors (the number of columns of X). PLSR is a multivariate statistical technique particularly useful in genomic studies in which a great number of variables are involved. It can overcome the strong collinearity between SNP variables in X or Y and, at the same time, maximize correlations between Y and X variables (Dimauro et al.

2011, Abdi 2003). A more detailed description of the PLSR imputation method can be found in Dimauro et al. (2011).

In the present work, each chromosome was processed independently and data were analyzed by using the PLS procedure of SAS® software (SAS® institute Inc., Cary, NC). Datasets were organized in a multivariate manner, having SNPs as columns and animals as rows. The 50K SNPs were divided into SNPs that have to be imputed (Y) and SNPs used as predictors (X). In particular, X contained only SNPs belonging to the 3K or 7K LDP. For animals in the PP, genotypes in Y were masked and constituted the SNPs to be predicted.

Genotype imputation from 3K (7K) LDP to the 50K SNP panel

The comparison of imputation performances from different publications is difficult due to the many differences between studies. TP size and number of markers in LDP heavily affect the accuracy of prediction. Moreover, the relationships between training and validation animals have an impact on imputation accuracies (Dassonneville et al. 2011). So, before applying the PLSR imputation method to our data, the method was tested on external data provided by Daetwyler et al. (2011) who exploited the ChromoPhase program (Daetwyler et al. 2011) to impute missing genotypes from low to high density SNP platforms. The data consisted of 1183 Holstein bulls genotyped with the Illumina 50K chip. Only the 2529 markers on chromosome 1 were available. A PP genotyped with the 3K chip (182 SNP) was simulated by masking the markers not present on the 3K chip. In particular, the PP was divided into non-founders (112 individuals that have at least one genotyped parent) and founders (212 animals that do not have a genotyped parent) and imputation accuracies were evaluated for both categories of animals. The PLSR method and Beagle (Browning et al. 2009) software were used to impute SNP genotypes in the PP and results were compared with accuracies obtained by Daetwyler et al. (2011). Population structure or pedigree was not used with either method.

In our experimental data, PLSR was first applied to the Holstein breed. Animals were ranked by age and divided in TP = 1993 (the older bulls) and PP = 100 (the younger) and both 3K and

7K scenarios were investigated. The Beagle software was applied to the same data. No pedigree information was used for either PLSR or Beagle.

On simulated data, Dimauro et al. (2011) demonstrated that, for each chromosome, the PLSR imputation accuracy improved as the number of variables contained in X increased. The reason is that when many variables have to be predicted (the columns of the Y matrix), the number of extracted latent factors should be large. The maximum number of possible latent factors is, however, less or equal to the number of variables in X. So, for chromosomes with a relatively low number of markers in X, a lower PLSR predictive ability is expected. This hypothesis can be easily tested by comparing the imputation accuracies obtained in the 3K and 7K scenarios. Moreover, a PLSR run using an X matrix obtained by combining SNPs belonging to chromosomes 26, 27 and 28, was carried out to test for possible improvement in genotype imputation accuracy when X is artificially enlarged.

Genotype imputation from 3K LDP to the 50K SNP panel for different breeds

The availability of a sufficiently large TP is a crucial factor for genotype imputation. Therefore, it is interesting to investigate if a multi-breed TP could enhance the accuracy of genotype predictions. Some authors (Kizilkaya et al. 2010, Pryce et al. 2011) reported a slight advantage of using a multi-breed TP to evaluate the genetic merit of animals under selection. However, Hayes et al. (2012) showed that, in sheep breeds, accuracy of imputation in single-breed analyses was higher than accuracy of imputation in a multi-breed analysis. To test the PLSR method in a multi-breed context, three groups of animals, one for each breed, were selected. Each group contained 479 bulls (the size of the Simmental population) and was split into a TP of 379 and a PP of 100 individuals. The imputation was first performed separately for each single breed and then by combining the three groups, thus obtaining a multi-breed dataset with TP = 1137 and PP = 300 bulls.

Evaluation of imputation accuracy

The ability of PLSR to impute SNP genotypes was quantified by considering the allele imputation error rate. This index represents the number of falsely imputed alleles divided by the total number of imputed alleles (Zhang et al. 2010). In practice, considering the real and the imputed genotypes, 0 error was counted if both genotypes were identical, 1 if the real genotype was homozygous and the imputed genotype heterozygous (or vice versa) and 2 if the real and imputed genotypes were both homozygous but different. The imputation accuracy (R), for each SNP, was equal to 1 minus allele error rate. The allele error rate and the related imputation accuracy were averaged both by chromosome and across all chromosomes.

The effect of SNP imputation on accuracy of DGV was also evaluated. DGV for milk yield, fat content and protein content were calculated using both the actual 50K markers (DGV) and the imputed genotypes (DGV_IMP). Briefly, effects of SNP genotypes on phenotypes in the TP population were estimated using a BLUP model (Meuwissen et al. 2001):

$$y = 1\mu + Zg + e$$

where y is the vector of polygenic breeding values, 1 is a vector of ones, μ is the overall mean, Z is the matrix of SNP scores, g is the vector of SNP regression coefficients assumed identically and normally distributed with $g_i \sim N\left(0, I\sigma^2_{g_i}\right)$ where $\sigma^2_{g_i} = \frac{\sigma^2_a}{k}$ (σ^2_a= additive genetic variance, *k* = number SNP), and *e* is the vector of random residuals. The overall mean ($\hat{\mu}$) and the vector ($\hat{g}$) of the marker effects estimated in the TP were used to calculate the DGV for PP as:

$$\hat{y} = \hat{\mu} + Z^* \hat{g}$$

where $\hat{y}$ is the vector of estimated DGV and Z^* is the matrix of SNP scores in PP. For each phenotype, both DGV and DGV_IMP were obtained and correlations between DGV and DGV_IMP were calculated (r).

Results

Results obtained by analyzing Daetwyler's data are reported in Table 1.

Table 1 Accuracy of genotype imputation from 3K to 50K with ChromoPhase, Beagle and PLSR algorithms for founders (F) and non-founders (NF)

	Imputation accuracy		
Type	ChromoPhase[1]	Beagle	PLSR
NF	0.925	0.926	0.929
F	0.728	0.868	0.924

[1] Values from Daetwyler et al. (2011).

Values of R for both PLSR and Beagle were higher than those obtained with ChromoPhase, especially for founder bulls. Nearly equal values were obtained by PLSR and Beagle for non-founder animals whereas for founders, imputation accuracy using PLSR was more than 5% higher than with Beagle.

Table 2 contains accuracies obtained with PLSR and Beagle for imputation from 3K and 7K SNP chips to 50K based on the 2093 Holstein bulls. The average R using PLSR was 89.6% (± 1.6%) and 94.2% (± 1.0%) for imputation from 3K and 7K chips, respectively. Accuracies obtained with PLSR were 4% higher than with Beagle for both LDP. As expected, R for each chromosome was higher for imputation from 7K than for imputation from 3K. For both LDP, imputation accuracies were higher for chromosomes with a high number of SNPs. For example, R was more than 4% higher for BTA1 than for BTA28, for imputation from 3K (Table 2). Finally, R obtained by combining SNPs on BTA27, 28 and 29 was 87.4%, which was nearly equal to the average R of the three chromosomes (87.3%), indicating that no advantage was obtained by combining markers from multiple chromosomes.

Imputation accuracies obtained by including the Brown Swiss and Simmental breeds, both for imputation within breed and in the multiple breed scenario, are reported in Table 3. For the 3K LDP, R was 0.88 and 0.89 for Holstein and Brown Swiss breeds, respectively, whereas R was equal to 0.83 for Simmental. Imputation accuracies from 7K to 50K were, on average, 4% higher than imputation accuracies from 3K to 50K. However, the multi-breed approach led to a considerable decrease in accuracy and to a reduction of differences in imputation accuracies between breeds, for imputation from both 3K and 7K.

Table 2 Number of SNPs per chromosome in the 50K, 3K and 7K SNP panels and the accuracy of imputation based on 3K and 7K panels with PLSR and Beagle

	Number of SNP				Imputation accuracy (PLSR)		Imputation accuracy (Beagle)	
Chromosome	**50K**	**3K**	**7K**		**3K**	**7K**	**3K**	**7K**
1	2814	146	320		0.916	0.953	0.876	0.919
2	2294	119	277		0.911	0.951	0.863	0.922
3	2191	107	261		0.897	0.944	0.846	0.898
4	2123	106	237		0.903	0.941	0.861	0.908
5	1812	107	233		0.912	0.948	0.872	0.912
6	2164	109	254		0.908	0.953	0.867	0.914
7	1876	95	215		0.908	0.949	0.858	0.915
8	2026	104	232		0.919	0.953	0.872	0.915
9	1708	92	214		0.904	0.949	0.851	0.909
10	1841	97	209		0.909	0.946	0.872	0.915
11	1913	91	222		0.901	0.947	0.862	0.914
12	1408	85	175		0.903	0.942	0.856	0.899
13	1486	75	166		0.910	0.949	0.860	0.911
14	1453	70	166		0.897	0.945	0.850	0.912
15	1427	74	167		0.898	0.945	0.864	0.915
16	1337	74	160		0.910	0.950	0.864	0.913
17	1367	65	156		0.888	0.936	0.842	0.900
18	1147	59	136		0.877	0.924	0.825	0.884
19	1164	56	143		0.878	0.935	0.827	0.895
20	1351	70	172		0.921	0.960	0.886	0.933
21	1170	58	134		0.881	0.934	0.832	0.899
22	1087	57	133		0.894	0.941	0.849	0.900
23	919	47	118		0.887	0.938	0.842	0.895
24	1072	54	135		0.888	0.941	0.842	0.903
25	831	41	109		0.865	0.926	0.816	0.887
26	905	45	102		0.889	0.931	0.841	0.890
27	834	41	100		0.872	0.924	0.832	0.890
28	806	46	99		0.871	0.922	0.826	0.879
29	901	47	110		0.875	0.934	0.828	0.888
Total SNP	43427	2237	5155	**Mean**	0.896	0.942	0.851	0.905

Table 3 Average accuracy of imputation from 3K and 7K to 50K panels using single-breed and multi-breed information

	Imputation accuracy			
	3K		**7K**	
Breed	**Single-breed**	**Multi-breed**	**Single-breed**	**Multi-breed**
Holstein	0.882	0.806	0.914	0.837
Brown Swiss	0.893	0.827	0.921	0.858
Simmental	0.826	0.788	0.854	0.817

Accuracies of DGV predictions were moderate (Table 4), in accordance with the low number of animals in TP. However, correlations between polygenic EBV and DGV ($r_{EBV,DGV}$) and correlations between EBV and DGV_IMP (r_{EBV,DGV_IMP}) were quite similar with actual and imputed data. This result is in agreement with the relatively high correlations between DGV and DGV_IMP (r_{DGV,DGV_IMP}), which were on average 0.96 across the three considered traits with the 7K LDP. However, r_{DGV,DGV_IMP} was lower when using the 3K LDP, for which rDGV,DGV_IMP was on average 0.89.

Table 4 Correlations of direct genetic values (DGV) with polygenic estimated breeding values (EBV) ($r_{EBV,DGV}$) and with DGV based on imputed genotypes (DGV_IMP) (r_{DGV,DGV_IMP}) for milk yield, fat content and protein content

Scenarios	Milk yield		Fat content		Protein content	
	$r_{EBV,DGV}$	r_{DGV,DGV_IMP}	$r_{EBV,DGV}$	r_{DGV,DGV_IMP}	$r_{EBV,DGV}$	r_{DGV,DGV_IMP}
Actual data (50K)	0.58		0.45		0.44	
Imputation from 7K	0.55	0.95	0.43	0.96	0.43	0.96
Imputation from 3K	0.52	0.89	0.42	0.93	0.38	0.86

Discussion

Results of PLSR applied to Daetwyler's data (Table 1) showed that the method did not produce different imputation accuracies for founders and non-founders, unlike ChromoPhase and, partly, Beagle. In our analyses, we never used pedigree information. As a consequence, both founders and non-founders were handled in the same manner. However, having a parent in the reference dataset seemed to be more important when using Beagle than when using PLSR. This is probably due to the different algorithms implemented in Beagle (Browning et al. 2009) and PLSR (Abdi 2003, Li et al. 2009).

PLSR imputation accuracies, from 3K and 7K LDP to the 50K panel, were higher than accuracies obtained with Beagle and ChromoPhase. These results indicate that, if no pedigree information is available, the PLSR method should be preferred over the other methods studied here when imputation is from 3K or 7K to 50K.

PLSR was further used to impute SNP genotypes both in single and multi-breed scenarios based on Holstein, Simmental and Brown Swiss data sets. No MAF threshold was applied in the editing procedure. To investigate whether differences in imputation accuracies between PLSR and the Beagle algorithms could arise with edits based on MAF, the impact of several MAF thresholds (no limit, 0.01, 0.05, 0.10) was evaluated. However, no differences in imputation accuracies were observed between the PLSR and Beagle results.

Mean R values obtained with PLSR in the single-breed scenario were 89.6% and 94.2% for the 3K and 7K LDP, respectively. It is worth mentioning that, in the present study, the ratio between the number of animals (n = 2179 Holstein bulls) involved in the study and the mean number of markers (m = 1497) on each chromosome, $R_{n/m}$, was 1.45. Dimauro et al. (2011), tested the PLSR imputation method on a simulated data set with m = 1000 markers on a chromosome and n = 5865 individuals. The resulting $R_{n/m}$ was 5.9. In ordinary statistics and, even more, in multivariate statistics, the availability of a larger number of observations guarantees more accurate results. Thus, Dimauro et al. (2011) applied the PLSR method in a more optimal dataset, obtaining an imputation accuracy of 0.86. Even if the latter study and the present research are difficult to compare, the large difference between $R_{n/m}$ ratios suggests that PLSR also works properly with actual data. This is an important result because, if a particular technique gives good results when applied to simulated data, it is not obvious that similar performances are obtained with actual data.

PLSR is an ordinary statistical technique included in the most popular commercial and free software packages that are currently used to perform genomic data analyses, such as SAS® and R. The PLSR approach could thus be easily implemented in software for genomic evaluations previously developed. Moreover, with PLSR, the computing time needed to impute SNP genotypes was, on average, around 10 times lower than with Beagle. For example, with the 7K LDP, PLSR took around 1 h to impute SNP genotypes for the first chromosome, whereas Beagle needed around 8 h. This aspect should not be underrated when an algorithm is chosen to perform imputation. In particular, PLSR could probably be used to impute SNP genotypes from the 50K chip to the denser Illumina 777K platform in a reasonable amount of time.

Imputation from 7K to 50K (R = 0.94) was more accurate than imputation from 3K to 50K (R = 0.90). This is an expected result and it is comparable to that obtained by Mulder et al. (2012), who found a mean imputation accuracy of around 88% for 3K and 92% for 7K, respectively. The mean R for each chromosome (Table 2) showed that genotype imputation accuracy depends strongly on the number of SNP variables in the X matrix. For example, in the 3K panel, BTA1 and BTA25 have 146 and 41 SNPs, respectively, and the related values of R were 0.92 and 0.87. Dimauro et al. (2011) found that imputation accuracy increases as the number of extracted latent factors in the PLSR procedure increases. The maximum number of possible latent factors is lower than or equal to the number of variables in X. This can explain the lower imputation accuracy for chromosomes with a lower number of markers. Moreover, the dimension of X cannot be artificially enlarged by using SNP from several chromosomes because it resulted in an accuracy that was equal to the mean of accuracies obtained with each chromosome. This result suggests that a chromosome can be considered as a genetically and statistically independent unit.

Results for imputation based on information from multiple breeds obtained in this study, basically confirm previous reports. Values of R using multi-breed information (Table 3) were considerably lower than R for imputation within breeds. Similarly, Hayes et al. (2012) obtained no advantage or, sometimes, worse results, for imputation based on information from multiple breeds, compared to single-breed information. Also, R for Simmental was lower than R for the other breeds. Dassonneville et al. (2012) also reported lower imputation accuracies in the French Blonde d’Aquitaine beef breed (around 5%) than in two dairy breeds. The lower imputation accuracy for Simmental may be partially explained by the fact that the Illumina 50K platform was not tested on the Simmental breed (Illumina 2011) and that the effective population size of the three breeds is very different, being higher for the Simmental than the other breeds (Medugorac et al. 2009, Hagger 2005, de Roos et al. 2008).

Differences in the underlying structure (Ajmone-Marsan et al. 2012) of the three populations may impact imputation accuracies. Finally, the use of a multi-breed TP also did not give better accuracies in GEBV prediction than the single-breed scenario (Pryce et al. 2012, Hayes et al. 2009).

The impact of the SNP genotype imputation on the accuracy of DGV was small. Correlations between DGV and DGV_IMP were, on average, 0.96 for all traits for imputation from 7K to 50K, and 0.89 for imputation from 3K to 50K. Similar results were obtained by Berry and Kearney (2011), who reported an average correlation of 0.97 across 15 traits for the 3K LDP. The lowest correlations between DGV and DGV_IMP were observed for imputation from 3K to 50K for protein content (0.86) and milk yield (0.89). The correlation between DGV and DGV_IMP was approximately the same (around 0.96) for all traits, when imputation was from 7K to 50K. Weigel et al. (2010) reported similar values, both for milk yield and protein content, and confirmed that DGV_IMP predictions improve if the number of SNPs on the LDP increases, both for protein content and milk yield. Therefore, the 7K chip seems to be an efficient imputation tool and the imputed genotypes could be used to correctly estimate DGV for milk yield, and fat and protein content.

Conclusions

This study demonstrates that the PLSR imputation method can efficiently impute missing genotypes from LDP to HDP. With this method, the same good results are obtained whether animals in the PP have parents in the TP or not. Moreover, the computing time was markedly lower than with Beagle. The PLSR method was applied chromosome-wise and the results indicate that imputation accuracies are higher when the number of SNPs in the X matrix is high. However, combining markers from several chromosomes did not increase the accuracy of imputation, which confirms that chromosomes are independent genetic and statistical units. The 7K LDP gave good results both in terms of R and DGV prediction. Similar to the 3K LDP, the multi-breed approach applied to the 7K scenario, did not yield better results than the single-breed approach.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

CD conceived the original ideas and wrote, under the supervision of NPPM and PAM, the first version of the SAS code. MC, RS and GG performed the analysis. GM contributed to the development of the ideas and algorithms. CD, MC and NPPM wrote the draft of the paper and all authors contributed in refining the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The research was funded by the Italian Ministry of Agriculture (Rome, Italy), grants SELMOL and INNOVAGEN, and from the PROZOO Project. Authors wish to acknowledge the Parco Tecnologico Padano and ANAFI for providing PROZOO data. Helpful comments and suggestions from the two anonymous reviewers are also acknowledged.

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Chapter 4

Maximum Difference Analysis: a new empirical method for genome-wide association studies

Abstract

The availability of high-density SNPs panels for humans and, recently, for several animal species has given a great impulse at genome-wide association studies toward the identification of genes associated with complex traits and diseases. Marker relevance is traditionally assessed by using the frequentist or the Bayesian approach. The first is the most used method being intuitive and easy whereas the second is more complicated than the former but has the advantage to verify prior information by a posterior probability of association. In this work we suggest a new empirical method for genome-wide studies that does not require explicit assumptions on data distribution and it solves the problem of false positive using a posterior probability that allows for the exclusion of random associations. This method, called Maximum Difference Analysis, was applied to find associations between single nucleotide polymorphisms and milk, fat and protein yield and fat and protein percentage in 2,093 Italian Holstein bulls. To validate the method, results were compared with annotated genes linked with traits under study and with results obtained in previous studies. The method was able to locate important gene as the *diacylglycerol O-acyltransferase 1* (*DGAT1*), the *ß-lactoglobulin* (*BLG*), the bovine casein gene cluster, the *prolactin receptor* (*PRLR*). These results confirm the ability of Maximum Difference Analysis to detect associations between markers and traits.

Introduction

The availability of high-density SNP panels has given a great impulse toward the identification of genomic regions associated to complex traits and diseases in humans and, recently, in several livestock species (Yang et al. 2010, Hayes and Goddard 2010). Even if SNPs are not always directly responsible for the observed phenotypic variation, they have been co-inherited together with unknown causal variants thus enabling the detection of genomic regions harboring the polymorphisms influencing traits or diseases. Cattle breeds are of particular interest for studying genetic differences due to the strong artificial selection they have been subjected (Hayes et al. 2009b, Qanbari et al 2010). Several genome-wide association studies (GWAS) pointed out associations between markers, production and functional traits in dairy breeds (Cole et al. 2009, Pryce et al. 2010, Hayes et al. 2010).

