



United States
Department of
Agriculture

National Institute
of Food and
Agriculture

Ovine FAANG Project: What's Newe?

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Objectives

1. Develop a deep and robust dataset of **transcribed** elements in the sheep genome.
2. Develop a deep and robust dataset of transcription **regulatory features** in the sheep genome.
3. Annotation of the ovine genome reference assembly.
4. Provide ovine FAANG data and support to the public.

New Reference Genome

- We generated a high quality genome sequence *de novo* assembled using (PacBio) sequence and Hi-C data.
 - The 2.87 Gb assembly (Oar_rambouillet_v1.0, GCA_002742125.1)
- 100 tissues collected (snap & slow frozen) from Benz 2616 in April, 2016 at USU
 - BioProject PRJNA414087
- FAANG Annotations from the same animal - Oar_rambouillet_v1.0

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Rambouillet ewes Benz 2616 (right)

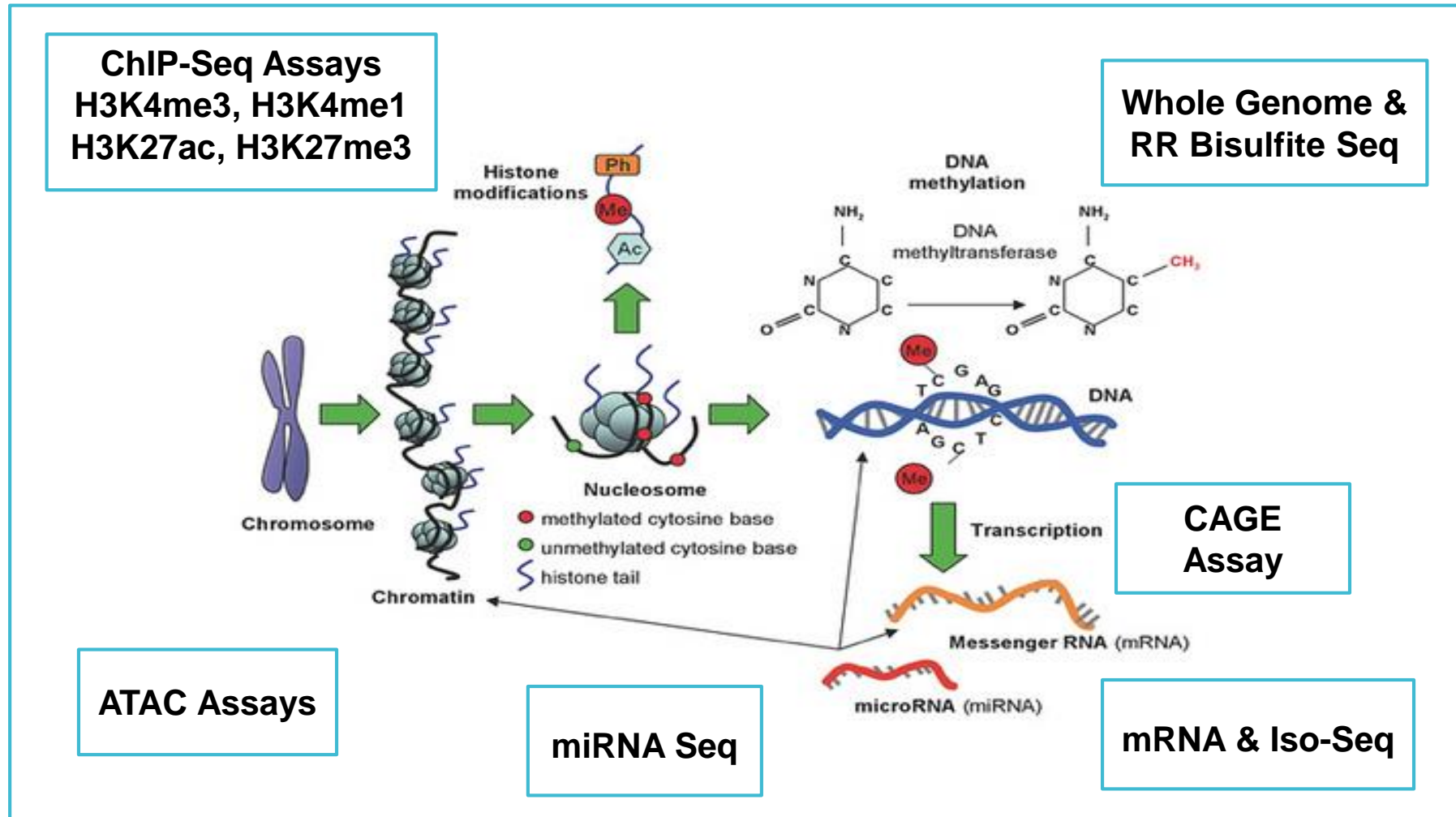
Tissues

- Tissue list for FAANG assays

Cerebral Cortex (frontal cortex)	Thyroid Gland	Tonsil – palatine
Cerebellum	Rumen – atrium	Tongue
Hypothalamus	Reticulum	Skin – non-haired
Abomasum	Omasum	Heart Atrioventricular Valve – left
Ileum	Abomasum - pylorus	Caudal vena cava
Liver	Duodenum	Retina
Kidney Cortex	Jejunum	Diaphragm
Ovary	Cecum	Gall Bladder
Hippocampus	Spiral Colon	Lymph Node – prescapular
Lung	Descending Colon	Urinary Bladder
Skeletal Muscle - biceps femoris	Rectum	Oviduct
Adrenal Cortex	Lymph Node – mesenteric	Parathyroid Gland
Spleen	Peyer's Patch	Esophagus
Adipose – subcutaneous	Kidney Medulla	Pons
Rumen – ventral	Adrenal Medulla	Soft palate
Mammary Gland	Uterus – caruncle	Thalamus
Alveolar Macrophages	Heart - left ventricle	Urethra
Skeletal Muscle – longissimus dorsi	Heart - right atrium	Uterus – intercaruncular
Spinal Cord – cervical	Heart - right ventricle	Vagina
Pituitary Gland	Pineal gland	Ureter

Bold = 58 tissues being used for CAGE

FAANG Assays



Objectives

1. Develop a deep and robust dataset of **transcribed** elements in the sheep genome.
 - Three types of transcriptome data on tissues
 - 1) Transcript sequencing using poly-A+ mRNAs
 - 2) Sequencing microRNAs
 - 3) Iso-Seq PacBio

Objectives

