TITLE: Ovine FAANG Project Harvest of Rambouillet Sheep Tissues from Benz2616

Collection: April 26, 2016 at Utah Veterinary Diagnostic Laboratory

*Protocol adapted from that written by Emily Clark at the Roslin Institute (May 29, 2014)

1 Purpose / Introduction

1.1 To collect tissues and fluids from the *de novo* reference genome sheep (OAR_USU_Benz2616), during post mortem dissection and examination, for subsequent extraction of RNA/DNA. These tissues will be used for epigenetic assays as part of the Ovine FAANG project.

2 Equipment/Reagents/Materials

2.1 Cryotubes for snap freezing tissues

Liquid Nitrogen dewars (insulated Styrofoam) filled with liquid nitrogen

Scalpel blade handles and sterile blades

Clean Forceps

Clean Scissors and Knives

Clean parchment paper sheets (non-coated)

Sterile PBS in liter bottles and 50 mL conical bottom falcon tubes

Beakers

5 liter bucket

250 mL glass vacuum bottle

Whirl-top bags

Chemical Waste Bags

1.5 mL screw top Eppendorf tubes

50 mL Falcon Tubes

Petri Dishes

Sharps Bins

Cryostor medium

EDTA 0.5 M, pH 8.0

16 gauge 1” needle

Blood collection set (tubing)

3 Principle

3.1 Post mortem examination is carried out by a team of six veterinary anatomic pathologists from Utah State University and Washington State University. Tissues are then handed to those on the tissue collection team, composed of biology researchers from Utah State University, Washington State University, University of Idaho, and Virginia State University, to cut into small pieces (approximately 0.5 cm diameter). For preservation of RNA and DNA the chopped up tissues are either snap frozen in liquid nitrogen immediately, processed to nuclei, or slowly frozen to -80° C.

4 Procedure

4.1 Euthanasia is accomplished according to current American Veterinary Medical Association recommended guidelines. The sheep is stunned with a penetrating captive bolt by a licensed veterinarian through the cerebrum and brainstem, immediately followed by exsanguination via incision of the carotid arteries of the neck.

4.2 Tissues are quickly collected from the carcass by the post mortem team as larger specimens and transferred to the cutting team on labelled clean pans or Petri dishes. Cutting teams are divided into six stations for efficiency of collection of individual tissue aliquots (Table 1). The tissue should be cleaned of any excess blood or gut contents (for GI tract tissues only) by dipping in sterile PBS using forceps and placed onto a fresh sheet of parchment paper. A clean scalpel should then be used to chop the tissue into small pieces that are approximately 0.5 cm diameter in any direction. Scalpel blades are either changed, or cleaned in water and alcohol between each tissue. To secure the tissue for chopping clean forceps can be used. Tissues should be placed singly and evenly in cryovials, which are immediately snap frozen in liquid nitrogen in approximately six to ten aliquots as organ size allows. Separate aliquots that are approximately the size of a grain of rice are placed into 1 mL of CryoStor preservative medium, transferred to a Mr. Frosty system (Thermo Fisher

Scientific) and slowly frozen to -80 °C to preserve nuclear integrity. At the end of the post mortem examination and collection, the snap frozen tissues are transferred carefully onto dry ice using a slotted spoon for transport to Utah State University or USDA U.S. Meat Animal Research Center for storage in -80 °C freezers.

4.3 Digital photographs of the collection location for each tissue type are taken after dissection from the carcass. These reference images are stored at Utah State University.

4.4 Representative samples from the major tissue types are collected, based on gross examination, from the same location as fresh aliquots for histological examination. Tissues specimens are sectioned less than 0.5 cm wide in one direction and placed into 4% neutral buffered formalin with at least a 1:20 ratio of tissue to formalin for adequate fixation. Labelled tissue cassettes are used for tissues with more than one type collected (skeletal muscle). Within two weeks of the collection, tissues are trimmed for routine histological processing and wet tissues are transferred into ethanol for long term storage. Processed tissues are paraffin embedded and routinely sectioned. Histological slides are examined by an anatomic pathologist for significant lesions.

4.5 Blood is collected into a 250 mL sterile bottle with EDTA while the sheep is alive. After captive bolt stunning and induction of unconsciousness by a licensed veterinarian, exsanguination is performed via incision through the jugular veins and carotid arteries. During exsanguination blood is collected into a five liter autoclaved metal bucket that contains EDTA. After collection blood is decanted into 50 mL falcon tubes under the laminar flow hood at Utah Veterinary Diagnostic Lab. Cells from whole blood are further processed as in the cell isolation protocols from the Ovine FAANG Project (ftp://ftp.faang.ebi.ac.uk/ftp/protocols/samples/).

4.6 For cell isolation, lungs are removed by the post mortem team and transferred to the collection station, flushed using sterile PBS and the contents decanted into 1 L sterile glass bottles. Decanting of the lavage fluid from the glass bottles

into 50 mL conical tubes for cell isolation is performed in a laminar flow hood. Cells from the lavage fluid are further processed as in the cell isolation protocols (ftp://ftp.faang.ebi.ac.uk/ftp/protocols/samples/).