In spite of a relevant amount of information on genes and genomic regions that could be implemented in animal breeding, several issues remain to be addressed in GWAS. A first point is represented by theoretical assumptions on the genetic architecture of the trait under study. Standard linear models of quantitative genetics assume additive effects not considering interactions between genes. This fact may result in false positive associations (Platt et al. 2010). On the other hand, inclusion of factors such as epistasis, lead to a highly parameterized model structure (Morota et al. 2013). A further cause of spurious associations can be found in the stratification that exists in cattle populations, due genetic drift or artificial selection (Ma et al. 2012). Moreover, the genetic variance explained by markers is usually lower than estimates obtained by classical quantitative genetics through the implementation of the polygenic models that fits the genetic (co)variance between individuals using pedigree relationships (van Binsbergen et al. 2012). Finally, the sampling effect should be mentioned: apart from associations that deal with genes with an assessed major effect on phenotypes such as the *DGAT1* for milk production traits, very often significant SNPs found in a sample of animals are not confirmed in an independent sample. For example, Chamberlain et al. (2012) recently tested in a validation population, 423 SNPs declared significantly associated with milk production traits in different screening experiments. The association of only 72 markers with milk traits was finally validated.

A key point for association studies is represented by the criteria used to declare a marker as significantly associated to a specific trait. Since the beginning of genome scans aimed at investigating QTLs in livestock by using microsatellites markers, the problem of assessing a suitable threshold for the test statistics has been pointed out by many researchers. The two main issues are represented by the approximation of the test statistics under the null hypothesis and by the multiple hypothesis testing, i.e. several tests are carried out for this purpose, but many of them are not independent (Churchill and Doerge 1994).

In frequentist methods, the issue of multiple testing can be addressed using the Bonferroni correction that, however, is extremely conservative and usually discards almost all detected associations (Baldin 2006). An alternative empirical procedure is the permutation test (Churchill and Doerge 1994). It is remarkably less stringent, but considering the large number of makers currently tested in GWAS (tens of thousands), a high risk of false positives could be hypothesized.

On the other hand, the Bayesian approach requires several explicit assumptions about the prior probability of association (π), the prior parameter distribution and the effect size at truly associated SNP. These assumptions are needed for calculating the Bayes factor (BF). However, small differences in r□ could result in very diffe probability of association (Stephens and Balding 2009). Moreover, the BF evaluation requires complex computational procedure as the resolution of high-dimensional integrals and the posterior density distribution is unknown.

So the BF is approximated by using the Markov chain Monte Carlo analysis which, however, requires long computing time.

In this paper, an empirical method is presented for testing associations between SNP genotypes and milk production traits in dairy cattle. This new proposed method is termed Maximum Difference Analysis (MDA) because it is based on the comparison of genotypic frequencies between two groups of animals ranked according to a specific phenotype. MDA could be considered a different option because does not rely on prior distributions of marker effects, it is not characterized by a complex mathematical structure, and the significance of marker association is evaluated by using a posterior probability distribution obtained with a bootstrap resampling procedure.

In this study, the MDA was used to detect possible associations between SNP-genotypes belonging to Italian Holstein bulls and five productive traits: milk (MY), fat (FY) and protein yield (PY), fat (FP) and protein percentage (PP). Results were compared with previous associations reported in literature (Pryce et al. 2010, Meredith et al. 2012, Jiang et al. 2010). The Python code of MDA method is provided in this work as supplemental material [S2]

Results

Significant associations

A large number of SNPs were initially declared candidate for possible associations with one of the 5 traits under study, i.e. with the $MDA_{k,j}$>1.66 for at least one resampling (Table 1). In particular, more than 30,000 for MY, around 29,000 for PY and around 31,000 for FY, FP and PP associations were pointed out. Most of them, however, were considered false positive associations. If the threshold value for posterior probability of bootstrap

(p_{boot}) was fixed at 0.95, only a range of 0.5% - 1.8% of the original associated SNP were confirmed (Table 1).

Table 1 Number of SNPs associated with the trait for different threshold values.

	MY	FY	PY	FP	PP	Total
N° SPNs with MDZ >1.66	30,295	31,148	29,337	31,845	31,662	
N° SNPs with p_{boot} > 0.95	542	360	684	143	246	1,432
N° SNPs with p_{boot} = 1	51	21	65	26	43	169

For a threshold p_{boot} = 1, fewest markers were selected for each trait (Table 1). For MY, most of the selected SNPs were located on BTAs 14 and 20. For FY, the 25% of SNPs with a p_{boot} =1 were located on BTA2. Chromosomes 4, 9 and 20 showed the 10% each of significant associated SNPs for PY. For FP and PP, over the 95% of SNPs with p_{boot} =1 were distributed on BTA14 and BTA20 respectively. It should be remembered that these SNP were associated to the trait in all 10,000 times in the resampling procedure. Therefore the reliability of a possible association of these markers with the trait could be considered very high. Considering threshold for p_{boot} > 0.95, the highest number of significantly associated SNPs with MY and FY were identified on BTA2 whereas the lowest number were detected on BTA26 and BTA27. For PY, the highest number of markers was identified on BTAs 1, 7 and 8, whereas the lowest number was on BTA 26. In the whole genome scan, BTA14 presented the largest number of significant SNPs for FP whereas there were several autosomes with only 0 or 1 significant SNPs. Finally, respect the five considered traits, yield traits exhibited the largest number of significant markers genome-wide whereas PP had the highest number for an autosome (BTA20) (Table 2).

Table 2 Distribution of SNPs significantly ($p_{boot} > 0.95$) associated with the 5 traits in the 29 autosomes.

BTA	MY	FY	PY	FP	PP
1	26	19	47	8	17
2	38	45	27	9	12
3	26	12	39	5	4
4	26	10	41	8	5
5	26	13	28	6	7
6	25	16	27	2	9
7	31	28	45	3	5
8	29	15	45	1	6
9	24	19	24	5	4
10	29	9	27	3	2
11	26	22	36	0	11
12	9	14	20	0	6
13	27	17	29	8	7
14	18	7	27	37	12
15	8	5	8	1	6
16	22	7	18	2	7
17	24	8	16	6	6
18	10	9	16	3	7
19	15	14	26	6	5
20	28	20	33	13	65
21	20	8	27	1	3
22	6	9	11	3	1
23	12	4	15	1	17
24	8	8	8	1	4
25	5	3	8	4	3
26	2	8	5	1	4
27	5	2	9	4	2
28	8	6	11	1	0
29	9	3	11	1	9
total	542	360	684	143	246

BTA27 resulted as the chromosome with less significant SNPs for all traits analyzed. Among the associated markers with $p_{boot} > 0.95$, several SNPs influenced more than one trait (Table 3).

Table 3 Number of SNPs associated with one or more traits.

N° of traits	N° of SNPs
1	1,166
2	221
3	44
4	1
5	0

In particular 221 SNPs were shared by two traits, 44 SNPs by three traits and 1 SNP was in common with four traits. No significant marker was associated with all the five considered traits.

The Manhattan plots for BTAs 6, 11, 14, and BTA20 are reported in Figures 1-4, respectively.

Figure 1 Plot of SNPs detected for traits and annotated genes on BTA6. The horizontal lines indicate $p_{boot} = 0.95$

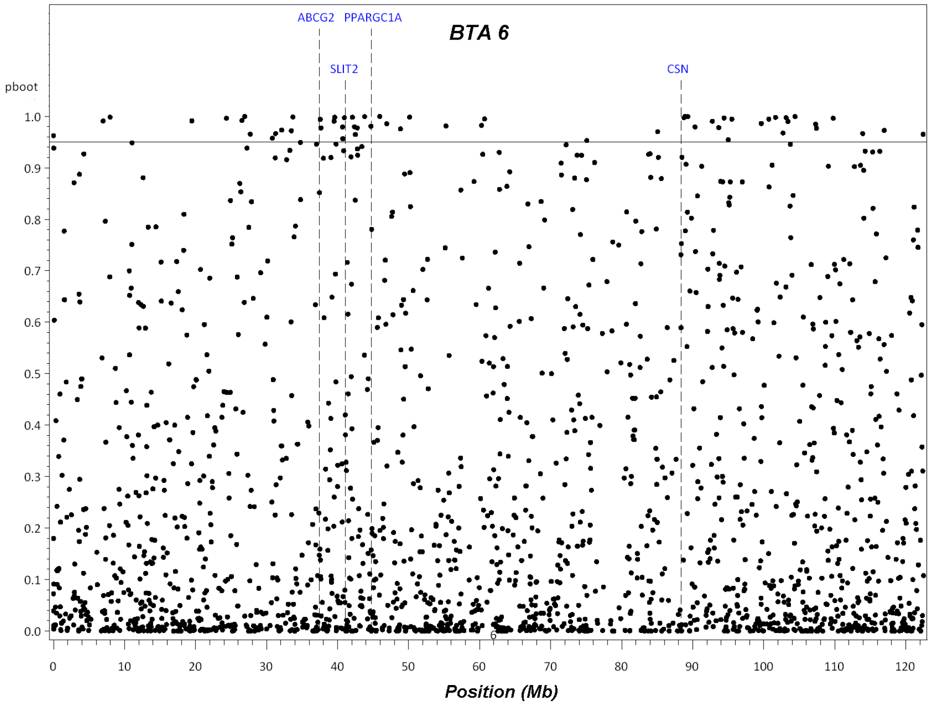


Figure 2 Plot of SNPs detected for traits and annotated genes on BTA11. The horizontal lines indicate p_{boot} = 0.95

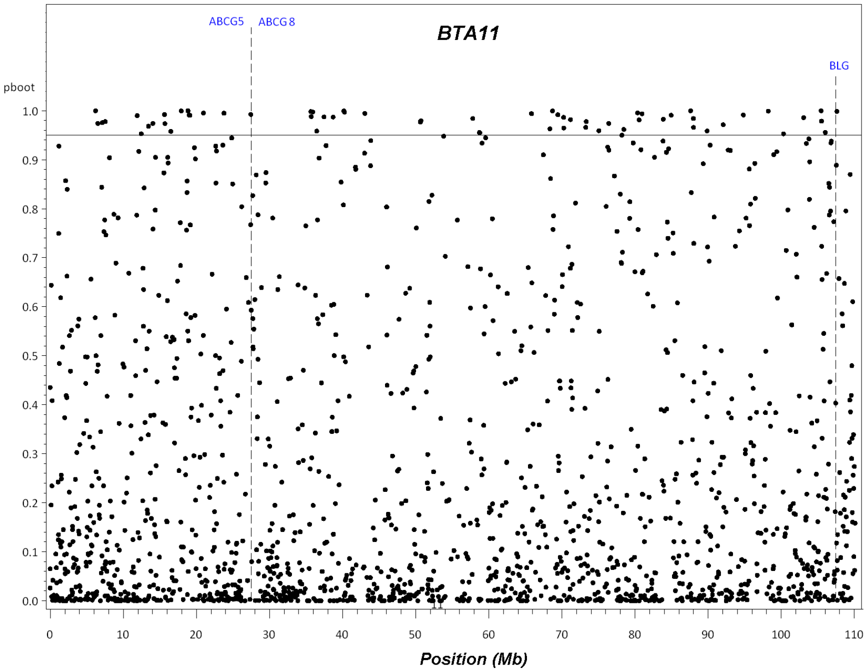


Figure 3 Plot of SNPs detected for traits and annotated genes on BTA14. The horizontal lines indicate p_{boot} = 0.95

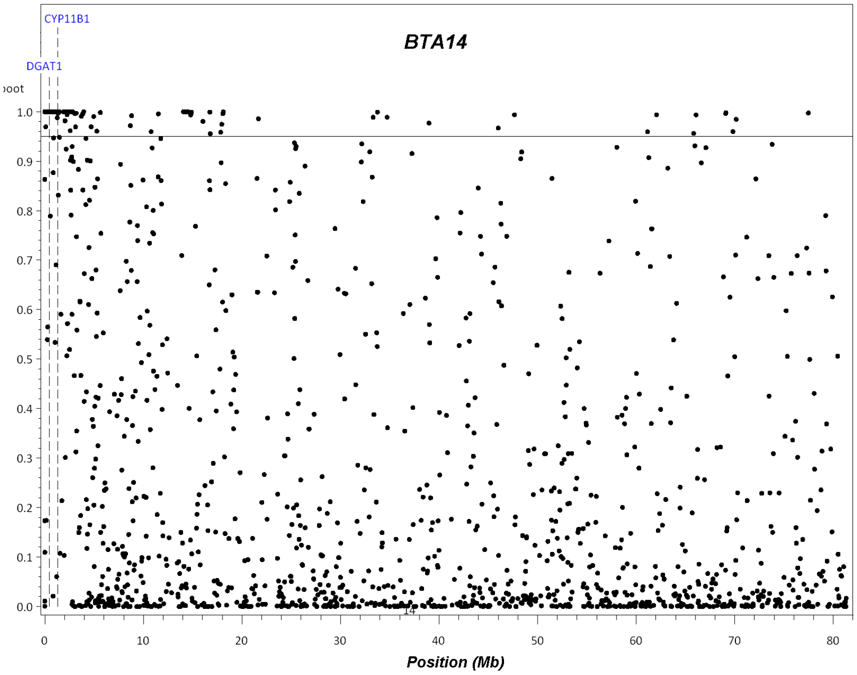
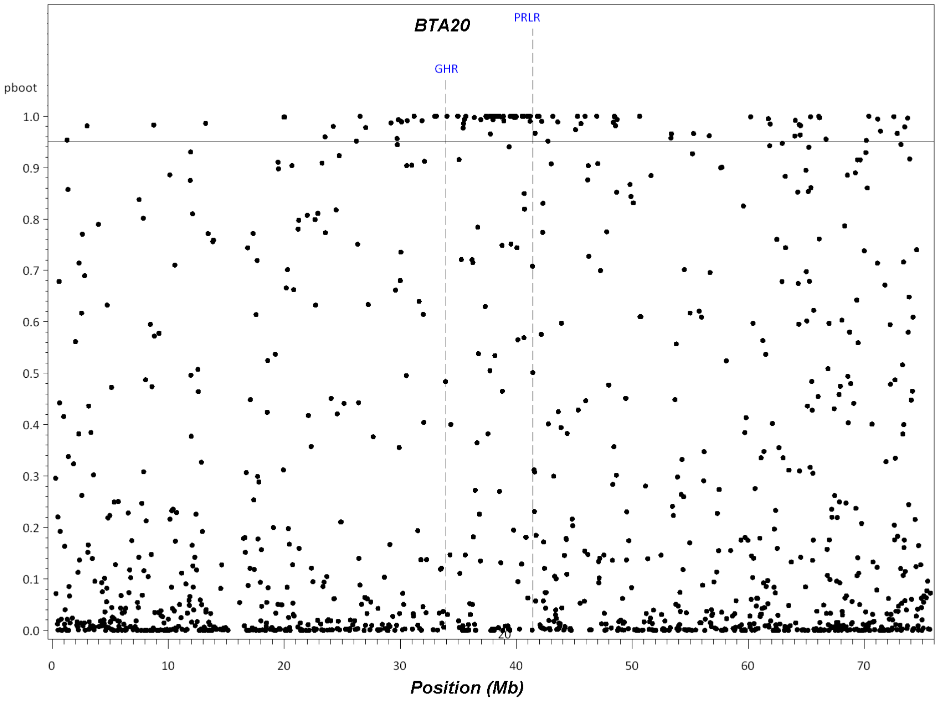


Figure 4 Plot of SNPs detected for traits and annotated genes on BTA20. The horizontal lines indicate p_{boot} = 0.95



A list of markers associated with all traits under study represented above the horizontal line (p_{boot} =0.95), is reported in Table S1 [Supplemental material].

SNPs declared associated with a trait in the MDA were used for a gene discovery study. In particular, MDA associated SNPs were compared with markers and annotated genes detected in previous association studies (Pryce et al. 2010, Meredith et al. 2012, Jiang et al. 2010, Hayes et al. 2009a, Flori et al. 2009, Cole et al. 2011).

Milk yield

On whole genome, a total of 542 SNPs with a p_{boot} > 0.95, were identified as significantly associated with MY (Table 1). Among these, on BTA14, 4 significant SNPs corresponded to markers detected by Jiang et al. (2010), 9 corresponded to markers detected by Meredith et al. (2012) and Pryce et al. (2010). Some of these markers are located in a region spanning from 76Kbp to 679Kb (Figure 3) that harbors the *diacylglycerol O-acyltransferase 1* (*DGAT1*) locus. Moreover, 8 significant SNPs located between 30-41 Mb on BTA20, were the same reported by Meredith et al. (2012) (Figure 4). MDA highlighted a SNPs associated with MY (p_{boot}>0.95) on BTA6. This marker was located at 37.5 Mb and it identifies a cluster of genes *ATP-binding cassette, sub-family G (WHITE), member 2* (*ABCG2*)*, polycystic kidney disease 2* (*PKD2*)*, secreted phosphoprotein 1* (*SPP1*) already proposed by several authors as candidates for milk QTL (Ron and Weller 2007). Moreover, the Hapmap 26848-BTC-038527 marker (44.7 Mb), highlighted on BTA6, was close to the *peroxisome proliferator-activated receptor gamma, coactivator 1 alpha* (*PPARGC1A*) gene, which has been reported to be associated to milk traits (Ogorevc et al. 2009). Three significant SNPs (Hapmap42161-BTA26363, BTA-92644-no-rs and ARS-BFGL-NGS-65409) from 41.2 Mb to 41.6 Mb were highlighted on BTA20, where *PRLR* locus maps. In this study the *PRLR* polymorphism is in agreement with the results of Zhang et al. (2007) in Chinese Holstein and Wang et al. (2012) in German Holstein-Frisian population.

Fat yield

The MDA method highlighted 360 SNPs (Table 1). Several of them were close to annotated genes known to affect lipid metabolism as *DGAT1*, *glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1* (*GRINA*), *alkylglycerone phosphate synthase* (*AGPS*), *vasoactive intestinal peptide* (*VIP*), *ATP-binding cassette, sub-family G (WHITE), member 5* (*ABGC5*), *ATP-binding cassette, sub-family G (WHITE), member 8* (*ABCG8), lysophosphatidylglycerol acyltransferase 1* (*LPGAT*1). Moreover, 4 SNPs on BTA14 and 8 SNPs on BTA 20 were the same SNPs declared associated with FY by Meredith et al. (2012) in Irish Holstein Friesian.

Protein yield

For the PY trait, 684 significant SNPS were detected (Table 1). Two SNPs were close to casein cluster on BTA6 (88.8 Mb), and one to the *β-lactoglobulin* locus on BTA11 (107.6 Mb). It is known that *caseins* (*CSNs)* and *β-lactoglobulin* genetic polymorphisms are related to milk production traits (Boettcher et al. 2004, Lunden et al. 1997). Four relevant SNPs nearby to the *DGAT1* gene were the same reported in previous studies (Pryce et al. 2010, Meredith et al. 2012, Jiang et al. 2010). In the central portion of BTA20, a well-known major QTL affecting the PY, but also PP and MY, was identified using the MDA. This relatively narrow region contains the *Growth hormone receptor* (*GHR*) and the *PRLR* loci. In particular the significant marker ARS-BFGL-NGS-118998 positioned at 34 Mb was found to fall within the *GHR* gene. This marker was the same reported by Jiang et al. (2010) for Chinese Holstein. The F279Y polymorphism in *GHR* was associated with a strong effect on milk yield and composition (Zhang et al. 2007) and it was considered responsible for the phenotypic variability in Holstein-Friesian milk (Plante et al. 2001, Blott et al. 2003).

Fat percentage

Most of 143 significant SNPs associated to FP (Table 1) were located on BTA14 and BTA20 (37 and 13 respectively). 34 out of 37detected SNPs on BTA14 and 5 out of 13 on BTA 20, respectively, were in common with the markers selected by Meredith et al. (2012) in Irish Holstein Friesian. 25 markers located on BTA14 were in common with SNPs detected by Jiang et al. (2010) in Chinese Holstein and 27on BTA 14 and 8 on BTA 20 were shared with Pryce et al. (2010) on American Holstein bulls, respectively. On BTA 14, all the significant SNPs detected were contained in a region spanning from 50 Kb to 5,000 Kb where a known QTL for milk traits was located. Figure 3 shows a region crowded of significant SNPs near the centromere where *DGAT1* locus was positioned.

Protein percentage

For PP a total of 246 significant SNPs were discovered (Table 1). 49 out of 65 and 45 out of 65 significant SNPs detected on BTA20 were in common with Meredith et al. (2012) and Pryce et al. (2010). Moreover, 13 out of 65 markers were in common with SNPs detected by Jiang et al. (2010). In Figure 4 a dense region of SNPs (between *GHR* and *PRLR* loci) could be observed. A considerable number of significant SNPs associated with PP were detected on BTA1 and BTA23 (Table S1).

