

Coat Color and Trait Certificate

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|-------------------------|--------------------------------|--------------------------|-----------------|
| Call Name: | Stevie | Laboratory #: | 196456 |
| Registered Name: | Delilahsway It was a landslide | Registration #: | DN64082204 |
| Breed: | Australian Shepherd | Microchip #: | 956000012739164 |
| Sex: | Female | Certificate Date: | Oct. 8, 2021 |
| DOB: | Sept. 2020 | | |

This canine's DNA showed the following genotype(s):

| Coat Color/Trait Test | Gene | Genotype | Interpretation |
|---|---------------|--------------------------------|---|
| A Locus (Agouti) | <i>ASIP</i> | a^t/a^t | Tricolor, black and tan |
| A ^S Locus (Saddle Tan) | <i>RALY</i> | N/N | No saddle tan/creeping tan |
| D Locus (Dilute) | <i>MLPH</i> | D/D | Non-dilute (does not carry dilute) |
| E Locus (Apricot/Yellow/Red) - e (Common Variant Found in Many Breeds) | <i>MC1R</i> | E/E | Black |
| E ^m Locus (Melanistic Mask) | <i>MC1R</i> | E ^m /E ^m | Melanistic mask |
| K Locus (Dominant Black) | <i>CBD103</i> | k^y/k^y | Agouti expression allowed |
| M Locus (Merle) | <i>PMEL</i> | m/M | *See detailed interpretation |
| S Locus (White Spotting, Parti, or Piebald) | <i>MITF</i> | S/S | No white spotting, flash, parti, or piebald |
| T Locus (Natural Bobtail) | <i>T</i> | t/T | Bobtail |

Interpretation:

This dog carries two copies of a^t which results in tan points and can also present as a black and tan or tricolor coat color. However, this dog's coat color is also dependent on the E, K, and B genes. The tan point coat color is only expressed if the dog is also E/E or E/e at the E locus and k^y/k^y at the K locus. This dog will pass on a^t to 100% of its offspring.

This dog carries two copies of the **N** allele, which is not associated with a saddle tan coat color. This dog's coat color is also dependent on the E, A, and K genes, among others. This dog will pass **N** to 100% of its offspring.

This dog does not carry any copies of the d^1 or d^2 mutations and has a D locus genotype of **D/D** which does not result in the "dilution" or lightening of the pigments that produce the dog's coat color. This dog will pass one copy of **D** to 100% of its offspring and cannot produce d/d dogs.

This dog carries two copies of **E** which allows for the production of black pigment. However, this dog's coat color is also dependent on the K, A, and B genes. This dog will pass on **E** to 100% of its offspring.

This dog carries two copies of **E^m** which results in a melanistic mask on the muzzle of the dog. However, a melanistic mask may be unrecognizable on a dog with a dark coat color. This dog will pass on **E^m** to 100% of its offspring and will produce only puppies with a melanistic mask.

This dog carries two copies of k^y which allows for the expression of the agouti gene (A locus) which can result in a variety of coat colors including sable/fawn, tricolor, tan points, black or brown. However, this dog's coat color is

dependent on its genotypes at the E, A and B genes. This dog will pass on **k^y** to 100% of its offspring.

M Locus Genotype: m/M²⁶⁸

This dog carries one copy of the **m** (non-merle, wild-type) allele and one copy of the **M** (merle insertion variant) allele of the *PMEL* gene. This dog will pass on one copy of the **m** (non-merle, wild-type) allele to 50% of its offspring and one copy of the **M** (merle insertion variant) allele to 50% of its offspring. The approximate size of the M allele of this dog (+/- 1 base pair) is listed in superscript in the genotype. Merle is inherited in a dominant fashion, meaning that only one copy of an M allele is necessary for a dog to display some variation of the merle coat color/pattern, which is marked by random dilution of eumelanin (black pigment) leaving patches of normal coat color within areas of diluted pigmentation.

