DNA TESTING

NEWS YOU NEED TO KNOW

By Dr. Doug Newcom

The National Swine Registry (NSR) has always adopted new DNA testing technologies as they have become available dating back to the Halothane “Stress” gene (HAL) in the early 2000s, as one method of maintaining breed integrity. The Yorkshire and Landrace breeds utilized physical test mating procedures to check each sire for the absence of non-white color. The Hampshire breed initially utilized physical test mating procedures to check for non-Hampshire color, but switched to a DNA-based test in the mid-2000s.

TABLE 1: SUCCESS AND FAILURE RATES FOR EACH SAMPLE TYPE

Sample type	Number of Samples	Successful	Unsuccessful	Success
Blood FTA Card	1,168	1,064	104	91.10%
Semen FTA Card	180	153	27	85%
Hair	115	109	6	94.78%
All	1,463	1,326	137	90.64%

In 2016, the American Yorkshire Club voted to implement DNA Breed Profile testing procedures, which utilizes DNA to determine breed composition and probability of carrying alleles for non-Yorkshire color. The American Landrace Association implemented the technology in 2017. The United Duroc Swine Registry and Hampshire Swine Registry boards voted to implement the technology at their annual meeting in March 2018. With this new technology comes the need for additional education regarding sample types, sample storage, and timeliness of sample submission.

The DNA Breed Profile Test is new technology that uses a low-density (LD) genotyping platform to determine breed composition and probability of non-breed color. This platform genotypes each animal for approximately 10,000 single-nucleotide polymorphisms (SNPs), or gene markers. Many of the SNPs simply tell us the sample is from a pig, but there are certain locations in the genome specific to each of our breeds. When you assign the probability that a particular SNP is associated with a particular breed and combine the effect of all the SNPs together, we can determine the composition of each breed within a given sample. Similarly, we combine the genomic results from areas of the genome known to impact coat color and determine the probability a white pig carries a gene for dark color (masked by the dominant gene for white color), or a Hampshire carries a gene for red or spotting color (masked by the dominant gene for black color). This technology allows for routine parentage verification for all sires and will be utilized for split-litter/questionable parentage testing and the clone verification procedure.

As you can see, this technology is much more expansive and a more accurate determinant of breed composition and color than the prior protocol using the physical test mating procedure. The physical test mating procedure for Yorkshires and Landrace required a boar to be mated to a dark colored sow and for the owner to fill out a form stating the colors of the offspring from the mating. This LD genotyping method is a quicker, more efficient and cost-effective method than mating a boar to produce a litter that may not have been beneficial besides fulfilling the physical test mating requirement.

While testing for HAL can be done in a couple of weeks, LD genotyping* takes comparably longer – however, it is considerably quicker than the previous physical test mating requirement. The agreement between NSR and the DNA testing lab states samples are shipped once weekly, Friday for normal business weeks. Once at the lab, samples are HAL tested, then put in the queue for LD genotyping. Each batch sent for LD genotyping must contain 24 samples, so when 24 samples are ready for LD testing they are genotyped. Genotypes for each batch are returned to the NSR for analysis upon completion and results are posted. When a sample fails genotyping quality control (QC) as 5 – 15% do, based on Table 1, it is retested with the next batch. If the sample fails QC a second time, the breeder is required to submit a new sample.

The genotyping lab maintains strict QC measures to ensure DNA quality and accurate genotypes. Table 1 shows the success and failure rates for each sample type, an indicator of DNA quality for LD genotyping.

As you can see, hair samples provide the greatest probability of supplying adequate DNA quality for LD genotyping, followed closely by blood on an FTA card. Hair samples must be pulled from the pig (not cut) and contain 30 – 50 follicles (hair root) to be useful in genotyping. Blood samples must fill each spot on the FTA card, soak completely through the felt paper on the card, and dry for 24 hours before closing the card in order to achieve maximum success rate. If you are determined to use semen make sure it is raw and not extended.

Please allow up to six weeks from the time the sample arrives at the lab, which can be up to two weeks after you have sent the sample to the NSR depending on mail service.

Things to remember when submitting your DNA samples to ensure you get your results as quickly as possible:

1. TIMELINESS OF SAMPLE SUBMISSION

a. With the time it takes to complete the required testing, samples should be submitted as soon as possible, preferably before using the boar. Any necessary retesting only adds to the timeline, and delays your ability to issue AI Certificates or record litters.

b. Fill out the DNA Testing Form as completely and accurately as possible. If you are unsure about the testing requirements, please contact DNA Secretary Whitney Webb.

c. For Parentage testing, you must submit a sample from the dam of the pigs you are testing and samples from each potential sire (or list of potential sires already DNA banked with the NSR).

d. If you are in need of testing other than Stress, DNA Breed Profile or Parentage (RN/ Napole, Seek-Gain Markers), please submit a second sample from your animal (you will only be charged to bank one sample).

