



Hybu Cig Cymru
Meat Promotion Wales

Genetic markers in beef and sheep breeding programmes

Unravelling the practicalities of DNA technologies



About HCC

Hybu Cig Cymru - Meat Promotion Wales (HCC) is the organisation responsible for the development, promotion and marketing of Welsh red meat. We work with all sectors of the Welsh red meat industry - from the farmers through to the retailers, to develop the industry itself as well as develop profitable markets for Welsh Lamb, Welsh Beef and pork from Wales.

This booklet forms part of a series of publications produced by HCC's Industry Development team.

The Industry Development team undertake a range of activities that include:

- Technology Transfer
- Research and Development
- Market Intelligence
- Training
- Benchmarking



This publication has received funding through the Rural Development Plan for Wales 2007 - 2013 which is funded by the Welsh Government and the European Union.

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Photos and diagrams courtesy of HCC, Innovis and IBERS, Signet Breeding Services

May 2013

Introduction

For more than 10 years, DNA technologies have promised beef and sheep breeders a revolution in genetic selection. The ability to test an animal's DNA to identify specific sequences that it is carrying offers the opportunity to obtain information about an animal's genetic make-up without having to wait for that particular trait to manifest itself.

However, the availability and use of genetic markers for sheep and cattle breeding in the UK is still extremely limited. Whilst a number of markers have been developed in Australia, New Zealand and America, few have been taken through to commercial development and validation in the UK, and the value and significance of these tests as part of UK breeding programmes is not clear.

The publication of the cattle and sheep genomes now opens up the possibility of genome wide selection as a tool that breeders can use as part of their breeding programmes. So what can DNA technologies really offer to sheep and beef farmers in Wales – now and in the future?



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Using DNA technologies in my flock / herd

Genetic markers provide a very useful, stand alone tool for:

- Parentage / breed verification
- Detecting single gene effects
- Detecting carriers of single gene recessive disorders

Where the effects have been validated, genetic markers can provide additional information which may be useful to help market animals or choose animals to meet different markets. They can also be used to choose commercial animals to store or finish on different feeding regimes.

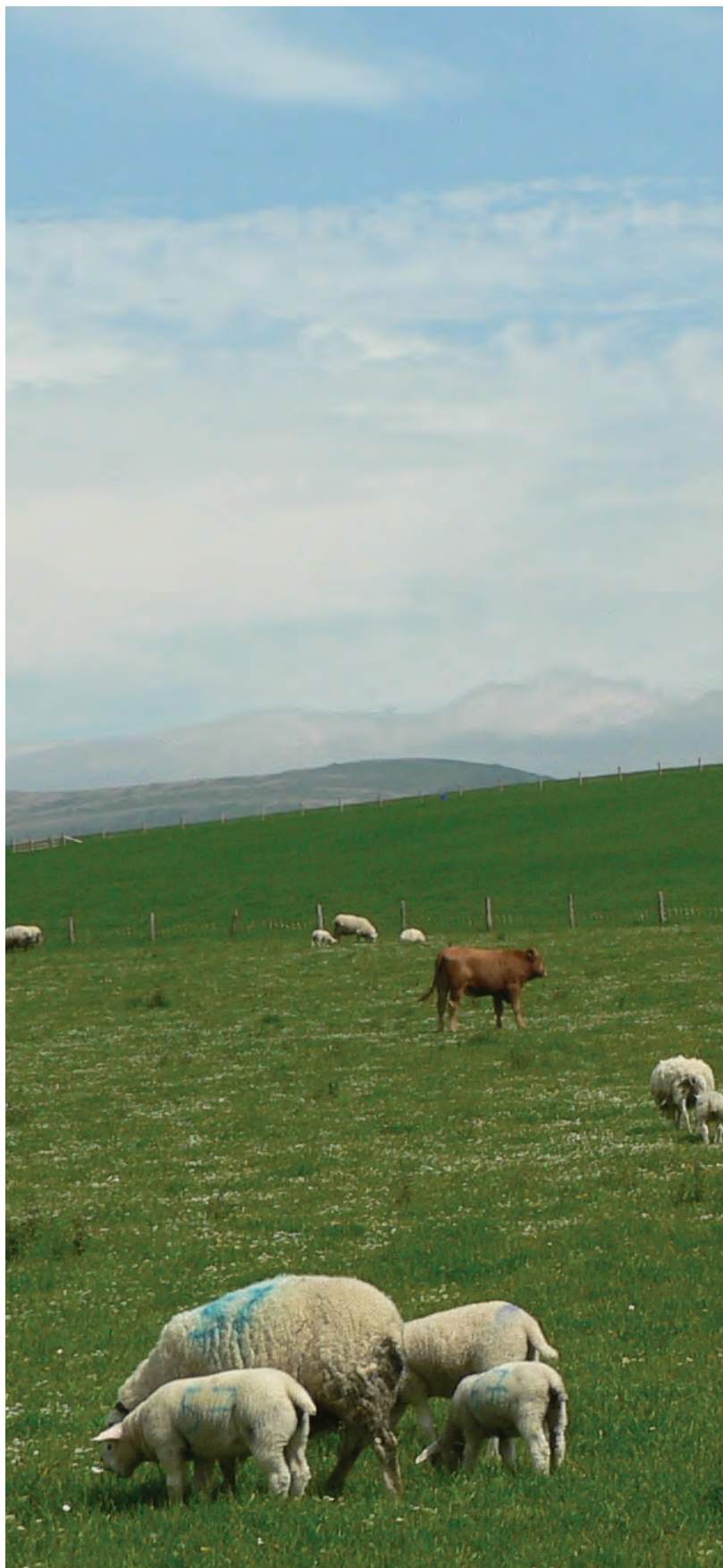
For production traits, EBVs with high accuracy should always be the first choice when selecting sheep or cattle for breeding.