Discussion

In the present work a method for GWAS was developed and tested on 2,093 Italian Holstein Frisian bulls for detecting associations between SNP markers and five dairy traits. The MDA approach was able to select 1,432 significant SNPs spanning the entire genome. This number of associated markers is comparable with results obtained in analogue studies developed by using common GWAS approaches (Pryce et al. 2010, Meredith et al. 2012, Jiang et al. 2010, Kolbehdari et al. 2009, Mai et al. 2010). The significant markers were distributed across all 29 autosomes and the positions were generally in agreement with those reported in literature (Meredith et al. 2012, Jiang et al. 2010, Khatkar et al. 2004, Smaragdov 2006). The number of significant markers reflected the assessed genetic architecture of traits: more relevant SNP were found for yield in comparison with composition traits. Actually it is well known that the genetic control of milk composition traits could be ascribed to a relatively small number of genes with a large to moderate effect (Hayes et al. 2010, Grisart et al. 2002) whereas a stronger polygenic background could be hypothesised for yield traits.

The whole genome scan confirmed, as expected, the important role of major QTLs for milk traits on BTA14 (Grisart et al. 2002, Bennewitz et al. 2003) and BTA20 (Blott et al. 2003). In addition, MDA highlighted candidate QTLs on BTA2 for MY and FY, and on BTA7 and BTA8 for PY. These three chromosomes have been recently investigated by other authors for association with milk traits (Buitenhuis et al. 2013, Gray et al. 2012).

BTA6 is one of the most studied chromosomes for milk QTLs within and between cattle breeds [37-41]. In a meta-analysis investigation, Khatkar et al. (2004) reported at least 77 QTLs on BTA6 with around 60% of them involved in milk production traits. The MDA was able to find, on BTA6, three significant SNPs mainly associated with PP were found at about 40Mb, where the *slit homolog 2 (Drosophila)* (*SLIT2*) gene maps (Figure 1). This locus encodes a protein expressed during neuronal development and also in mammary gland during ductal morphogenesis (Strickland et al. 2006).

On BTA11, MDA detected one SNP associated with FY, (BTB-01550704) located close to *ABCG5* and *ABCG8* at 27.4 Mb. These genes are believed to be involved in the mammalian cholesterol balance and in the physiology of intracellular lipid transport (Schmitz et al. 2001). Viturro et al. (2006) hypothesized their potential role in lipid trafficking and excretion during lactation. Many association studies identified QTLs affecting FY and FP in the centromeric region of BTA14 (Meredith et al. 2012, Jiang et al. 2010, Ogorevc et al. 2009, Viitala et al. 2003). The *DGAT1* locus is an enzyme that catalyzes the synthesis of diacylglycerols involved in several biological processes (Mai et al, 2010). The association between polymorphisms in the *DGAT1* gene and milk fat content in dairy cattle has been evidenced in several breeds (Grisart et al. 2002). To explain the genetic variability presented by milk production traits Bennewitz et al. (2003) hypothesized the existence of a further QTL with possible epistatic effects in linkage with the *DGAT1* locus. This second QTL was localized closely to the gene *cytochrome P450, family 11, subfamily B, polypeptide 1* (*CYP11B1*) (Mai et al, 2010). In cattle this enzyme is involved in the lipogenesis and lipolysis mediated by corticosteroids (Kaupe et al. 2007). For all five milk traits considered in this study, MDA highlighted, on BTA14, several significant SNPs in the region where *DGAT1* and *CYP11B1* loci are located. These SNPs were the same observed by Jiang et al. (2010) in Chinese Holstein population, Pryce et al. (2010) in bulls of American Holstein and Meredith et al. (2012) in Irish Holstein-Frisian. Moreover, other six significant SNPs, delimited a QTL region spanning from 62Mb to 69 Mb, associated to PY, PP and MY phenotypes were found when MDA was applied on BTA14. Within this genomic segment a QTL affecting production traits in Holstein cattle was already detected (Heyen et al 1999, Ashwell et al. 2004).

On BTA22, at 55.7 Mb, the *Ghrelin-obestatin prepropeptide* (*GHRL)* (Hapmap41094-BTA83358), associated with FP trait, was pointed out. This gene encodes a precursor that generates two hormones: ghrelin and obestatin. The first molecule is involved in the regulation of the growth hormone release and influences the body general metabolism. Recently, *GHRL* was proposed as candidate gene for milk production traits (Gil et al. 2011). Indeed, a polymorphism affected FY, FP and PP was observed in water buffalo and Polish Holstein-Friesian (Gil et al. 2011, Kowalewska-Luczak et al. 2011).

In addition to the QTLs discussed above, MDA method confirmed two QTLs affecting milk traits previously reported in literature. The significant marker Hapmap43212-BTA-23629 on BTA4 pointed out the *CD36 molecule (thrombospondin receptor)* (*CD36*) locus already reported by Lemay et al. (2009) in an analysis of genes expressed in cattle during lactation. The Hapmap41328-BTA-66089 on BTA29 focused the *fibroblast growth factor 4*

(*FGF4*) gene. Hayes et al. (2009a) speculate about the presence of a QTL for MY in BTA29 asserting that the strongest candidate gene for harboring a mutation affecting the trait was *FGF4*. Also Pryce et al. (2010) considered this region like an area for further investigation in Holstein and Jersey cattle breeds. Indeed, during mammary gland morphogenesis and involution this gene regulates the apoptosis and induces the end of lactation (Monks and Henson 2009). Using MDA two new intriguing QTLs not previously associated to milk production traits were detected. On BTA2 the marker ARS-BFGL-N GS-110442 was significantly associated with FY. This marker is located at 137 Mb where the *phospholipase A2* gene cluster containing the *phospholipase A2, group IIA (platelets, synovial fluid)* (*PLA2G2*) maps. This gene cluster encodes for a group of enzymes involved in the hydrolysis of phospholipids into fatty acids and other lipophilic molecules. The expression level of transcripts varied between dry period and lactation in mammary gland (Golik et al. 2006).

On BTA24 two significant markers, the BTB-00885200 and BTB-00885058 were associated with MY. These SNPs were positioned close the *Aquaporin 4* (*AQP4*) gene. Aquaporins (AQPs) is a family of ubiquitous membrane proteins involved in the transport of water and a wide range of solutes (Gomes et al. 2009). Recently, a functional role for *AQP1, AQP3, AQP4, AQP5* and *AQP7* during the production and secretion of bovine milk was confirmed in an immunohistochemical study conducted by Mobasheri et al. (2011). Therefore, on the basis of results of the present study and of previous investigations, *PLA2G2* and *AQP4* could be considered as potential candidate genes for dairy traits in cattle.

In the present work, as in many previous studies (Pryce et al. 2010, Mai et al. 2010, Smaragdov 2006), 266 SNPs showing significant effects on more than one trait have been detected. The genetic correlation can be the result of pleiotropic effects of single QTL affecting more than one trait or of linkage disequilibrium between two or more QTLs each affecting one trait only (Bolormaa et al. 2010). Therefore, the pleiotropic action of QTLs should be considered when animal will be selected for a particular breeding goal. More detailed investigations, such the use of much denser marker map, will be necessary to move from the marker associations toward the discovery of causal mutations underlying economically important traits in dairy cattle.

Materials and Methods

The data

Data consisted of SNPs genotypes belonging to 2,093 Italian Holstein bulls, born between 1979 and 2007. Animals were genotyped with the Illumina 50K BeadChip. Only SNPs located in the 29 autosomes, with a call rate higher than 2.5% were retained for the analysis. Missing genotypes in each single SNP were imputed according to the most frequent allele at that locus. After editing, 49,933 SNPs were retained. Genotypes were coded as the number of copies of one SNP allele it carries, i.e. 0 and 2 for homozygous alleles, 1 for heterozygous alleles. Phenotypes were polygenic estimated breeding values for milk yield (MY), protein yield (PY) fat yield (FY), fat percentage (FP) and protein percentage (PP) supplied by the Italian Holstein Association (ANAFI).

The MDA method

MDA is an empirical method based on the comparison of the genotypic frequencies recorded in two different groups of animals ranked according to a particular trait (T).

Let *n* the number of animals in whole data set (A) and S a subset containing *p* individuals (*p* < *n*) randomly sampled from A. The MDA starts by sorting animals in S according to T. Two groups, each with p_{bw} individuals ($p_{bw} << p$) are then selected from S. They consist of the top (B) and bottom (W) ranked animals. B and W are, therefore, two disjoint subsets of S which contain animals with a different genetic merit for T. Thus animals belonging to B and W should be genetically more similar within each group than between groups. The next step is the calculation of the genotypic frequencies for each SNP, both in B and W, and the identification of the genotype having the largest frequency (f_B) of animals in B . The maximum difference is then calculated as the difference between f_B and the frequency of the same genotype in W (f_W). An example is reported in (Table 5). SNP_1 has the maximum frequency for the genotype 2 (f_B= 58), while in W, the frequency of the same genotype is f_W= 26. Thirty-two represents the maximum difference (MD) between the genotypic frequencies for the SNP_1

$$MD = f_B - f_W = 58 - 26 = 32$$

Table 5 Genotypic frequencies evaluated both for SNP in best (B) and worst (W) subset. The maximum difference (MD) between genotypic frequencies in B and W is also reported.

Subset	Genotype	Snp_1	Snp_2	Snp_3	Snp_4	…
B	0	12	**78**	20	**40**	…
	1	30	20	**65**	38	…
	2	**58**	2	15	22	…
W	0	20	**40**	25	**75**	…
	1	54	51	**65**	15	…
	2	**26**	9	10	10	…
	MD	32	36	0	-35	…

A marker *i*, located on autosome *k* with a large value for $MD_{i,k}$ (max value equal to p_{bw}) is considered a putative candidate for association with T. Low or negative MD values may indicate that the locus is not involved the genetic determinism of the trait (genotypic frequencies f_B and f_W are similar) or that the predominant allelic combination at that locus is not favorable for T. After standardization, according to MD mean and standard deviation of the *k-th* chromosome, $MD_{k,i}$ can be considered a random variable approximately normally distributed with mean zero and standard deviation 1. In the present paper, a marker was declared positively associated with T if its standardized $MD_{k,i}$ value was greater than 1.66.

A test for possible false positive associations of candidate SNPs found in the previous step was then developed by using a bootstrap resampling procedure without replacement. The size of the S subset was fixed at p=1,500 whereas the dimension of both B and W groups was set at p_{bw}=100. For each marker, N=10,000 randomly subset S were generated by resampling and the MDA was calculated each time. At the end of the resampling procedure, a frequency value, f_i, was calculated for each SNP. This value indicates how many times a marker was flagged as associated to T (MD >1.66) in the bootstrap procedure. The posterior probability (p_{boot}) of association between T and the i^{th} marker was the calculated as:

$$p_{boot_i} = \frac{f_i}{N}$$

A level of 0.95 of significance for p_{boot}, was considered indicating association between markers and traits.

The MDA procedure was applied on whole genome and to the goodness of method was mainly evaluated performing the analysis on four chromosomes (BTA6, BTA11, BTA14 and BTA20) known to harbor genes affecting milk production traits. Results obtained confirmed the effectiveness of the MDA procedure. The Baylor release BTAU_4.0 assembly, (http://genome.ucsc.edu/cgi-bin/hgGateway?org=cow) was used to locate the genes position and detected SNPs were considered associated to a gene if the locus was contained within a window of 250 Kb upstream and downstream the marker position.

Conclusions

MDA is a new empirical method able to discover associations between SNPs and quantitative traits. This technique was applied on a population of Italian Holstein bulls born between 1979 and 2007. Some among selected SNPs were detected close to well-known genes that affect milk production traits. Moreover, the MDA detected numerous markers in common with other association studies. These results confirmed that the MDA should be used to perform GWAS analysis.

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Chapter 5

Prediction of direct genomic values by using a restricted pool of SNP selected by maximum difference analysis

Introduction

In the last few years, several national breeding organizations have implemented genomic selection (GS) programmes for dairy cattle. Expected results are an acceleration in the breeding cycle and a gain in reliability of the genomic breeding value (GEBV) estimation (Van Raden and Sullivan 2010) compared to traditional EBV. However, genotyping costs and computational difficulties are two of the most important constraints that limit a wider diffusion of the GS. Several researches demonstrated that accuracy of genomic predictions strongly depend on the size of the training population (TP) that should be as large as possible (Goddard and Hayes 2009), and on the SNP platform density (Solberg et al. 2008, Habier et al. 2009). Actually, the Illumina 50K BeadChip high-density platform (HDP), is the most widely used chip in bovine GS programs. Costs for genotyping in a large population are, however, still high and become prohibitive when HDPs are used to genotype animals belonging to species as chicken, rabbit or sheep whose individuals have a lower economic importance. Moreover, the combination of a large genotyped population size and a high number of SNP variables requires huge amount of computer resources and long computational time.

Most of these problems could be partially overcome by using a reduced number of markers able to produce genomic predictions with good reliabilities. Actually, some low density SNP panels (LDP), cheaper than the 50K chip, are commercially available (the Illumina Bovine3K Genotyping BeadChip or the Illumina BovineLD Bead-Chip, for example) (Boichard et al. 2012). These panels have offered new opportunities to increase the number of animals involved in genomic selection programs. The resulting GEBV reliabilities are, however, lower than accuracies obtained by using the 50K platform (Solberg et al. 2008, Habier et al. 2009). For this reason, genotypes obtained from a commercial LDP are usually imputed to HDP by using suitable algorithms. Dimauro et al. (2013), for example, obtained up to 95% of reliability in DGV evaluation by using data imputed from the 7K to the 50K Illumina's chips for milk, protein and fat yield in Italian Holstein bulls. In a similar scenario, Segelke et al. (2012)

reported a negligible reduction in reliability of genomic predictions, averaged over 12 traits, of around 1% by using the Beagle package (Browning and Browning, 2009).

Several authors have proposed different strategies to select, for each trait under study, a suitable restricted pool of SNP from a HDP. This approach should assure that the pool of selected markers is the smallest as possible and that it is specific for the population and the trait under study. Vazquez et al. (2010), starting from the 50K Illumina's BeadChip, selected several SNP subsets that could be used to develop a LDP. Two strategies were adopted. In the first, evenly spaced SNP across the genome were selected; in the second, "best" SNP were chosen on the basis of their estimated effects on six traits of economic interest. Results indicated that LDP including "best" SNP outperformed predictions based on evenly spaced SNP. With 2,000 "best" SNP, the 95% of the predictive ability provided by the HDP was reached. Similar results were obtained by Zhang et al. (2011) who exploited simulated data to obtain the best combination of the number of SNP in LDP and the effective population size to respect a specific trait. As before 95% of reliability obtained by using an HDP was reached with the "best" combination.

In the present research, an alternative strategy for selecting a reduced number of SNP significantly associated with some traits from a HDP, is developed. The method was called Maximum Difference Analysis (MDA) and the association with traits was assessed on the basis of the differences between the genotypic frequencies of each SNP. The selected markers could be used to produce a custom low cost breed-specific assay to genotype animals involved in GS programs.

Aim of this work was 1) to assess the ability of MDA to detect SNP significantly associated with five productive traits, 2) to compare the direct genomic value (DGV) of the involved animals obtained by using both the MDA selected markers and the full original marker set.

Materials and methods

The data

Data consisted of SNP genotypes belonging to 2,054 Italian Holstein bulls genotyped with the Illumina's 50K BeadChip. Genotypes were generated into two research projects: SELMOL and PROZOO, funded by the Italian Ministry of Agriculture and Fondazione CARIPLO, respectively. Animals were ranked according with age: the 204 youngest bulls were flagged as prediction population (PP), whereas the remaining animals were considered as training population (TP). PP animals were excluded from the original dataset and used only in the direct genomic value (DGV) evaluation. Only markers located on the 29 autosomes were considered. Non mapped SNP, monomorfic markers and SNP with more than 2.5% missing values were removed. At the end of the data editing 39,555 SNP were retained. Missing genotypes at each single locus were imputed according to the most frequent allele. Genotypes were coded as the number of copies of one SNP allele it carries, i.e. 0 (homozygous for allele A), 1 (heterozygous) or 2 (homozygous for allele B). Phenotypes were deregressed proofs for milk (MY), fat (FY) and protein (PY) yield, fat (F%) and protein (P%) percentage calculated by the Italian Holstein Association (ANAFI)

The MDA approach

MDA is an empirical method based on the comparison of the genotypic frequencies recorded in two different groups of individuals selected to respect a particular trait T.

Let n the number of the involved animals and S a subset containing p-animals (p < n) randomly selected from n. The MDA starts with the sorting of S animals by T. Two groups, each with pb individuals (pb << p) are selected. The first group, named best (B), consists of the top ranked animals for T. On the contrary, the second group, named worst (W), contains individuals with the lowest values of T. B and W are, therefore, two disjoint subsets of S and the two groups contain animals whose T values are very different. As a consequence, we assume that animals belonging to B and W are genetically more similar within groups than between groups. In other words, B and W bulls should have allele combinations positively (B) or negatively (W) associated with the trait under study, respectively. To detect positively (P_SNP) and negatively (N_SNP) associated markers, the genotypic frequencies for each SNP are calculated both in B and W, respectively, and then compared. Table 1 shows an example of genotypic frequencies evaluated for some markers.

Table 1 Genotypic frequencies evaluated both for best (B) and worst (W) dataset. The maximum difference (MD) between genotypic frequencies in B and W is also reported.

Subset	Genotype	Snp$_1$	Snp$_2$	Snp$_3$	Snp$_4$	…
B	0	12	**78**	20	**40**	…
	1	30	20	**65**	38	…
	2	**58**	2	15	22	…
W	0	20	**40**	25	**75**	…
	1	54	51	**65**	15	…
	2	**26**	9	10	10	…
	MD	32	36	0	-35	…

P_SNP for a particular T are detected by considering, for each marker, the maximum genotypic frequency in B. For SNP1 (Table 1), for example, the maximum frequency, fB= 58, is obtained for genotype=2. In W, for the same genotype=2, the frequency is fW= 26. The difference MD1 = fB- fW = 32 represents the maximum difference (MD) between the genotypic frequencies for the SNP1. The MDs were evaluated for each SNP into a chromosome and for all chromosomes. MD can be considered a random variable approximately normally distributed and, after standardization within each chromosome, with mean zero and standard deviation one. Markers with high MD (max value equal to pb) are considered as P_SNP, whereas markers with low or negative MD indicate that the marker does not positively influence T. The i-th marker is considered positively associated with T if its MDi value is greater than 1,66. A test for possible false positive associations is then developed by using a bootstrap procedure to generate a posterior probability distribution. The original animals are N=10,000 times resampled. At each resample, the subset S which contains p <n individuals, is generated. In the present study, p was fixed equal to 1,220 and pb equal to 100. The MDA procedure was run on all the 10,000 S-subsets and SNP with MDi

>1,66 were retained. At the end of the resampling procedure, a frequency value (fi) was assigned to each SNP. This value indicated how many times a marker was flagged as a P_SNP in the bootstrap procedure. The posterior probability (pboot) of association between T and the i-th marker is calculated as:

$$pboot_i = \frac{f_i}{N}$$

At the end of the procedure only the P_SNP with a pboot greater than 0.80 were retained.

To select the N_SNP associated with T, the MDA was completely redeveloped simply changing the group where the MD is evaluated. In other words, if we consider the former example (Table 1), for SNP1 in W, the maximum frequency, fW= 54, is obtained for genotype=1. In B, for the same genotype=1, the frequency is fB= 30. The MD value fW-fB = 24 is calculated and the entire MDA procedure is repeated. At the end, a pool of N_SNP is selected.

Direct genomic value evaluation

DGV for milk, fat and protein yield, fat and protein content were calculated using both the about 40K original markers and the P_SNP+N_SNP selected in the MDA procedure. Effects of SNP markers on phenotypes in the TP population were estimated by using the following BLUP model:

$$y = 1\mu + Xg + e$$

where y is the vector of the deregressed proofs, 1s is a vector of ones, μ is the overall mean, X is the matrix of SNP genotypes, g is the vector of SNP regression coefficients treated as random, and e is the vector of random residuals. The overall mean (μ) and the vector (ĝ) of the marker effects estimated in the TP were used to calculate the DGV for PP as:

$$\hat{\mathrm{y}} = \mathrm{X}^{*}\hat{\mathrm{g}}$$

where $\hat{y}$ is the vector of estimated DGV and $\boldsymbol{X}^*$ is the matrix of SNP genotypes in PP. For each phenotype, the DGV for the PP was evaluated by using both all original markers and the P_SNP+N_SNP. Moreover, a number of evenly spaced markers equal to the MDA selected SNP were chosen across the entire genome. These SNP were used to evaluate the DGV of the PP to test the goodness of MDA SNP selection. Accuracies in DGV predictions were assessed calculating the Pearson correlations between the evaluated DGVs and the original deregressed proofs.

