Let's Grow, American Sheep Industry Association (ASI)
Project Final Performance Report

Project Title: Engaging the New Biology: establishing the foundation for genome-enhanced breeding values in the U.S. sheep industry

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Final Report

A. Comparison of timeline, tasks and objectives outlined in the proposal as compared to actual performance.

Objective One: Provide Producer Education

The first objective of this project was to develop and deliver an educational program focused on state-of-the-art technologies in animal genomics, and their potential impacts on traditional genetic evaluation programs in the U.S. sheep industry.

This objective was accomplished in three ways. Firstly, Dr. Ron Lewis (University of Nebraska-Lincoln (UNL)), the project leader, presented a webinar entitled "A Journey: The Opportunities and Challenges of Meddling Genomics into the U.S. Sheep Breeding Programs" as part of the Sheep Producer Webinar Education Program hosted by Dr. Jay Parsons (UNL). It was broadcasted on May 24, 2016, and was attended by 83 people representing 34 states and 3 Canadian provinces. The webinar was posted for streaming on the Educational Webinars page on the American Sheep Industry (ASI) Association website (http://sheepusa.org/Growourflock_Resources_EducationalWebinars). So far, it has been viewed by 305 individuals.

Secondly, as part of three regional meetings hosted by the National Sheep Improvement Program (NSIP), Dr. Lewis presented seminars describing the future of genomic technologies in the U.S. sheep industry. Those meetings were: (i) the National Polypay Show and Sale, Springfield, IL, on June 18, 2016 (approximately 20 people attended); (ii) the 10th Annual Center of the Nation NSIP Sale, Spencer, IA, on July 22, 2016 (approximately 40 people attended); and, (iii) the 12th Annual Katahdin Hair Sheep International Expo and Sale, Cookeville, TN, on August 4, 2016 (approximately 80 people attended). In addition to the seminar, he demonstrated jugular blood sampling and the process for preparing blood collection cards.

Lastly, a training video was prepared to demonstrate the process of jugular blood sampling and the preparation of blood cards. The video was filmed on May 26, 2016, at the Dry Sandy Sheep Company, Alexandria, NE, which is owned and operated by Matt and Amy Beals. It was narrated by Dr. Kelly Heath, attending veterinarian at UNL. It was posted on the ASI website for online streaming (http://sheepusa.org/Growourflock_Resources_EducationalWebinars). Written guidelines targeted at producers participating in the project were also prepared to complement the video (Appendix I).

Objective Two: Devise Efficient Strategies to Collect Genomic Data

The second project objective was to devise an efficient strategy to collect genomic data. In other livestock industries, the animals initially genotyped were mostly chosen *ad hoc* and generally represented one extreme trail of the distribution of Estimated Breeding Values (EBV). The later was because only animals with desirable EBV would produce enough progeny to reach high levels of accuracy. This strategy of selecting animals for genotyping disregarded i) the potential bias created by the animals being genotyped not fully reflecting the additive variation present in a population or, more specifically, a breed, and ii) the strength of the genetic relationships or connectivity of animals genotyped to the rest of the animals in the population.

More closely related or connected flocks typically share more ancestors in common. As a consequence, they also are genetically more similar at an allelic (genotypic) level. Therefore, when wishing to fully describe the genetic profile of a population or breed, collecting DNA on flocks and thereby animals that are disconnected is valuable.

In order to collect DNA on relevant animals as the foundation of a genomic repository in two breeds – Suffolk and Rambouillet – a comprehensive sampling strategy was devised. It was based on evaluating pedigree and performance data on flocks in these breeds that were recording with the National Sheep Improvement Program (NSIP). Based on their connectedness with other flocks, the aim was to choose those flocks for sampling that would be most valuably contribute to the establishment of a DNA repository.

Suffolk sheep. Pedigree data on 98,016 Suffolk sheep from 105 flocks born between 1960 and 2016 were considered, which included 2,989 sires and 13,534 dams. Median, average, and standard deviation (SD) in sire family size were 5, 16.8, and 29.4 progeny, respectively. One ram sired 355 lambs. Weaning weights collected between 1983 and 2016 on 36,409 lambs from 101 Suffolk flocks also were available, and contained 2,058 contemporary groups. At weaning, average age was 63.0 (SD 10.7) days and average weight was 67.6 (SD 11.7) lb. Among these flocks, 18 submitted data to NSIP between 2014 and 2016, and were classified as active.

