Paw Print Genetics
A division of Genetic Veterinary Sciences, Inc.

Coat Color and Trait Certificate

Call Name:	Ivy	**Laboratory #:**	131880
Registered Name:	Fullerton Poison Ivy	**Registration #:**	ASDT-NE-2113325
Breed:	Toy Australian Shepherd	**Microchip #:**	990000004565262
Sex:	Female	**Certificate Date:**	Jan. 28, 2022
DOB:	June 2021		

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

Coat Color/Trait Test	Gene	Genotype	Interpretation
A Locus (Agouti)	*ASIP*	a^t/a^t	Tricolor, black and tan
B Locus (Brown)	*TYRP1*	B/b or b/b	Black or brown coat, nose and foot pads (carries at least one copy of brown)
Chondrodysplasia (CDPA)	*CFA18 FGF4*	cd/cd	No Leg Shortening Associated with CDPA
D Locus (Dilute)	*MLPH*	D/D	Non-dilute (does not carry dilute)
E Locus (Apricot/Yellow/Red) - e (Common Variant Found in Many Breeds)	*MC1R*	E/e	Black (carries yellow/red)
E^m Locus (Melanistic Mask)	*MC1R*	E^m/N	Melanistic mask (carrier)
K Locus (Dominant Black)	*CBD103*	k^y/k^y	Agouti expression allowed
M Locus (Merle)	*PMEL*	m/M	*See detailed interpretation
S Locus (White Spotting, Parti, or Piebald)	*MITF*	S/S	No white spotting, flash, parti, or piebald

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 one or more copies of the four possible b mutations and has a B locus genotype of **B/b** or **b/b** that cannot be distinguished without additional testing of parental samples or by examining the coat, nose and footpad color of the dog. Dogs inherit two copies of the B locus, one from each parent. Because there are four different B locus mutations that can potentially be identified, as well as some limitations inherent to genetic testing methodologies currently available, a result of "B/b or b/b" means that it cannot be determined if the b mutations identified in this dog are present on the same copy of the B locus inherited from one parent or if they occur on separate copies of the B locus inherited from each of the parents. If the mutations identified are all present on the same copy of the B locus, this dog will have a **B/b** genotype and typically will have a black coat, nose and footpads. If the mutations identified are present on different copies of the B locus, this dog will have a **b/b** genotype and may have a brown coat, and will typically have a brown nose and footpads. Depending on the breed, b/b dogs may be referred to as brown, chocolate, liver or red. However, this dog's coat color is dependent on the genotypes of many other genes. The B locus genotype for this dog can be inferred without the need for parental testing by evaluating the color of this dog's nose. If this dog's nose is brown, the B locus genotype of this dog must be **b/b** and this dog will pass one copy of **b** to 100% of its offspring. If this dog's nose is black, the final B

locus genotype of this dog must be **B/b** and this dog will pass one copy of **B** to 50% of its offspring and one copy of **b** to 50% of its offspring. In either case, this dog carries at least one copy of **b** and can produce b/b offspring if bred to a dog that is also a carrier of a b mutation (B/b or b/b).

Two genetic mutations are associated with shortened legs in dogs. Both mutations consist of copied sections (duplication) of the canine *FGF4* gene (called an *FGF4*-retrogene) that have been inserted into two aberrant locations in the genome; one in chromosome 12 (*CFA12 FGF4*; associated with CDDY and IVDD risk) and one in chromosome 18 (*CFA18 FGF4*; associated with chondrodysplasia [CDPA], but not associated with IVDD). Appropriate breeding decisions regarding dogs which have inherited the *CFA12 FGF4* mutation (WT/M or M/M) need to address both the potential loss of genetic diversity in a population which would occur if dogs with this mutation were prohibited from breeding as well as the loss of the short-legged appearance that is a defining physical characteristic for some breeds. In breeds which inherit both mutations, breeders may use genetic testing results to selectively breed for the CDPA (*CFA18 FGF4*) mutation while breeding away from the CDDY and IVDD risk (*CFA12 FGF4*) mutation to reduce IVDD risk and retain the short-legged appearance. However, the frequency of each mutation varies between breeds and, in some cases, may not be conducive to such a breeding strategy. For example, breeds with extreme limb shortening (e.g. Basset hound, Dachshund, Corgi) typically develop their appearance due to inheritance of both the *CFA12 FGF4* and *CFA18 FGF4* mutations. In addition, depending on the breed, offspring born without either the *CFA12 FGF4* or *CFA18 FGF4* mutations may display longer limbs than cohorts and, therefore, not meet specific breed standards.

This dog carries two copies of the **cd** allele which does not result in leg shortening. However, the actual leg length of the dog is a result of a combination of factors including the mutation associated with CDDY and IVDD risk (*CFA12 FGF4*) as well as variants in other genes. This dog will pass one copy of **cd** 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 one copy of **E** and one copy 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 **E** on to 50% of its offspring and **e** to 50% of its offspring, which can produce a yellow/red coat (including shades of white, cream, yellow, apricot or red) if inherited with another copy of **e**.

This dog carries one copy of $\mathbf{E^m}$ and one copy of **N** 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 $\mathbf{E^m}$ to 50% of its offspring and **N** to 50% of its offspring.

This dog carries two copies of $\mathbf{k^y}$ 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 $\mathbf{k^y}$ to 100% of its offspring.

M Locus Genotype: m/M^{266}

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

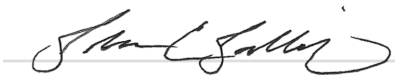
coat color/pattern associations found in the various M allele bin sizes listed here have been identified. Therefore, care should be taken when correlating M allele sizes with anticipated coat color/pattern outcomes. These bin sizes should not be interpreted as having discrete boundaries but should be 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.

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.



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