Genetic markers can be a useful, second stage tool, especially for difficult to measure traits.

Current gene tests explain only a small proportion of the total variation so they cannot substitute for performance recording.

The presence of a specific variation of a gene does not guarantee phenotypic effects (e.g. the myostatin mutation in Belgian Blue and Highland cattle).

New approaches may provide a way of combining information in an optimum way but it will need good cooperation and coordination between breeders to achieve substantial benefits.



Choosing genetic markers

When choosing genetic markers for your flock / herd there are a number of things to consider first:

- **Has it been validated in UK populations?**
- **Will it work in your breed?**
- **Timing – when do you need to make your selection decisions**
Allow enough time for samples to be sent away for analysis and results to come back
- **Will you need help in interpreting results?**
- **Who will derive the benefit and who will pay for those benefits?**

disease resistance (e.g. Scrapie):

farmer will benefit if disease is present

muscularity (e.g. MyoMax® and LoinMax®):

for MyoMax® both farmer (better grades) and processor (increased lean meat yield) will benefit as well as retailer and consumer

for LoinMax® only the processor will benefit using current grading systems

some benefits can be identified through less expensive recording methods e.g. ultrasound scanning, so what real benefit does gene testing provide?

- **Why would you want to use them?**

Will they help to introduce a trait that you want in your herd / flock?

Will they help you to manage or eliminate an undesirable trait in your herd / flock?

- **Will they help you to market your stock?**

This booklet aims to provide breeders with a practical understanding of gene marker technologies and an objective view of how they could be used effectively as part of a breeding programme. In this way, breeders in Wales will be able to make informed decisions about the choice of tools available, and their use, to benefit their own beef and sheep enterprises.





Fulfilling potential – traditional selective breeding

Livestock breeding is all about choosing the animals with the 'best' genes for the traits we want to improve and mating these animals together.

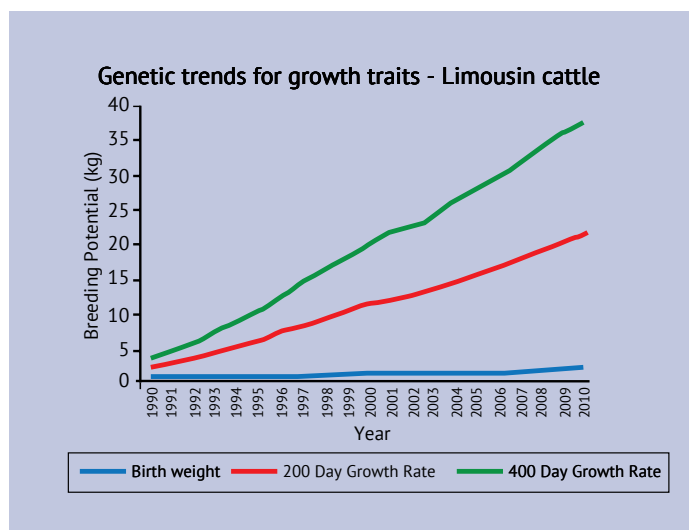
In the past, the only way we have been able to identify the individuals with the 'best' genes is by looking at their own performance or appearance and the performance and appearance of their relatives. Pedigree and performance recording, and the use of Estimated Breeding Values (EBVs) and index scores, is an objective way of doing this.

For many of the important and highly heritable **production traits**, such as growth rates, eye muscle and fat depth where relatively simple measurements can be taken on farm, excellent progress has been made and this approach has resulted in ever increasing and permanent genetic gains. Performance recording is the simplest and most cost effective approach for genetic improvement and will remain so for many years to come.