The strength of genetic relatedness (connectedness) was determined between all pairs of the 105 Suffolk flocks for weaning weight. The heritability for weaning weight was 0.15, which is the value used by LambPlan in the genetic evaluation of this trait in the Suffolk breed.

Connectedness was summarized among the 18 active flocks by linking together or clustering those flocks that were more closely related genetically. The results of those analyses are shown as a tree diagram (dendrogram) in Figure 1. There were 3 clusters of strongly inter-related flocks consisting of 5, 4 and 2 flocks. However, those 3 clusters were relatively weakly inter-connected. Furthermore, the remaining 7 flocks were weakly connected to the rest.

From these results, it appeared that ancestries differed among the various groupings of Suffolk flocks, which also may coincide with genetic differences. Therefore, in order to fully reflect the genetic diversity across Suffolk flocks recording in NSIP, it was key to collect DNA samples from the genetically less related flocks as well as from those flocks within the three clusters. Such a strategy was adopted when contacting flocks to participate in genomic sampling.

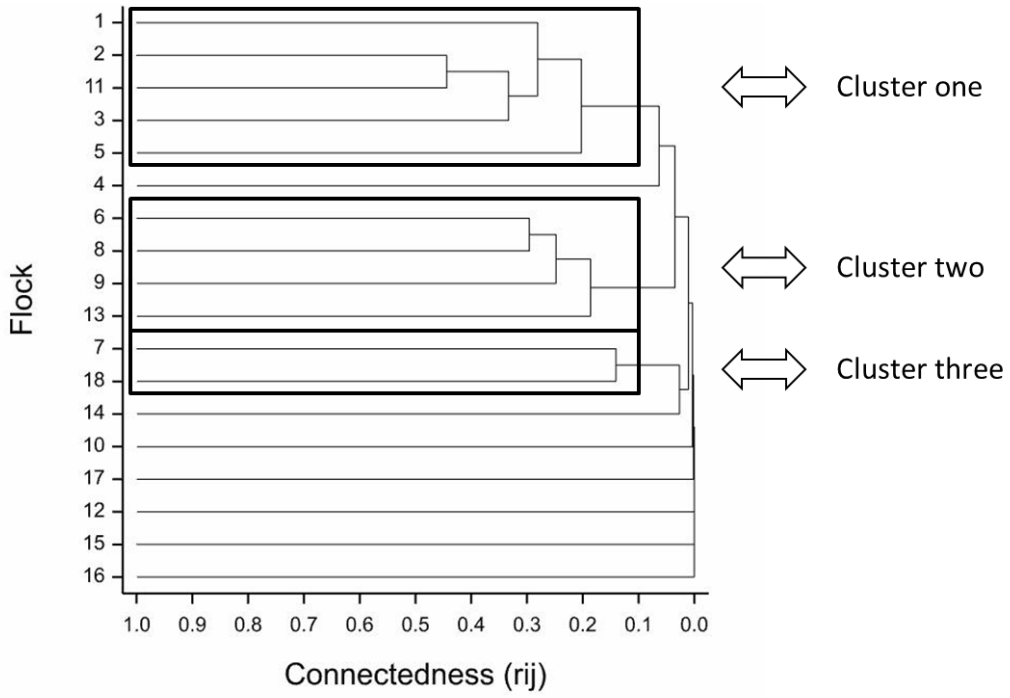

Figure 1. *Cluster analysis (tree diagram) of 18 Suffolk flocks with weaning weights recorded between 2014 and 2016 in the National Sheep Improvement Program. Three strongly inner-connected clusters are highlighted.*

Rambouillet sheep. Pedigree data on 108,749 Rambouillet sheep from 10 flocks born between 1977 and 2016 were considered, which included 304 sires and 3,594 dams. Median, average, and SD in sire family size were 38.5, 51.9, and 52.3 progeny, respectively. One ram sired 304 lambs. Post-weaning weights collected between 1983 and 2016 on 12,483 lambs from 9 Rambouillet flocks also were available, and contained 156 contemporary groups. At post-weaning, average age was 152.1 (SD 36.1) days and average weight was 81.9 (13.1 SD) lb. All 9 of these flocks submitted data to NSIP between 2015 and 2016. However, weight data from the additional flock was pending, and therefore it too was considered active for these analyses.

