Inherited Recessive Genetic Testing for U.S. Red and Black Wagyu Cattle

A Fact Sheet and Guide for Producers – REVISED 11/01/2014

Inherited Recessive Traits and the Five Disorders

What is a Recessive Trait?

Many physical traits can be linked directly to a specific genetic variant (mutation) in the DNA, and these variants can be passed from parent to offspring. Because an animal inherits a copy of DNA from each parent, a mutation can be transmitted from either the sire or the dam.

A "dominant" trait is one that is physically observable in an animal when it inherits only one copy from either its sire or dam. It only takes one copy of the mutation to change the physical appearance of that animal. An example of this is Black hair color.

A "recessive" trait is one that is only physically observable when a calf inherits a mutated copy of the DNA from both parents. An example of a recessive trait is red coat color. Note that not all recessive traits are bad or of an economical problem for the animal and some may in fact have a selective advantage. Two copies of the mutation are necessary to change the physical appearance of that animal. For this reason, an animal with only one copy of the recessive gene will typically not show any sign of the trait. An animal with two copies of the recessive mutation will show the trait. Only a cross between two Carrier parents or between Carrier and Affected parents can create an Affected animal.

Why have these Recessive Traits recently shown up in Wagyu cattle?

Most animals are actually Carriers of a mutation somewhere in their DNA for one or many recessive traits. Because an animal must inherit two copies of a given recessive mutation to be Affected, and with only a few animals typically sharing the same mutation in the whole population, there is rarely a mating cross that has the potential to create Affected offspring under natural selection. Most abnormal recessive traits are never even discovered.

However, under the selection pressure of most current breeding strategies, and given the relatively small founding population of Wagyu cattle outside of Japan, it is common to utilize a backcross and line breeding with a highly regarded sire line. If the original sire was a Carrier, there is a percentage of its generational offspring that will also be Carriers. In this case, it creates a higher-than-average frequency of Carriers and potentially Affected cattle in the population. Artificial insemination of a popular sire which is a carrier of a recessive gene is capable of spreading this gene throughout a population much faster than when bulls are only used in natural breeding conditions. This has also happened in the Wagyu breed in Japan, Australia, and the United States.

What are the Five Recessive Disorders?

In the last 10 years, several Recessive Disorders have been shown to be caused by recessive traits (mutations) in the Wagyu breed:

- **Erythrocyte Membrane Protein Band III Deficiency (Spherocytosis) (Band 3)**
  Affected cattle (cattle with two copies of the causative mutation) are morbidly anemic. The mutations affect a protein necessary for proper shape and function of red blood cells.

Calves are typically born weak and small (40-55 lbs birth weight) with severe anemia, labored breathing, palpitations, and not able to stand or suckle at birth. This disorder is often lethal, but some affected cattle survive to adulthood, although with severely retarded growth.

- **Claudin 16 Deficiency (CL16)**
  This mutation causes a buildup of fibrous tissue in the kidneys as well as other tissues. Affected cattle suffer from a severe risk of kidney failure throughout their lives. Other symptoms include growth retardation, increased blood urea nitrogen and creatinine values, diarrhea and overgrowth of hooves. It may or may not be lethal, but affected cattle tend to have atypically short lives.

- **Chediak-Higashi Syndrome (CHS)**
  Affected cattle have a deficiency in cells that make up a functional immune system. As a result, these calves are often more susceptible to disease and infection. These cattle may also have a light coat color, and slight coagulation problems (hemorrhaging). This disorder is usually not lethal.

- **Bovine Blood Coagulation Factor XIII Deficiency (F13)**
  This disorder is where one of the proteins needed to form blood clots is missing or reduced. Symptoms include severely prolonged bleeding time, bruising from castration/branding, and severe anemia. Death occurs in most cases.

- **Factor XI Deficiency (F11)**
  This mutation affects the efficiency of the clotting factor F11. Affected cattle suffer from mild hemophilia-like bleeding tendencies, either spontaneously or following trauma and surgical procedures. It is also possible that Carrier x Carrier mating have increased difficulty producing viable fertilized embryos and full-term pregnancies and are often Repeat Breeders[1]. Normal repeat breeding may be considered 40% with 60% conception being an industry average. It has been reported that factor 11 increased rebreeding by 50% in the Canadian Holstein breed, so now instead of 60% conception we will get 40% conception with 60% of the animals open to be rebred.

[1] Repeat Breeders are Cows that are cycling normally, with no clinical abnormalities, which have failed to conceive after at least two successive inseminations or embryo transfers. From a clinical perspective, there are two types of repeat breeders:

1. **Early repeats -** Cows that come into heat within 17-24 days after insemination or embryo transfer. In these animals the luteal function has been shorter than normal or typical for the physiological estrus cycle in non-bred cows. In these cows the most probable event is either failure of fertilization (delayed ovulation, poor semen quality etc.) or early embryonic death (delayed ovulation, poor embryo quality, unfavorable uterine environment, precocious luteolysis).