However,

- we still don't have EBVs for all the more complex traits (e.g. meat eating quality, feed efficiency, disease resistance) that might be of interest;

- some records aren't available until late in the animal's life (e.g. longevity);
- if genetics accounts for only a small proportion of the variation seen in a particular trait, then many records are needed to predict the outcome with any significant level of accuracy (e.g. calving ease, reproductive rate).



Graph courtesy of Signet Breeding Services

A role for genetic markers in livestock breeding programmes

Advances in genetics are giving us an ever-increasing understanding of how alleles (variations of a gene) relate to an animal's performance, its appearance, or its susceptibility to disease.

The ability to test the DNA of an animal and identify those animals that carry either specific alleles or the characteristic markers associated with those alleles means that we can now identify animals with the 'best' genes for some of the complex traits more accurately.

The large scale breeding programmes and recording of millions of animals in controlled environments over successive generations has meant that significant genetic progress has already been made in pigs and poultry, and DNA testing is now routinely used in these species as well as in dairy cattle. The challenge now is for the beef and sheep sectors to embrace the potential benefits of these technologies.



Slow progress....

In contrast to the pig and poultry sectors, the low litter sizes, long generation intervals and small flock/herd sizes in the pedigree sheep and beef sectors, together with the limited levels of recording, means that genetic progress has been (and continues to be) relatively slow. This is particularly so with some breeds (e.g. hill sheep) where parentage is also difficult to record.

Furthermore, given that assessing some performance traits in a bull may rely on measuring outcomes in his progeny and this takes on average around 5.5 years, it is easy to see why progress has been relatively slow.

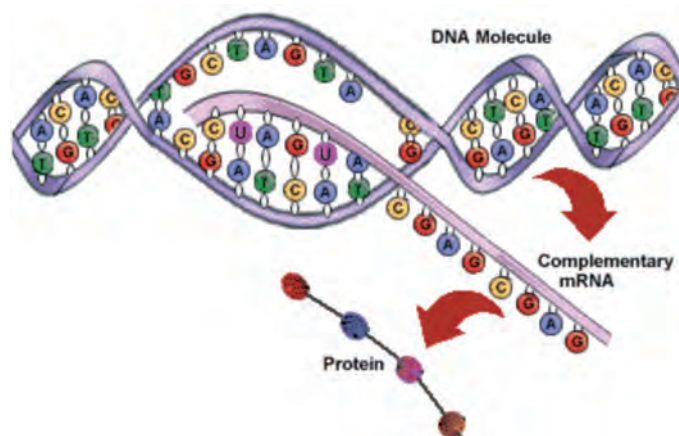
Nevertheless, some genetic markers that can help to speed up genetic progress are commercially available and, in particular situations, these can be used to support sheep and beef breeding programmes.

The majority of the markers that are available have been identified and developed in Australia, New Zealand and America where much larger flocks and herds are recorded. Several thousands of animals need to be recorded to properly validate a DNA test, and this means that only a few of these have been taken through to commercial development and validation in UK breeds.



Genes and gene markers – the basics

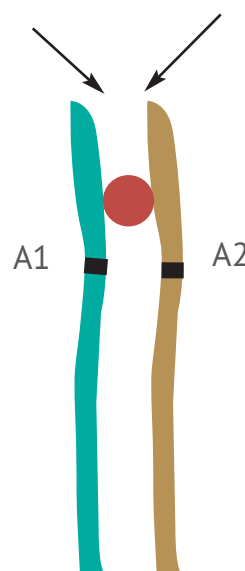
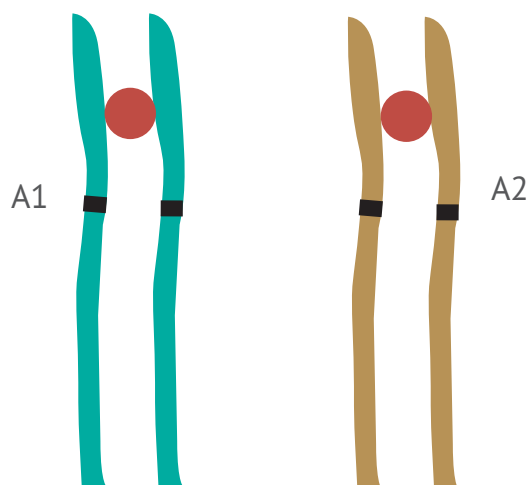
A **gene** is a stretch of DNA on an individual chromosome that carries instructions for the animal's cells to make a specific protein that is required for the healthy growth and development of that animal. All animals carry two copies of every chromosome, one inherited from the dam and one from the sire (humans have 23 pairs of chromosomes while cattle have 30 pairs and sheep 27 pairs). One pair is the 'sex chromosomes', with females having two 'X chromosomes' and males one 'X' and one 'Y chromosome'.