5 Results

5.1 The snap frozen samples are carefully catalogued into the -80 °C freezers, split between U.S. MARC and USU and the database is updated after samples are taken out of the archive. Tubes are labelled via barcode on the external surface of the tube and with a human readable abbreviation for each tissue type as listed in the EMBL-EBI Biosamples submission and in Table 1 below. The tissue type label is duplicated on laminated sterile plastic labels within each tube (Table 1).

5.2 A major thank you goes out to our entire team who helped to plan, collect tissues, perform assays, procure the sheep, and care for the sheep during this extensive process. Please see Table 2 for those involved and their roles.

Table 1. List of tissues collected, organized by collection station, and the abbreviations and barcodes on the tubes.

Tissue (sorted by collection stations)	**Abbreviation on tube label**	**Barcode**
HEAD		
frontal cortex	Cor	1001-1010
cerebellum	Cer	1011-1020
medulla oblongata	Med	1021-1030
pituitary gland	Pit	1031-1036
hippocampus	Hipp	1037-1046
hypothalamus	Hyp	1047-1056
olfactory bulb	Olf	1057-1066
pons	Pns	1067-1076
pineal gland	Pin	1088-1090
thalamus	Tha	1091-1100
corpus callosum	CCa	1101-1108
trigeminal ganglion	Trg	1109-1116
mesencephalon (midbrain)	Mid	1077-1087
retina (eye)	Eye	1193-1200
tongue*	Ton	1155-1162
palatine tonsil*	Tng	1147-1154
epiglottis	Car-E	1163-1172
soft palate	Spal	1173-1182
hard palate	Hpal	1183-1192
mandibular lymph node	LN-Man	1117-1126
submandibular salivary gland	Slv	1127-1136
lip skin (non-haired)	Skn-NH	1137-1146
GUT	**Abbreviation on tube label**	**Barcode**
rumen-atrium	Rum-A	2011-2020
rumen-dorsal	Rum-D	2021-2030
rumen-ventral	Rum-V	2031-2040

reticulum	Ret	2041-2050
omasum	Oma	2051-2060
abomasum	Abo	2061-2070
pylorus (of abomasum)	Abo-P	2071-2080
duodenum	Duo	2081-2090
jejunum	Jej	2091-2100
ileum	Ile	2111-2120
cecum	Cec	2131-2140
spiral colon	SCol	2141-2150
descending colon	DCol	2151-2160
rectum	Rec	2161-2170
pancreas	Pan	2001-2010
Peyer's patch	Ile-PP	2121-2130
ABDOMINAL	**Abbreviation on tube label**	**Barcode**
liver	Liv	3011-3020
gall bladder	Gal	3001-3010
perirenal fat	Adi-P	3051-3060
adrenal cortex	Adn-C	3031-3040
adrenal medulla	Adn-M	3041-3050
spleen	Spl	3021-3030
UROGENITAL	**Abbreviation on tube label**	**Barcode**
kidney cortex	Kid-C	4001-4010
kidney medulla	Kid-M	4011-4020
urinary bladder	Bla	4031-4040
urethra	Uta	4041-4050
ureter	Urt	4021-4030
ovary (left)	Ovy	4051-4060
uterus (caruncule)	Ute-C	4061-4070
uterus (intercaruncular)	Ute-T	4071-4080
mammary gland	Mam	4107-4116

uterine tube (oviduct)	Ovi	4081-4086
vagina	Vag	4087-4096
uterine cervix	Cvl	4097-4106
THORAX	**Abbreviation on tube label**	**Barcode**
mediastinal lymph node	LN-med	5021-5030
heart left ventricle	Hrt-LV	5041-5050
heart right atrium	Hrt-RA	5067-5076
heart right ventricle	Hrt-RV	5077-5086
heart left atrium	Hrt-LA	5051-5060
atrioventricular valve (left)	AV-L	5061-5066
atrioventricular valve (right)	AV-R	5087-5092
semi-lunar valve (aortic)*	Aor	5119-5128
caudal vena cava	Vn-C	5099-5108
pulmonary vein	Vn-P	5109-5118
aorta (thoracic)*	Aor-va	5093-5098
lung	Lng	5031-5040
trachea	Trac	5129-5138
bronchus	Brnc	5139-5148
skeletal muscle - diaphragm	Dpm	5149-5158
thoracic part of esophagus	Eso	5159-5168
CARCASS	**Abbreviation on tube label**	**Barcode**
haired skin - dorsum	Skn-HS	6021-6030
subcutaneous adipose tissue (back fat)	Adi-SC	6011-6020
skeletal muscle - longissimus dorsi	Mus-LD	6001-6010
skeletal muscle - semimembranosus	Mus-SM	6041-6050
skeletal muscle - supraspinatus	Mus-SS	6071-6080
skeletal muscle - biceps femoris	Mus-BF	6051-6060
lymph node - mesenteric	LN-mes	2101-2110
lymph node - prescapular	LN-ps	6091-6100
spinal cord - cervical	SC	6131-6140