Results

Results of the MDA procedure are reported in Table 2 where, for each T, the selected P_SNP, N_SNP and their common markers into traits are displayed. Moreover, some identically markers were detected among two or more traits and, considering them only one time, the little number of 2,213 different markers were selected for all the involved traits.

Table 2 Number of MDA selected markers positively (P_SNP) and negatively (N_SNP) associated to each trait. The number of SNPs associated both positively and negatively (P_SNP+N_SNP) and the number of common SNPs between P_SPN and N_SNP are also displayed for each trait.

Trait	P_SNP	N_SNP	P_SNP+N_SNP	Common SNP
Milk yield	478	346	763	61
Fat yield	300	297	557	40
Protein yield	512	377	823	66
Fat %	215	210	380	45
Protein %	286	264	515	35

DGV accuracies for the PP evaluated by using all markers (All_SNP) of the chip after editing, the MDA selected SNP and an equal number of evenly spaced markers are displayed in Table 3.

Table 3 Direct genomic values (DGV) accuracies evaluated by using the MDA selected SNP (P_SNP+N_SNP), all the original SNP (All_SNP) and 2,200 evenly spaced SNP.

Markers	DGV accuracies for				
	Milk yield	Fat yield	Protein yield	Fat %	Protein %
All_SNP	0.43	0.41	0.39	0.44	0.51
P_SNP+N_SNP	0.45	0.51	0.39	0.61	0.57
Evenly spaced	0.41	0.25	0.24	0.35	0.31

For each trait, accuracies in DGV prediction for P_SNP+N_SNP were greater or nearly equal than values obtained with All_SNP. In particular, accuracies for fat percentage and fat yield were around 0.17 and 0.10 greater than results obtained with All_SNP, respectively. Finally, DGV accuracies obtained by using 2,200 evenly spaced markers were lower than values obtained both with All_SNP and P+N_SNP.

Discussion

The MDA procedure was able to select a reduced pool of associated markers for each trait. The number of the N_SNP was nearly equal for every T, apart from for protein yield, where the number of P_SNP was 25% greater than the number of N_SNP. Moreover, F% shows the lowest number of both P_SNP and N_SNP respect to the number of associated markers for the other traits. Particularly important are markers in common to P_SNP and N_SNP. These markers have both a positive and a negative impact on the trait. All common SNP are homozygous with genotypes, for example, AA in P_SNP and BB in N_SNP or vice-versa. In consequence, these common SNP have a positive influence on the trait for the best animals, negative in worst animals. Among the P_SNP+N_SNP selected for each T, several markers are common to two or more traits and, in consequence, the total number of selected SNP is lower than the simple sum of P_SNP+N_SNP across the traits. Our study suggests that 2,213 markers could be enough to turn out a custom LDP to genotype Italian Holstein bulls. The obtained data could be used to evaluate the genetic merit of the involved animals to respect the six traits used in selecting markers with the MDA procedure. This procedure could be useful to lay out a GS program for livestock species different from bovine. First, a TP genotyped with a HDP should be created. Then, a restricted pool of markers should be selected by using the MDA procedure. A PP would be created by using the LDP which contains the MDA selected markers. At the end, the overall costs of the genomic breeding program should be reduced.

DGV accuracies obtained by using the P_SNP+N_SNP (table 3) were on average nearly equal or, sometimes, greater than accuracies obtained by using all SNP. In particular accuracies for fat and, partially, for protein percentage are considerably greater than values obtained with all original SNP. Moreover, the number of P_SNP+N_SNP selected for the two percentage traits is the lowest among the traits under study.

Conclusion

The MDA method applied to 2,054 Italian Holstein bulls selected 2,213 markers that could be used to develop a LDP to genotype animals under selection. Accuracies of the estimated DGV were equal or greater than accuracies obtained by using all SNP. Therefore, no SNP imputation to a HDP is required if the MDA selected markers are used. This results in a considerable reduction in the computational time as well as a reduction costs.

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Chapter 6

Conclusions

One of the most important issues in genomic selection is the estimation of the effects of tens of thousands of SNPs by using only few thousands of genotyped animals. Multivariate dimension reduction techniques, such as the principal component analysis (PCA), could be an alternative approach to other methods, such as BayesB and BayesL. Using the PCA, the contribution of each marker is estimated taking into consideration the total SNP variance structure, whereas the reduction of both data dimensionality and computational complexity do not decrease the accuracy of GEBV evaluation (Macciotta et al. 2010). All PCAs start from the variance-covariance matrix obtained from the X matrix of data. In Chapter 2, the impact of the rank of the variance-covariance matrix on GEBV accuracy is studied when the PCA technique is used to reduce the dimensionality of the data. Results indicated that, if the variance-covariance matrix has a full rank, the reduction of the data dimensionality by using the PCA does not worsen the accuracy of GEBV predictions. In particular, the study evaluated the accuracy of GEBV when the number of animals in a reference population decreased comparing two scenarios: one where the PCs were extracted genome-wide (ALL) and another where PCs were extracted separately by chromosome (CHR). In ALL, the GEBV accuracies became soon unsettled as the number of animals decreased because the SNP variance-covariance matrix (S) was singular. Differently, in CHR, the S matrix of each chromosome had a full rank and, consequently, the GEBV accuracy remained stable as long as the number of animals remained greater than or equal to the number of SNPs in the chromosomes. Moreover, obtained GEBV accuracies were always better for CHR than for ALL. Results of the present study can be used to fix the size of the reference population at a value nearly equal to the number of SNPs in the largest chromosome when the PCA technique is used.

Another important issue that affects the genomic selection is the low number of animals involved in selection programs. Generally, only males and elite females are genotyped by using high-density platforms (Weigel et al. 2010). The reason is that their commercial price is high, thus limiting their use only to animal population with high economic value, such as cattle or swine. To increase the number of animals involved in breeding programs, cheaper low-density panels (LDP) could be used. However, to avoid a reduction in the accuracy of GEBV estimation, markers not present in economic chips are currently imputed to HDP. In Chapter 3, the partial least squared regression (PLSR) is proposed to impute missing genotypes from a LDP to a HDP. The study demonstrates that the PLSR imputation method can efficiently impute missing genotypes from LDP to HDP and requires much less time than the commonly used methods.

The study was performed on a single-breed and on a multi-breed and tested the ability of PLSR to impute from a LDP of 3K and 7K to a HDP with 50K SNP. In the single-breed approach, the accuracy of imputation using PLSR was approximately 90 and 94% for the 3K and 7K platforms, respectively; whereas the corresponding accuracies obtained with Beagle were approximately 85% and 90%. Moreover, computing time using the PLSR method was on average around 10 times lower than the computing time required by Beagle. Imputation accuracy obtained with PLSR was lower in the multi-breed than in the single-breed data. Moreover, in the single-breed approach, the impact of the SNP-genotype imputation on the accuracy of GEBV was small and the correlation between estimates of genetic merit obtained by using imputed versus SNPs of HDP was around 0.96 for the 7K chip.

In Chapter 4, a new empirical approach for GWAS is proposed. The method called Maximum Difference Analysis (MDA) could be an alternative to the frequentist and Bayesian methods that are usually used. MDA does not need any assumptions about genome architecture or data distribution. The obtained results were validated by comparing them with those published in other studies which used both frequentist and Bayesian approaches. MDA was applied to find associations between SNP and five quantitative traits: milk, fat and protein yield and fat and protein percentage. The MDA method was able to locate some well-known genes that affect milk production, such as *diacylglycerol O-acyltransferase 1* (*DGAT1*), *β-lactoglobulin* (*BLG*), bovine casein gene cluster, and *prolactin receptor* (*PRLR*). In addition, some hardly identified genes in other studies were located by MDA. For example, on BTA4, MDA located the *CD36 molecule (thrombospondin receptor)* (*CD36*) locus previously reported by Lemay et al. (2009) in an analysis of genes expressed in cattle during lactation. Moreover, on BTA29, MDA identified the *fibroblast growth factor 4* (*FGF4*) gene. Hayes et al. (2009) speculated about the presence of a QTL for milk yield in BTA29 asserting that the strongest candidate gene for harboring a mutation affecting the trait was *FGF4*. The results demonstrated the ability of MDA to detect associations between markers and traits.

Results obtained in Chapter 4 were then used to reduce the dimensionality of the data in a study proposed in Chapter 5. In this research, markers selected by MDA were used to evaluate the GEBV of the animals involved. Results indicate that accuracies obtained with the MDA selected SNPs are comparable with and sometimes better than results obtained by using all 54K markers. In particular, accuracies for fat percentage and fat yield were around 0.17 and 0.10 percentage units greater than the accuracy obtained with all SNPs, respectively. These results were obtained using 380 and 555 selected SNPs for fat percentage and fat yield, respectively, instead of the 39,555 SNPs available in HDP. The selected SNPs could be implemented in a cheaper customized LDP that could be used instead of a HDP. The results obtained in this chapter confirmed the goodness of MDA to select SNPs.

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Supplemental material (Chapter 4)

Table S1 List of significant SNPs detected using MDA method (pboot>0.95) for milk yield (MY), fat yield (FY) protein yield(PY) fat percentage (FP) and protein percentage (PP).

chr	position (Kp)	marker	Trait				
1	29	Hapmap52416-rs29016842	MY				
1	1,480	ARS-BFGL-NGS-39992		FY	PY		
1	1,937	BTA-120704-no-rs		FY			
1	2,486	ARS-BFGL-NGS-79093			PY		
1	2,581	BTB-00001612		FY			
1	3,211	ARS-BFGL-NGS-108686		FY			
1	4,071	BTB-01747944	MY	FY			
1	4,827	ARS-BFGL-NGS-111125	MY		PY		
1	9,576	ARS-BFGL-NGS-113570				FP	
1	10,144	Hapmap51183-BTA-19351				FP	
1	11,132	Hapmap38737-BTA-22640				FP	
1	14,322	Hapmap24335-BTA-127763			PY		
1	14,841	BTA-28028-no-rs			PY		
1	15,464	ARS-BFGL-BAC-13008				FP	
1	16,276	Hapmap41782-BTA-16216		FY			
1	16,444	BTB-01084253			PY		
1	16,958	Hapmap60239-rs29019581		FY			
1	17,455	Hapmap49012-BTA-109196		FY			
1	17,516	Hapmap48613-BTA-112066		FY			
1	17,699	Hapmap44269-BTA-67047			PY		
1	23,763	Hapmap32844-BTA-151959		FY	PY		
1	24,040	BTB-00010021		FY			
1	25,183	ARS-BFGL-BAC-6737		FY	PY		
1	25,510	BTA-49289-no-rs			PY		
1	25,790	BTA-49283-no-rs		FY		FP	
1	27,135	ARS-BFGL-BAC-5834			PY		
1	31,801	BTB-01335860				FP	
1	40,996	ARS-BFGL-NGS-20360	MY		PY		
1	41,169	BTB-01249999			PY		
1	42,390	Hapmap23514-BTA-150593			PY		
1	53,341	Hapmap38361-BTA-93866		FY			
1	54,738	Hapmap48975-BTA-99363	MY				
1	67,948	BTA-05186-no-rs			PY		
1	76,506	ARS-BFGL-NGS-116528			PY		
1	76,557	ARS-BFGL-NGS-15456			PY		
1	84,394	Hapmap40421-BTA-39479	MY		PY		
1	84,416	ARS-BFGL-NGS-69661	MY		PY		
1	93,284	Hapmap41804-BTA-24071	MY				
1	98,640	ARS-BFGL-NGS-96389				FP	
1	117,880	Hapmap24434-BTA-48171					PP
1	118,986	ARS-BFGL-NGS-10545		FY			
1	120,397	BTB-02013809	MY		PY		
1	120,444	BTB-01877866	MY				
1	121,510	BTB-00052125	MY		PY		
1	121,532	BTB-01476130	MY				
1	121,811	ARS-BFGL-BAC-13578	MY				
1	123,610	Hapmap43795-BTA-16918	MY				
1	123,918	BTA-49414-no-rs					PP

1	124,766	UA-IFASA-8594					PP
1	124,845	BTB-00055741					PP
1	124,989	ARS-BFGL-BAC-14851			PY		
1	125,016	Hapmap38963-BTA-50274			PY		
1	127,445	Hapmap41768-BTA-120174	MY				
1	127,680	ARS-BFGL-NGS-98257	MY				
1	128,191	ARS-BFGL-NGS-27011	MY		PY		
1	130,259	ARS-BFGL-NGS-116868			PY		
1	130,335	ARS-BFGL-NGS-99827	MY				
1	131,506	BTB-01662109					PP
1	131,552	Hapmap38448-BTA-92131					PP
1	131,657	Hapmap35746-SCAFFOLD181011_3284					PP
1	134,026	BTB-00059569	MY		PY		
1	136,344	Hapmap35582-SCAFFOLD40562_2432	MY				
1	137,001	ARS-BFGL-NGS-72308			PY		
1	137,924	ARS-BFGL-NGS-107390		FY			
1	140,707	ARS-BFGL-NGS-82122					PP
1	141,310	ARS-BFGL-NGS-73455					PP
1	141,469	ARS-BFGL-NGS-14502			PY		
1	141,510	ARS-BFGL-NGS-104662			PY		PP
1	142,224	Hapmap41574-BTA-54365			PY		PP
1	142,643	ARS-BFGL-NGS-22768					PP
1	144,559	ARS-BFGL-NGS-31728					PP
1	145,522	ARS-BFGL-NGS-106222					PP
1	145,578	BTB-00068200					PP
1	146,075	ARS-BFGL-NGS-82590	MY				
1	148,570	ARS-BFGL-NGS-65139			PY		
1	148,765	Hapmap47854-BTA-119090			PY		
1	148,854	ARS-BFGL-NGS-25873	MY				
1	148,912	ARS-BFGL-NGS-30170					PP
1	149,025	ARS-BFGL-BAC-12960			PY		
1	149,865	BTA-58315-no-rs	MY	FY	PY		
1	150,396	BTB-01975281			PY		
1	150,807	ARS-BFGL-BAC-5688		FY			
1	151,530	ARS-BFGL-NGS-105124			PY		
1	152,228	ARS-BFGL-NGS-110653			PY		
1	153,237	ARS-BFGL-NGS-105623			PY		
1	153,609	Hapmap60790-rs29024220					PP
1	154,731	ARS-BFGL-NGS-45342			PY		
1	155,843	ARS-BFGL-NGS-95240			PY		
1	157,424	Hapmap60257-rs29016165				FP	
2	373	ARS-BFGL-NGS-11180	MY				
2	1,030	Hapmap55208-ss46526613			PY		
2	2,241	ARS-BFGL-NGS-113652	MY		PY		
2	7,564	ARS-BFGL-NGS-90839			PY		
2	7,745	Hapmap60397-ss46527095			PY		
2	8,882	ARS-BFGL-NGS-37283				FP	
2	9,505	BTB-02094616	MY		PY		
2	9,590	Hapmap43273-BTA-47993			PY		
2	11,032	BTA-23383-no-rs			PY		
2	16,525	BTB-00080812		FY			
2	16,561	ARS-BFGL-NGS-100666					PP
2	16,632	Hapmap35360-SCAFFOLD145911_8451					PP
2	17,552	BTA-49719-no-rs	MY		PY		
2	17,932	BTA-04435-no-rs			PY		
2	18,171	ARS-BFGL-NGS-24246		FY			

2	19,105	ARS-BFGL-BAC-35137		FY			
2	19,202	Hapmap53232-rs29020795			PY		
2	19,256	Hapmap43615-BTA-54400	MY		PY		
2	20,045	ARS-BFGL-NGS-23300		FY			
2	20,235	Hapmap59161-rs29014139					PP
2	20,505	BTB-01112976		FY			
2	20,738	BTB-00083220		FY			
2	21,841	UA-IFASA-1574		FY			
2	21,961	ARS-BFGL-NGS-119036		FY			
2	22,381	Hapmap50971-BTA-46778		FY			
2	22,512	BTB-00085286		FY			
2	22,556	ARS-BFGL-NGS-32709		FY			
2	26,218	Hapmap51238-BTA-46810		FY			
2	26,428	Hapmap43586-BTA-46818		FY			
2	26,937	BTB-00088008	MY	FY	PY		
2	28,144	BTB-00091356		FY			
2	35,032	BTB-00088434			PY		
2	38,338	BTA-55603-no-rs	MY				
2	39,159	ARS-BFGL-NGS-28859	MY				
2	40,697	ARS-BFGL-NGS-60043				FP	
2	42,702	Hapmap38483-BTA-25757	MY				
2	42,771	BTB-01341517	MY				
2	43,208	BTA-47440-no-rs	MY				
2	43,229	Hapmap50154-BTA-91586	MY				
2	43,875	BTB-01242184	MY	FY	PY		
2	44,195	ARS-BFGL-NGS-115659	MY				
2	46,284	ARS-BFGL-NGS-93283					PP
2	48,820	Hapmap57575-rs29011345	MY				
2	52,255	BTA-47682-no-rs				FP	
2	53,269	BTA-47612-no-rs	MY		PY		
2	53,307	BTB-00098202	MY		PY		
2	54,237	BTB-00098707	MY	FY			
2	54,258	BTB-00098730	MY	FY			
2	54,637	BTB-00098773				FP	
2	55,505	ARS-BFGL-NGS-49789	MY				
2	56,762	Hapmap34718-BES7_Contig295_922	MY		PY		
2	58,164	BTB-01160816		FY			
2	59,718	BTA-19224-no-rs		FY			
2	64,245	ARS-BFGL-NGS-109852	MY	FY	PY		
2	65,110	ARS-BFGL-NGS-12099	MY				
2	65,525	ARS-BFGL-NGS-102253		FY			
2	65,624	Hapmap39338-BTA-47826			PY		
2	66,145	ARS-BFGL-NGS-100643	MY				
2	71,513	BTB-01941823	MY		PY		
2	74,662	ARS-BFGL-NGS-105719	MY				
2	79,981	BTB-02066351	MY				
2	80,592	BTA-48073-no-rs	MY				
2	80,678	BTB-00103137	MY				
2	80,701	ARS-BFGL-NGS-111158	MY				
2	83,588	ARS-BFGL-NGS-38368					PP
2	83,641	ARS-BFGL-NGS-114651				FP	
2	88,599	Hapmap47638-BTA-47957	MY				
2	97,062	BTA-24303-no-rs	MY	FY			
2	98,989	ARS-BFGL-NGS-2970		FY			
2	101,231	Hapmap51953-BTA-48787	MY	FY			
2	101,681	BTA-48456-no-rs					PP
2	101,850	Hapmap44082-BTA-48435					PP
2	101,883	ARS-BFGL-NGS-31792					PP
2	102,811	ARS-BFGL-NGS-5965				FP	