Similar to the analyses of the Suffolk sheep, genetic connectedness was summarized among the 10 active Rambouillet flocks. The heritability for post-weaning weight was 0.10, which is the value used by LambPlan in the genetic evaluation of this trait in the Rambouillet breed.

Connectedness was summarized among the 10 flocks through a clustering analysis. The results are provided in Figure 2. There were four clusters of strongly inter-related flocks consisting mainly of pairs of flocks. However, these 4 clusters were relatively weakly inter-connected with each other; the remaining flock also was largely unrelated. Since there was no clear pattern of connectedness among these Rambouillet flocks, collecting DNA samples from all 10 flocks was deemed a priority.

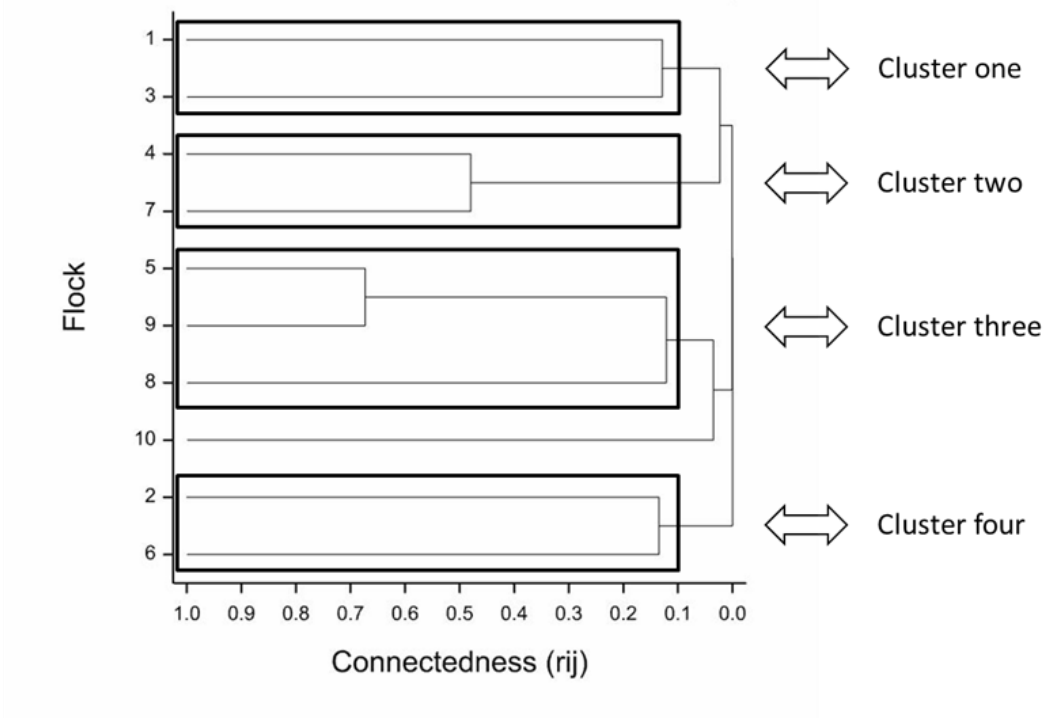

Figure 2. *Cluster analysis (tree diagram) of 10 Rambouillet flocks with pedigree data submitted to the National Sheep Improvement Program. Post-weaning weights were available on 9 of these flocks between 2015 and 2016. Four strongly inner-connected clusters are highlighted.*

Objective Three. The third project objective is to establish a reservoir of genomic (DNA) samples on sheep in NSIP recorded Suffolk and Rambouillet flocks.