Every animal has two sets of instructions (**genes**) for every one of thousands of proteins that are required by the body. For any specific gene an animal will carry two copies (**alleles**) of each gene that are either **identical (homozygous)** or are slightly **different to each other (heterozygous)**.

When an animal reproduces it passes one of its two alleles for each protein to its offspring. Which allele is passed on by the sire and dam is completely random for every offspring produced.

Homozygous sire Homozygous dam

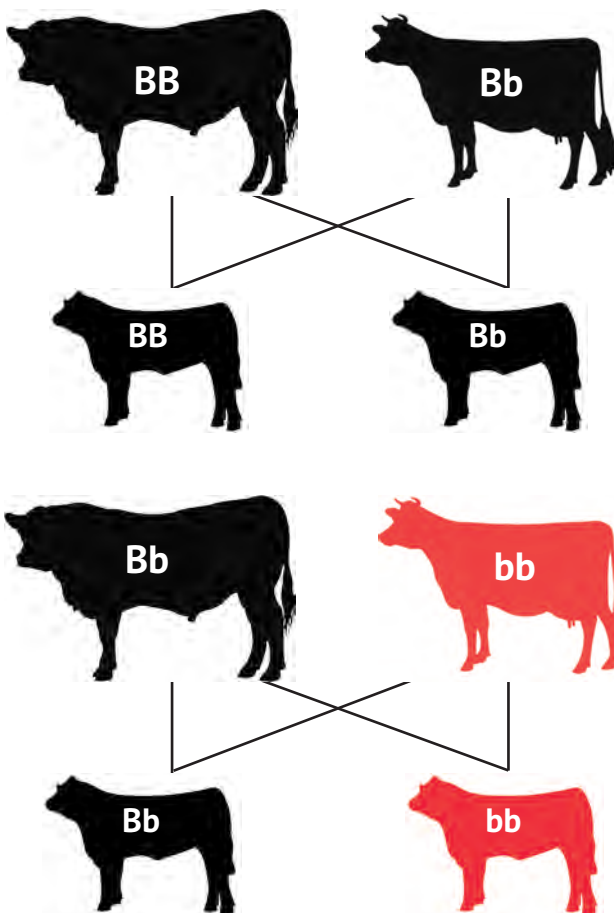


Heterozygous offspring

Variations in the DNA code, that occur naturally over time to create different alleles, may mean that the animal produces a slight variant of the normal protein or produces it in different quantities or at different times. In some cases, the change in the DNA code may mean that the protein cannot be produced at all.

It will also depend on whether that allele is dominant (always expressed) or recessive (only expressed if not paired with the dominant allele).

For example, black coat colour (B) in cattle is dominant over others, so animals carrying BB or Bb alleles have black coats and only animals with 2 copies of the recessive b allele (bb) are red coated.



The impact of these changes depends on what the gene controls and the effects can be either:

Minor e.g. coat colour in cattle

or

Major e.g. dwarfism and bulldog calf syndrome.



Red coat colour



Black coat colour



Dwarfism in Dexter cattle is due to a single copy of the dwarfing allele



2 copies of the dwarfing allele result in the lethal bulldog calf syndrome

10 Genes and gene markers – the basics

Which allele is expressed in a particular animal can also depend on other factors.

Specific alleles will not necessarily produce the same effect in all breeds and in all populations, so they may be expressed in some breeds but not others.

In some cases the expression of a particular gene depends on the presence or absence of other related alleles which may prevent the gene from being expressed. One example of this is the allelic variation of the Myostatin gene that is associated with double muscling in the Belgian Blue breed. This gene is also found in Highland cattle but is not expressed. So Highland cattle carrying the allele are NOT double muscled.



Double muscling in the Belgian Blue breed



Highland cattle

Some traits are controlled by many genes

Whilst some traits are controlled by a single gene with just one pair of alleles (like coat colour), many of the traits of interest in livestock breeding are influenced by many different genes (like growth rates, meat quality etc.). These are known as **polygenic** or **quantitative** traits.