2	103,004	ARS-BFGL-NGS-107381				FP	
2	106,249	BTA-27937-no-rs		FY			
2	111,235	ARS-BFGL-NGS-108395		FY			
2	111,917	BTA-48867-no-rs		FY			
2	112,139	ARS-BFGL-NGS-82228		FY			
2	113,354	BTB-00111019		FY			
2	113,572	Hapmap48786-BTA-49002		FY			
2	113,931	Hapmap59378-rs29018764		FY			
2	116,200	Hapmap39834-BTA-49029		FY			PP
2	116,462	ARS-BFGL-NGS-274		FY			
2	116,798	ARS-BFGL-NGS-12225					PP
2	117,480	UA-IFASA-2047				FP	
2	118,686	Hapmap43218-BTA-26258					PP
2	122,549	ARS-BFGL-NGS-110996		FY	PY		
2	127,173	ARS-BFGL-NGS-109716				FP	
2	130,628	ARS-BFGL-NGS-118505	MY	FY	PY		
2	131,281	ARS-BFGL-NGS-34317		FY			
2	131,318	ARS-BFGL-NGS-21994		FY			
2	132,409	ARS-BFGL-NGS-41994	MY				
2	133,769	ARS-BFGL-NGS-36151		FY			
2	133,982	BTA-49769-no-rs					PP
2	134,028	ARS-BFGL-NGS-33709		FY	PY		
2	135,336	ARS-BFGL-NGS-110186			PY		
2	135,752	BTB-01978832		FY	PY		
2	136,681	ARS-BFGL-NGS-100214			PY		
2	137,038	ARS-BFGL-NGS-110442		FY			
3	7,009	BTB-01678060		FY			
3	31,193	ARS-BFGL-NGS-112694					PP
3	33,866	ARS-BFGL-NGS-113746	MY				
3	34,191	ARS-BFGL-NGS-40213	MY				
3	36,915	Hapmap41054-BTA-67528			PY		
3	37,863	ARS-BFGL-NGS-14022	MY				
3	39,299	ARS-BFGL-NGS-23295			PY		
3	39,339	ARS-BFGL-NGS-117495			PY		
3	40,024	ARS-BFGL-NGS-1886			PY		
3	44,394	Hapmap60335-rs29018229					PP
3	47,248	Hapmap32570-BTA-141315			PY		
3	49,688	Hapmap38207-BTA-19427			PY		
3	50,463	BTA-18980-no-rs	MY		PY		
3	50,486	Hapmap42865-BTA-18979	MY				
3	53,427	ARS-BFGL-NGS-23466	MY		PY		
3	54,013	Hapmap57732-rs29023272			PY		
3	56,417	Hapmap43965-BTA-89883			PY		
3	60,237	ARS-BFGL-NGS-103935			PY		
3	60,523	Hapmap43156-BTA-112841			PY		
3	60,996	Hapmap44119-BTA-67972			PY		
3	61,622	Hapmap43441-BTA-103289			PY		
3	62,908	ARS-BFGL-NGS-16054			PY		
3	65,463	BTA-68142-no-rs			PY		
3	65,706	BTB-00131364	MY	FY	PY		
3	65,833	BTB-01587097	MY		PY		
3	65,860	BTB-01587043	MY		PY		
3	66,290	Hapmap51550-BTA-111095	MY				
3	89,434	Hapmap47699-BTA-68564		FY			
3	91,680	ARS-BFGL-NGS-11694			PY		
3	93,948	Hapmap32684-BTA-89476			PY		
3	94,618	BTB-00141843			PY		

3	95,082	BTB-00143272			PY		
3	95,291	ARS-BFGL-NGS-100400			PY		
3	95,509	Hapmap41332-BTA-68635				FP	
3	95,552	BTB-00142550			PY		
3	95,572	BTB-00142497			PY		
3	97,188	Hapmap32622-BTA-155129			PY		
3	97,233	ARS-BFGL-NGS-40591	MY	FY	PY		
3	97,485	ARS-BFGL-NGS-73518		FY			
3	99,280	ARS-BFGL-NGS-111451					PP
3	103,040	Hapmap52129-rs29016142			PY		
3	103,945	BTB-00147905	MY		PY		
3	105,270	BTB-00150138	MY		PY		
3	108,063	ARS-BFGL-NGS-3713	MY		PY		
3	108,917	ARS-BFGL-NGS-32606					PP
3	109,147	ARS-BFGL-NGS-102829			PY		
3	109,604	ARS-BFGL-NGS-118597	MY				
3	111,281	ARS-BFGL-NGS-55043	MY				
3	111,321	ARS-BFGL-NGS-1038	MY				
3	111,371	BTB-00154062	MY				
3	112,790	BTB-00148908	MY				
3	114,946	Hapmap35089-BES2_Contig293_493				FP	
3	114,969	ARS-BFGL-NGS-66328				FP	
3	115,221	ARS-BFGL-NGS-117810				FP	
3	115,721	ARS-BFGL-NGS-87394			PY		
3	116,604	ARS-BFGL-NGS-34881				FP	
3	120,475	Hapmap56950-ss46526304	MY	FY	PY		
3	120,899	ARS-BFGL-NGS-32060		FY			
3	122,299	ARS-BFGL-NGS-115542	MY		PY		

3	125,025	ARS-BFGL-NGS-111207	MY				
3	125,046	ARS-BFGL-NGS-101315	MY				
3	125,114	ARS-BFGL-NGS-90439	MY				
3	127,818	ARS-BFGL-NGS-114675		FY			
4	21	Hapmap38667-BTA-28216			PY		
4	5,129	ARS-BFGL-NGS-91047				FP	
4	8,952	ARS-BFGL-NGS-106242				FP	
4	14,620	BTA-70786-no-rs	MY		PY		
4	14,645	ARS-BFGL-NGS-113152	MY		PY		
4	23,086	Hapmap33790-BTA-159878			PY		
4	23,125	Hapmap27025-BTA-159880		FY			
4	28,917	Hapmap48233-BTA-16470		FY			
4	29,093	BTB-01114634		FY			
4	36,814	Hapmap44123-BTA-70017			PY		
4	37,909	Hapmap34749-BES4_Contig461_1146			PY		
4	40,236	BTB-01885061			PY		
4	40,280	Hapmap24263-BTA-161141			PY		
4	41,684	Hapmap43212-BTA-23629			PY		
4	42,073	BTB-00176150	MY		PY		
4	42,107	Hapmap43659-BTA-70032		FY	PY		
4	42,909	BTB-01927917			PY		
4	43,207	BTB-00178712			PY		
4	44,482	BTA-70272-no-rs				FP	
4	44,896	ARS-BFGL-NGS-113663	MY				
4	46,361	Hapmap38427-BTA-70434	MY	FY	PY		
4	46,393	Hapmap49715-BTA-70437	MY	FY	PY		
4	48,626	ARS-BFGL-NGS-104842					PP
4	62,192	ARS-BFGL-NGS-71481				FP	

4	63,586	BTB-00192005				FP	
4	63,721	ARS-BFGL-NGS-3438				FP	
4	63,774	BTB-00191572				FP	
4	74,927	Hapmap42065-BTA-111154	MY				
4	75,091	BTB-01595788	MY				
4	75,135	BTB-01708864	MY				
4	76,782	ARS-BFGL-NGS-55672	MY				
4	78,755	ARS-BFGL-NGS-112329	MY				
4	82,824	Hapmap46191-BTA-101479	MY		PY		
4	84,629	BTB-01497290	MY				
4	84,788	BTB-00566744	MY				
4	86,308	BTA-96855-no-rs			PY		
4	86,342	BTB-01142755			PY		
4	86,402	BTA-96837-no-rs					PP
4	87,190	BTB-01278461			PY		
4	87,452	BTB-01443627	MY				
4	88,270	BTB-01257567					PP
4	89,021	BTA-65916-no-rs			PY		
4	91,593	ARS-BFGL-NGS-26218			PY		
4	94,254	ARS-BFGL-NGS-109045			PY		
4	94,520	ARS-BFGL-NGS-118100				FP	
4	95,049	ARS-BFGL-NGS-114724	MY		PY		
4	95,125	Hapmap32136-BTA-160383	MY		PY		
4	96,632	Hapmap25269-BTA-142380			PY		
4	96,652	ARS-BFGL-NGS-110997			PY		
4	97,467	BTB-00203494	MY		PY		
4	97,734	BTB-01502164	MY	FY	PY		
4	99,412	ARS-BFGL-NGS-38881	MY		PY		
4	99,532	ARS-BFGL-NGS-103036			PY		
4	99,587	ARS-BFGL-NGS-13008			PY		
4	99,998	Hapmap50564-BTA-110789	MY		PY		
4	100,994	ARS-BFGL-NGS-52947	MY		PY		
4	101,912	ARS-BFGL-NGS-77010			PY		
4	105,002	ARS-BFGL-NGS-36185			PY		
4	105,339	ARS-BFGL-NGS-25648			PY		
4	108,845	ARS-BFGL-NGS-5899			PY		
4	108,885	ARS-BFGL-NGS-76596			PY		
4	109,186	ARS-BFGL-NGS-3479			PY		
4	111,350	ARS-BFGL-NGS-39879					PP
4	117,784	ARS-BFGL-NGS-119857					PP
5	1,751	ARS-BFGL-NGS-109950	MY				
5	1,792	ARS-BFGL-NGS-104371	MY		PY		
5	1,905	BTB-01252633	MY		PY		
5	3,785	BTB-01357570			PY		
5	3,951	Hapmap55203-rs29023737					PP
5	12,945	BTA-23621-no-rs		FY	PY		
5	15,738	BTA-72768-no-rs			PY		
5	16,101	Hapmap36482-SCAFFOLD163485_1458			PY		
5	17,250	BTA-05007-rs29019174		FY			
5	20,301	Hapmap45956-BTA-74297			PY		
5	20,328	BTA-74300-no-rs			PY		
5	22,523	BTA-27242-no-rs	MY		PY		
5	23,091	BTA-06872-rs29021228	MY				
5	25,064	ARS-BFGL-NGS-44305	MY				
5	25,740	ARS-BFGL-NGS-29300	MY		PY		
5	36,496	BTB-01226567					PP
5	36,959	BTB-01496004	MY				

5	39,604	BTB-00225371	MY				
5	40,261	BTB-01635088			PY		
5	40,346	BTB-01832706			PY		
5	45,449	ARS-BFGL-NGS-18989					PP
5	46,500	BTB-00226702	MY				
5	46,856	BTB-01675520					PP
5	50,023	ARS-BFGL-NGS-98210	MY		PY		
5	50,069	ARS-BFGL-NGS-114616			PY		
5	54,953	ARS-BFGL-NGS-114918					PP
5	58,821	BTA-54940-no-rs			PY		
5	59,065	ARS-BFGL-NGS-3921			PY		
5	60,764	ARS-BFGL-NGS-4763	MY		PY		
5	64,566	Hapmap53993-rs29024740			PY		
5	64,749	ARS-BFGL-NGS-5504		FY			
5	70,327	ARS-BFGL-NGS-20849		FY			
5	72,205	ARS-BFGL-NGS-28026		FY			
5	72,980	Hapmap49622-BTA-46973		FY			
5	84,392	Hapmap57435-rs29016994					PP
5	86,756	BTA-74203-no-rs			PY		
5	88,530	Hapmap49859-BTA-109537					PP
5	91,221	ARS-BFGL-NGS-71971	MY	FY			
5	93,640	ARS-BFGL-NGS-11173			PY		
5	94,607	BTB-01602960	MY				
5	94,733	BTB-01278306		FY			
5	96,688	Hapmap50624-BTA-22932	MY				
5	97,370	Hapmap23365-BTA-156277		FY			
5	98,725	Hapmap33512-BTA-158274		FY		FP	
5	103,348	ARS-BFGL-NGS-29237	MY				
5	105,028	Hapmap59520-rs29021624				FP	
5	105,238	Hapmap46939-BTA-114206			PY		
5	108,587	ARS-BFGL-NGS-81143		FY		FP	
5	108,769	Hapmap36373-SCAFFOLD248777_1273				FP	
5	114,329	ARS-BFGL-NGS-118406		FY			
5	114,799	BTA-74965-no-rs	MY				
5	116,803	ARS-BFGL-NGS-6829				FP	
5	116,877	ARS-BFGL-NGS-32908				FP	
5	118,958	BTA-75110-no-rs	MY		PY		
5	119,005	Hapmap23876-BTA-143610	MY				
5	122,834	ARS-BFGL-NGS-78419			PY		
5	123,572	ARS-BFGL-NGS-36365			PY		
5	123,841	ARS-BFGL-NGS-1089	MY				
6	2	Hapmap27542-BTC-062507		FY			
6	6,995	ARS-BFGL-NGS-104900			PY		
6	7,962	BTB-00242529			PY		
6	19,485	Hapmap57362-rs29014889	MY				
6	24,357	Hapmap49541-BTA-24412	MY				
6	26,537	Hapmap27407-BTA-143867			PY		
6	26,946	ARS-BFGL-NGS-22019	MY	FY	PY		
6	27,720	ARS-BFGL-NGS-10082		FY			
6	30,817	Hapmap53749-rs29023061		FY			
6	31,265	ARS-BFGL-NGS-103412	MY		PY		
6	32,130	BTA-120439-no-rs					PP
6	33,499	Hapmap41633-BTA-75713			PY		
6	33,720	Hapmap27945-BTC-073459			PY		

6	37,564	Hapmap27503-BTC-033786	MY				
6	37,670	Hapmap26259-BTC-033526			PY		
6	39,509	BTB-00260450			PY		
6	39,604	Hapmap27818-BTC-035199		FY			
6	40,700	Hapmap60113-rs29017603					PP
6	40,741	BTB-00252917					PP
6	40,967	Hapmap57625-rs29027071		FY	PY		PP
6	42,099	BTB-00251852		FY			
6	42,376	Hapmap44280-BTA-75941		FY	PY		
6	42,512	Hapmap43676-BTA-75936			PY		
6	42,787	BTA-95818-no-rs			PY		
6	43,805	ARS-BFGL-NGS-42501		FY	PY		
6	44,699	Hapmap26848-BTC-038527	MY				
6	45,960	Hapmap49746-BTA-76106	MY		PY		
6	46,921	BTA-76116-no-rs					PP
6	48,864	Hapmap39620-BTA-113785					PP
6	50,150	BTA-18812-no-rs			PY		
6	55,267	BTB-00843793					PP
6	60,289	ARS-BFGL-NGS-106371			PY		PP
6	60,704	BTA-97854-no-rs		FY			
6	75,093	BTA-76827-no-rs				FP	
6	85,083	Hapmap43417-BTA-96760					PP
6	88,807	ARS-BFGL-NGS-82008	MY				
6	88,947	Hapmap57014-rs29019575	MY		PY		
6	89,355	ARS-BFGL-NGS-54753	MY				
6	90,356	Hapmap51409-BTA-122717	MY	FY	PY		
6	92,788	Hapmap40845-BTA-97263	MY				
6	93,683	BTB-01428718	MY				
6	94,434	ARS-BFGL-NGS-83066	MY				
6	95,043	BTA-77154-no-rs			PY		
6	95,528	ARS-BFGL-NGS-100802	MY				
6	99,688	BTB-00274080	MY		PY		
6	100,740	Hapmap10869-BTA-77464	MY				
6	101,684	Hapmap30053-BTA-161410	MY				
6	102,756	BTB-01791461	MY				
6	103,177	Hapmap50779-BTA-77533			PY		
6	103,431	ARS-BFGL-NGS-114582			PY		
6	104,437	ARS-BFGL-NGS-93120	MY	FY	PY		
6	107,336	Hapmap53924-rs29022499	MY				
6	107,444	ARS-BFGL-NGS-116512			PY		
6	109,808	ARS-BFGL-NGS-10777		FY			
6	113,960	BTB-01754370				FP	
6	116,998	Hapmap55397-rs29017692	MY				
6	122,474	ARS-BFGL-NGS-29384	MY				
7	15,513	Hapmap60436-ss46526689					PP
7	18,373	BTB-00296617			PY		
7	23,021	BTA-78558-no-rs					PP
7	23,447	Hapmap59434-rs29012267					PP
7	30,629	BTB-00549060	MY				
7	30,891	ARS-BFGL-NGS-21597	MY		PY		
7	33,067	UA-IFASA-4938		FY			
7	36,876	ARS-BFGL-NGS-17959	MY				
7	36,967	Hapmap32661-BTA-28979	MY				
7	36,997	ARS-BFGL-NGS-18669	MY				

7	37,319	ARS-BFGL-NGS-119880	MY				
7	37,339	ARS-BFGL-NGS-35666	MY		PY		
7	39,761	ARS-BFGL-NGS-30468			PY		
7	40,196	BTB-00368665				FP	
7	44,805	BTB-00309643	MY		PY		
7	45,473	BTB-00310653		FY			
7	50,743	ARS-BFGL-NGS-96012	MY		PY		
7	53,416	Hapmap58262-rs29024901					PP
7	55,024	Hapmap54976-rs29019286					PP
7	55,357	BTB-01961486			PY		
7	57,095	Hapmap52252-rs29011665				FP	
7	57,365	BTB-00311684			PY		
7	57,651	ARS-BFGL-NGS-33432	MY		PY		
7	57,712	BTB-00311926			PY		
7	57,747	BTB-00311957	MY		PY		
7	58,158	BTB-00313206			PY		
7	58,355	BTB-00314357	MY	FY	PY		
7	62,700	BTB-00316348	MY				
7	63,358	Hapmap36214-SCAFFOLD145184_7453			PY		
7	63,609	ARS-BFGL-NGS-113819	MY				
7	63,664	ARS-BFGL-NGS-109819	MY	FY			
7	65,093	ARS-BFGL-NGS-42452	MY	FY	PY		
7	69,587	BTB-00318531	MY	FY	PY		
7	72,070	BTA-112613-no-rs			PY		
7	72,456	BTB-01217472		FY	PY		
7	72,746	Hapmap48995-BTA-103787		FY	PY		
7	72,792	BTB-01557864		FY	PY		
7	72,871	ARS-BFGL-NGS-89239			PY		
7	73,749	ARS-BFGL-NGS-26484			PY		
7	74,122	ARS-BFGL-NGS-23727	MY				
7	77,583	BTB-01339356		FY			
7	78,201	ARS-BFGL-NGS-31863	MY	FY			
7	78,653	BTB-01273562		FY			
7	81,598	ARS-BFGL-NGS-11872	MY				
7	81,753	BTB-01514268	MY				
7	84,571	Hapmap51053-BTA-80120	MY		PY		
7	84,684	ARS-BFGL-NGS-103162	MY		PY		
7	84,854	BTB-01363214			PY		
7	86,472	BTB-01455682	MY				
7	86,515	ARS-BFGL-NGS-110503	MY				
7	88,937	Hapmap43690-BTA-80156		FY			
7	89,538	Hapmap39294-BTA-80145		FY			
7	94,536	ARS-BFGL-NGS-43916	MY		PY		
7	95,187	Hapmap46388-BTA-93108			PY		
7	95,640	ARS-BFGL-NGS-113774			PY		
7	96,469	Hapmap47490-BTA-108189			PY		
7	96,893	Hapmap48501-BTA-87072			PY		
7	96,986	ARS-BFGL-NGS-68719		FY			
7	97,011	Hapmap24200-BTA-147598		FY	PY		
7	98,261	ARS-BFGL-NGS-94147			PY		
7	99,797	ARS-BFGL-NGS-70915		FY	PY		
7	99,898	ARS-BFGL-NGS-70114		FY			
7	100,457	BTA-87872-no-rs			PY		
7	102,077	Hapmap31054-BTA-112283		FY			
7	102,166	ARS-BFGL-NGS-4342				FP	
7	103,092	BTA-80441-no-rs		FY			
7	105,245	BTB-00955215			PY		

7	106,689	Hapmap50111-BTA-80468			PY		
7	106,918	Hapmap48479-BTA-80447		FY			
7	107,109	ARS-BFGL-NGS-69739		FY			
7	109,340	Hapmap44412-BTA-80524		FY			
8	1	Hapmap42099-BTA-120289			PY		
8	4,169	Hapmap54974-rs29015318		FY	PY		
8	4,251	BTA-86031-no-rs			PY		
8	4,287	BTB-01956236			PY		
8	4,999	ARS-BFGL-NGS-68739			PY		
8	6,369	Hapmap44053-BTA-28733		FY	PY		
8	6,585	Hapmap43365-BTA-81894			PY		
8	7,456	ARS-BFGL-NGS-20843					PP
8	14,685	BTA-92138-no-rs					PP
8	16,151	Hapmap51695-BTA-16700					PP
8	16,988	Hapmap53455-rs29027941	MY				
8	18,616	Hapmap50115-BTA-80812					PP
8	22,250	ARS-BFGL-NGS-3384			PY		
8	22,833	BTB-01168801			PY		
8	22,856	ARS-BFGL-NGS-34771			PY		
8	26,936	ARS-BFGL-NGS-24524					PP
8	33,339	BTB-01469421	MY				
8	33,664	Hapmap54720-rs29023017			PY		
8	35,520	Hapmap23351-BTA-123397			PY		
8	41,832	Hapmap32013-BTA-104628			PY		
8	42,208	ARS-BFGL-NGS-82111				FP	
8	43,646	ARS-BFGL-NGS-30070			PY		
8	43,709	BTA-80993-no-rs		FY	PY		
8	44,047	Hapmap52331-rs29021338	MY		PY		
8	45,276	ARS-BFGL-NGS-86183		FY			
8	46,413	Hapmap54235-rs29024181			PY		
8	47,990	ARS-BFGL-NGS-113176			PY		
8	51,778	Hapmap42685-BTA-81134	MY		PY		
8	53,071	ARS-BFGL-NGS-10990	MY				
8	61,711	ARS-BFGL-NGS-118882	MY		PY		
8	62,535	ARS-BFGL-NGS-100613	MY		PY		
8	64,072	BTB-00105019			PY		
8	64,104	ARS-BFGL-NGS-118369			PY		
8	64,140	BTB-00351490			PY		
8	64,871	ARS-BFGL-NGS-97020			PY		
8	66,814	ARS-BFGL-NGS-16925			PY		
8	67,247	Hapmap43062-BTA-81698			PY		
8	67,282	Hapmap44415-BTA-81700			PY		
8	67,320	ARS-BFGL-NGS-66565	MY		PY		
8	67,350	Hapmap30871-BTA-158348	MY		PY		
8	67,696	BTA-19348-no-rs		FY			
8	68,392	ARS-BFGL-NGS-24979			PY		
8	69,841	ARS-BFGL-NGS-29663	MY				
8	70,711	Hapmap59270-rs29027144	MY		PY		
8	71,591	Hapmap25871-BTA-152798			PY		
8	75,261	BTB-01227548	MY				
8	75,485	ARS-BFGL-NGS-16507	MY				
8	75,556	ARS-BFGL-NGS-29576	MY				
8	77,113	ARS-BFGL-NGS-5294			PY		
8	80,713	ARS-BFGL-NGS-29876	MY				
8	88,798	ARS-BFGL-NGS-26532			PY		