Genomic sample collection. Based on the results of Objective two, select Suffolk and Rambouillet breeders were contacted. In Suffolk, 11 flock owners were contacted all of whom agreed to participate in the project. The 7 flocks omitted had recently disbanded or ceased recording, or had comparatively few animals being recorded. In Rambouillet, all 10 flock owners were contacted of which 9 agreed to participate.

The flock owners were provided supplies for collecting DNA (i.e., syringes and needles; blood cards), a data sheet for keeping track of samples, guidelines for jugular blood sampling and blood card preparation, and packaging and postage for return shipment of the blood cards. Sufficient supplies were sent to allow collection of 2 blood cards on each animal. Such redundancy allowed backup in case of contamination of a sample. Sufficient supplies were sent to collect DNA on approximately 1,000 Suffolk and 1,300 Rambouillet sheep.

The participants were asked to collect DNA on all ewe lambs and their sires born in their most recent lamb crop, and on a subset of their ram lambs. This strategy ensured that the breadth of genetic diversity within a flock and breed was captured. Seven of the participating flocks sought assistance in collecting blood samples. In those cases, either Dr. Ron Lewis or Russell Burgett (Program Director, NSIP) visited the operation and assisted in blood collection. As part of those visits, they often trained a member of the producer's family to collect jugular blood samples.

Genomic repository. To date blood cards have been received on 820 animals from 9 Suffolk flocks, and on 195 animals from 3 Rambouillet flocks. Additional DNA samples have been collected on, particularly, Rambouillet sheep that have yet to be returned by participating flock owners.

An electronic database was established to inventory the DNA samples. Bar codes on the blood cards were scanned and loaded into a purpose-built Microsoft Access database. Identifier information including the animal's NSIP identification, flock, sex, and birth and DNA sampling date were entered. The database was constructed to facilitate finding samples on individual animals, including keeping track of the whereabouts of those samples when sent for commercial genotyping. Since 2 blood cards were collected on most animals, they were split between 2 locations for their bio-secure long-term storage.

B. If the stated objectives in the project were met, give a summary of your experiences in meeting those objectives.

The objectives of the project were met given that additional blood cards are received from the participating breeders. Meeting the project objectives was an extremely positive experience. It provided the opportunity to interact with innovative sheep breeders willing to both engage in the educational programming provided and to incorporate genomic sampling into their routine flock husbandry. This required both their time and flexibility.

One aspect of the project was unexpected. The DNA samples were collected through jugular blood sampling, with preparation of blood cards. That choice was in large part due to the ease of preparation of blood cards, and their durability for long-term storage. The process for collecting jugular blood samples was demonstrated at regional meetings, through a training video, and with written guidelines. However, it became clear that some producers remained uncomfortable with collecting a jugular sample. Since the incorporation of genomic technologies into genetic evaluation programs will require routine collection of high-quality DNA samples, either additional training in jugular blood sampling or alternative approaches for sample collection may well be needed (e.g., tissue sample via ear punch).

C. Identify if the project is complete or ongoing and what you see as the next step.

With the exception of receiving additional DNA samples, the objectives of the project are complete. An educational program was implement. A strategy for identifying flocks for genomic sampling was developed. A DNA repository was established in both the Suffolk and Rambouillet breeds in flocks recording in NSIP. Currently, no funds are available to genotype test the samples collected. However, by having a repository of well-characterized genomic samples available, NSIP and the sheep industry generally is in a much stronger position to leverage financial support for further integration of genomic information into genetic evaluation programs.

Still, if genomic information is to be used effectively within sheep breeding programs, on-farm performance recording and genotyping will need to become a routine part of sheep enterprises. For that opportunity to be realized, an industry-wide initiative is needed to:

i. develop a long-term repository for accumulating, inventorying and storing DNA samples. It seems most sensible that a single industry-based organization assume that responsibility. As

options, this repository could be established and managed directly by NSIP, or a reliable outside vendor;

ii. define the type(s) of DNA samples collected. There are several types of DNA samples that could be collected for genomic testing (e.g., blood, ear punch, wool fibers), that differ in their ease of collection, storage and shipping, durability and cost. The type of sample(s) to be collected need to be considered when defining the characteristics of a long-term repository;

iii. define one or more dates during the year by which samples would need to be provided to a central entity to be included in the batch sent for DNA testing;

iv. define the genotype testing to be conducted (e.g., parentage and simply-inherited genetic conditions or defects; low density panel; high density panel); and,

v. define and negotiate price with potential commercial vendors providing genotyping services.