2. **Late repeats -** Cows that come into heat later than 25 days after insemination or embryo transfer. In these animals the luteal function was maintained for longer than the physiological luteal phase in non-bred cows. Fertilization and initial recognition of pregnancy probably took place but for some reason (inadequate luteal function, inadequate embryo signaling, infectious diseases, induced luteolysis) luteolysis was induced and pregnancy lost.

Genetic Testing Status and Offspring Distribution Predictions

The genetic status of each tested animal will be reported as one of the four following results:

Free (F)	•Means the animal has <u>two</u> copies of the normal gene •Also referred to as Normal and Unaffected
Carrier (C)	•Means the animal has <u>one</u> copy of the normal gene and <u>one</u> copy of the mutated gene •Also referred to as Positive
Affected (A)	•Means the animal has <u>two</u> copies of the mutated gene
No Result (NR)	•Means the DNA sample was good but did not yield a result under the applicable test protocol

The Science of Genetics Predicts the Following Results from Each Type of Mating:

Mating	Offspring Distribution		
	Free	Carrier	Affected
Free x Free	100%		
Free x Carrier	50%	50%	
Carrier x Carrier	25%	50%	25%
Free x Affected		100%	
Carrier x Affected		50%	50%
Affected x Affected			100%

Managing Recessive Genetic Disorders in Fullblood, Purebred, and Percentage U.S. Red and Black Wagyu Cattle

For proper herd management it is important for breeders to have an accurate understanding of the status of their cattle with respect to the genetic disorders. Without knowing the Free, Carrier, and/or Affected cattle in a breeder's herd, it is impossible to eliminate or reduce the risk of propagating the disorders in future generations of cattle. Actively addressing these genetic disorders today will pay dividends in the near future to both the breeder and collectively for the Wagyu breed.

Methods for managing recessive genetic disorders are breeder specific and depend on the type of cattle operation, i.e., registered, commercial, fullblood, purebred, percentage, seedstock, beef production, etc. Below are some suggested methods for breeders to consider. It should be noted that this list is not all inclusive.

1. Test all animals and remove Affected animals from the herd. Always use Free animals to mate with any Carrier animals remaining in the herd. A commitment must be made to test all offspring from Carrier animals that will remain in the breeding herd. The Carrier rate will be reduced over time in future generations.
2. Test all animals and remove all Affected animals and Carrier sires. Use only Free sires in the breeding program going forward. A commitment must be made to test all offspring from Carrier dams that will remain in the breeding herd. This will reduce the Carrier rate the same as in Method 1 above.
3. Test and remove Carrier and Affected animals from the herd. Only use Free animals in the breeding program going forward. No further testing will be required. This Method will ensure a totally Free herd going forward.
4. Test all animals and use Carrier and/or Affected[2] animals ONLY in a terminal breeding program.
5. Test all animals and use Carrier and/or Affected[2] animals as recipients. If a cleanup bull is used, it should be Free. Offspring DNA verified to the cleanup bull must be tested for any animals that will remain in the breeding herd.
6. These are just five examples of management methods that can be used exclusively or in combination.

As discussed above, proper management has a major impact on reducing the frequency of Carrier and Affected animals in a breeder's herd. **Table 1** demonstrates the reduction in the Carrier rate in future generations when consistently using Free sires on Carrier dams and their future generational offspring.

Table 1 – Generation 1: Free Sire x Carrier Dam

Generation	Offspring Distribution		
	Free	Carrier	Affected
1st	50.00%	50.00%	
2nd	75.00%	25.00%	
3rd	87.50%	12.50%	
4th	93.75%	6.25%	
5th	96.88%	3.12%	
6th	98.44%	1.56%	
7th	99.22%	0.78%	

As you can see the Carrier percentage is basically eliminated by the seventh generation and no Affected cattle are in the offspring distribution.

However, if a Carrier sire were reintroduced to the fourth generation dams, the fifth generation offspring would be 48.44% Free vs. 96.88, 50% Carrier vs. 3.12%, and 1.56% Affected vs. 0%.

[2]According to research in the Holstein breed, Affected F11 animals have a tendency to be repeat breeders. It is anticipated that a study will be conducted to determine if there is a correlation between Affected F11 Wagyu and Repeat Breeders.

Conversely, **Table 2** shows the offspring distribution when a Carrier sire is mated to a Carrier dam, and Carrier sires are used to mate with the future generational offspring dams. The offspring distribution remains constant through all future generations. Having 25% Affected offspring annually is a costly endeavor for any breeder to experience.

Table 2 – Carrier Sire x Carrier Dam

Generation	Offspring Distribution		
	Free	Carrier	Affected
1st	25.00%	50.00%	25.00%
2nd	25.00%	50.00%	25.00%
3rd	25.00%	50.00%	25.00%
4th	25.00%	50.00%	25.00%
5th	25.00%	50.00%	25.00%
6th	25.00%	50.00%	25.00%
7th	25.00%	50.00%	25.00%

Testing Policy

Required Testing

All live cattle and genetics sold at Association sanctioned production sales must be tested as well and the results must be published in the sale catalog for all potential buyers to review prior to the sale.