1. Develop a deep and robust dataset of **transcribed** elements in the sheep genome.
 - RNA sequencing and transcriptome analyses is in progress.

Transcript	Tissues run by Kim Worley	Tissues run by Tim Smith	Total Tissues
mRNA	29	31	60
miRNA	30	-	30
Iso-Seq	5	10	15

Objectives

1. Develop a deep and robust dataset of **transcribed elements** in the sheep genome.
2. Develop a deep and robust dataset of transcription **regulatory features** in the sheep genome.
 - 1) CAGE Sequencing
 - 2) Chromatin accessibility – ATAC-Seq
 - 3) ChIP-Seq – H3K4me3, H3K4me1, H3K27me3 & H3K27ac
 - 4) DNA methylation – RR Bisulfite and WG Bisulfite sequencing

Regulatory Feature Assays

- CAGE assay & analyses, lead by Emily Clark and Alan Archibald is currently in progress.
 - In total 59 samples of mRNA were shipped to Scotland
 - 48 libraries have been prepared
 - Sequenced on the HiSeq 2500 platform
 - Libraries for the remaining samples being prepared and 8 duplicate libraries ready for a second HiSeq run
 - The duplicate libraries will help us test reproducibility over sequencing runs
 - Data is expected in very soon
 - Data will be processed using CAGEr to generate a global picture of transcription start sites, and promotor and enhancer usage

Regulatory Feature Assays

- ChIP-Seq & ATAC-Seq, are in progress in collaboration with Brenda Murdoch, Stephen White and Michelle Mousel.
- Separate pilot projects are underway to ensure protocols are working for sheep tissues.
 - Initial pilot experiments yielded inconsistent results.
 - New protocol is working much better!



Regulatory Feature Assays

- ATAC-Seq is being lead by Stephen White and Michelle Mousel.

1) DNA from nuclei was collected from 11 samples at the time of slaughter – in progress

2) Nuclei from 18 tissues were isolated and slow frozen

3) Remaining tissues were slow frozen as tissues

Nuclei isolated fresh samples	Nuclei isolated from fresh tissues then slow frozen	
Liver	Cerebral Cortex	Cardiac Ventricle
Spleen	Hypothalamus	Lung
Adipose	Duodenum	Bone marrow
PBMC	Jejunum	Uterus Caruncle
Ileum	Cecum	WC1 cells
Cerebellum	Pancreas	CD4 T cells
Rumen Atrium	Mammary gland	CD8 T cells
Abomasum	Kidney Cortex	CD14 monocytes
Longissimus dorsi	Mesenteric lymph node	Alveolar macrophage
Spiral Colon		
Abomasum		