2. SAMPLE TYPE

a. Hair – Pull from the pig, 30-50 hair follicles (hair root), 2nd place in the hair card. When pulling hair from more than one pig at a time, be careful to avoid cross contamination from one pig's hair sample to the next.

b. Blood on FTA card – Fill each spot with blood, make sure the blood soaks through the felt paper on the card, and the card needs to dry for 24 hours prior to closing

c. Semen – Raw semen on an FTA card, fill each spot on the card, make sure the semen soaks through the felt paper on the card, and the card needs to dry for 24 hours prior to closing – DO NOT SEND EXTENDED SEMEN

d. When using FTA DNA cards, be sure you are using cards that have the National Swine Registry logo and have 4 sample spots inside them. The NSR FTA cards that have 4 sample spots allow for a larger amount of blood or semen to be collected on the card compared to the old smaller FTA cards that only had one spot for sample collection.

3. SAMPLE CARE

a. Keep FTA and hair cards clean and dry prior to use

b. Make sure blood or semen on FTA cards soaks through the felt paper and has 24 hours to dry before closing them up

c. Keep collected samples out of extremely hot temperatures, including but not limited to:

i. Vehicle dashboard, car trunk and direct sunlight

d. Write the pig's breed, ear notch, and registration number on the DNA card prior to collecting DNA. As you collect DNA, double check the pig's ear notch to ensure you are collecting the correct animal's DNA.

4. TESTING COSTS

a. Sample DNA banking - $6 per sample

b. Stress (HAL) testing - $25 per sample

c. DNA Breed Profile testing - $80 per animal (failed DNA quality control will result in re-testing and additional test fee of $80 for subsequent re-test)

d. Parentage testing - $58 per sample

In addition to having a DNA sample on file and a negative test result for the Halothane (stress) gene, each NSR breed has implemented the following policies regarding the DNA Breed Profile Test:

UNITED DUROC SWINE REGISTRY

Any boar farrowed on or after November 1, 2017 must pass the DNA Breed Profile Test in order to be used as the sire of a registered litter.

HAMPSHIRE SWINE REGISTRY

The Hampshire Swine Registry Board of Directors voted to switch from the current DNA Hampshire Color testing platform to the new DNA Breed Profile Test, effective July 1, 2018.

AMERICAN LANDRACE ASSOCIATION

ALL Landrace sires must pass the DNA Breed Profile Test before any litters can be recorded, effective with litters farrowed on or after March 1, 2018.

AMERICAN YORKSHIRE CLUB

ALL Yorkshire sires must pass the DNA Breed Profile Test before any litters can be recorded, effective with all DNA samples submitted on or after July 1, 2016.

DNA Sample Collection Procedures

This instruction sheet is intended to assist National Swine Registry members in collecting DNA samples for submission to the NSR office and to meet DNA requirements for sires when registering purebred Duroc, Hampshire, Landrace and Yorkshire litters. **Only OFFICIAL NSR BLOTTER/HAIR CARDS will be accepted.** Cards must be purchased from the NSR office for $6 each.

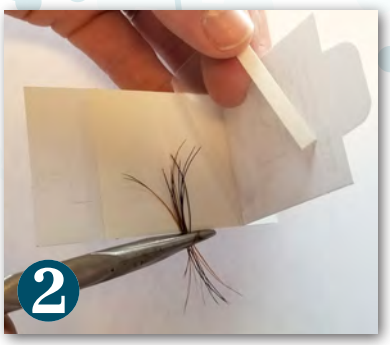
Blood Collection

Locate vein on ear. Clean area with moistened cloth (water or rubbing alcohol). Wipe dry. Pierce vein with sharp end of the disposable lancet (provided) or a clean, sterile hypodermic needle.

Withdraw lancet. Allow blood to collect on skin's surface. Allow blood to drip onto all four blotter card indicator circles. Make sure blood soaks through felt paper. Let card dry for 24 hours.

Hair Collection

Using pliers, pull hair making sure follicle (root) is intact.



Place hair on NSR-approved hair card. Make sure follicles are under the clear tab. Trim off excess hair outside the card. Make sure there are 30-50 hair follicles – it may take 4-6 hair pulls to get enough.

Blotter Card

Hair Card

Complete the space on the card for owner, breed, ear notch and registration number immediately to assure that each sample is properly identified. Complete the breeder information on the NSR Testing Form with the sample ID and breed.

3. Make certain that the sample is correctly identified with the owner, breed, ear notch and registration number.

4. Return the BLOTTTER or HAIR card to the NSR office with the DNA Testing Form, and indicate the sample banking and/or diagnostic tests requested for the corresponding sample.

Type of samples accepted:

- **BLOOD**
- **RAW SEMEN** *DO NOT USE EXTENDED SEMEN* (same as blood card method • Note on sample it is semen)
- **HAIR**