Suid-Afrikaanse Stamboek- en Diereverbeteringsvereniging
(Geregistreer in terme van Wet 62 van 1998)

South African Stud Book and Animal Improvement Association
(Registered in terms of Act 62 of 1998)

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VAN GROOTVEE- EN KLEINVEETELERSGENOOTSKAPPE

GENOMIKA INLIGTINGSTUK

Hiermee 'n baie insiggewende inligtingstuk oor genomika wat op hierdie stadium op almal se lippe in die veebedryf is, maar waaroor daar ook ongelukkig baie misverstande en selfs verkeerde interpretasies in die bedryf rondswerf.

Dat genomika beslis 'n omwenteling in die veebedryf as hulpmiddel ten opsigte van die genetiese verbetering van ons diere kan meebring, is nie te betwyfel nie. Dat daar egter met omsigtigheid en verantwoordelikheid hiermee gewerk moet word en met die regte leiding, is ook 'n feit.

Daarom dat ons span genetici by Stamboek met die samewerking van die Departement Veekunde, Universiteit van Pretoria hierdie inligtingstuk saamgestel het wat ons graag aan al ons telers wil versprei. Ons versoek u dus vriendelik om hierdie stuk aan u telers te stuur en indien daar enige navrae is kan u met enige van die persone, soos aangedui, skakel.

Stamboek groete

DR PIERRE VAN ROOYEN
HOOFBESTUURDER

Genomic selection

A proposed first strategy for South African Breed Societies, breeders and industry role players

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Brief summary and action steps

The **aim of this document** is to provide an **overview** the importance **genomic selection** to South African breeders and how to go about putting a strategy in place. Genomic selection in broad terms implies that we will be able to use DNA information to assist us in selection of our livestock. Detail is provided in the next section. The first step now is for Breed Societies should start by **identifying breeding animals** that had (and still has) **large impact** on their breed locally. Typically that would be local or imported **AI bulls** or bulls with **many measured** (recorded) **progeny in South Africa**. **Biological samples** (semen, tail hairs with follicles, frozen blood or frozen body tissue or nose swabs) of these animals should be collected and stored to be used (now or in future) for genomic analyses. Only when many genomic profiles of such animals have been obtained and correlated to their BLUP breeding values can proper genomically enhanced breeding value predictions (GEBVs) take place. Only **"raw" genomic profiles** done with the **Illumina 50K SNP chip** (or the Illumina HD SNP chip) can be used for the (international) exchange of information and the development of a local system where genome information can be used to **increase the reliability of breeding value predictions of young animals**. Genome testing has **no value** if the breeding value predictions emanating from these tests are **not related to the local breeding value predictions** (heritabilities, genetic correlations, BLUP models, contemporary group deviation, base year or units of measurement). Tests based on SNP chips **other than Illumina** will also result in SNP **information that cannot be exchanged** with other groups, breed societies or used in relating genome information to genetic merit (breeding values). **Genomic breeding values** (GBVs) calculated on properly functioning systems of **countries outside South Africa** only have value if **converted to fit into the local comparisons** making use of the **Interbull conversion formulas** and cannot directly be used.

Breeders' Societies, other industry role players and individual breeders are welcome to contact any of the authors for more information, uncertainties or questions. This is especially encouraged BEFORE any offered genome service for the predicting of breeding values (genetic merit) is considered or any other non South African base or system is applied to market semen or breeding animals locally.