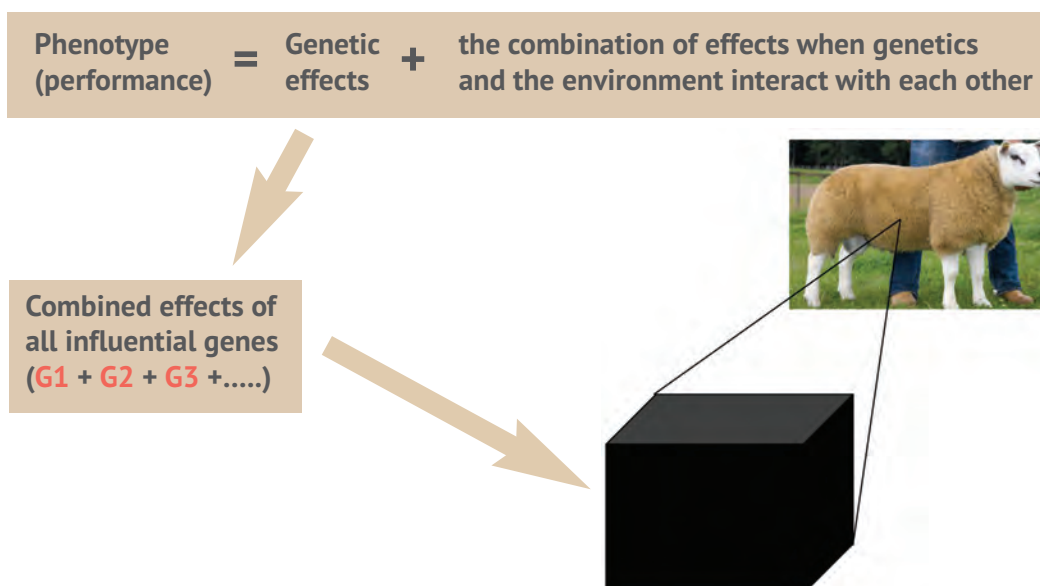
Examples of polygenic traits are:

- Muscling genes such as MyoMax® and Loinmax® in sheep, and the double muscling genes found in cattle.
- Prolificacy genes in sheep such as Inverdale® and Booroola.

Some genes can also influence several different traits e.g. the effect of growth hormone on growth rates and carcass composition.

These complex traits are usually also influenced by the animal's environment and its management.

For polygenic traits that are influenced by many genes and the animal's environment, we may be able to identify individual pairs of genes that have a relatively large influence on the trait, but these will explain only a proportion of the differences observed between individual animals.



Some genes have similar effects but they don't always work in the same way

A number of different alleles can increase ovulation rates in sheep.

The **Inverdale®** gene is **sex linked** (carried on the X chromosome) and animals inheriting one copy from their sire will be more prolific. The Inverdale effect is self-limiting in that animals inheriting a copy from both sire and dam will be sterile. In the UK this is managed by a commercial arrangement that retains control of the sires to prevent the breeding of double copy females in breeding populations.

In contrast, the **Booroola** gene, which is not carried on the X chromosome, has additive effects which can increase ovulation rates from 1-2 eggs (non-carrier) to 2-3 eggs per ovulation (one copy of Booroola) to 4-5 eggs per ovulation in animals with 2 copies.



So what are genetic markers?

Genetic markers are specific changes in the DNA that are located either within the gene or very close to it. They are tightly associated with the specific variants of the gene (alleles) and this means that they will almost always be inherited together so, once these markers have been identified and validated, they can be used to detect which particular alleles are carried by the animal.

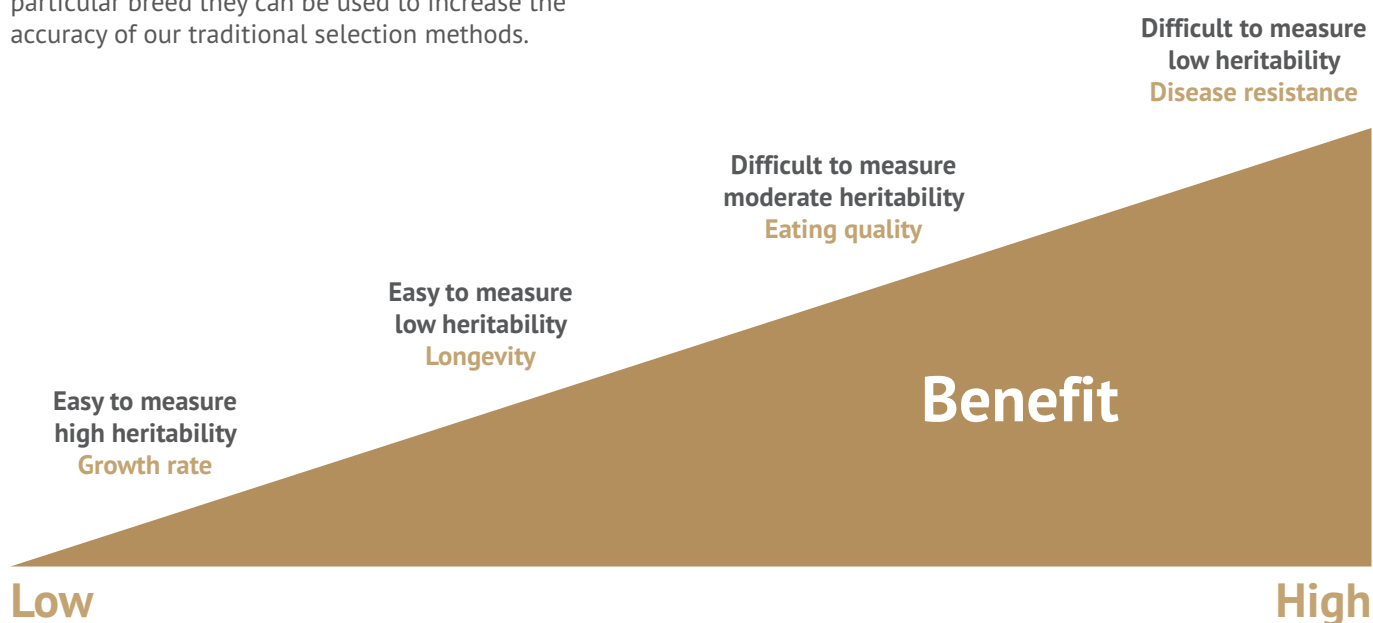
They then act like flags – pieces of DNA that we can easily recognize and which can then be used as the basis for laboratory tests.