A genomics subcommittee that includes sheep producers and scientists has been established within NSIP to begin to consider these issues.

D. Project summary suitable for posting on the ASI Let's Grow website.

The efficiency of lamb and wool production has increased substantially by applying quantitative genetic principles in sheep breeding programs. Accelerating those gains depends on melding state-of-the-art technologies in animal genomics with quantitative genetics approaches to more accurately identify high merit animals. In this project, three key steps necessary for the U.S. sheep industry to combine molecular and quantitative tools in genetic improvement programs were taken.

Firstly, an educational program was developed and implemented focused on the opportunities and limits of genomics, and the practices needed in order to collect molecular information to obtain more accurate estimates of genetic merit. This involved i) a webinar, ii) three regional sheep meetings, and iii) a training video that demonstrated jugular blood sampling and blood card preparation as a vehicle for DNA sample collection and long-term storage. The webinar and training video are available at http://sheepusa.org/Growourflock_Resources_EducationalWebinars.

The second achievement was to devise a strategy to collect genomic data i) that fully reflected the genetic variation present within a sheep population or, more specifically, a breed, and ii) that accounted for the closeness of the genetic relationships, or connectivity, of animals genotyped to the rest of the population. Using pedigree and performance data available through the National Sheep Improvement Program (NSIP), the connectivity among flocks within the Suffolk and Rambouillet breeds recording in NSIP was determined. Based on those analyses, a strategy was established that identified flocks that reflect the genetic diversity present with these two breeds for genomic (DNA) sampling.

The final accomplishment was to establish a reservoir of genomic samples on sheep in NSIP recorded Suffolk and Rambouillet flocks. Using the genomic sampling strategy established, 11 Suffolk and 9 Rambouillet flocks were recruited. Supplies we provided to collect DNA samples on at least 1,000 animals per breed. That target number was nearly achieved in Suffolk sheep, with additional sampling still underway in Rambouillet sheep.

Importantly, the outcomes of this project are simply a starting point. If genomic information is to be used effectively within the U.S. sheep industry, on-farm performance recording and genotyping will need to become a routine part of sheep enterprises. For that to occur, an industry-wide initiative is

needed to both encourage and coordinate ongoing genomic sampling and testing, accompanied by continued extensive performance recording.

Appendix I.
Guidelines for collection of jugular blood sample
and for preparing blood collection card

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Sponsor: Let's Grow, American Sheep Industry Association

Project abstract

The efficiency of lamb and wool production has increased substantially by applying quantitative genetic principles in sheep breeding programs. Accelerating those gains depends on **melding state-of-the-art technologies in animal genomics with quantitative genetics approaches** to more accurately identify high merit animals. This project focuses on three key steps for the U.S. sheep industry to combine molecular and quantitative tools in genetic improvement programs: (i) **provide producer education** clarifying the opportunities and limits of genomics, and the practices needed in order to collect molecular information to obtain more accurate estimates of genetic merit (genome-enhanced estimated breeding values); (ii**) devise efficient strategies to collect genomic data**; and, (iii) **establish a reservoir of genomic samples** (DNA) on well-chosen performance-recorded animals through collaboration with the National Sheep Improvement Program (NSIP); such is necessary to develop procedures for their incorporation in genetic improvement programs.

In order to establish the reservoir of genomic samples, NSIP members in the Suffolk and Rambouillet breeds are being asked to collect jugular blood samples and to prepare blood collection cards on lambs and their sires (more specifics will be provided to the individual breeders on animals to sample). Using the collected blood sample on each animal, two blood collection cards are to be prepared. Supplies for bleeding animals and blood collection cards are being provided. Further guidelines follow. A video demonstration also is available at http://sheepusa.org/Growourflock_Resources_EducationalWebinars.