Kort opsomming en aksiestappe

Die **doel van hierdie document** is om ‘n kort **oorsig** te gee van die belangrikheid van **genomiese seleksie** vir Suid-Afrikaanse telers en hoe om te werk te gaan om ‘n strategie daar te stel. Genomiese seleksie in breë terme behels dat ons DNA inligting sal kan gebruik om ons behulpsaam te wees in die seleksie van ons veestapel. Detail word voorsien in die onderstaande gedeelte. Die eerste stap is nou dat **Telersgenootskappe** reeds moet begin om **diere te identifiseer** wat **plaaslik groot impak gemaak** het of steeds maak in die ras. Dit is gewoonlik plaaslike sowel as internasionale **KI bulle** en ander bulle met **baie plaaslik gemete nageslag**. **Biologiese materiaal** (semen, sterthare met haarwortels, gevriesde bloed en liggaamsweefsel of neusepiteel-weefsel) van hierdie diere moet bymekaar gemaak en geberg word vir genoomontledings, nou en moontlik vir die toekoms. Slegs wanneer ’n voldoende hoeveelheid van hierdie diere se genoominligting bekend is en hierdie waardes met hulle onderskeie BLUP teelwaardes gekorreleer is kan sinvolle genoom-verreikte teelwaardes (GEBV) bereken word. Slegs **rou genoom-profiele** verkry uit ontledings met die **Illumina 50K SNP skyfie** (of die Illumina HD SNP skyfie) is geskik vir (internasionale) **uitruiling** van genoominligting en vir die ontwikkeling van ’n plaaslike stelsel waar hierdie inligting kan bydra tot meer betroubare teelwaarde-voorspelling vir jong diere. **Genoom-toetsing is waardeloos** indien dit **nie verbind** kan word aan die **plaaslike teelwaardes nie** (erfbaarhede, genetiese korrelasies, BLUP modelle, behandelingsgroep-afwykings, basisjaar en metings-eenhede). Toetse wat ook **nie met die Illumina SNP** skyfies gedoen is nie lei daartoe dat inligting **nie-uitruilbaar** is met ander groepe of telersgenootskappe nie. Die waardes is ook nutteloos vir gebruik om genetiese meriete (teelwaardes) na behore te voorspel. Genomiese teelwaardes (GBVs) wat op wetenskaplik-gefundeerde stelsels in ander lande berus het **slegs waarde** indien hulle eers **omgeskakel word** om in te pas by die plaaslike vergelykings deur gebruik te maak van die **Interbull omsettings-formules**.

Telersgenootskappe, ander rolspelers in die industrie en individuele telers is welkom om een van die outeurs te kontak vir meer inligting, onsekerhede of vrae. Dit word veral aangemoedig VOOR enige diens oorweeg word wat teelwaarde voorspellings (genetiese meriete) baseer op genoom-inligting of wat semen of teeldiere wil verhandel met gepubliseerde teelwaardes wat bereken word op ’n nie-Suid-Afrikaanse basis of stelsel.

Brief background and descriptions

Genomically enhanced breeding value predictions, especially for dairy cattle, have recently become more widely used. The primary advantage in applying this technology is to increase the **reliability of breeding value predictions for young animals**, usually for sex limited traits (such as milk production and reproduction), traits only measurable at a late stage of life (like productive herd life) and traits that are less commonly recorded (like disease resistance and feed efficiency).

Genome testing of animals requires a biological sample with an adequate amount of body tissue to enable the extraction of DNA to be used to establish the nucleotide sequence (single nucleotide polymorphism or SNP) of the particular animal. This sequence gives a glimpse into the genome of the animal. The development of this technology is the result of the "Human Genome Project" carried out in the 1980s that was followed by a series of DNA sequencing of other species, among them the bovine where, initially a Hereford cow and later a Holstein cow, were sequenced (since then many more cattle were sequenced for the development of the SNP bead chips used in genomically enhanced breeding value predictions). The size of the bovine genome is 3.5 Gb (3.5 billion [thousand million] **base pairs**). It contains approximately 22 000 genes (14 000 are common to all mammalian species). Figure 1 gives an illustration of this genetic material in the nucleus of each cell.

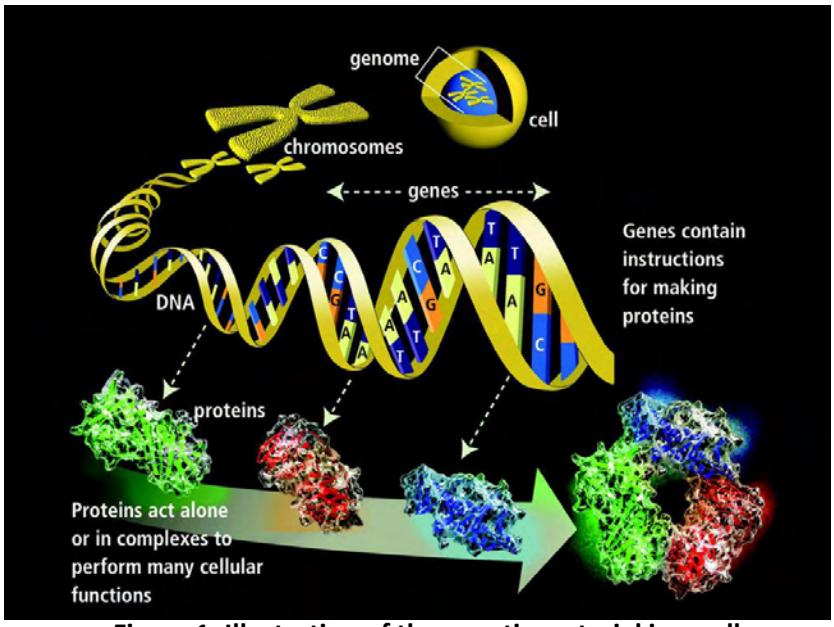

Figure 1. Illustration of the genetic material in a cell.
(http://www.broadinstitute.org/education/glossary/genome)