As an exception to Required Testing, if both the sire and dam of the offspring are Free from all five of the genetic disorders set out above, the offspring will be exempt from testing and will be given "Free" status by the Association for all five genetic disorders.

Publication of Test Results

Test results that were submitted to the AWA will be will be posted on the Association website. Once the GENEPROB implementation is complete the information will be tracked and updated in the database and be available to the public.

Authorized Lab

The following US Based lab is authorized by the American Wagyu Association to conduct testing for the genetic disorders set out in this document.

GeneSeek, a Neogen company
4665 Innovation Drive, (Suite 120)
LINCOLN, NE 68521
Phone: (402) 435-0665
Fax: (402) 435-0664
Website: www.neogen.com

The AWA website has all the information and documents you will need from ordering your sample collector cards to receiving your results.

Important Information

IGenix, Inc will cease operations in early 2013 and GeneSeek will be the new service provider to the AWA.

In December, 2012 GeneSeek acquired Scidera (formerly MMI) who was AWA's primary service provider for DNA parentage.
As of Jan 1, 2013 all Wagyu sample orders and testing is done through GeneSeek. The current storage of Wagyu samples existing at the Davis lab will be relocated to the GeneSeek Nebraska lab mid to late 2013. The primary purpose of this storage is for extracting parent information (if viable and sufficient quantity) for SNP profiling.

The **preferred** sample type is HAIR and DNA sample collector cards are available through the AWA office and 'How to Collect' instructions are available via the AWA website. Blood, semen and tissue is also acceptable for DNA testing but maybe subject to surcharge. Please contact the AWA if you have any questions.

PLEASE NOTE that all DNA ordering and payments are handled through the AWA Office. All samples are to be sent directly to the NE address (above) and clearly labeled.

Frequently Asked Questions

Q. What affect do the genetic disorders have on Wagyu meat products?

A. Absolutely no affect. Beef products from Carrier and/or Affected cattle have no impact on the quality, safety, and health of the end product.

Q. Do I have to test all of my animals?

A. No, you are only required to test your AI sires, embryo donor dams, and animals/genetics sold in Association sanctioned production sales. However, it is a good idea to test all of your breeding stock so you can effectively manage your breeding program and reduce the risk of propagating the genetic disorders throughout your herd and having Affected offspring.

Q. Do I have to test offspring from Free parents?

A. No, if both of the parents are Free, then the offspring will be Free as well.

Q. Do I have to destroy carrier animals?

A. Absolutely No. Each animal should be judged on all of its phenotypic qualities. If the animal is excellent in many characteristics but happens to be a carrier for one of the disorders, you can still use the animal for breeding stock, including as a donor. However, you will need to be responsible for testing all of the offspring and typically keeping the Free animals for your breeding program and using the Carrier animals in your terminal beef program either as cows or for harvest.

Q. If I've not ever experienced any of the symptoms of the disorders in my herd, does that mean my herd is Free?

A. Not necessarily. You could have Carrier animals in your herd and not know it, because a Carrier does not display any symptoms of the disorder. You will only see the symptoms described in this document with Affected cattle.

Q. If I breed a Carrier cow to a Free bull, what is the chance of having an Affected calf?

A. 0% chance. However, you have a 50% chance of having a Carrier calf and a 50% chance of having a Free calf.

Q. Why are Carriers of genetic disorders important?

A. Carriers of genetic disorders used in breeding programs (registered or commercial) are responsible for propagating the recessive mutation within the cattle population.

Q. What does a Carrier of a genetic disorder look like?

A. A Carrier of any genetic disorder looks perfectly normal; there is nothing in the way an animal looks (its phenotype) that indicates that the animal is a Carrier of a genetic mutation.

Q. If a cow has an Affected calf, what does that mean?

A. If a cow has an Affected calf, and it is the cow's natural calf, it means the cow is a Carrier of the applicable genetic mutation and the sire of the calf is also a Carrier.

Q. If a recipient cow has an Affected calf, what does that mean?

A. If a recipient cow has an Affected calf, and the calf is not the result of a cleanup bull being used after ET, then it means that both the donor cow and the sire are both Carriers of the applicable genetic mutation. If the calf is the result of the cleanup bull, it means that both the recipient cow and the cleanup bull are both Carriers of the applicable genetic mutation.

Q. What do I do with Non-Carrier females in my herd?

A. If the females tested Free and are bred to Free bulls, they will never produce Affected or Carrier calves. This is really ideal and these females can be used throughout your breeding program with no risk of propagating the genetic mutations**.**

Contact Information

Should you have any questions, please contact the AWA Office on (208) 2623-8100 or office@wagyu.org

DISCLAIMER - Genetic test results are based on samples provided by breeders. The American Wagyu Association AND the Board of Directors make no statements, representations or warranties about the accuracy or completeness of, any information relating to the status of a particular animal; and, disclaims all responsibility for information and all liability (including without limitation, liability in negligence) for all expenses, losses, damages, and costs you may incur as a result of information being inaccurate or incomplete in any way for any reason.