8	88,843	ARS-BFGL-NGS-104204			PY		
8	90,328	ARS-BFGL-NGS-119337	MY				
8	91,547	ARS-BFGL-NGS-104416			PY		
8	92,786	ARS-BFGL-NGS-33495			PY		
8	95,575	Hapmap48568-BTA-103950		FY			
8	96,576	BTB-01864543		FY	PY		
8	97,070	BTA-101737-no-rs		FY	PY		
8	97,099	BTA-101724-no-rs		FY	PY		
8	97,973	BTB-01734135	MY	FY			
8	98,726	ARS-BFGL-NGS-52705	MY				
8	99,390	ARS-BFGL-NGS-113098			PY		
8	108,182	BTB-00369009	MY				
8	112,918	Hapmap49333-BTA-82773	MY				
8	113,162	BTB-01515798	MY				
8	114,396	ARS-BFGL-NGS-33591	MY		PY		
8	114,480	Hapmap49395-BTA-98771	MY				
8	115,942	Hapmap53326-rs29023047	MY				
9	2,289	Hapmap36664-SCAFFOLD50340_7682	MY	FY			
9	9,412	ARS-BFGL-NGS-57285				FP	
9	9,957	ARS-BFGL-NGS-10202	MY				
9	12,088	ARS-BFGL-NGS-59162	MY	FY	PY		
9	13,730	BTA-91270-no-rs	MY		PY		
9	15,209	Hapmap28752-BTA-146270	MY				
9	15,958	BTB-01407982	MY				
9	19,432	UA-IFASA-1686			PY		
9	19,560	Hapmap47550-BTA-25655			PY		
9	21,423	BTB-01095008					PP
9	21,474	BTA-20861-no-rs		FY			
9	23,736	ARS-BFGL-NGS-74851					PP
9	24,624	BTB-01362120		FY			
9	27,740	Hapmap31053-BTA-111664			PY		
9	28,519	ARS-BFGL-NGS-79864			PY		
9	30,185	BTB-00387060	MY				
9	34,031	ARS-BFGL-NGS-14098	MY				
9	34,706	UA-IFASA-814	MY				
9	40,130	Hapmap29482-BTA-146449			PY		
9	42,326	ARS-BFGL-NGS-72216			PY		
9	42,423	ARS-BFGL-NGS-13783			PY		
9	44,569	UA-IFASA-4157		FY			
9	46,601	BTA-10828-no-rs	MY		PY		
9	48,193	Hapmap34923-BES9_Contig458_891	MY				
9	50,230	ARS-BFGL-NGS-27097			PY		
9	52,206	ARS-BFGL-NGS-22125		FY			
9	52,314	UA-IFASA-4980				FP	
9	57,732	BTB-01828494		FY			
9	58,626	BTB-01151441			PY		
9	58,723	Hapmap49396-BTA-98905			PY		
9	59,861	BTA-104917-no-rs	MY	FY	PY		
9	59,894	BTB-01604502	MY	FY	PY		
9	62,055	Hapmap49337-BTA-83888	MY				
9	62,195	ARS-BFGL-NGS-107809	MY				
9	65,149	ARS-BFGL-NGS-36482				FP	
9	65,181	BTB-01673493				FP	
9	67,711	BTA-33284-no-rs		FY			
9	72,409	Hapmap49339-BTA-84110	MY				
9	73,068	ARS-BFGL-NGS-78172			PY		
9	75,732	ARS-BFGL-NGS-36451	MY		PY		

9	75,802	BTA-84237-no-rs	MY		PY		
9	75,843	Hapmap42339-BTA-84231	MY		PY		
9	83,201	BTB-01182727	MY				
9	84,177	ARS-BFGL-NGS-99576		FY	PY		
9	85,451	ARS-BFGL-NGS-25441	MY				
9	89,138	ARS-BFGL-NGS-43711		FY			
9	89,501	Hapmap54036-ss46525997		FY			
9	92,155	BTB-00404735				FP	
9	92,490	ARS-BFGL-NGS-46105		FY			
9	92,776	BTB-00404235		FY			
9	93,253	BTB-01839335		FY			
9	95,864	ARS-BFGL-NGS-112933					PP
9	96,229	Hapmap44147-BTA-84872			PY		
9	105,894	ARS-BFGL-NGS-87714					PP
10	2,690	ARS-BFGL-NGS-118679					PP
10	8,136	ARS-BFGL-NGS-115023	MY		PY		
10	11,075	BTB-00407977			PY		
10	13,344	ARS-BFGL-BAC-13545			PY		
10	13,592	ARS-BFGL-NGS-71024			PY		
10	13,840	ARS-BFGL-NGS-100004			PY		
10	20,945	Hapmap57100-rs29013509		FY			
10	31,421	BTB-00416806	MY		PY		
10	31,807	BTB-00415821	MY				
10	31,948	BTB-00416033	MY		PY		
10	31,973	BTB-00416055			PY		
10	34,700	Hapmap25237-BTA-125338		FY			
10	35,862	Hapmap34243-BES6_Contig306_1185	MY		PY		
10	35,929	Hapmap55209-rs29013243	MY				
10	36,426	ARS-BFGL-NGS-35605			PY		
10	36,557	Hapmap53714-rs29017586	MY				
10	38,937	ARS-BFGL-BAC-12872	MY		PY		
10	39,506	BTB-00093532	MY		PY		
10	40,770	BTA-122483-no-rs			PY		
10	41,245	BTB-01700213			PY		
10	45,088	ARS-BFGL-NGS-16794			PY		
10	46,922	ARS-BFGL-NGS-15826	MY				
10	47,637	ARS-BFGL-NGS-36243	MY				
10	47,879	ARS-BFGL-NGS-103757			PY		
10	48,053	ARS-BFGL-NGS-104551	MY				
10	49,231	ARS-BFGL-BAC-11657		FY	PY		
10	54,386	BTB-01137783	MY		PY		
10	54,632	Hapmap47128-BTA-89018	MY				
10	54,740	BTB-01137914	MY		PY		
10	54,810	BTA-95978-no-rs	MY		PY		
10	61,188	Hapmap57627-rs29027143	MY		PY		
10	62,511	ARS-BFGL-NGS-1410		FY	PY		
10	66,285	ARS-BFGL-NGS-69379	MY		PY		
10	68,128	BTA-100674-no-rs			PY		
10	69,724	ARS-BFGL-NGS-110711	MY		PY		
10	70,431	ARS-BFGL-NGS-57077	MY				
10	70,455	Hapmap50263-BTA-122214	MY				
10	71,120	ARS-BFGL-NGS-12520	MY				
10	71,842	BTA-74271-no-rs	MY				
10	86,227	BTB-00436473	MY				
10	86,940	ARS-BFGL-NGS-117016				FP	
10	88,110	ARS-BFGL-NGS-16573	MY				
10	92,399	Hapmap39512-BTA-79353	MY				

10	93,400	ARS-BFGL-NGS-26052				FP	
10	93,838	BTB-00445081		FY			
10	95,836	ARS-BFGL-NGS-74928	MY	FY	PY		
10	96,040	Hapmap54178-rs29021913				FP	
10	96,098	BTB-00446145		FY			
10	96,955	Hapmap50620-BTA-21279		FY			
10	105,001	BTA-83475-no-rs					PP
11	6,209	ARS-BFGL-NGS-91251	MY	FY	PY		
11	6,516	BTB-00454142			PY		
11	7,134	ARS-BFGL-NGS-47869		FY			
11	7,545	BTA-101065-no-rs		FY	PY		
11	11,906	ARS-BFGL-NGS-19864		FY			
11	12,468	ARS-BFGL-BAC-13568			PY		
11	13,451	BTA-85470-no-rs					PP
11	14,044	ARS-BFGL-NGS-13679			PY		
11	15,627	BTB-00483333	MY				
11	15,648	BTB-00461989	MY				
11	15,690	ARS-BFGL-NGS-74492			PY		
11	16,521	ARS-BFGL-BAC-14856		FY			
11	17,940	BTB-01391227			PY		
11	18,851	BTB-01934985			PY		
11	18,999	BTB-01679746			PY		
11	19,023	BTB-01940421			PY		
11	19,130	BTB-01913936			PY		
11	20,980	ARS-BFGL-NGS-104435			PY		
11	23,779	ARS-BFGL-NGS-43804	MY				
11	27,454	BTB-01550704		FY			
11	35,658	BTB-01431917			PY		
11	35,716	BTB-01293391			PY		PP
11	35,947	BTB-02040693			PY		
11	36,475	BTA-91929-no-rs					PP
11	37,465	ARS-BFGL-NGS-112269	MY				
11	38,704	ARS-BFGL-NGS-32737		FY			
11	40,185	ARS-BFGL-NGS-118144					PP
11	40,276	ARS-BFGL-BAC-14233					PP
11	43,053	ARS-BFGL-NGS-14714					PP
11	50,639	ARS-BFGL-NGS-68510			PY		
11	50,695	ARS-BFGL-NGS-108232			PY		
11	50,727	Hapmap59833-rs29027583			PY		
11	57,819	BTA-32746-no-rs		FY			
11	58,725	BTB-01079189					PP
11	58,778	BTB-00475277					PP
11	65,840	Hapmap34879-BES7_Contig396_841			PY		
11	65,871	ARS-BFGL-NGS-100607			PY		
11	68,383	BTA-101061-no-rs		FY			
11	68,724	ARS-BFGL-NGS-109780		FY	PY		
11	69,456	ARS-BFGL-NGS-18300	MY				
11	70,246	Hapmap34845-BES7_Contig520_696		FY	PY		
11	70,268	Hapmap12055-BTA-86516	MY		PY		
11	71,197	Hapmap27139-BTA-102152		FY			PP
11	73,281	ARS-BFGL-NGS-110450	MY		PY		
11	73,342	ARS-BFGL-NGS-20385		FY			
11	75,076	ARS-BFGL-NGS-74702	MY	FY			
11	76,426	ARS-BFGL-NGS-95312	MY		PY		
11	78,221	Hapmap25799-BTA-126762		FY			
11	78,484	ARS-BFGL-NGS-112276	MY				
11	80,363	ARS-BFGL-NGS-61477	MY				
11	80,576	ARS-BFGL-NGS-73132	MY				

11	80,973	ARS-BFGL-NGS-32286			PY		
11	83,876	ARS-BFGL-NGS-105586	MY	FY			
11	84,956	Hapmap46768-BTA-117394	MY	FY			
11	87,632	ARS-BFGL-NGS-83288	MY		PY		
11	88,003	ARS-BFGL-NGS-107825	MY		PY		
11	88,023	Hapmap43168-BTA-119307	MY		PY		
11	89,891	ARS-BFGL-NGS-33784	MY				
11	92,078	Hapmap42125-BTA-19379	MY		PY		
11	94,840	ARS-BFGL-NGS-14308			PY		
11	98,252	Hapmap41435-BTA-115556	MY		PY		
11	100,331	ARS-BFGL-NGS-113879	MY				
11	103,079	ARS-BFGL-NGS-114094	MY		PY		
11	105,482	ARS-BFGL-NGS-39065	MY		PY		
11	105,535	ARS-BFGL-NGS-102267	MY				
11	106,046	ARS-BFGL-NGS-22188			PY		
11	107,651	ARS-BFGL-NGS-114744			PY		
12	126	ARS-BFGL-NGS-104447		FY			
12	5,492	BTA-17590-no-rs		FY			
12	6,221	ARS-BFGL-NGS-28486			PY		
12	8,964	ARS-BFGL-NGS-112946	MY				
12	11,872	Hapmap50654-BTA-31559	MY				
12	12,313	ARS-BFGL-NGS-31202		FY			
12	14,213	ARS-BFGL-NGS-16501			PY		
12	14,373	BTA-31571-no-rs			PY		
12	14,511	ARS-BFGL-NGS-42200	MY		PY		
12	15,522	ARS-BFGL-NGS-43671	MY	FY	PY		
12	18,461	ARS-BFGL-NGS-62217		FY			
12	21,939	BTA-120906-no-rs		FY			
12	22,947	ARS-BFGL-NGS-51613		FY			
12	52,640	Hapmap43521-BTA-23812		FY			
12	52,880	Hapmap59400-rs29023728					PP
12	53,843	BTB-00496702			PY		
12	55,173	ARS-BFGL-NGS-2151					PP
12	55,793	BTB-00499073			PY		
12	55,813	Hapmap56826-rs29013564			PY		
12	57,806	BTB-01839492					PP
12	65,371	BTB-01337869			PY		
12	65,419	BTB-01337853	MY		PY		
12	65,741	ARS-BFGL-BAC-14364			PY		
12	65,797	ARS-BFGL-NGS-90411			PY		
12	67,030	ARS-BFGL-NGS-54132	MY	FY	PY		
12	68,594	BTB-00503215			PY		
12	73,402	UA-IFASA-2256	MY	FY	PY		
12	73,480	ARS-BFGL-NGS-19305	MY	FY	PY		
12	75,467	ARS-BFGL-NGS-12480			PY		
12	78,334	Hapmap42176-BTA-31298					PP
12	78,512	Hapmap59799-rs29010339		FY	PY		
12	80,766	ARS-BFGL-NGS-53938		FY			
12	80,870	ARS-BFGL-NGS-107794		FY			
12	81,408	BTB-01315661	MY				
12	81,705	ARS-BFGL-NGS-41933					PP
12	82,410	BTB-01198427			PY		
13	680	BTB-01141508		FY	PY		
13	1,122	BTA-122179-no-rs			PY		
13	1,311	ARS-BFGL-BAC-12483	MY				
13	1,372	BTA-15911-no-rs	MY				
13	1,498	Hapmap45253-BTA-15908		FY			
13	3,111	Hapmap35931-			PY		

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13	3,251	ARS-BFGL-BAC-15070	MY		PY		
13	3,483	ARS-BFGL-NGS-4272			PY		
13	4,058	ARS-BFGL-NGS-62490		FY	PY		
13	4,078	ARS-BFGL-NGS-105636	MY				
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13	5,019	ARS-BFGL-NGS-98610		FY	PY		
13	5,082	ARS-BFGL-NGS-92938	MY				
13	5,660	ARS-BFGL-NGS-84327	MY	FY	PY		
13	6,459	ARS-BFGL-NGS-105883			PY		
13	8,968	ARS-BFGL-NGS-93056		FY			
13	12,438	Hapmap50305-BTA-27942		FY			
13	13,139	ARS-BFGL-NGS-114459			PY		PP
13	13,408	ARS-BFGL-NGS-109071			PY		
13	13,909	ARS-BFGL-NGS-113489		FY			
13	15,059	ARS-BFGL-NGS-92946				FP	
13	16,262	Hapmap39397-BTA-31932	MY				
13	16,285	Hapmap42509-BTA-31930	MY				
13	24,489	Hapmap42181-BTA-31908		FY			
13	24,517	ARS-BFGL-NGS-104788	MY				
13	25,233	BTA-31957-no-rs	MY				
13	26,814	Hapmap25132-BTA-96391		FY	PY		
13	32,893	Hapmap40947-BTA-32313	MY				
13	33,741	Hapmap57166-rs29020401	MY		PY		
13	35,765	BTB-00517708			PY		
13	36,033	BTB-00517668			PY		
13	38,193	BTA-32346-no-rs	MY		PY		
13	39,178	ARS-BFGL-NGS-110611					PP
13	39,371	ARS-BFGL-BAC-14448			PY		
13	41,409	BTB-00522444				FP	
13	42,319	Hapmap51209-BTA-32563	MY		PY		
13	42,702	ARS-BFGL-NGS-57335	MY				
13	44,016	ARS-BFGL-NGS-5872				FP	
13	44,039	ARS-BFGL-NGS-104720				FP	
13	44,982	BTB-01376014	MY		PY		
13	45,361	BTB-01505690					PP
13	46,536	ARS-BFGL-NGS-97782					PP
13	47,301	ARS-BFGL-NGS-80072				FP	PP
13	48,171	Hapmap54365-rs29014934			PY		
13	48,188	BTB-01718516			PY		
13	48,300	ARS-BFGL-NGS-3711	MY		PY		
13	48,393	BTB-00527671					PP
13	55,371	BTB-00529185	MY		PY		
13	55,818	Hapmap40246-BTA-32935			PY		
13	56,446	ARS-BFGL-NGS-1365	MY				
13	61,718	ARS-BFGL-NGS-83014				FP	
13	72,684	ARS-BFGL-NGS-81880				FP	
13	77,103	ARS-BFGL-NGS-104779		FY			
13	78,470	ARS-BFGL-NGS-18031	MY	FY			
13	79,539	ARS-BFGL-NGS-16572			PY		
13	82,440	ARS-BFGL-NGS-56575				FP	
14	5	Hapmap29758-BTC-003619				FP	
14	51	Hapmap30381-BTC-005750				FP	
14	77	Hapmap30383-BTC-005848	MY		PY		
14	101	BTA-34956-no-rs				FP	
14	237	ARS-BFGL-NGS-57820	MY		PY	FP	
14	444	ARS-BFGL-NGS-4939	MY		PY	FP	

14	596	ARS-BFGL-NGS-71749				FP	
14	680	ARS-BFGL-NGS-107379	MY	FY		FP	PP
14	742	ARS-BFGL-NGS-18365				FP	
14	763	Hapmap30922-BTC-002021				FP	
14	812	UA-IFASA-8997				FP	
14	931	ARS-BFGL-NGS-101653				FP	
14	997	ARS-BFGL-NGS-26520				FP	
14	1,154	Hapmap29888-BTC-003509				FP	
14	1,264	ARS-BFGL-NGS-3122				FP	
14	1,285	Hapmap25486-BTC-072553				FP	
14	1,308	ARS-BFGL-NGS-31471		FY		FP	
14	1,409	ARS-BFGL-NGS-41248				FP	
14	1,461	Hapmap30646-BTC-002054				FP	
14	1,889	ARS-BFGL-NGS-74378	MY			FP	
14	1,913	ARS-BFGL-NGS-117542				FP	
14	2,011	ARS-BFGL-BAC-1511					PP
14	2,049	Hapmap30730-BTC-064822				FP	
14	2,131	ARS-BFGL-NGS-33248				FP	
14	2,202	UA-IFASA-9288				FP	
14	2,262	Hapmap24777-BTC-064977				FP	
14	2,347	ARS-BFGL-NGS-22111				FP	
14	2,370	UA-IFASA-7269				FP	
14	2,392	Hapmap26072-BTC-065132				FP	
14	2,419	Hapmap26527-BTC-005059				FP	
14	2,580	ARS-BFGL-NGS-56327		FY			
14	2,712	UA-IFASA-5306				FP	
14	2,826	Hapmap27703-BTC-053907				FP	
14	3,019	Hapmap22692-BTC-068210	MY				
14	3,100	Hapmap23302-BTC-052123	MY				
14	3,122	ARS-BFGL-NGS-113309					PP
14	3,189	Hapmap25217-BTC-067767				FP	
14	3,698	ARS-BFGL-NGS-78318					PP
14	3,834	Hapmap32262-BTC-066621				FP	
14	3,941	Hapmap30091-BTC-005211				FP	
14	4,694	Hapmap30988-BTC-056315				FP	
14	4,956	ARS-BFGL-NGS-112858				FP	
14	5,282	ARS-BFGL-NGS-110894				FP	
14	5,640	Hapmap32234-BTC-048199			PY		
14	8,692	ARS-BFGL-NGS-28580			PY		
14	8,810	Hapmap25450-BTC-055819			PY		
14	10,792	ARS-BFGL-NGS-119373	MY				
14	11,525	Hapmap57409-rs29021898			PY		
14	14,073	ARS-BFGL-NGS-33755			PY		
14	14,132	ARS-BFGL-NGS-117354			PY		
14	14,409	ARS-BFGL-NGS-549			PY		
14	14,560	UA-IFASA-5528			PY		
14	14,806	BTA-122375-no-rs			PY		
14	14,884	Hapmap60993-rs29025756			PY		
14	16,048	BTB-00553468			PY		
14	16,746	Hapmap33723-BTA-156547	MY		PY		
14	16,788	UA-IFASA-9744	MY				

