How Embark's Age Test Uses DNA Methylation to Determine Your Dog's Calendar Age

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Embark's Age Test can determine a dog's calendar age using proven technologies in the cutting-edge field of epigenetics, specifically DNA methylation. The test can provide a highly accurate estimate of a dog's birthdate. This knowledge empowers dog owners to provide personalized pet care based on age. For veterinarians with patients of previously unknown ages, knowing the calendar age based on the Age Test will assist with age-appropriate recommendations such as diet, exercise, or preventive care.

Introduction

Cells within an individual have the same DNA sequence, yet the genes that are used change based on development, aging, and environmental factors. These changes in gene expression are caused by epigenetics. Epigenetic modifications affect which regions of DNA are accessible for RNA transcription, which impacts the cell's physiologic function.

One example of an epigenetic modification is DNA methylation. A methyl group ($-CH_3$) can be attached to cytosines that are adjacent to guanosines (CpGs), which then signals that the genomic region should be less accessible, and nearby genes should be shut off. The methylation status of an individual CpG can be measured by sequencing and microarray technologies due to a biochemical reaction: when DNA is treated with sodium bisulfite, cytosines are converted into uracils unless that particular cytosine is methylated. The ratio of cytosine to uracil therefore is a readout of DNA methylation at a particular site.

Methylation level is impacted by a variety of factors, including cell type, disease status, environmental factors, and age. As an animal ages, methylation changes in a predictable pattern, and aging clocks have been developed in a wide variety of species[1]. Multiple dog age clocks exist, based on both microarray and sequencing technologies[2−5]. Embark is now offering a calendar Age Test for dogs, based on these proven technologies.

An accurate calendar age test has strong clinical utility

Age is a helpful input for preventative screenings and veterinary health care decision making – especially for diseases and conditions that tend to present later in a dog's life. Physical examinations can provide an age range within several months for puppies, but those same physical parameters are not similarly informative for adults. A reputable dog calendar age product can enable owners of dogs with unknown birthdates from shelters and rescues to make more accurate age-informed lifestyle and care decisions.

This test was developed using a wide variety of dogs

To create the most accurate Age Test possible, we collected genome-wide methylation data from >500 dogs of known dates of birth. These dogs span ages from four months to 18 years (Fig. 1A), which covers most of a dog's lifespan. Methylation is impacted by many factors, which if unaccounted for could create artifacts in our age algorithm. For that reason, we selected similar numbers of males and females (Fig. 1B), as well as dogs

with genetic weights spanning 5-140 pounds (Fig. 1C). Another factor that impacts methylation is life history, including intrauterine environment, traumatic experiences, and social environment. While we did not intentionally recruit dogs who were from shelters or from breeders, we did include a mixture of mixed and purebred dogs (Fig. 1D). By including this diversity of dogs, we expect that
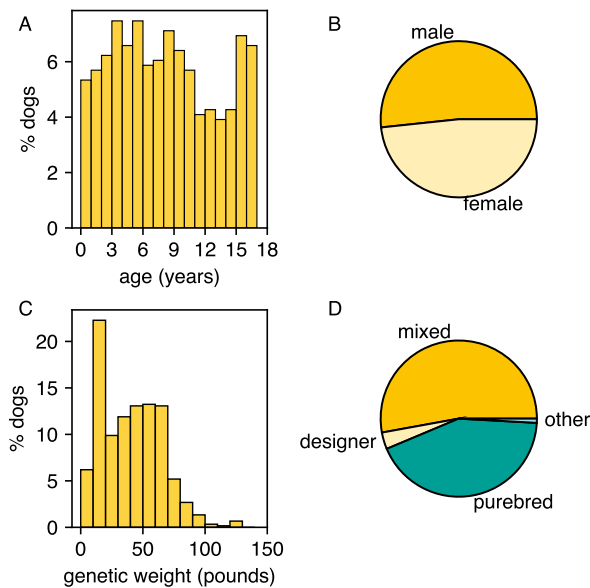

Figure 1: Dogs included in the Age Test model building.

methylation signatures caused by sex, size, and life history will not have any affect on our age estimates.

Genome-wide methylation data were used to train the age model

To measure DNA methylation we used the Embark Methylation Array, which has thousands of canid-specific probes in addition to all probes present on a pan-mammalian methylation chip[6]. A saliva sample was collected from each of the dogs selected for the age model training, and DNA was extracted. After bisulfite conversion, the DNA was hybridized to the Embark Methylation Array and imaged. Raw data was converted to normalized methylation values and quality information. Samples and probes with high quality were included in model training. Two algorithms

were trained for age prediction, using the Python package Sci-Kit Learn: 1) a generalized linear regression with Lasso regularization, and 2) a random forest regression model.

The Age Test is highly accurate

We tested our models on the dogs in the training set and found that they have a median absolute error of 3.2 months (Fig. 2). We compared the percentage of
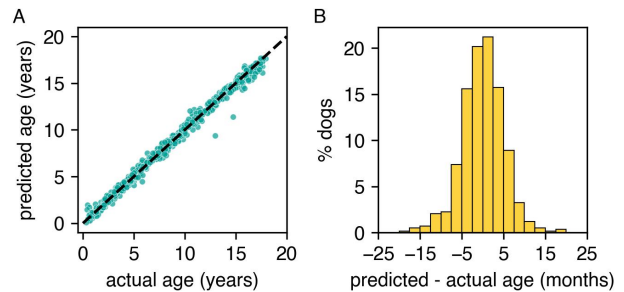

Figure 2: Accuracy of the Age Test.

dogs whose results fall within different ranges, from 1 month to 1 year. Our predictions were within $\pm$ 5 months for 70% of dogs and within $\pm$ 10 months for 92% of dogs. With this degree of accuracy, dogs can be confidently assigned to the correct adult life stage, which enables more personalized care plans.

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The Embark Dog Age Test is available through embarkvet.com. If you have any questions about our methodology or individual test results, reach out to Customer Service, howdy@embarkvet.com.