Attached to each sugar (the "backbone" of the nucleotide strand) is one of four types of molecules called nucleotides (sometimes called nucleobases or informally bases). It is the sequence of these four nucleotides along the backbone that encodes information. This information is read using the genetic code, which specifies the sequence of the amino acids within proteins (and therefore an individual animal's genetic merit). Base pairs link the two chromatin strands (to construct the chromosome) and consist of one of four possible nucleotides that will specifically link. Only two combinations are possible as Thymine will always bond to Adenine and Cytosine to Guanine. Figure 2 depicts the molecular structure.

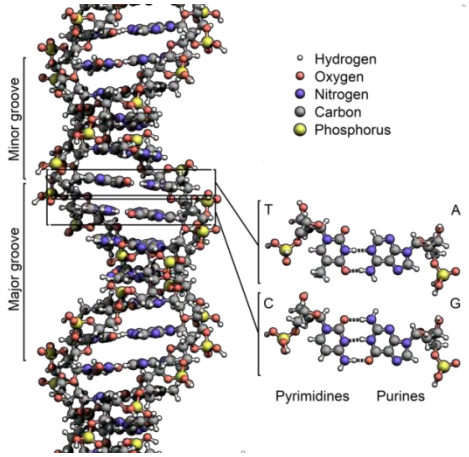

Figure 2. Part of the molecular structure of the chromosome
(http://en.wikipedia.org/wiki/DNA)

The bovine genome is therefore made up of 3 billion "codes" of T, A, C and G in a specific unique sequence for each animal. A series of such a sequence (allocated on the chromosome) is called a gene, the part carrying the genetic code for protein syntheses.

The developed SNP technology only reads part of this sequence. In the case of the 50K SNP Chip a total of 50 000 evenly spaced SNPs (base pairs) of the total genome are read (one every 60 000). It therefore serves as "road markers" along the long strands of the chromosomes. Figure 3 is an illustration of the differences that can occur between two animals at exactly the same location on the chromosome. Animal 1 has the G – C combination and animal 2 the T – A combination.

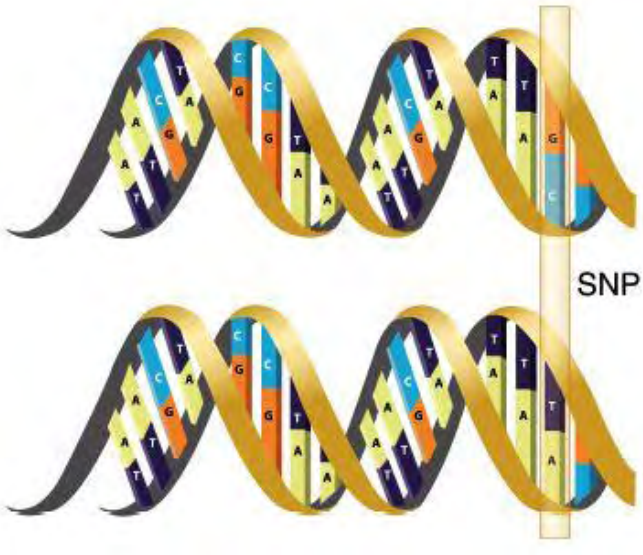

Figure 3. SNP reading at the same location for two different animals.
(http://www.mdsupport.org/library/genetics.html)

Linking SNP information to genetic merit.

Since it is known that the sequence of base pairs carry the genetic code for production and other traits in farm animals and the SNP information obtained from one of the bead chips serve as indicators of this, the next obvious step was to try and link the two sources of information. Please note that it is, in most cases, still not known exactly where and in what sequence a specific gene is located (only a few are known). Researchers therefore employed mathematical techniques used for probability prediction in achieving the link between SNP information and genetic merit.