14	17,851	UA-IFASA-6305					PP
14	17,956	BTB-01720377			PY		
14	18,078	BTB-00555233			PY		
14	18,116	ARS-BFGL-NGS-100788			PY		
14	21,668	UA-IFASA-7382			PY		
14	33,321	Hapmap40239-BTA-20881			PY		
14	33,756	Hapmap49579-BTA-34549			PY		
14	34,728	ARS-BFGL-NGS-72344		FY			
14	38,982	ARS-BFGL-BAC-21453					PP
14	46,013	BTB-01223066		FY			
14	47,657	ARS-BFGL-NGS-3879				FP	
14	61,165	ARS-BFGL-NGS-112068			PY		
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14	65,845	ARS-BFGL-NGS-119102					PP
14	66,091	ARS-BFGL-NGS-32742			PY		
14	69,097	ARS-BFGL-NGS-3717	MY				
14	69,119	ARS-BFGL-NGS-69078	MY		PY		
14	69,828	BTA-35465-no-rs			PY		
14	70,164	UA-IFASA-7141	MY				
14	77,487	ARS-BFGL-BAC-26943	MY				
15	1,808	ARS-BFGL-NGS-101623	MY				
15	3,801	Hapmap42143-BTA-23359		FY			
15	11,900	Hapmap45702-BTA-93884	MY				
15	14,307	ARS-BFGL-NGS-100235			PY		
15	14,339	Hapmap44375-BTA-37785			PY		
15	20,916	ARS-BFGL-NGS-73400		FY			
15	23,112	Hapmap42921-BTA-36160	MY				
15	31,000	BTA-09703-rs29025860			PY		
15	31,441	ARS-BFGL-BAC-35396					PP
15	31,586	ARS-BFGL-NGS-107321	MY				
15	32,753	ARS-BFGL-NGS-2713	MY				
15	34,467	BTB-01444556		FY			
15	35,145	BTB-01559217			PY		
15	44,055	UA-IFASA-2402			PY		
15	44,672	ARS-BFGL-BAC-19395	MY				
15	44,705	BTB-01459155		FY			
15	47,944	ARS-BFGL-BAC-21163					PP
15	58,052	Hapmap53286-rs29015961					PP
15	58,948	Hapmap57960-rs29017396				FP	
15	61,155	BTB-01177461					PP
15	61,202	BTB-01177436					PP
15	68,125	ARS-BFGL-NGS-101744			PY		
15	72,891	BTA-98582-no-rs			PY		
15	73,800	BTB-00479196			PY		
15	75,599	ARS-BFGL-NGS-31754					PP
16	2,468	ARS-BFGL-NGS-22265					PP
16	2,656	ARS-BFGL-NGS-21426					PP
16	9,738	BTB-01698088			PY		PP
16	14,324	BTA-40290-no-rs	MY		PY		
16	17,950	Hapmap42200-BTA-40314				FP	
16	19,001	ARS-BFGL-NGS-35246	MY				
16	27,619	ARS-BFGL-NGS-41039	MY		PY		
16	31,374	BTB-00636189	MY				
16	33,890	Hapmap42928-BTA-38715	MY				
16	34,941	BTA-38771-no-rs	MY				
16	35,329	Hapmap47936-BTA-38791	MY				
16	35,581	ARS-BFGL-NGS-117892	MY				
16	35,606	BTB-00639530	MY				
16	43,454	ARS-BFGL-NGS-110930			PY		

16	44,730	ARS-BFGL-NGS-111082			PY		
16	46,838	ARS-BFGL-NGS-59272			PY		
16	47,763	Hapmap39327-BTA-39134		FY	PY		
16	48,117	ARS-BFGL-NGS-18487			PY		
16	48,227	BTB-00646159			PY		
16	49,781	ARS-BFGL-NGS-63175	MY		PY		
16	49,945	ARS-BFGL-NGS-111268			PY		
16	50,801	ARS-BFGL-NGS-29043	MY				
16	55,747	BTB-00648059	MY				
16	55,769	BTB-00648053	MY				
16	57,448	BTB-01492749			PY		
16	62,401	ARS-BFGL-NGS-61156	MY				
16	62,593	ARS-BFGL-NGS-101997		FY			
16	62,931	Hapmap59629-rs29013680	MY	FY	PY		
16	63,025	ARS-BFGL-NGS-113169	MY	FY	PY		
16	66,661	BTA-39971-no-rs	MY	FY	PY		
16	68,149	BTB-00659112					PP
16	69,413	ARS-BFGL-NGS-36241					PP
16	69,702	BTB-00660988		FY	PY		
16	70,486	UA-IFASA-8461		FY			
16	70,546	ARS-BFGL-NGS-32521					PP
16	71,333	Hapmap39023-BTA-39937					PP
16	71,857	ARS-BFGL-NGS-112904	MY				
16	72,921	ARS-BFGL-NGS-117855				FP	
17	102	BTB-01851867	MY	FY	PY		
17	139	BTB-01927707			PY		
17	474	BTB-00666435	MY				
17	1,325	ARS-BFGL-NGS-45119			PY		
17	3,253	BTA-109611-no-rs		FY			
17	6,783	BTB-00669395	MY				
17	6,811	Hapmap47945-BTA-41852	MY				
17	6,838	BTB-00669586	MY				
17	7,693	Hapmap54786-rs29011077	MY				
17	7,809	Hapmap28805-BTA-147247	MY				
17	7,976	BTB-01381100	MY				
17	11,307	Hapmap52387-rs29021226	MY				
17	11,991	Hapmap47504-BTA-111690	MY		PY		
17	13,429	BTA-42193-no-rs	MY				
17	13,548	ARS-BFGL-NGS-96040			PY		
17	14,162	Hapmap24693-BTA-156848				FP	
17	14,209	Hapmap26095-BTA-113931				FP	
17	14,231	Hapmap28090-BTA-113932				FP	
17	14,607	ARS-BFGL-NGS-22135				FP	
17	16,137	ARS-BFGL-NGS-29973		FY			
17	20,351	ARS-BFGL-BAC-34046	MY				
17	27,083	BTA-22770-no-rs					PP
17	29,262	BTA-40721-no-rs	MY				
17	29,830	Hapmap58096-rs29011314					PP
17	33,515	ARS-BFGL-NGS-38157	MY				
17	47,053	ARS-BFGL-NGS-11160			PY		
17	48,762	BTA-117207-no-rs			PY		
17	60,835	ARS-BFGL-NGS-10055	MY				
17	61,232	ARS-BFGL-NGS-118636	MY				
17	61,413	ARS-BFGL-NGS-3759	MY				
17	62,375	ARS-BFGL-NGS-26121			PY		
17	63,451	Hapmap51186-BTA-21161	MY		PY		

17	63,727	Hapmap49912-BTA-21169	MY				
17	64,993	BTB-01301223				FP	
17	65,228	BTA-41643-no-rs			PY		
17	65,255	ARS-BFGL-NGS-39284			PY		
17	65,289	ARS-BFGL-NGS-50172	MY				
17	66,561	ARS-BFGL-NGS-34489		FY			PP
17	68,948	ARS-BFGL-NGS-118351		FY	PY		
17	69,005	ARS-BFGL-NGS-118399			PY		
17	69,246	BTA-41779-no-rs					PP
17	69,280	ARS-BFGL-NGS-114711					PP
17	72,467	ARS-BFGL-NGS-70175					PP
17	72,850	ARS-BFGL-NGS-116537				FP	
18	2,773	BTB-00691673			PY		
18	2,822	BTB-01590114	MY		PY		
18	6,448	BTB-00695596					PP
18	8,739	ARS-BFGL-NGS-1116	MY		PY		
18	12,987	ARS-BFGL-NGS-25688	MY				
18	21,508	Hapmap51449-BTA-42665		FY			
18	23,970	ARS-BFGL-NGS-32550		FY			
18	25,961	ARS-BFGL-NGS-66258			PY		
18	28,275	ARS-BFGL-NGS-99463				FP	
18	34,256	ARS-BFGL-NGS-23693		FY			
18	37,107	Hapmap45736-BTA-43103					PP
18	39,837	ARS-BFGL-NGS-88483				FP	PP
18	40,852	ARS-BFGL-NGS-63087			PY		
18	41,399	BTA-42967-no-rs	MY		PY		
18	41,453	BTA-23408-no-rs			PY		
18	41,828	ARS-BFGL-NGS-112414			PY		
18	41,887	Hapmap40976-BTA-43213		FY			
18	43,246	UA-IFASA-8905					PP
18	43,330	ARS-BFGL-NGS-113354					PP
18	43,604	ARS-BFGL-NGS-3258					PP
18	43,660	ARS-BFGL-NGS-75672					PP
18	46,112	UA-IFASA-2345		FY	PY		
18	47,572	ARS-BFGL-NGS-110180	MY				
18	48,909	ARS-BFGL-BAC-35461	MY				
18	51,133	Hapmap49609-BTA-43790			PY		
18	52,162	ARS-BFGL-NGS-114962				FP	
18	52,355	UA-IFASA-9064			PY		
18	53,069	ARS-BFGL-NGS-10036	MY		PY		
18	53,126	ARS-BFGL-NGS-116232	MY		PY		
18	54,290	ARS-BFGL-NGS-100074			PY		
18	55,150	BTA-43890-no-rs	MY				
18	55,626	BTA-43831-no-rs		FY			
18	55,862	ARS-BFGL-NGS-25104		FY			
18	61,209	ARS-BFGL-NGS-49873			PY		
19	1,965	Hapmap50697-BTA-44862		FY			
19	16,212	ARS-BFGL-NGS-6298	MY				
19	18,879	ARS-BFGL-NGS-82757			PY		
19	21,097	Hapmap53206-rs29014774		FY			
19	21,681	Hapmap41542-BTA-44740			PY		
19	23,211	ARS-BFGL-NGS-4411			PY		
19	24,364	ARS-BFGL-NGS-4744			PY		
19	24,407	ARS-BFGL-NGS-81462			PY		
19	25,075	ARS-BFGL-NGS-103353			PY		
19	25,556	ARS-BFGL-NGS-101545			PY		
19	25,806	Hapmap46758-BTA-108921			PY		
19	26,253	ARS-BFGL-NGS-1837			PY		
19	31,896	ARS-BFGL-NGS-57209					PP

19	31,954	ARS-BFGL-NGS-39118	MY		PY		
19	32,590	ARS-BFGL-NGS-103323	MY				
19	34,150	BTA-45034-no-rs			PY		
19	34,230	ARS-BFGL-BAC-33744				FP	
19	40,563	ARS-BFGL-NGS-119404	MY	FY	PY		
19	44,019	ARS-BFGL-NGS-28651				FP	
19	46,188	BTB-00753901				FP	
19	46,499	ARS-BFGL-NGS-31468	MY				
19	46,829	ARS-BFGL-BAC-2364				FP	
19	48,216	BTA-23253-no-rs				FP	
19	51,542	BTA-45898-no-rs	MY	FY			
19	51,635	ARS-BFGL-NGS-105988	MY				
19	51,667	ARS-BFGL-NGS-83703		FY			
19	51,692	ARS-BFGL-NGS-102298		FY			
19	61,273	UA-IFASA-8477			PY		
19	61,456	Hapmap32800-BTA-133450			PY		
19	61,653	ARS-BFGL-NGS-111401				FP	
19	62,807	ARS-BFGL-NGS-116261	MY		PY		
19	62,832	Hapmap43271-BTA-46356		FY	PY		
19	63,214	ARS-BFGL-BAC-32334		FY	PY		
19	63,380	ARS-BFGL-NGS-88748		FY			
19	63,763	ARS-BFGL-NGS-39527	MY				
19	64,258	BTB-01987097		FY			
19	64,283	ARS-BFGL-NGS-101226		FY	PY		
19	64,446	ARS-BFGL-NGS-54958	MY				
19	64,517	ARS-BFGL-NGS-43321			PY		
19	64,590	ARS-BFGL-NGS-72483			PY		
19	64,618	ARS-BFGL-NGS-108629			PY		
19	64,648	ARS-BFGL-NGS-32846		FY	PY		
19	65,133	ARS-BFGL-NGS-18449			PY		
20	1,291	ARS-BFGL-NGS-17557	MY				
20	3,014	ARS-BFGL-NGS-23863		FY			
20	8,767	ARS-BFGL-NGS-44829	MY				
20	13,234	ARS-BFGL-NGS-12791		FY			
20	19,982	Hapmap50241-BTA-115966	MY				
20	20,006	ARS-BFGL-NGS-110436	MY				
20	20,041	BTA-115956-no-rs	MY				
20	23,528	ARS-BFGL-NGS-110975			PY		
20	24,231	Hapmap50712-BTA-50068		FY	PY		
20	26,229	ARS-BFGL-NGS-108866			PY		
20	26,556	ARS-BFGL-NGS-18978					PP
20	27,037	ARS-BFGL-NGS-38132					PP
20	29,212	ARS-BFGL-BAC-36217					PP
20	29,734	ARS-BFGL-NGS-17586			PY		
20	29,838	BTA-50190-no-rs			PY		
20	30,094	ARS-BFGL-NGS-31598	MY				
20	30,129	ARS-BFGL-BAC-27914					PP
20	30,613	BTB-01328684					PP
20	31,203	ARS-BFGL-BAC-27930					PP
20	31,886	ARS-BFGL-NGS-16297					PP
20	32,980	BTA-103550-no-rs					PP
20	33,014	Hapmap59121-rs29022980					PP
20	33,079	Hapmap54258-rs29018641					PP
20	33,122	UA-IFASA-9183					PP
20	34,037	ARS-BFGL-NGS-118998			PY		PP
20	34,954	Hapmap39724-BTA-122305					PP
20	34,983	ARS-BFGL-NGS-89478					PP
20	35,433	Hapmap39811-BTA-122745				FP	

20	35,457	BTB-01888575				FP	
20	35,552	ARS-BFGL-BAC-2469				FP	
20	35,671	ARS-BFGL-NGS-26909				FP	PP
20	36,394	Hapmap54938-rs29013720			PY		
20	36,956	Hapmap57531-rs29013890					PP
20	37,399	BTB-00778154		FY		FP	PP
20	37,443	BTB-00778141				FP	PP
20	37,479	ARS-BFGL-NGS-34049			PY		
20	37,708	ARS-BFGL-NGS-38482					PP
20	37,785	ARS-BFGL-NGS-84088					PP
20	37,866	Hapmap39660-BTA-50453					PP
20	37,946	BTB-00779241	MY				
20	38,002	BTB-00780234	MY				
20	38,076	BTB-00780124	MY				
20	38,201	Hapmap52690-ss46526609					PP
20	38,296	BTA-50420-no-rs					PP
20	38,381	BTB-01912756					PP
20	38,519	ARS-BFGL-NGS-13317		FY	PY		PP
20	38,540	ARS-BFGL-NGS-11884		FY			PP
20	38,590	ARS-BFGL-NGS-63936			PY		PP
20	38,741	ARS-BFGL-NGS-2860					PP
20	38,900	ARS-BFGL-NGS-22355					PP
20	38,936	Hapmap51600-BTA-50467	MY				
20	39,486	BTB-00782435		FY	PY		PP
20	39,519	BTA-13793-rs29018751		FY	PY		PP
20	39,601	BTB-01842107		FY	PY		PP
20	39,639	Hapmap53888-rs29021190					PP
20	39,667	INRA-620		FY	PY		PP
20	39,698	Hapmap38412-BTA-50496			PY		PP
20	39,728	Hapmap53199-rs29014437			PY		PP
20	39,826	Hapmap57276-ss46526009	MY				
20	39,861	Hapmap42572-BTA-50505			PY		PP
20	39,950	BTA-50515-no-rs					PP
20	40,005	BTB-00781699					PP
20	40,519	ARS-BFGL-NGS-38574	MY				
20	40,634	ARS-BFGL-NGS-91540					PP
20	40,923	BTB-01423688					PP
20	41,064	BTB-01163526		FY			PP
20	41,189	Hapmap42161-BTA-26363	MY				
20	41,217	BTA-92644-no-rs					PP
20	41,633	ARS-BFGL-NGS-65409	MY				
20	41,818	BTB-01898603	MY				
20	41,861	ARS-BFGL-BAC-34879	MY				
20	41,923	ARS-BFGL-NGS-36606					PP
20	41,947	BTA-102910-no-rs					PP
20	41,976	Hapmap42401-BTA-102906	MY				
20	42,197	ARS-BFGL-NGS-73590					PP
20	42,740	ARS-BFGL-BAC-33668					PP
20	43,164	BTB-01410122					PP
20	43,585	Hapmap43599-BTA-50578	MY				
20	45,121	Hapmap38112-BTA-50631			PY		
20	45,288	BTB-01263010					PP
20	45,582	BTB-01263230	MY				
20	45,936	BTA-50635-no-rs					PP
20	46,950	Hapmap50991-BTA-50645					PP
20	48,368	ARS-BFGL-NGS-37203	MY				
20	48,464	BTB-00785931					PP

20	48,504	Hapmap43873-BTA-50695					PP
20	48,572	ARS-BFGL-NGS-57668	MY				
20	48,703	BTB-00786292			PY		
20	50,644	BTB-00411452					PP
20	53,350	UA-IFASA-2994					PP
20	53,387	Hapmap54729-rs29023630					PP
20	55,293	BTB-02040655					PP
20	56,645	Hapmap40003-BTA-50839					PP
20	60,208	BTA-50852-no-rs					PP
20	61,736	BTB-01648514			PY		
20	61,903	ARS-BFGL-NGS-111931			PY		
20	64,019	BTB-01340958					PP
20	64,066	BTB-01341053			PY		
20	64,397	BTB-01580948			PY		
20	64,482	BTB-01456930					PP
20	64,508	BTB-01899482					PP
20	65,379	ARS-BFGL-BAC-34915					PP
20	66,093	BTB-00793280			PY		
20	66,150	ARS-BFGL-BAC-36223			PY		
20	66,705	ARS-BFGL-NGS-17058					PP
20	70,190	ARS-BFGL-NGS-41833	MY				
20	70,409	ARS-BFGL-NGS-118449	MY	FY	PY		
20	71,165	ARS-BFGL-NGS-109799	MY				
20	71,407	BTB-01525417	MY				
20	72,558	ARS-BFGL-NGS-34321	MY				
20	72,851	ARS-BFGL-NGS-29478	MY				
20	73,497	ARS-BFGL-NGS-117598		FY			
20	73,749	BTA-51296-no-rs	MY				
21	4,359	Hapmap50019-BTA-52721	MY		PY		
21	4,500	ARS-BFGL-NGS-44523			PY		
21	4,657	ARS-BFGL-NGS-34864			PY		
21	6,007	Hapmap38507-BTA-52931			PY		
21	6,464	ARS-BFGL-NGS-46597					PP
21	7,947	ARS-BFGL-NGS-118623	MY				
21	8,284	Hapmap47860-BTA-120557			PY		
21	8,336	ARS-BFGL-NGS-21637			PY		
21	9,707	ARS-BFGL-NGS-8069			PY		
21	12,697	ARS-BFGL-NGS-109184	MY				
21	13,539	BTB-01258471	MY				
21	13,564	ARS-BFGL-NGS-86644		FY			
21	14,914	ARS-BFGL-NGS-25378	MY		PY		
21	15,037	ARS-BFGL-NGS-42615	MY				
21	15,058	BTA-53495-no-rs			PY		
21	15,142	ARS-BFGL-NGS-54451	MY		PY		
21	16,338	ARS-BFGL-NGS-30546	MY		PY		
21	16,994	ARS-BFGL-NGS-33483		FY	PY		
21	17,382	ARS-BFGL-NGS-79733			PY		
21	18,331	BTB-00808681	MY		PY		
21	18,843	ARS-BFGL-NGS-41922			PY		
21	19,075	ARS-BFGL-BAC-33343	MY				
21	22,397	ARS-BFGL-NGS-69585			PY		
21	23,030	ARS-BFGL-NGS-28785			PY		
21	24,164	ARS-BFGL-NGS-99587			PY		
21	24,974	Hapmap53212-rs29015272			PY		PP
21	26,007	ARS-BFGL-BAC-33968			PY		
21	26,070	BTA-51988-no-rs		FY	PY		
21	26,661	Hapmap60593-rs29025761		FY	PY		
21	26,782	BTA-51981-no-rs			PY		
21	30,629	Hapmap46427-BTA-51697	MY				

