EVG molekularna diagnostika d.o.o.
Taborska ulica 8 · 2000 Maribor · Slovenia
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REFERENCE NO.: 2019 - 30103
OWNER:
NICOLE KELPEN
SCHLACKENWERTHER STR. 13
DE-76437 RASTATT
GERMANY

NAME/LABEL:
AZULIAN CATCH YOUR DREAMS
SPECIES: DOG
BREED: SHETLAND SHEEPDOG
SEX: MALE
MICROCHIP NO.: 985113002089467
TATOO NO.: NOT PROVIDED
PEDIGREE NO.: NOT PROVIDED

GENETIC REPORT

SAMPLE: BUCCAL SWAB

SAMPLE TAKEN BY: MARCUS ERBEN, DVM

REQUESTED TEST: COLLIE EYE ANOMALY (CEA)

RESULT: CLEAR (WT/WT)

COMMENT :
The test examines presence or absence of NHEJ1 gene mutation (c.588+462_588+8260del7799bp) described as the cause for collie eye anomaly (CEA) in several dog breeds. The disease is characterized by different level of impairment of retina and choroid sclera that occurs during development of the eye. Collie eye anomaly is inherited as an autosomal recessive trait.

Regarding to the presence of tested mutation animals are classified in three groups:

- Clear (wt/wt) - mutation is not present, normal genotype
- Carrier (mut/wt) - one of two alleles carries tested mutation, disease is not clinically manifested
- Affected (mut/mut) - both alleles carry tested mutation, disease is clinically manifested

For each group different breeding strategies should be followed. Breeding of affected and carrier animals should be avoided. If particularly valuable animal is classified as affected, it should be bred only with clear animal. In such case, all first generation siblings will be carriers. If a carrier is bred with clear animal, 50% of siblings are expected to be clear. In case two carriers are bred, 25% of siblings are expected to be clear and 50% are expected to be carriers. However, 25% of siblings are expected to be affected, therefore such breeding practice is discouraged.

AUTHORIZED SIGNATURE:

MARIBOR, 28.11.2019

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PEDIGREE NO.: NOT PROVIDED

GENETIC REPORT

SAMPLE: BUCCAL SWAB

SAMPLE TAKEN BY: MARCUS ERBEN, DVM

REQUESTED TEST: MULTI DRUG RESISTANCE (IVERMECTIN SENSITIVITY, MDR1)

RESULT: CLEAR (WT/WT)

COMMENT :
The test examines presence or absence of MDR1/ABCB1 gene mutation (c.295_298del) described as the cause of multi drug resistance (MDR) in several dog breeds. The condition is characterized by increased susceptibility to neurotoxic side effects of several drugs including Ivermectin. MDR1 gene defect is inherited as an autosomal recessive trait.

Regarding to the presence of tested mutation animals are classified in three groups:

- Clear (wt/wt) - mutation is not present, normal genotype
- Carrier (mut/wt) - one of two alleles carries tested mutation, disease is not clinically manifested
- Affected (mut/mut) - both alleles carry tested mutation, disease is clinically manifested

For each group different breeding strategies should be followed. Breeding of affected and carrier animals should be avoided. If particularly valuable animal is classified as affected, it should be bred only with clear animal. In such case, all first generation siblings will be carriers. If a carrier is bred with clear animal, 50% of siblings are expected to be clear. In case two carriers are bred, 25% of siblings are expected to be clear and 50% are expected to be carriers. However, 25% of siblings are expected to be affected, therefore such breeding practice is discouraged.

AUTHORIZED SIGNATURE:

MARIBOR, 28.11.2019

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DOGDNATEST

OWNER:
NICOLE KELPEN
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DE-76437 RASTATT
GERMANY

DATE:28.11.2019

TEST REPORT NO. 202692

TEST: DEGENERATIVE MYELOPATHY (DM)

MUTATION: c.118G>A in SOD1 gene

RESULT: CARRIER (NORMAL/MUTATION) (NORMAL/MUTATION)

ANIMAL NAME: AZULIAN CATCH YOUR DREAMS

SPECIES: DOG

BREED: SHETLAND SHEEPDOG

MICROCHIP NO.: 985113002089467

PEDIGREE NO.: NOT PROVIDED

SAMPLE TYPE: BUCCAL SWAB

SAMPLE TAKEN BY: MARCUS ERBEN, DVM

RESULT COMMENT:

Clear (normal/normal): tested mutation is not present, normal genotype.

Carrier (normal/mutation): one allele carries tested mutation, disease is not clinically manifested.

Affected (mutation/mutation): both alleles carry tested mutation, disease is clinically manifested.

AUTHORIZED SIGNATURE:

DOGDNATEST

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SEX: MALE
MICROCHIP NO.: 985113002089467
TATOO NO.: NOT PROVIDED
PEDIGREE NO.: NOT PROVIDED

GENETIC REPORT

SAMPLE: BUCCAL SWAB

SAMPLE TAKEN BY: MARCUS ERBEN, DVM

REQUESTED TEST: PROGRESSIVE RETINAL ATROPHY (CNGA1-PRA)

RESULT: CLEAR (WT/WT)

COMMENT :
The test examines presence or absence of CNGA1 gene mutation (c.1752_1755delAACT) described as the cause of CNGA1 progressive retinal atrophy (CNGA1 - PRA) in Shetland Sheepdog. The disease is characterized by progressive degeneration of retinal cells. CNGA1 gene defect is inherited as an autosomal recessive trait.