The method of choice in predicting genetic merit is the employment of mixed model methodology resulting in BLUP (best linear unbiased prediction) breeding values. Like all methodology the reliability of predictions are dictated by the contribution of useful information (own recordings and those of relatives) to the predictions. In the case of dairy related traits, sex-limited traits are only recorded for females. Linking reliable predicted breeding values to genome information is the first step in setting up a system where breeding value prediction reliability can be enhanced with SNP information.

The following steps should be followed:

- Identification of as many as possible animals in a population (a specific breed within a country or animals that has been grouped together in one genetic – BLUP – evaluation) with predicted breeding values of (very) high reliability. In most cases this will be bulls with a large number of recorded progeny, such as AI bulls. These animals are called the R***eference Population***.
- Genome testing of these animals with a SNP chip of the highest affordable density, but not lower than 50K (the only acceptable exchange of "raw" genome information should take place with **raw information** obtained with the **Illumina** SNP chips).
- Establishing the relationship ("correlation") between the SNP (genome) information of the individual animals and their respective (very reliable) BLUP breeding values. This sets the basis for using genome information for the prediction of breeding values on younger animals in future.
- To be able to establish the enhancement in reliability, the relationship between BLUP EBVs and genomically enhanced EBVs (GEBVs) of the Reference Population first need to be tested on a so called ***Training Population***. These animals are a second group with BLUP EBVs with a lower reliability. The accurate establishment of the correlations on the Reference Population will determine the increase in reliability when genome information is added.
- **Only when these tests show that the calculated correlation between the SNP information and animals with reliable EBVs exceed a certain level, can this technology be implemented on that specific population**.
- Genome (SNP) raw information will always have to be incorporated in the local breeding value prediction program, because it is an extra source of information. The other sources (through recording) are parent information (the EBVs of the parents), own information (where the trait can be measured on the animal) and recording of progeny. In the case of a young animal (especially a young dairy bull, for instance) the only source of information will

be the EBVs of the parents, meaning that the EBV will only be based on the parent average (half the EBVs of each parent) and the random sample of genes (on the chromosomes) will not be considered. This is where genomically enhanced breeding value predictions are of value.

- Once this system has been tested and established, other (mostly cheaper) methods can be used to do wider screens of the population to identify young animals that can be potential sires (or dams) of significance. Illumina has developed SNP chips of lesser density for this purpose (and other purposes). The 3K, and lately 6K chips are used. These chips are "scaled down" versions of the 50K chip and, if used correctly the results from them can be **imputed** to resemble (very close) the 50K chip.

Common questions and answers.

1. **There are so many terms and acronyms used in breeding value predictions, what are their meanings and what are the differences among them?**

   Acronyms and terms in breeding value predictions usually refer to the method or the available information used in the prediction of the breeding value. A brief explanation of some of the most important terms and acronyms:

   Breeding value

   Could be described as the value of an animal as a parent, therefore the difference in performance of its progeny compared to the progeny of other parents. The true breeding value is never known and accurate prediction depends on the value and amount of useful information used in the prediction. A (predicted) breeding value is also dependant on the population it is expressed in. In breeding and genetics a breeding value is also referred to as the additive (genetic) value as it only involves additive genes.

   EBV

   This term refers to the "estimated breeding value" (in some circles "expected breeding value") and is the outcome of applying mixed model methodology (BLUP) in the prediction of breeding values. The synonym of EBV is PBV (predicted breeding value) that is very seldom used. Please note that the EBV refers to the genetic merit (breeding value prediction) of the animal itself and not the expected progeny difference (half the EBV).

   EPD and ETA

   The acronym for "expected progeny difference". An EPD value is exactly half an EBV and refers to the "transmitting ability", therefore it is synonymous with **ETA** (expected/estimated transmitting ability).

   Reliability and accuracy (of breeding value prediction)

The reliability of breeding value prediction refers to the relationship between the number of (reliable) sources of information, heritability of the trait and the error (pev = prediction error variance) associated with the prediction. Reliability and accuracy are different sides of the same coin (reliability is accuracy squared). A property of breeding values predicted with mixed models (BLUP) is that predictions with (very) low reliability will regress to the parent average. In applying mixed model (BLUP) technology, three major sources of information (besides genetic parameters, namely heritabilities and genetic correlations, and pedigree information) are the recordings (measurements) of an animal's parents, the animal itself and its progeny. The only source of information on a young, un-recorded animal is therefore the information from its parents, limiting the reliability of the prediction until such time as an own recording and that of its progeny is considered in the breeding value prediction.

