

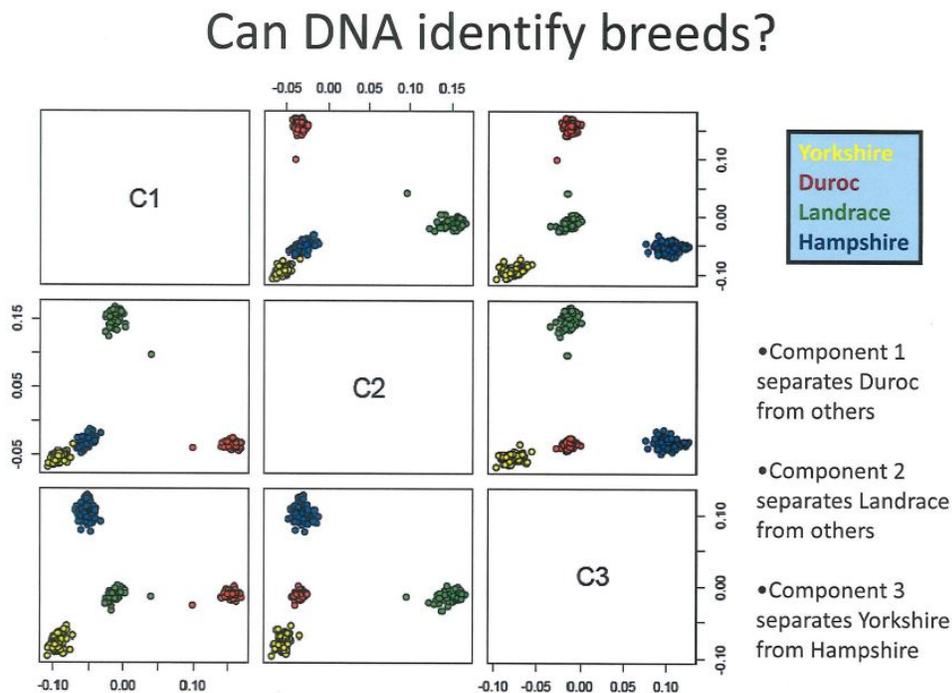
DNA Breed Profile Testing FAQ – April 2020

What is the history behind breed composition testing?

Breed composition testing, or breed integrity testing has been in existence for many years. Hampshire, Landrace, and Yorkshire members were once required to perform a physical test mating with their sires to ensure the boar carried no non-breed color. This process took 3 months, 3 weeks, and 3 days for the test litter to be born to verify a sire was free from non-breed color. DNA testing for Halothane 1843 testing, often referred to as the Stress Gene, has been utilized to maintain breed integrity as well, with the NSR Stress Policy dating back to the early 2000's. In 2004, the Hampshire Swine Registry changed from a physical test mating requirement to a DNA test developed by PIC to ensure no non-Hampshire color existed in AI sires. This policy was amended in 2014 whereby all Hampshire sires had to be tested and pass the DNA Hampshire Color Test. Transitioning to a DNA based-test shortened the time required from ~16 weeks to ~6 weeks.

The DNA Breed Profile Test concept began as a collaboration between Michigan State University, the American Yorkshire Club, the National Swine Registry, and USDA Meat Animal Research Center, conducting multiple genotyping projects with varying goals. One such project had a simple goal: Can DNA technology be utilized to eliminate the need for physical test matings for Yorkshire sires just as the PIC Hampshire Color DNA Test eliminated the need to perform physical test matings on Hampshire sires.

Initial results showed that not only could DNA technology eliminate the need to perform physical test matings on Yorkshire sires, it could also be used as a tool to cluster animals of the same breed into groups and determine breed composition. Below is a graph that shows visually how animals cluster together when DNA information is analyzed with a statistical model:



The processes and procedures utilized in DNA Breed Profile Testing have been well documented in peer-reviewed scientific journal articles.

Badke, Y. M., Bates, R. O., Ernst, C. W., Schwab, C., Fix, J., Van Tassell, C. P., & Steibel, J. P. (2013). Methods of tagSNP selection and other variables affecting imputation accuracy in swine. BMC genetics, 14(1), 8.

Funkhouser, S. A., Bates, R. O., Ernst, C. W., Newcom, D., & Steibel, J. P. (2017). Estimation of genome-wide and locus-specific breed composition in pigs. Translational Animal Science, 1(1), 36-44.

Huang, Y., Bates, R. O., Ernst, C. W., Fix, J. S., & Steibel, J. P. (2014). Estimation of US Yorkshire breed composition using genomic data. Journal of animal science, 92(4), 1395-1404.

Ramos, A. M., Crooijmans, R. P., Affara, N. A., Amaral, A. J., Archibald, A. L., Beever, J. E., ... & Hansen, M. S. (2009). Design of a high density SNP genotyping assay in the pig using SNPs identified and characterized by next generation sequencing technology. PloS one, 4(8), e6524.

Is this a new test?

Development of the DNA Breed Profile Test began in 2009. Development of the Reference Panel and validation from multiple sources of genotyping data led to the American Yorkshire Club Board of Directors voting to implement the technology in 2016.

What boars are being tested?

All Duroc sires born on or after November 1, 2017 must pass the Duroc Breed Profile Test. Sires born prior to November 1, 2017 are grandfathered into the system.

All Hampshire litters born on or after June 1, 2019 must be sired by a boar that has passed their Hampshire Breed Profile Test

All Landrace litters born on or after March 1, 2018 must be sired by a boar that has passed their Landrace Breed Profile Test. Sires meeting previous color requirements (physical test mating) were permitted to sire litters until February 28, 2018.