Regarding to the presence of tested mutation animals are classified in three groups:

- Clear (wt/wt) - mutation is not present, normal genotype
- Carrier (mut/wt) - one of two alleles carries tested mutation, disease is not clinically manifested
- Affected (mut/mut) - both alleles carry tested mutation, disease is clinically manifested

For each group different breeding strategies should be followed. Breeding of affected and carrier animals should be avoided. If particularly valuable animal is classified as affected, it should be bred only with clear animal. In such case, all first generation siblings will be carriers. If a carrier is bred with clear animal, 50% of siblings are expected to be clear. In case two carriers are bred, 25% of siblings are expected to be clear and 50% are expected to be carriers. However, 25% of siblings are expected to be affected, therefore such breeding practice is discouraged.

MARIBOR, 28.11.2019

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PEDIGREE NO.: NOT PROVIDED

GENETIC REPORT

SAMPLE: BUCCAL SWAB

SAMPLE TAKEN BY: MARCUS ERBEN, DVM

REQUESTED TEST: VON WILLEBRAND DISEASE TYPE III (VWDIII)

RESULT: CLEAR (WT/WT)

COMMENT :

The test examines presence or absence of VWF gene mutation (c.937delT) described as the cause of von Willebrand's disease type III (vWD III) in Shetland Sheepdog. This type of vWD is characterized by full absence of plasma von Willebrand factor responsible for blood coagulation process. Affected animals experience episodes of life threatening bleeding. Tested VWF gene defect is inherited as an autosomal recessive trait.
Regarding to the presence of tested mutation animals are classified in three groups:

- Clear (wt/wt) - mutation is not present, normal genotype
- Carrier (mut/wt) - one of two alleles carries tested mutation, disease is not clinically manifested
- Affected (mut/mut) - both alleles carry tested mutation, disease is clinically manifested

For each group different breeding strategies should be followed. Breeding of affected and carrier animals should be avoided. If particularly valuable animal is classified as affected, it should be bred only with clear animal. In such case, all first generation siblings will be carriers. If a carrier is bred with clear animal, 50% of siblings are expected to be clear. In case two carriers are bred, 25% of siblings are expected to be clear and 50% are expected to be carriers. However, 25% of siblings are expected to be affected, therefore such breeding practice is discouraged.

AUTHORIZED SIGNATURE:

MARIBOR, 28.11.2019

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PEDIGREE NO.: NOT PROVIDED

GENETIC REPORT

SAMPLE: BUCCAL SWAB

SAMPLE TAKEN BY: MARCUS ERBEN, DVM

REQUESTED TEST: DERMATOMYOSITIS (DMS)

RESULT:

PAN2:	A/a
MAP3K7CL:	b/b
DLA-DRB1:	002:01/002:01 (C/C)

ASSOCIATED RISK: *LOW RISK*

COMMENT :

The test examines presence or absence of mutations in *PAN2* (locus A), *MAP3K7CL* (locus B), and *DLA-DRB1* (locus C) genes associated with increased risk for development of dermatomyositis (DMS) in Shetland sheepdog and Collie. Dermatomyositis is an autoimmune disease of the skin and muscle caused by a combination of environmental and genetic factors. Skin lesions consist of hair loss and crusts on areas with minimal muscle overlying the bone such as the face, ear tips, legs and feet, and the tip of the tail. Onset of lesions may occur as early as 12 weeks of age or in mature dogs. Definitive diagnosis can only be made with a skin biopsy.

Genotypes of *PAN2*, *MAP3K7CL*, and *DLA-DRB1* are considered together. According to the scientific literature detected genotypes are classified in three groups:

- *HIGH RISK* – 90% of dogs with these genotypes developed DMS
- *MODERATE RISK* - 33%-50% of dogs with these genotypes developed DMS
- *LOW RISK* - 0-5% of dogs with these genotypes developed DMS

It is recommended that breeding pairs are selected on their genotypes at locus A and B. Ideally, matings that could produce puppies with high-risk genotypes should be avoided. More information on how to select mating pairs based on test results can be found on our website.

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PEDIGREE NO.: NOT PROVIDED

GENETIC REPORT

SAMPLE: BUCCAL SWAB

SAMPLE TAKEN BY: MARCUS ERBEN, DVM

REQUESTED TEST: MALIGNANT HYPERTHERMIA (MH)

RESULT: CLEAR (WT/WT)

COMMENT :
The test examines presence or absence of RYR1 gene mutation (c.1640T>C) described as the cause of malignant hyperthermia (MH) in many dog breeds. The disease is a pharmacogenetic disorder of skeletal muscle elicited by exposure to volatile anaesthetics and depolarizing muscle relaxants. Malignant hyperthermia is inherited as an autosomal dominant trait.