Parent average and Mendelian sampling

Parent average refers to the average breeding values of the parents of an animal. Where an animal has not yet been measured (or a measurement is not possible, such as milk production or maternal value for bulls) and no progeny recordings are considered, the parent average is used as the EBV. The Mendelian sampling refers to the random sample of genes each of the progeny of a specific sire and dam will receive at conception. Figure 4 depicts the expected outcome where two parents, differing in EBVs, are mated.

The first EBV prediction on a young animal is therefore the parent average. This prediction will be altered as more information becomes available, either as a result of an own recording and/or recordings of progeny. The use of molecular (genome) information, also assists in predicting the Mendelian sampling, but do not negate the parent average value.

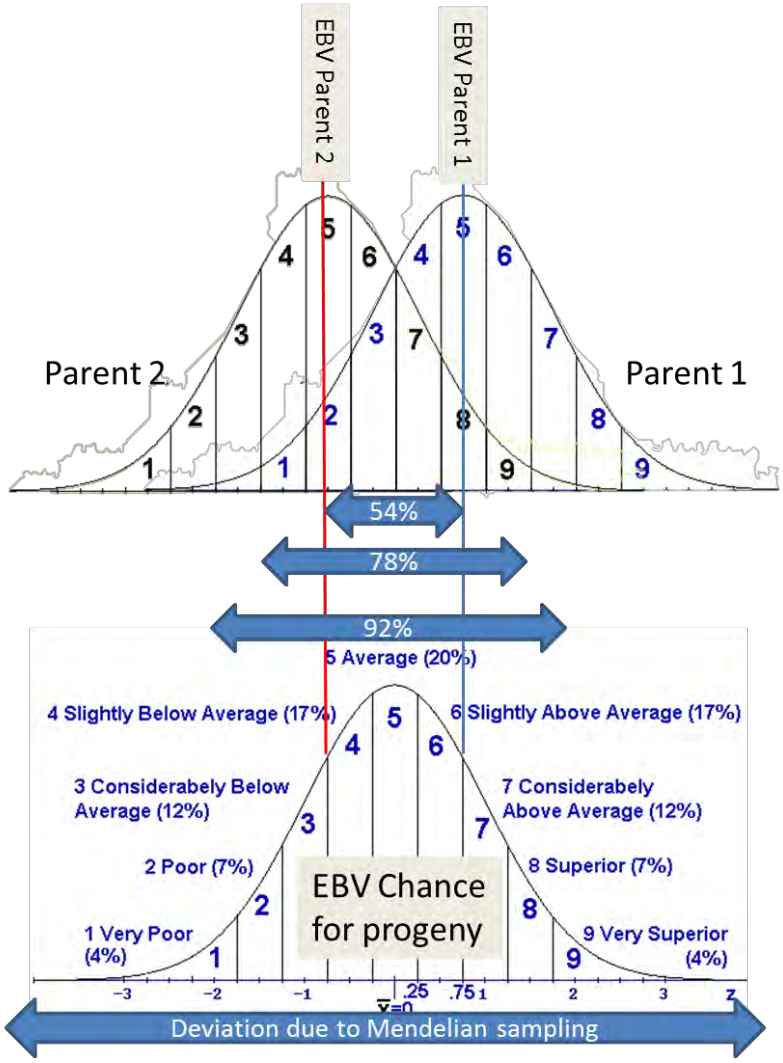

Figure 4. Expected EBV of progeny born from parents differing in EBVs (Adapted from http://www.mathnstuff.com/math/spoken/here/2class/90/stanine.htm)

GEBV and GBV

The Genomic EBV (GEBV) is a breeding value prediction where the genomic values are "blended" with the EBVs obtained from applying mixed model equations (BLUP). In the case of a young animal without own or progeny recordings, this will include the parent average and a genomic prediction indicating the Mendelian sample the animal received. If applied properly and in the population where the Reference Population has been established, the reliability of the GEBV might be as high as the same level where 10 progeny (daughters in the case of dairy bulls) were recorded. The GBV or direct genomic value usually refers to the genomic value without taking the parent average into account and therefore has a lower reliability of prediction (compared to the GEBV). As in the case of the GEBVs, the GBV values can only be used for comparison within the population where the Reference Population has been established. Obviously, as more daughters of a bull are recorded, the value of genomically enhanced EBVs becomes less.