All Yorkshire sires tested on or after July 1, 2016 must pass the Yorkshire Breed Profile test. Boars meeting previous color requirements (physical test mating) were grandfathered into the system.

What is the criteria for animals to Pass versus Fail?

Yorkshire, Landrace, and Hampshire sires are required to pass both a Breed Composition component and Color component. Duroc sires are required to pass a Breed Composition component. The Breed Composition component is reported as the Whole-Genome Breed Composition (WGBC), which is the percentage purebred an animal is based on its 10K gene marker genotype. The Color component is based on probability of non-breed color at gene locations known to control color: for Yorkshire and Landrace the area of note are genes around *KIT* and for Hampshires the area of note are genes around *MC1R*.

Will the Whole-Genome Breed Composition (WGBC) percentages of animals be shared?

Breeders of animals that have passed the Hampshire Composition test will be informed of results and then the results will be posted online for the membership. Breeders of animals that have failed will be informed of the results and the percentage at which the animal has failed. Duroc, Landrace and Yorkshire results are reported as Pass/Fail.

Are junior hogs required to be tested for exhibition?

No. Juniors are not required to test their animals before exhibiting their animals. Champions and reserve champions, including Bred and Owned, will however, be tested for parentage, stress, and Hampshire color. All junior champions and reserve champions, including Bred and Owned, will be required to pass their respective breeds' Breed Profile test effective January 1, 2021.

What is a "Reference Panel"?

A Reference Panel, Reference Population, or as some describe as the "Breed Standard", is a group of registered purebred animals, as unrelated as possible, and representative of the entire population, that future test samples are compared against.

How was the Reference Panel developed for the NSR Breed Profile Test?

The NSR Reference Panel was developed as part of a larger research project conducted by Michigan State University to determine Linkage Disequilibrium in swine. Linkage Disequilibrium is the non-random association of gametes at different genetic locations, or more simply stated, how genes closer to each other in the genome move from generation to generation.

The initial NSR Reference Population consisted of 25 – 30 Sire-Dam-Offspring Trios for each NSR breed (Duroc, Hampshire, Landrace, Yorkshire). The trios were selected to be as unrelated as possible, where possible sharing no ancestry two generations back in the pedigree and as representative of each breed as possible. An effort was made to have representation from different "lines" or segments of each breed: show-oriented, commercially-oriented, "Old-line", etc. In total, 117 trios across the four breeds were evaluated. Key to note, the NSR Reference Panel is built around representative animals from all four breeds. From the NSR Reference Panel, the probability that a particular gene marker is associated with a particular breed was determined – combining each of these probabilities across all ~10,000 gene markers to develop the algorithm used to calculate the breed composition of future samples.

The reference panel is updated annually with animals that have passed the test in the past year.

How was the test validated?

Initial validation was carried out utilizing sires from each NSR breed (already genotyped for a different research project) that had been selected for having large numbers of daughter records, thereby being influential in their respective breed. In addition, crossbred and non-registered Yorkshire animals that were known to be crossbreds or had failed a physical test mating were also evaluated. Also, a large number of Yorkshire sires genotyped in conjunction with a genomic selection project were also utilized. Results of this validation are available from:

Huang, Y., Bates, R. O., Ernst, C. W., Fix, J. S., & Steibel, J. P. (2014). Estimation of US Yorkshire breed composition using genomic data. *Journal of animal science*, 92(4), 1395-1404.

How did the Breed Boards decide to implement the test?

Prior to presenting results to each breed board for implementation, Michigan State researchers determined there was sufficient coverage of the diversity within the breed from all the animals genotyped. Each breed Board of Directors was presented background information regarding the science behind the technology, development of the Reference Panel, and validation results from the test.

For each breed, a Validation group of animals was selected to determine if the test could detect both purebred and crossbred animals. Purebred animals, influential sires, known (by pedigree) and unknown (look-a-lines) crossbreds, and animals that had failed previous tests (physical test mating, Stress, Hampshire Color Test) were genotyped and evaluated using the DNA Breed Profile Test procedure. Results from these validation tests were presented to each breed board, recommendations for Pass/Fail criteria were made, and the requirements for implementation were voted upon.

The pedigrees from each breed were adequately represented in the reference population to a degree that the Michigan State researchers determined that the test for each breed was reliable as confirmed by the validation testing.

Can two animals which pass the DNA Breed Profile Test produce offspring that fail?

Yes, two parents which both pass the DNA Breed Profile Test may produce offspring which fail. Except in the case of identical twins, no two siblings (full-sibs or littermates) receive the exact same 50% of genes from their sire and dam. Genes segregate randomly when sperm and eggs are developed, so siblings may receive more or less non-breed genes.

This situation is similar to two parents, both with 6-6 underlines, producing an offspring with a 5-5 or 6-5 underline – both of which are ineligible to exhibit as a breeding animal (and results in pedigree cancellation in the Yorkshire and Landrace breeds). In addition, two perfectly belted Hampshire parents can produce offspring with broken/off-belted appearance or too much white color, both of which make an animal ineligible to be exhibited as a breeding animal.

How long does it take to receive results from testing?

Conservatively it takes 6 – 8 weeks to receive results. However, depending on timing the process can take as few as 4 weeks. NSR ships samples to the lab each Friday; stress testing is completed the following week. Samples are then grouped into batches of 24 for genotyping and the genotyping process takes 10 business day turnaround time. Factors that affect timing are mail schedule, holidays, sample quality, and how long it takes to group 24 samples for genotyping.