Regarding to the presence of tested mutation animals are classified in three groups:

- Clear (wt/wt) - mutation is not present, normal genotype
- Single affected (mut/wt) - one of two alleles carries a mutation, disease is clinically manifested
- Double affected (mut/mut) - both alleles carry mutations, disease is clinically manifested

Because of autosomal dominant mode of inheritance the disease is clinically manifested in all animals that carry a mutation (one or both affected alleles). When double positive animal is bred with clear animal all siblings will be single affected with clinical manifestation of the disease. When single positive and clear animals are bred 50% of siblings will be clear and 50% will be single affected. With the aim of disease eradication and prevention of possible animal suffering it is advised to avoid breeding of double affected and single affected animals.

AUTHORIZED SIGNATURE:

MARIBOR, 02.04.2020

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BREED: SHETLAND SHEEPDOG
SEX: MALE
MICROCHIP NO.: 985113002089467
TATOO NO.: NOT PROVIDED
PEDIGREE NO.: NOT PROVIDED

GENETIC REPORT

SAMPLE: BUCCAL SWAB

SAMPLE TAKEN BY: MARCUS ERBEN, DVM

REQUESTED TEST: HYPERURICOSURIA (HU)

RESULT: CLEAR (WT/WT)

COMMENT :
The test examines presence or absence of SLC2A9 gene mutation (c.616G>T) described as the cause of hyperuricosuria (HU) in many dog breeds. The disease is characterized by an excessive excretion of uric acid into urine, leading to formation of uric acid stones. Hyperuricosuria is inherited as an autosomal recessive trait.

Regarding to the presence of tested mutation animals are classified in three groups:

- Clear (wt/wt) - mutation is not present, normal genotype
- Carrier (mut/wt) - one of two alleles carries tested mutation, disease is not clinically manifested
- Affected (mut/mut) - both alleles carry tested mutation, disease is clinically manifested

For each group different breeding strategies should be followed. Breeding of affected and carrier animals should be avoided. If particularly valuable animal is classified as affected, it should be bred only with clear animal. In such case, all first generation siblings will be carriers. If a carrier is bred with clear animal, 50% of siblings are expected to be clear. In case two carriers are bred, 25% of siblings are expected to be clear and 50% are expected to be carriers. However, 25% of siblings are expected to be affected, therefore such breeding practice is discouraged.

MARIBOR, 02.04.2020

AUTHORIZED SIGNATURE:

EVG Molekularna diagnostika
EVG d.o.o. Taborska ulica 8, SI-2000 Maribor

Certificate of DNA analysis of an animal in professional breeding programs

Owner / Breeder

Surname and name: Kelpen Nicole
Address: Schlackenwerther Str. 13
76437 Rastatt
Phone: 0049 17620973022
E-mail: Deav_Nicole@web.de

Animal and Sample:

Species: Dog
Breed: Shetland Sheepdog
Name of the animal: **AZULIAN CATCH YOUR DREAMS**
Sex: Male **Date of birth:** 21.5.2019
Chip / Ring Nr.: 985 113 002 089 **Tattoo number:**
Date of sample receipt: 14.5.2020 00:00:00 **Reg. number:**
Date of result release: 19.5.2020 12:10:14
Biological material: Hair
Sampled by: Owner

At the time of sampling the identity of the animal has not been verified.

Description of the molecular technology used:
Total DNA has been isolated from the sampled biological material. PCR amplification of all specified molecular targets (see table of this Document) has been followed by fragment analysis using ABI3500 capillary sequencer and bioinformatic analysis.

Precise specification of the analyzed genetic abnormalities, results and their interpretation:

Genetically determined disease	Associated gene	Specification of the mutation	Genetic description of the result
Merle coat pattern	SILV	SINE insertion (m/Mc/Mc+/Ma/Ma+/M/Mh)	m/m

Interpretation of the genetic description and terminology:
Wild type: m/m – neither of the investigated alleles of the gene harbor SINE insertion
Heterozygous mutation - one of the investigated alleles of the gene harbor a SINE insertion, the other is wild type (without SINE insertion)
Compound heterozygous mutation - both investigated alleles of the gene harbor a SINE insertion
Homozygous mutation - both investigated alleles of the gene harbor the same SINE insertion

Cryptic Merle: Mc – the length of SINE insertion ranges from 200 to 230 bp
Mc+ – the length of SINE insertion ranges from 231 to 246 bp
Atypical Merle: Ma – the length of SINE insertion ranges from 247 to 254 bp
Ma+ – the length of SINE insertion ranges from 255 to 264 bp
Merle: M – the length of SINE insertion ranges from 265 to 268 bp
Harlequin Merle: Mh – the length of SINE insertion ranges from 269 to 300 bp

The result has been approved by:
MUDr. Peková Soňa , Ph.D.
TILIA LABORATORIES s.r.o.
Laboratoř Molekulární Diagnostiky
phone: +420 605 335 953
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Result number: **BH386/11220**
Date of result release: 19.5.2020