MACE and GMACE

Interbull is the international body established to develop and apply breeding value predictions to compare dairy bulls across populations (countries) This body has developed MACE, Multiple Across Country Genetic Evaluations. MACE is calculated for each country based on the performance of the bull's daughters in his country of birth and genetic correlations among the participating countries. MACE is expressed on the units of measurement, genetic variances and base year applied in each country – each participating country therefore receives its own set of MACE breeding values which is directly comparable to that country's national EBVs. As the use of genome information to enhance EBVs has become more widespread, Interbull has developed methodology to include these values in the MACE sent to participants. These predictions are known as GMACE.

2. **Can the GEBV or GBV values for young bulls from one country directly be used in another country?**

   No, even if the genomically enhanced breeding value predictions of the original country are based on the best methodology and built on a sound Reference Population, **breeding value predictions can never be directly compared over countries** as the correlation among populations in different countries is always less than one (100%). The only basis for comparison of young animals is to apply the Interbull developed conversion factors based on a regression. If the parents of such animals have already been included in the receiving country's EBV predictions, the parent average value can also be included with the converted value.

   Even in the case of bulls with very reliable EBVs, these predictions in the country of origin first need conversion to that of the import country by applying MACE.

3. **Services are often rendered where direct genomic values are predicted without any reference to a (local) population. Are these predictions of any value?**

   No, there are no short cuts. The only way to apply genomically enhanced breeding value predictions, is to apply the principles of first using performance recordings based on local animals, predicting breeding values and comparing the reliable EBVs with the SNP information. Research has shown that there is a very poor, if any, correlation between animal populations. This means that SNP results from one country or breed cannot be used for another.

4. **Can the SNP (raw) values estimated elsewhere be used locally?**

   Yes, but only if included in the local evaluation system. Many countries have joined exchange programs to share SNP information of prominent bulls with other countries. The information is then used as part of building up the correlation with local EBVs in the case where the bull has many measured progeny locally or to use the SNP information to obtain GEBVs (on the local scale) for young bulls. Please note that the **raw information is exchanged** and NOT the genomic predictions from the country of origin. Due to standardising the exchange of (raw) genomic information, the global community had to

decide on one format (and specific SNP positions on the chromosomes). This has led to the acceptance of the **50K Illumina SNP chip** results as the **industry standard**.

5. **Can SNP chips of varying density be used in genomically enhanced breeding value predictions.**

   Illumina has also developed other SNP chips of varing density (number of SNPs and therefore the distance between SNPs) making sure that overlaps are meticulously done among them. The 50k SNP chip still remains the industry standard, but Illumina has also developed a HD (high density) bovine chip representing in the order of 700 000 SNPs (700K). This much denser chip is currently assisting researchers to make some comparisons over populations (breeds or countries) and can play a major role in future. Having higher genome coverage, however, means that the cost involved is higher and more (computer) storage space is needed for profiles. Illumina has also developed SNP chips of much lower density. The first was the 3K SNP chip that is recently replaced by the 6K SNP chip. The advantage of using a less dense chip, is the lower cost of analysis but unfortunately results in giving up some predictive reliability. Scientists have developed mathematical methodology to predict profiles resembling higher density chips for analyses carried out with lower density SNP chips (3K or 6K). This methodology is called **imputing**. The accuracy of imputing relies on the number of family members with known genomic profiles. Typically the 3K or 6K SNP chip will be used on young bull calves and females to search the population for prospective AI bulls or bull mothers. Once young bull calves of merit have been identified, they will be tested with the 50K SNP chip. The 3K or 6K chip used on sire dams also assist in eliminating the possibility of bias due to non-recorded preferential treatment. Figure 5 gives an illustration of the comparisons among results from using SNP chips of varying density, as well as the use of imputing to derive at values of higher density but using a less dense SNP chip.