Guidelines

Supplies needed

- 20 gauge 1-inch needle with 3 ml syringe (provided to participants)
- Blood collection card (provided to participants)
- Data collection sheets (provided to participants)
- Rubbing alcohol
- Gauze
- Electric shears
- Ink pen or marker
- Re-sealable plastic storage bags (quart size)
- Shipping envelope or small box

Blood sampling

- Clip the animal's neck to clearly see the jugular vein. Use electric shears to shave a patch approximately 4 inches wide by 8 inches long. Shaving an area allows for easier viewing of the vein

and provides a clean area in order to minimize the chance of introducing dirt or bacteria into the vein with the needle.

- Blood sampling can be done with assistance or alone. However, producers who are new at blood sampling will benefit from having assistance. The assistant should turn the head of the animal at a 30-degree angle to the side by holding the animal under its jaw to allow for easy access to the vein. The animal's body may also need to be restrained.
- The easiest way to locate the vein is to draw an imaginary line from the middle of the animal's eye down the side of its neck. The vein can be located by applying pressure with the thumb or fingers in the groove on either side of the trachea and below the half-way point of the shaved area. The pressure will cause the vein to pop up and be easy to see.
- Once the vein has been located, the area needs to be properly cleaned to keep bacteria out of the needle insertion site. This is most easily accomplished using (rubbing) alcohol. Apply a small amount of the alcohol to a few pieces of gauze. The area should be cleaned by starting in the center and working out toward the edge in a circular motion. Never go back over a place that has already been wiped, which may allow bacteria to be carried back into the disinfected area.
- Once the area has been cleaned and the vein has been located, the blood can be drawn. This can be done using a needle and syringe. Be sure to use a new needle and syringe for each animal.
- The vein should be easy to see and feel. Try to aim for the center so you will have more of a chance of placing the needle within the vein. Angle the needle at about 25 degrees when inserting. Inserting the needle does not require great force. Apply just enough pressure to break through the skin and enter into the jugular vein.
- Once the needle is in place, slowly draw back the plunger. Blood will immediately start to fill the syringe. If this does not happen, gently withdraw the needle so that the tip comes to the outside of the wall of the vein and re-insert. Only a small amount of blood needs to be collected (½ ml).
- Before removing the needle, remove thumb or fingers pressure on the animal's neck. Press your fingertip over the area where the needle was inserted. Remove the needle and, for a few seconds, maintain light pressure. Gently rub the area. A small red dot may still appear on the animal's neck from the needle insertion site. This is normal and is nothing to be concerned about.

Preparation of blood collection card

- Complete the information on the blood collection card in the space provided: your name, animal's 16-digit NSIP ID number, your signature, and the date the blood sample was collected. Also, please complete the data collection sheet accompanying the supplies provided.
- Prepare two collection cards for each animal from the single blood draw.
- Apply only enough blood to the collection card to fill the circle outlined on the tissue paper, but not so much that the card is soggy and overly saturated. Two or three heavy drops of blood is plenty.
- The collection card should then be immediately "tented" by sliding the tab on the collector into the slot closest to the circle tissue paper. This will create a bow in the collector allowing air to dry the sample. After the circle tissue paper has fully dried, the tab can be moved to the second slit at the end of the collector for flat storage.
- Make sure the collection cards do not touch, as this is a possible source of cross-contamination. Also do not dry the cards in direct sunlight, as UV radiation impairs the quality of the sample.
- Once dry, rubber band together the closed blood collection cards in batches of 10-15 and combine several batches together in a re-sealable plastic storage bag.
- Store the samples in a cool location out of the sun (refrigeration is not needed) until shipped.

Disposal of bleeding equipment and clean-up

- Once the blood collection cards have been prepared, promptly dispose of all sharps (needles) in a rigid sharps container. Carefully remove the needle from the syringe (pliers are recommended) and drop it into the sharps container. If preferred, the combined needle and syringe can instead be dropped into the container.
- An empty laundry detergent or laundry softener container works well as a re-purposed sharps container. Do not use a lightweight plastic beverage container since they may be punctured by the needles.
- The re-purposed sharps container should be labeled “Sharps” and “Do Not Recycle”. For proper disposal, once the container is half-full of sharps, add sufficient encasement compound such as concrete or cement to entrap the sharps. By filing the container only half full, there is room to do mixing within the same container. Then seal the container for disposal.