However, we cannot be 100% certain that an animal carrying the markers has also inherited the specific allele associated with it. The closeness of association between presence of the marker and presence of the associated allele can differ from breed to breed. This means that the results of the DNA test may be less reliable in breeds that have not been used in the development of the test.



Marker assisted selection

Advances in DNA technology have allowed us to identify some alleles that have a relatively large effect on a polygenic/quantitative trait, and hence some genetic markers are associated with improved performance in sheep and cattle. Once these have been validated in a particular breed they can be used to increase the accuracy of our traditional selection methods.



Markers are particularly beneficial in helping to select for traits that are difficult to measure and that have a moderate to low level of heritability which would otherwise make progress very slow.

For example, the markers identifying the different alleles which code for the PrP protein and which control scrapie resistance in sheep have enabled breeders to remove susceptible stock and select for scrapie resistance in a way and a timeframe that would not have been possible by traditional selection alone.

Because DNA tests can be done at birth they have the potential to increase the accuracy of our genetic predictions of young animals and this is where their appeal and potential value lies.

It was initially thought that just a few markers would be sufficient to identify a significant proportion of the genetic variation in traits that are economically significant. But we now know that it's not quite as simple as that....

Nevertheless a number of gene tests have been developed. These tests are based on either:

- markers for single alleles that have a relatively large effect on performance;
- markers for single alleles that result in inherited disorders
- markers that identify groups of genes that combined together have an effect on performance.

The added information that DNA tests can bring to performance traits depends on the extent to which performance is influenced by that particular collection of genes. The tests explain only a very small amount of the total variation between animals and so they are always best used in conjunction with EBVs.

For many traits, individual alleles or groups of alleles that can explain economically important differences in the performance of individual animals have still not been identified. It may take the next generation of genetic/genomic technologies to enable us to make significant progress with these.

Commercially available marker tests

Traits controlled/influenced by single genes

The most obvious DNA markers to exploit and the easiest to test for are those involving a single variation (allele) that has a relatively large effect on the expression of that gene.

As a result, a number of DNA tests have been developed for sheep and cattle that can identify animals carrying specific alleles for simple traits such as coat colour, the presence or absence of horns, or some inherited diseases.

These DNA tests can be a useful tool for identifying breeding animals with the desired alleles for use in a breeding programme. They can be used as part of eradication programmes as in the case of Scrapie in sheep (see also inherited recessive disorders in the next section) and as tools to help introduce specific desirable traits into other breeds which do not normally carry those traits (e.g. polling into horned cattle breeds).

Some of these tests may be specific to the breeds in which the test has been developed, and so it is important to ask the test provider about the validity of the test in your breed, about data that is available from the UK, and about the mode of inheritance of the allele in your particular breed.

In some cases (e.g. polling in cattle), the desirable strain can be established without using genetic markers but the backcrossing programme to completely breed out the recessive trait (in this case horned) from the population is time consuming and often expensive. Gene markers can help to speed up this process by distinguishing between homozygous animals that carry two copies of the desirable allele and heterozygous animals that only carry one copy.



Commercially available tests based on single gene traits

Sheep	Cattle
Scrapie resistance Tests identify the 3 key alleles of the PrP protein that are known to affect scrapie resistance and susceptibility in the UK (e.g. ARR, ARQ, ARH, AHQ, VRQ).	Coat colour There are a number of genes and gene tests available depending on breed. Breeds most commonly tested include Simmental, Limousin, and composite or hybrid animals from a combination of breeds that have red and black ancestry.
	Polling Carriers (P) do not grow horns. Various tests are available for Charolais, Hereford, Limousin, Salers and Simmental cattle to distinguish between homozygous PP and heterozygous Pp polled individuals.

Inherited Recessive Disorders

A number of disorders in both cattle and sheep are caused by animals carrying two copies of a defective allele. Affected animals usually die from the disease or are culled when symptoms become evident.