Specific sizes of the M allele have been associated with the potential to produce “classic” merle patterning or other M-associated coat color variations. Merle is most appropriately viewed as a spectrum of coat colors/patterns and the size of the variant M allele is associated with a coat color/pattern somewhere within that spectrum. Although some coat color/pattern variations have been associated with specific sizes of the M allele in certain breeds, referred to here as a ‘bin’, the size of the M allele does not guarantee a specific outcome. In general, dogs with M allele sizes between 200 – 246 base pairs (bp) have been associated with non-merle or minimal-merle coat colors/patterns and are often referred to as “cryptic” merle; M allele sizes between 247 – 264 bp have been associated with “atypical” or “diluted” coat colors/patterns; M allele sizes between 265 – 269 bp have been associated with the “classic” merle coat colors/patterns; and M allele sizes between 270 – 280 bp have been associated with a “tweed”, “harlequin” or “patchwork” merle coat colors/patterns. Many exceptions to the coat color/pattern associations found in the various M allele bin sizes listed here have been identified. Therefore, care should be taken when correlating M allele sizes with anticipated coat color/pattern outcomes. These bin sizes should not be interpreted as having discrete boundaries but should be viewed as a range within which specific coat colors and patterns are likely. Variations in genetic background between breeds and in individual dogs within a breed may result in the identification of different coat colors/patterns not typically found in a given bin, especially when the size of an M allele is at the border between bins. Furthermore, due to the complex nature of the merle insertion variant and the limitations of currently available molecular technologies, precise sizing of the merle insertion variant is challenging. However, the sizing of the merle insertion variant in our laboratory has been validated to be accurate to within +/- 1 bp which, nevertheless, makes correlations between genotype and coat color/pattern of dogs close to the boundaries of a specific bin potentially problematic. In addition, the M allele bins defined here are only relevant to test results generated by Paw Print Genetics. The variable nature of the M gene variant and subtle differences in methodologies used by each laboratory precludes strict interlaboratory genotype comparisons. Therefore, in some cases, it may be prudent to test related dogs in a single laboratory if comparisons across related dogs or dogs within a breed are desired.

The inherent instability of the M insertion variant makes it susceptible to further mutation events that can result in “mosaicism” whereby more than one version (allele) of the M insertion variant of a potentially different size is found in various cells throughout a dog’s body. Indeed, mosaicism is likely what gives a merle dog its variable coat color/pattern with some cells having a copy of one M variant allele that results in altered pigmentation while other cells may have a different sized insertion resulting in an alternate form of the M variant allele that may express the coat color or pattern differently. It has also been documented that, due to the inherent instability of the M insertion variant, changes to the M insertion variant size can occur during the replication of each M allele, which may result in subtle changes in M allele size from cell to cell (mosaicism) and even from one generation to the next if present in egg or sperm cells (germ cells or gonads). However, current evidence suggests that lengthening of the M insertion variant is less likely to occur than shortening, although either event is theoretically possible. Thus, this “mosaicism” may result in different alleles of the M insertion variant being present in different cell lines or tissue types in the same dog. Importantly, if the mosaicism occurs in the germ cells (sperm or eggs) of a dog, the different alleles of the M insertion variant may be passed on to offspring. Furthermore, this mosaicism may be found in only a small percentage of cells and may not be present in the cells from which a given sample is obtained from a dog for genetic testing, making it difficult to always reliably detect mosaicism. Thus, all levels of mosaicism may not be detected by this test. If identified, mosaic M alleles at ~5% or greater of the total M alleles detected will be reported in the final genotype with the approximate percentage of each M allele identified in brackets.

Dogs that are identified as having a mosaic genotype may pass on each of their mosaic M alleles if they are also present in the germ cells of the dog. Germline mosaicism can only be confirmed by testing samples obtained from sperm or eggs. Dogs that inherit two copies of the M insertion variant are at an increased risk of being mostly white with hearing and/or vision deficits. To avoid producing “double merle” (M/M) puppies, dogs with a

copy of M (particularly those with M alleles near the size which is likely to produce the classic merle coat color/pattern) should only be bred to dogs that do not have a copy of the M allele. Dogs related to this dog have an increased chance of carrying an M allele. Testing for the M allele is indicated for related dogs.

This dog carries two copies of **S** which results in a solid coat with no white spotting, flash, parti, or piebald coat color. This dog will pass on one copy of **S** to 100% of its offspring.

This dog carries one copy of the dominant **T** allele and one copy of the recessive **t** allele which produces a natural bobtail. This dog will pass on the **T** allele to 50% of its offspring and the **t** allele to 50% of its offspring.

Paw Print Genetics® has genetic counseling available to you at no additional charge to answer any questions about these test results, their implications and potential outcomes in breeding this dog.



Blake C Ballif, PhD
Laboratory & Scientific Director



Christina J Ramirez, PhD, DVM, DACVP
Medical Director

Normal results do not exclude inherited mutations not tested in these or other genes that may cause medical problems or may be passed on to offspring. These tests were developed and their performance determined by Paw Print Genetics®. This laboratory has established and verified the tests' accuracy and precision. Because all tests performed are DNA-based, rare genomic variations may interfere with the performance of some tests producing false results. If you think these results are in error, please contact the laboratory immediately for further evaluation. In the event of a valid dispute of results claim, Paw Print Genetics will do its best to resolve such a claim to the customer's satisfaction. If no resolution is possible after investigation by Paw Print Genetics with the cooperation of the customer, the extent of the customer's sole remedy is a refund of the fee paid. In no event shall Paw Print Genetics be liable for indirect, consequential or incidental damages of any kind. Any claim must be asserted within 60 days of the report of the test results.