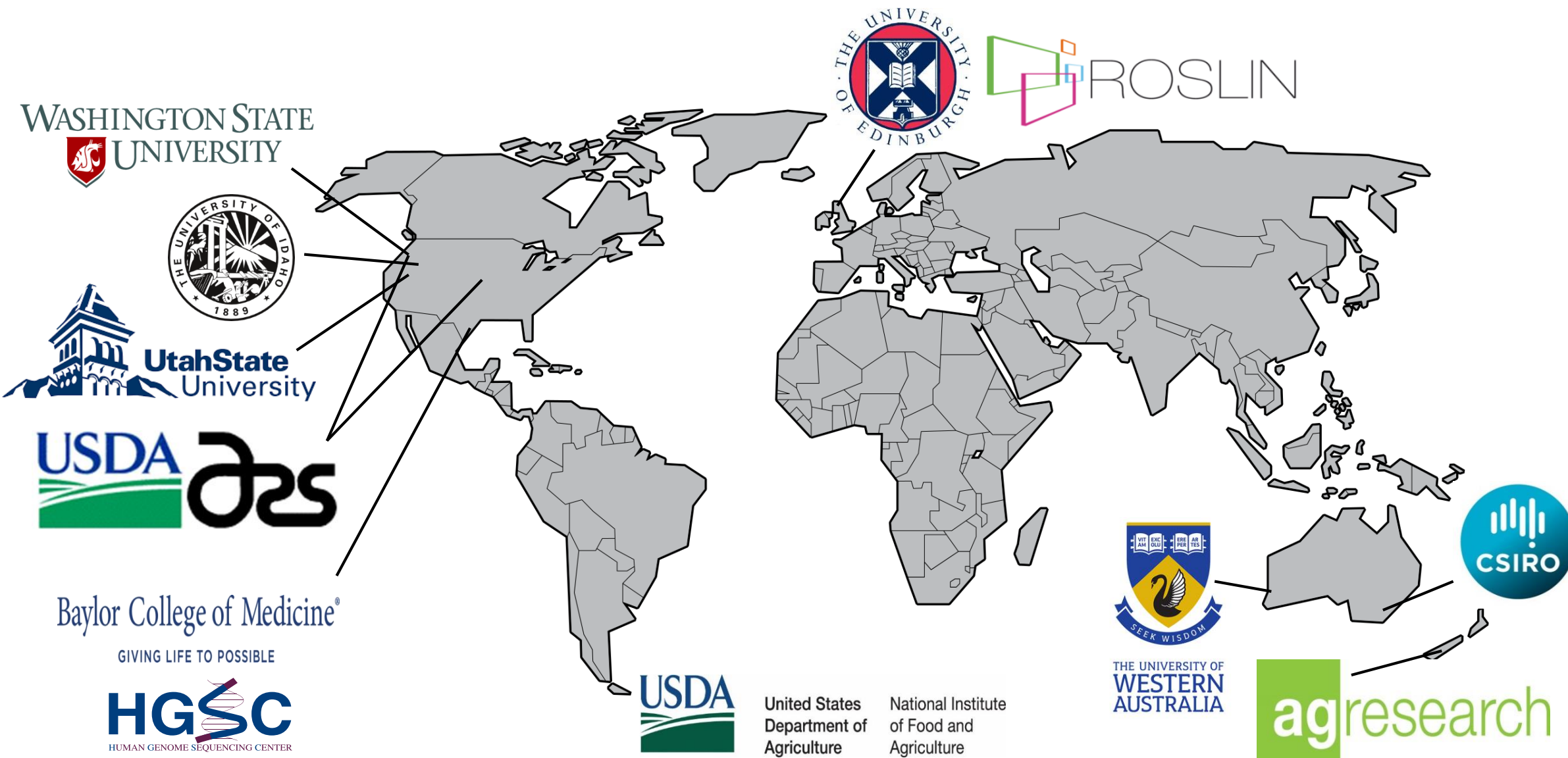
Regulatory Feature Assays

- DNA methylation, is being lead by Shannon Clarke and Rudiger Brauning.
 - Reduced Representation Bisulfite Sequencing
 - on the same 60 tissues
 - Whole Genome Bisulfite Sequencing
 - for 22 core FAANG tissues

Summary

1. Develop a deep and robust dataset of **transcribed** elements in the sheep genome.
 - transcriptomic data for 60 tissues
2. Develop a deep and robust dataset of transcription **regulatory features** in the sheep genome.
 - CAGE for 58 tissues are being sequenced
3. Annotation of the ovine genome reference assembly.
4. Provide ovine FAANG data and support to the public.

Acknowledgements



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