These disorders can be difficult to eradicate from the breeding population because often the allele causing them is recessive. Animals that only carry one copy of the defective recessive allele are perfectly healthy, but they have a 50% chance of passing the defective allele to their offspring. If the offspring also inherits a defective allele from its other parent it will be affected by the disorder.

This makes it very difficult to eliminate these disorders from our herds or flocks without DNA testing

DNA testing allows us to identify potential breeding animals that carry one copy of a defective recessive allele (carriers) these animals can then be either eliminated from the breeding programme or mated to animals that are known to carry NO defective alleles.

Gene marker tests have been developed for some inherited diseases that can be used to identify carrier

animals and thus prevent them from being used for breeding. Use of these tests can be a cost effective way of eradicating the disorder from a herd or flock. Most of the tests available have been developed in the US and New Zealand and are for disorders that are not common in the the UK or their manifestation is not considered sufficient to require testing.

Breeding Results for Recessive Disorders

Sire	Dam	Progeny
Clear	Clear	Clear
Clear	Carrier	50% Clear
		50% Carrier
Carrier	Clear	50% Clear
		50% Carrier
Carrier	Carrier	25% Clear
		50% Carrier
		25% Affected

Commercially available tests to identify carriers of Inherited Recessive Disorders

Sheep	Cattle
Micro-ophthalmia Characterised by lambs having small or absent eyes. Affected lambs are blind. Not considered a major problem in UK breeds but has been seen in some UK flocks.	Curly Calf Syndrome Arthrogryposis Multiplex (AM) A disorder affecting mainly Angus cattle in the USA. Affected calves have a bent and twisted spine, little muscle development and rigid legs.
Dermatosparaxis A relatively recent discovery in White Dorper sheep in SA, Aus and USA causing very fragile skin that often tears near the legs in wrinkled areas.	Fawn Calf Syndrome Contractural Arachnodactyly (CA) This condition is rarely fatal but affected calves are weaker and have limited joint motion. They may have trouble suckling. Recorded in US Angus.
Spider Lamb Syndrome Affects the growth of cartilage and bone such that limbs of affected animals are thin, elongated, and "spider-like". Found mainly in Suffolk and Hampshire sheep originating from the USA.	Water Head Neuropathic Hydrocephalus Affected calves have an enlarged head due to the accumulation of fluid and are usually born dead or die shortly after birth. Recorded in US Angus.
	Tibial Hemimelia (TH) Affected calves have malformed or absent leg bones and abdominal hernia. Found in US Shorthorn and Galloway cattle.
	Mannosidosis Affected calves are uncoordinated, have head tremors, aggressive, and finally paralysed. Recorded in Angus and Galloway populations in NZ and Australia.
	Crooked Tail Syndrome (CTS) This manifests itself as crooked tails in Belgian Blues and has been associated with increased muscling but also causes reduced growth and musculo-skeletal problems.

Performance, health and fitness

For polygenic traits that are controlled by many genes and also influenced by the animal's environment, DNA tests may provide **additional information that can be used alongside EBVs** to help make better breeding decisions.

These DNA tests may identify combinations of markers that are known to be associated with improved performance, even though the specific alleles that influence the trait may not have been identified. These results are usually presented as a score or rating that indicates the likely benefits associated with the markers that are present in that animal. These results should be used in a similar way to EBVs within a breeding programme.

If many different genes influence a trait it is particularly important that the effect of the specific markers you are

identifying with a DNA test have been validated in the breed/population you are interested in. Sometimes specific markers may be associated with a relatively large effect in one breed or one environment but not in other breeds or environments.

Tests are available for a number of production / quality traits in sheep and cattle. These tend to use a single marker for specific alleles (e.g. MyoMAX or Cold Tolerance) or small numbers of markers (10-12) that are known to have an effect across a number of breeds (e.g. the Genestar and Igenity suites of markers).

However, they tend only to account for a relatively small proportion of the total genetic variation for the particular trait of interest and hence their accuracy is often relatively low in terms of EBV equivalents.

DNA tests available to give additional information on performance, health and fitness traits

Sheep

- **WormSTAR®** - Parasite resistance (must be used alongside FEC testing)
- **MyoMAX®** - Improves conformation and lean content, reduces fat. Homozygous in more than 90% of Texel sheep and present to varying degrees in some other UK breeds (e.g. Lley & Charollais) and absent in others (e.g. Suffolk)
- **LoinMAX®** - Increases size of loin muscle. Identified originally in Poll Dorset sheep in Aus and NZ
- **Cold Tolerance** - Identifies alleles that are associated with lower lamb mortality due to hypothermia in NZ. Is being used by some breeders in the UK
- **Footrot resistance** - identifies alleles that are associated with lower incidences of footrot in NZ sheep. Tests in the UK have so far failed to confirm this association.