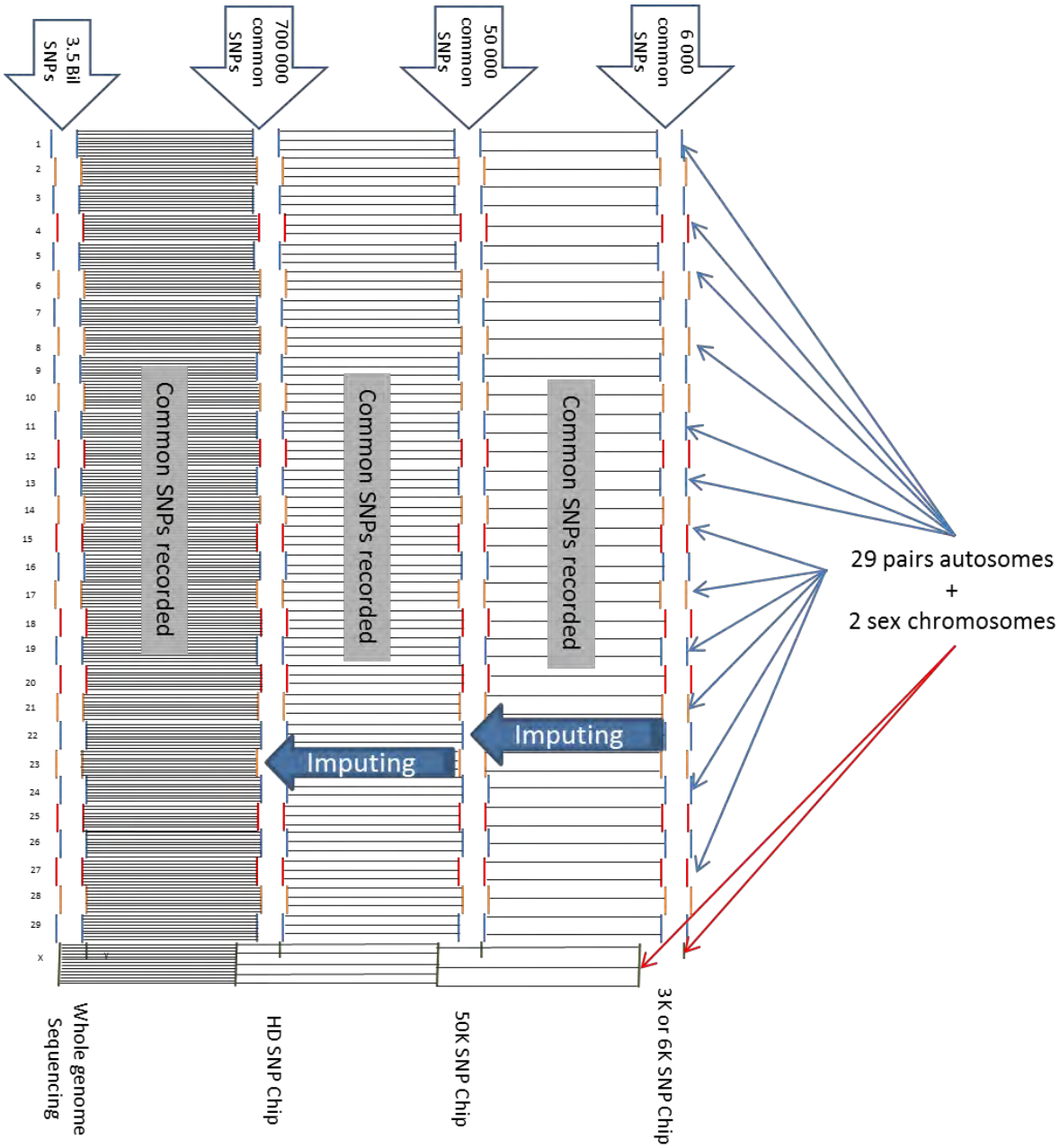

Figure 5. Illustration of the amount of information recorded on the same genome using different density Illumina SNP chips.

6. How many genome profiles of animals are needed for a Reference Population?

The general rule is to have as many as possible. Some global groups have built up Reference Populations of several thousand (within a breed) through cooperative agreements. If possible, a Reference Population of more than a thousand bulls of the same breed is needed if based on the 50K SNP chip. The development of the HD SNP chip that has more common SNPs over breeds, might assist in using information over breeds in future (especially if many breeds are included in one common BLUP breeding value prediction). Although speculative, the minimum number of animals as reference should exceed 800 to 1 000. Based on the current fee structure this will need a budget of at least R800 000 to R1 million.

7. How can the South African livestock industry ensure that genome information can become part of breeding value predictions in future?

7.1 Collecting and storing biological material

Countries that use genome information successfully have been collecting biological samples (semen, hair follicles, blood and/or other tissues) from animals of significance. The focus is mainly on animals with many recorded (measured) progeny. South African breeders' societies should all engage in actions to firstly **identify** these **animals by using progeny lists** and then actively start to source samples from them. A rule of thumb would be to target bulls with more than 50 recorded progeny and even cows that impacted significantly in a breed.