21	32,960	ARS-BFGL-NGS-104404	MY				
21	37,998	ARS-BFGL-NGS-119377			PY		
21	40,026	BTB-01533089	MY		PY		
21	40,230	Hapmap35241-BES8_Contig395_800			PY		
21	40,877	BTB-00818669	MY				
21	49,392	BTA-52470-no-rs	MY				
21	65,869	ARS-BFGL-NGS-2582				FP	
22	84	Hapmap46833-BTA-54748		FY			
22	982	ARS-BFGL-NGS-103852					PP
22	1,071	ARS-BFGL-NGS-39898			PY		
22	1,159	BTB-01355483			PY		
22	1,317	ARS-BFGL-NGS-118681			PY		
22	3,945	Hapmap60454-rs29020896			PY		
22	4,862	Hapmap46936-BTA-113993			PY		
22	6,126	BTA-08756-no-rs	MY				
22	6,168	ARS-BFGL-NGS-66672	MY				
22	6,574	BTB-01641930	MY				
22	14,514	ARS-BFGL-NGS-74971	MY				
22	19,979	ARS-BFGL-NGS-114883			PY		
22	38,334	ARS-BFGL-NGS-87577		FY			
22	51,758	Hapmap58292-rs29023404		FY	PY		
22	51,812	ARS-BFGL-NGS-111216		FY			
22	51,910	ARS-BFGL-NGS-102411		FY	PY		
22	54,992	Hapmap60563-ss46526220		FY			
22	55,641	Hapmap41094-BTA-83358				FP	
22	57,441	BTB-00855998				FP	
22	58,128	BTA-109257-no-rs				FP	
22	60,851	ARS-BFGL-NGS-54563	MY				
22	61,419	Hapmap39470-BTA-121373		FY			
22	61,644	ARS-BFGL-NGS-41433			PY		
23	2,821	BTA-55567-no-rs		FY			
23	3,017	ARS-BFGL-NGS-15303		FY			
23	7,611	Hapmap50393-BTA-57089	MY				
23	7,809	ARS-BFGL-NGS-112194	MY				
23	8,319	ARS-BFGL-NGS-44219					PP
23	8,838	BTA-57141-no-rs					PP
23	9,244	Hapmap23991-BTA-137000	MY		PY		
23	14,993	ARS-BFGL-NGS-8960	MY				
23	16,666	ARS-BFGL-NGS-34042	MY		PY		
23	17,631	ARS-BFGL-NGS-114979				FP	
23	22,371	ARS-BFGL-NGS-20819			PY		
23	22,681	UA-IFASA-5859			PY		
23	24,748	UA-IFASA-8890					PP
23	25,816	Hapmap28130-BTA-137222	MY				
23	26,060	ARS-BFGL-NGS-117031	MY				
23	27,197	Hapmap47328-BTA-56087	MY				
23	29,745	ARS-BFGL-NGS-109612	MY				
23	38,839	ARS-BFGL-NGS-88425					PP
23	39,049	BTA-56563-no-rs					PP
23	40,052	BTA-01409-rs29012374					PP
23	40,288	Hapmap57401-rs29021597		FY			
23	42,523	Hapmap59016-rs29021748					PP
23	43,007	BTA-56863-no-rs					PP
23	43,029	ARS-BFGL-NGS-95117					PP
23	43,195	UA-IFASA-4209					PP
23	43,681	Hapmap42978-BTA-56919					PP

23	44,801	ARS-BFGL-NGS-105406					PP
23	45,179	Hapmap47993-BTA-56668					PP
23	45,589	BTB-00869928			PY		
23	45,686	ARS-BFGL-NGS-84634					PP
23	45,727	ARS-BFGL-NGS-41732					PP
23	46,011	BTA-56731-no-rs					PP
23	46,217	ARS-BFGL-NGS-104353					PP
23	49,704	ARS-BFGL-NGS-108142			PY		
23	50,770	ARS-BFGL-NGS-119306			PY		
23	51,015	Hapmap39230-BTA-56961			PY		
23	51,536	ARS-BFGL-NGS-11502			PY		
23	51,584	ARS-BFGL-NGS-118139			PY		
23	51,691	ARS-BFGL-NGS-112069			PY		
23	52,506	BTB-01381524			PY		
23	52,611	ARS-BFGL-NGS-17155			PY		
23	53,092	Hapmap57192-rs29027634			PY		
24	2,619	ARS-BFGL-NGS-108020	MY				
24	7,657	BTB-01414130	MY	FY	PY		
24	21,679	Hapmap59517-rs29027550		FY			
24	22,361	ARS-BFGL-NGS-1701		FY			
24	25,540	ARS-BFGL-NGS-108732	MY	FY			
24	29,475	ARS-BFGL-NGS-5141	MY				
24	30,667	BTB-00885200	MY				
24	30,726	BTB-00885058	MY				
24	34,936	ARS-BFGL-NGS-116211			PY		
24	35,638	BTB-00886759			PY		
24	37,929	BTB-01978737		FY			
24	38,800	ARS-BFGL-NGS-49210	MY		PY		
24	42,553	ARS-BFGL-NGS-73693			PY		
24	46,427	Hapmap33939-BES5_Contig460_1314			PY		
24	47,271	Hapmap56316-rs29025240					PP
24	47,359	Hapmap44102-BTA-58355		FY			
24	53,329	ARS-BFGL-NGS-19883				FP	
24	60,413	ARS-BFGL-NGS-45332					PP
24	62,662	ARS-BFGL-NGS-112116			PY		
24	64,042	BTB-00893217					PP
25	4,361	ARS-BFGL-NGS-16204	MY		PY		
25	4,393	Hapmap30941-BTC-018717	MY		PY		
25	4,426	Hapmap23660-BTC-018762	MY		PY		
25	16,557	ARS-BFGL-NGS-18399			PY		
25	17,233	ARS-BFGL-NGS-16007	MY		PY		
25	17,349	ARS-BFGL-NGS-74312				FP	
25	17,784	ARS-BFGL-NGS-102125		FY			
25	22,525	ARS-BFGL-NGS-57864					PP
25	23,954	ARS-BFGL-NGS-117215					PP
25	24,531	ARS-BFGL-NGS-15260	MY				
25	26,103	ARS-BFGL-NGS-31959				FP	
25	26,138	ARS-BFGL-NGS-42319				FP	
25	26,240	ARS-BFGL-NGS-1148				FP	
25	28,024	ARS-BFGL-BAC-42500		FY			
25	30,630	BTB-01701816		FY			
25	32,150	ARS-BFGL-NGS-103963					PP
25	33,271	Hapmap31673-BTC-065823			PY		
25	38,858	ARS-BFGL-NGS-76406			PY		
25	41,134	ARS-BFGL-NGS-42041			PY		
26	8,781	ARS-BFGL-NGS-37164					PP
26	9,468	BTB-01211987	MY		PY		

26	11,300	BTA-62062-no-rs					PP
26	20,477	ARS-BFGL-NGS-111739					PP
26	28,969	Hapmap50547-BTA-102741		FY			
26	29,484	BTB-01619529		FY			
26	29,566	ARS-BFGL-NGS-43819		FY			
26	29,590	Hapmap44427-BTA-92700		FY			
26	31,529	ARS-BFGL-NGS-91860		FY			
26	32,420	ARS-BFGL-NGS-22409				FP	
26	32,480	ARS-BFGL-NGS-89840		FY			
26	32,708	BTA-61163-no-rs		FY			
26	36,834	ARS-BFGL-NGS-36795			PY		
26	41,317	ARS-BFGL-NGS-111901			PY		
26	41,545	ARS-BFGL-NGS-10498			PY		
26	41,950	ARS-BFGL-NGS-33804			PY		
26	43,017	INRA-573	MY				
26	46,189	ARS-BFGL-NGS-35886		FY			
27	990	ARS-BFGL-NGS-102273			PY		
27	11,888	BTB-01753761			PY		
27	12,293	BTB-01581312		FY			
27	12,324	BTB-01581416		FY	PY		
27	12,829	Hapmap24215-BTA-163266	MY				
27	13,049	ARS-BFGL-NGS-21780	MY				
27	13,929	BTB-00953522			PY		
27	19,314	Hapmap42678-BTA-79248	MY				
27	21,188	ARS-BFGL-NGS-110610			PY		
27	27,098	ARS-BFGL-NGS-102382			PY		
27	29,065	ARS-BFGL-NGS-339				FP	
27	29,087	ARS-BFGL-NGS-110867				FP	
27	30,697	Hapmap42020-BTA-97693	MY				
27	36,004	ARS-BFGL-NGS-35260					PP
27	36,527	Hapmap35718-SCAFFOLD271203_2920			PY		
27	40,301	BTA-121522-no-rs					PP
27	44,368	ARS-BFGL-NGS-64852			PY		
27	44,540	Hapmap41400-BTA-101218			PY		
27	46,730	ARS-BFGL-NGS-112603				FP	
27	46,768	ARS-BFGL-NGS-116840				FP	
28	608	BTA-64665-no-rs			PY		
28	3,488	ARS-BFGL-NGS-114198	MY	FY	PY		
28	6,185	ARS-BFGL-NGS-43798	MY		PY		
28	6,469	BTB-00974967	MY		PY		
28	7,858	Hapmap57617-rs29026743			PY		
28	10,431	Hapmap55640-rs29014036			PY		
28	12,845	ARS-BFGL-NGS-42033			PY		
28	14,380	Hapmap50823-BTA-92119		FY			
28	15,987	ARS-BFGL-NGS-105316	MY		PY		
28	16,091	ARS-BFGL-NGS-1363				FP	
28	19,213	Hapmap48416-BTA-63708	MY				
28	19,697	Hapmap48125-BTA-92753	MY				
28	25,440	ARS-BFGL-NGS-109305		FY			
28	27,975	Hapmap55318-rs29013309		FY			
28	37,463	BTB-01640085			PY		
28	41,598	BTA-99379-no-rs			PY		
28	43,076	ARS-BFGL-NGS-116671	MY		PY		
29	2,118	ARS-BFGL-NGS-13527			PY		
29	4,211	BTB-01360311					PP
29	4,598	ARS-BFGL-NGS-18177					PP
29	6,275	ARS-BFGL-NGS-86658					PP

29	6,415	ARS-BFGL-NGS-112954					PP
29	6,750	ARS-BFGL-NGS-35685					PP
29	7,164	BTB-01892890					PP
29	7,693	ARS-BFGL-NGS-35993					PP
29	12,323	BTB-01007059			PY		
29	15,449	BTB-00426200			PY		
29	23,628	UA-IFASA-7930					PP
29	26,295	ARS-BFGL-NGS-64656					PP
29	29,653	Hapmap54158-rs29026721			PY		
29	29,797	Hapmap40781-BTA-65234	MY				
29	30,945	ARS-BFGL-NGS-119428			PY		
29	32,144	ARS-BFGL-NGS-98534			PY		
29	32,284	Hapmap50431-BTA-65530			PY		
29	33,147	Hapmap42287-BTA-65439	MY				
29	33,423	ARS-BFGL-NGS-109714	MY				
29	35,829	Hapmap38768-BTA-66476	MY				
29	37,061	ARS-BFGL-NGS-101872				FP	
29	41,336	UA-IFASA-9622			PY		
29	42,982	ARS-BFGL-NGS-85356			PY		
29	43,970	Hapmap34333-BES2_Contig145_646			PY		
29	48,975	Hapmap41328-BTA-66089	MY				
29	49,317	Hapmap24835-BTA-140780		FY			
29	51,788	ARS-BFGL-NGS-14481	MY		PY		

[S2] Python Script for MDA method

```
'''
Created on 18/apr/2013

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'''
# How to use MDA program:
# In the script's folder must be present:
#    the files, termed cromo1.txt,  cromo2.txt, ...., cromo29.txt, where the data are stored (animal_name SNP1 SNP2, ...
SNPN)
#    1 file (ebv.txt) where EBVs are stored (animal_name trait1 trait2, ... )
#    a folder for each trait with the same name of the trait specified into ebv.txt file (trait1, trait2, ....).
#
#    file cromoN.txt (all variables have to be separated by a space)
#    animal_name snp1 snp2 snp3 snp4
#    Plate24-A01 0 1 2 0
#    Plate24-A04 0 1 0 0
#
#    file ebv.txt (all variables have to be separated by a space)
#    animal_name MY FY PY FP PP
#    Plate24-A01 -2154.7 -63.12 -74.27 0.1976 -0.0281
#    Plate24-A04 -895.2 -10.7 -45.09 0.2543 -0.1603
#
# Results will be stored into sub-folders MY, FY, ... (these names have to be the same of the traits into the ebv.txt file)
# MDA generates files QTL_B1.txt, QTL_B2.txt, ..., QTL_B29.txt, one for each chromosome.
#      nSnp gtype diffMean freqB pboot
#      1 0 0.0 0 0.0
#      2 0 1.8 1 0.1
#      3 0 0.0 0 0.0
# where:
# nSnp is the considered SNP
# gtype is the genotype of maximum difference selected by MDA
# diffMean is not used
# freqB specify how many times the SNP is associated to the trait
# pboot is the posterior probability of bootstrap

import sys, os
from operator import itemgetter
import random
import datetime
from math import sqrt

def main(argv):
    global _trait_, _ebv_, _nboot_, _nds_, _best_
    if len(argv)>=2:
        _trait_=argv[0]
        _ebv_=argv[1]
        if len(argv)>=3:
            _nds_=float(argv[2])
```

```
         if len(argv)>=4:
            _nboot_=int(argv[3])
            if len(argv)==5:
               if (argv[4]=='True') or (argv[4]=='true') or (argv[4]=='T') or (argv[4]=='t'):
                  _best_=True
               else:
                  _best_=False
            else:
               _best_=True
         else:
            _nboot_=5000
            _best_=True
      else:
         _nds_=1.66
         _nboot_=5000
         _best_=True
   else:
      print("syntax:")
      print ("python MDA.py trait fileEbv [nds=1.66] [nboot=5000] [best=True[True/False]]")
      print ("Example python MDA.py milk ebv.txt")
      print ("Example python MDA.py FC ebv.txt 1.96")
      print ("Example python MDA.py protein ebv.txt 1.96 1000")
      sys.exit(2)


def loadSetting(folderIn, folderOut, trait):
   global pathIn, pathOut, feno
   pathIn=folderIn+'/'
   pathOut = folderOut+'/'+trait+'/'
   feno=trait



def leggiTrait(fIn):
#legge il file dei trait e restituisce 2 liste: la prima con i nomi degli animali e la seconda con i trait
   fp=pathIn+fIn
   f = open(fp, 'r')

   head = f.readline().split()
   for ncol in range(0, len(head)):
      if head[ncol] == feno:
         nT=ncol
         break

   nomi=[]
   trait={}
   for row in f:
      nomi.append(row.split()[0])
      trait[row.split()[0]]= float(row.split()[nT])
   f.close()
   return nomi, trait

def leggiBTA(nBTA):
   fp=pathIn+'cromo'+repr(nBTA)+'.txt'
   f = open(fp, 'r')
   f.readline().split()  # si legge la prima riga contenente le intestazioni
   dati=[]
   for row in f:
      dati.append(row.split())
   f.close()
   return dati # snp



def getDiffMda(primo, secondo):
# restituisce una lista di liste. [nSnp, gtype, diff]  nSnp parte da 1!!!!
   diff=[]
   seq=[]
   for i in range(1,len(primo[0])-1):  # si parte da 1 perche il primo e' il nome e l'ultimo e' il trait
      zp=up=dp=zs=us=ds=0
      for j in range(0,len(primo)):
         if primo[j][i]=='0':
            zp+=1
         elif primo[j][i]=='1':
            up+=1
         elif primo[j][i]=='2':
            dp+=1
         if secondo[j][i]=='0':
            zs+=1
         elif secondo[j][i]=='1':
            us+=1
         elif secondo[j][i]=='2':
            ds+=1
      if (zp>=up):
         if (zp>dp):
            diff.append([i,0,zp-zs])
            seq.append(zp-zs)
         else:
            diff.append([i, 2, dp-ds])
            seq.append(dp-ds)
      elif (up>dp):
         diff.append([i,1, up-us])
         seq.append(up-us)
      else:
         diff.append([i,2, dp-ds])
   return media(seq), devStd(seq), diff

def media(a):
   n=float(sum(a))
   return n/len(a)

def varianza(sequence):
   #Calcola la varianza della sequenza.
   med = media(sequence)
   return sum([(x-med)**2 for x in sequence]) / len(sequence)

def devStd(sequence):
   #Calcola la deviazione standard della sequenza.
   return sqrt(varianza(sequence))

def scriviQTL(ris, ncrom, Best):
   if Best:
      fp=pathOut+'QTL_B'+repr(ncrom)+'.txt'
   else:
      fp=pathOut+'QTL_W'+repr(ncrom)+'.txt'
```

```
    f = open(fp, 'w')
    f.write("nSnp gtype diffMean freqB pboot\n")
    for line in ris:
        for x in line:
            f.write("%s " % x)
        f.write("\n")
    f.close()

def getNBW(numC):
    if numC < 1000:
        return int(numC *0.1)
    elif numC < 1500:
        return int(numC * 0.09)
    elif numC < 2000:
        return int(numC * 0.085)
    elif numC < 3000:
        return int(numC * 0.08)
    elif numC < 4000:
        return int(numC * 0.07)
    elif numC < 5000:
        return int(numC * 0.06)
    elif numC < 7000:
        return int(numC * 0.05)
    else:
        return int(numC * 0.45)

def getNumCampione(numPop):
    if numPop<1000:
        return int(numPop * 0.7)
    elif numPop<2000:
        return int(numPop * 0.66)
    elif numPop<5000:
        return int(numPop * 0.6)
    elif numPop<8000:
        return int(numPop * 0.55)
    else:
        return int(numPop * 0.5)


def bootQtl(nboot, chrIni, chrFin, nds, Best, fileTrain, numC=-1, nBW=-1):
    now=datetime.datetime.now()
    h=now.hour*100
    m=now.minute
    seed=h+m
    random.seed(seed)

    nomiAn, t=leggiTrait(fileTrain)
    if numC == -1:
        numC=getNumCampione(len(nomiAn))
    if nBW == -1:
        nBW=getNBW(numC)

    for ncrom in range(chrIni,chrFin+1):
        print "BTA N.", ncrom, "...", datetime.datetime.now()
        d=leggiBTA(nBTA=ncrom)

        datiChr=[]       # prendo in esame solo gli animali di training
```

```
      for i in range(0,len(d)):
         if d[i][0] in nomiAn:
            d[i].append(t.get(d[i][0])) # nell'ultimo elemento abbiamo ebv
            datiChr.append(d[i])

      nr=len(datiChr)  # nr contiene il numero di animali
      for nb in range(0,nboot):
         lrnd=random.sample(xrange(nr), numC)
         lrnd=sorted(lrnd)
         datiRnd=[]
         for i in lrnd:
            datiRnd.append(datiChr[i])
         t_ord= sorted(datiRnd, key=itemgetter(len(datiRnd[0])-1))
         chrW = t_ord[:nBW]
         chrB = t_ord[-nBW:]  # si toglie l'ultimo elemento che e' l'EBV

         if Best:
            mean, ds, diffBW = getDiffMda(chrB, chrW)
         else:
            mean, ds, diffBW = getDiffMda(chrW, chrB)

         soglia=mean+ds*nds
         for i in diffBW:
            if i[2]<soglia:
               i[2]=0
               i.append(0)
            else:
               i.append(1)
         # risMDA e' una lista di liste del tipo [nSnp, gtype, diff, freq]
         if nb == 0:
            risMDA = diffBW[:]
         else:
            for i in range(len(risMDA)):
               if diffBW[i][2]>0 and (risMDA[i][1]==diffBW[i][1] or risMDA[i][2]==0):  # stesso genotipo
                  risMDA[i][2]+=diffBW[i][2]    # si sommano le diff
                  risMDA[i][3]+=diffBW[i][3]    # is incrementa freq

      for snp in risMDA:
         snp.append(float(snp[3])/nboot)
         snp[2]=float(snp[2])/nboot           # si aggiunge la colonna pboot

      scriviQTL(risMDA, ncrom, Best)


# ---------------------------- MAIN PROGRAM -----------------------------
if __name__ == '__main__':
   main(sys.argv[1:])


loadSetting(folderIn=os.getcwd(), folderOut=os.getcwd(), trait=_trait_)

bootQtl(nboot=_nboot_, chrIni=1, chrFin=29, nds=_nds_, Best=_best_, fileTrain=_ebv_)
```