Cattle

- **Feed Efficiency**
- **Marbling/Tenderness**
- **Meat Yield**
- **Maternal Traits**
- **Docility**

Tests are available (**Genestar** and **Igenity**) that include several of these traits tested using a single DNA sample

Validation has been based primarily on US and Australian populations. Tests are being used by some breeders in the UK.



Parentage / Breed verification

DNA information can be used to provide information about parentage of animals. This may be useful in situations where single sire mating has not been possible, or there is doubt about the sire (or dam) of an individual. If the parentage of the animal is unknown it may not be eligible for pedigree registration and will be disadvantaged when EBVs are calculated.

For situations where parentage recording is not cost effective (e.g. in large, extensive hill flocks), technologies are available that allow the calculation of EBVs based purely on performance records and DNA samples for pedigree recording.

Technological developments mean that DNA tests for parentage verification are now far more cost effective than before. However, these tests are usually based on a small number of markers and so parentage cannot be assigned unequivocally - generally only 80-90% of individuals can be assigned a single set of parents. These tests are used most effectively when there is a need to prove/disprove parentage or choose amongst a small number of potential parents.

Parentage verification tests are commercially available for sheep and cattle.



Breed verification

A limited number of breed verification tests are available. They are often based on a small number of markers and tend to be tailor made for those breeds rather than openly available.

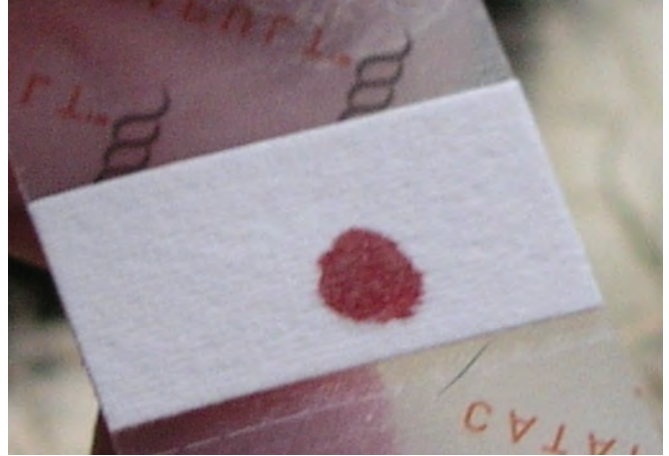
Breed verification tests can also be used on meat products as well as on the animal itself.



Collecting DNA samples from sheep and cattle

DNA is present in most cells of the animal's body. Most DNA tests can be carried out from a sample of the animal's blood, from semen or from hair follicles. In some cases tests have been developed that can use samples collected from nasal swabs, saliva swabs or from samples of tissue (e.g. ear punches). Some tests require a veterinary surgeon to take the samples, others can be done by the farmer.

- For reliable DNA tests it is important the samples are collected carefully following the instructions supplied by the test provider - especially since in some cases it may not be possible to go back and re-sample animals.
- Avoid cross-contamination of DNA samples between animals by always cleaning equipment between animals.
- It is important that all samples are marked clearly with the identification of the animal (e.g. Tag Number)
- If sending tissue sample abroad for testing you may be required to include an import permit.



DNA can be extracted from blood



or from tail hair samples



Genomics – the future of DNA technologies

The genomes of sheep and cattle contain a lot of information. These DNA sequences are made up of almost 1,000,000,000 individual genes and this information, uncompressed, wouldn't fit onto a CD.

Recent developments in DNA testing technologies now allow very small changes in the DNA code of an animal (Single Nucleotide Polymorphisms or SNPs) to be efficiently identified in thousands of sites right across the genome.

If the association of these tiny DNA changes with an animal's performance or appearance can be established in a well recorded population, the information can then be used to predict the breeding value of other animals.

Breeding values predicted in this way are known as Genomic Breeding Values and can be used in conjunction with Estimated Breeding Values (EBVs) to improve the accuracy with which we identify the best animals to use in our breeding programmes.

Genomic breeding values are likely to be most useful for traits that are difficult or expensive to record in an animal you may want to breed from (e.g. carcass and meat quality traits), can only be measured in one sex (e.g. milk yield or prolificacy) or can only be measured accurately later in an animal's life (e.g. longevity).



The Geneseek 80,000 SNP array for cattle

Good performance data from several thousand animals will be vital for calibrating Genomic Breeding Values for these new genomic tools to be useful. The associations between performance records and SNPs will also need to be recalibrated every few (5 or 6) generations. As such, genomic tests will always be an addition to and not a replacement for performance recording.



Further information

Please contact HCC's Industry Development team
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For further information on this booklet or the work of HCC please visit www.hccmpw.org.uk