If hairs are collected it is suggested that at least **40 tail hairs** with very distinct follicles are stored. One frozen semen straw also contains an adequate amount of DNA. It is suggested that at least two semen straws of prominent bulls (especially AI bulls) are kept for this purpose. Blood could also be stored. **Fresh blood** should be kept **frozen** at minus 40°C until the DNA extraction can take place. An alternative is the use of **FTA® cards** where blood can be stored at room temperature for many years without any harm to the DNA. Other **body tissue**, such as notches from ears should also be kept **deep frozen** (like fresh blood) at minus 40°C.

Currently a reliable system is in the development phase where different types of biological material will be accepted and a secure database kept to locate (and use) it.

7.2 Joining one of or more of the international groups.

Three major groups, namely the North American Consortium, EuroGenomics and IgenoP were established to exchange genome profiles of globally used sires based on the Illumina SNP chips. Through the Interbull contacts South Africa should join one of the groups. The most likely choice is IgenoP due to its open approach in the exchange of profiles. International bulls bred elsewhere but with many measured daughters, are likely to have at least a 50K SNP profile in one of the participating countries, meaning that there is no need to duplicate.

7.3 Making use of genome and other information from imported semen, embryos and animals.

It is very likely that the Illumina 50K SNP data will be available for all (especially dairy) bulls from the prominent export countries. Breed societies should **insist** on this information **prior to any approvals for imports** of semen (or embryos). Societies should also consider cooperating with societies in other countries in drawing up lists of all animals with SNP profiles and to set up exchange mechanisms to make this information available to everyone willing to take part. This will speed up the setting up of Reference Populations and avoiding the duplication currently taking place.

The reliability of breeding value predictions will also be enhanced if local breeders' societies insist on **at least a five generation pedigree** on all imports and all animal identifications linked to their **Interbull numbers**.

7.4 Establishing a permanent work group of local scientists in collaboration with the livestock industry.

Currently there is a lack of true direction in applying genome information in breeding value predictions. In the meantime genomic products not based on sound scientific principles and of extreme doubtful value are marketed to the breeding industry causing false hope and confusion. Many local scientists are also not clear on the use of genomic information in sound breeding value predictions.

The establishment of a permanent working group in the use of genome information in breeding value predictions will ensure that:

- There is a common understanding among scientists in the use of this technology.
- The industry will be guided in the future (and current) use of genomic information.
- Local scientists will stand a better chance to jointly secure funding for research and development.
- Current practices by other countries will be better utilised, these include the application of Interbull's conversion factors for GBVs based on one of the foreign countries' systems or the application of GMACE on the South African scale.

8. In some cases breeders, AI companies and other groups are already testing local animals to obtain genomic information. What data and information should be made available to be of any value?

Current Genome testing **only have value of these tests are carried out with either the 50K or HD (700K) Illumina SNP chip**, the GBV **expressed on the scale of one of the countries with a proper genome based breeding value system AND** the **GBVs converted to the South African scale** by making use of the **Interbull conversion factors (only available for dairy cattle)**.

In all other cases the value of the genome based breeding value predictions are in serious doubt (non-exchangeable SNP information, eg. where raw data are not supplied by the laboratory, analyses are not based on Illumina SNP chips, no referral to a well-defined reference population is done and direct genome values are not converted to the local breeding value prediction system).

Where genome analyses are conducted with the Illumina SNP chips, **the following (raw data) information should also be insisted on:**

The Final Report

Contains the actual genotype calls.
Lists the SNP name, Sample ID, Allele1-Forward call, Allele2-Forward call, the GenCall score (a quality score), and the raw intensities of the X and Y.

DNA Report:

Lists the summaries of each DNA sample with call rates and allele frequencies. It also indicates the Gencall scores at the 50th and 10th percentiles.

Locus Summary:

Lists the summary statistics broken down by SNP. Gentrain is a quality score similar to GenCall. This file contains statistics about the clustering and calls which are too numerous to break down individually.

Locus X-DNA:

Lists the calls broken down by sample and locus in a matrix format with A,B,H notation for the genotypes.

Sample Map:

Lists all of samples present in the reports and their physical positions on the Illumina chips.

SNP Map:

Lists the Markers and their positions in the panel.

9. More information?

For more information contact:

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