brachial nerve plexus	Nrv-BP	6101-6110
sciatic nerve	Nrv-S	6061-6070
cartilage - xiphoid	Car-S	6167-6176
adipose-mesenteric	Adi-M	2171-2180
psoas major muscle	Mus-PM	6111-6120
gluteus medius	Mus-GM	6031-6040
intercostal muscle	Mus-CS	6121-6130
infraspinatus muscle	Mus-IS	6081-6090
tendon (forelimb superficial digital and deep digital flexor)	Ten	6185-6194
parathyroid gland	PThr	6151-6156
thyroid gland	Thr	6141-6150
bone marrow	Mar	6157-6166
bone	Bn	6177-6184
blood	Bld	+
CELL ISOLATION	**Abbreviation on tube label**	**Barcode**
peripheral blood mononuclear cells	Bld-PBMC	7033-7042
CD14 positive monocytes	Bld-CD14	7001-7008
CD8 positive T cells	Bld-CD8	7009-7016
CD4 positive T cells	Bld-CD4	7017-7024
WC1 positive gamma-delta T cells	Bld-WC1	7025-7032
alveolar macrophages	Mac-al	7053-7062

*These tissues were collected into the wrong tubes and recorded on the day of collection; abbreviations and barcode numbers listed here correctly correspond to the tissues listed.

+This specimen was processed for individual cell isolations.

Table 2. Members of the team for the Ovine FAANG project, thank you to all involved.

Last Name	First Name	Email	Role
Archibald	Alan	alan.archibald@roslin.ed.ac.uk	1, 3, 4
Baldwin	Thomas	tom.baldwin@usu.edu	1, 2
Birge	Caylee	cbirge@vetmed.wsu.edu	4
Brauning	Rudiger	Rudiger.Brauning@agresearch.co.nz	3, 4
Clark	Emily	Emily.Clark@roslin.ed.ac.uk	1, 3, 4
Clarke	Shannon	Shannon.Clarke@agresearch.co.nz	3, 4
Cockett	Noelle	Noelle.Cockett@usu.edu	1, 2, 3, 4, 5
Dalrymple	Brian	Brian.Dalrymple@uwa.edu.au	1, 3, 4
Durfee	Codie	Codie.Durfee@ars.usda.gov	1, 2, 4, 7
Eisenhauer	Jessica	Jessica.Eisenhauer@ARS.USDA.GOV	5
Forrester	Dave	dave.forrester@usu.edu	2, 5
Hadfield	Tracy	tracy.hadfield@usu.edu	1, 2, 3, 4, 5
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Heaton	Mike	Mike.Heaton@ARS.USDA.GOV	1, 3, 5, 6
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Hullinger	Gordon	gordon.hullinger@usu.edu	2
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Lee	Bob	Bob.Lee@ARS.USDA.GOV	5
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Mousel	Michelle	michelle.mousel@ars.usda.gov	1, 2, 3, 4
Murdoch	Brenda	bmurdoch@uidaho.edu	1, 2, 3, 4
Rood	Kerry	kerry.rood@usu.edu	2
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Stott	Rusty	rusty.stott@usu.edu	2, 5
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White	Stephen	stephen.white@ars.usda.gov	1, 3, 4
Worley	Kim	kworley@bcm.edu	1, 3, 4, 6

Table legend:

1. Collection planning
2. Sample collection
3. Investigator
4. Assays
5. Sheep provision and care
6. Biosequence provider
7. EMBL-EBI Biosamples submitter

6 Risk assessment

- Care should be taken in the use of sharps. Needles and scalpels are both easily capable of puncturing the skin and causing a significant wound. The associated risk is greatly dependent upon the exact procedure being performed (i.e. if a puncture wound occurs, what the sharp is contaminated with – e.g. animal blood/cells). As a result, both needles and scalpels should be handled appropriately and carefully as single use items (DO NOT REUSE), and should be disposed of into a yellow sharps container immediately after use. SCALPEL BLADES SHOULD ONLY BE REMOVED USING THE BLADE BINS LOCATED IN THE LAB. NEEDLES SHOULD NOT BE REMOVED FROM SYRINGE AFTER USE – DISPOSE OF NEEDLE ATTACHED TO SYRINGE AS A SINGLE UNIT INTO SHARPS BIN.
- The use of safety glasses when using a needle and syringe is recommended.
- Surgical scissors should be thoroughly cleaned after use to reduce the risk of exposure to hazardous substances in the instance of a puncture wound (and don't run with them).
- Care should also be taken not to slip or fall on any blood or animal waste that may end up on the floor.
- Expectant mothers should avoid close contact with sheep due to the risk of transmissible infections so are advised NOT to participate in sheep tissue harvests.
- Safe working with liquid nitrogen is essential. Liquid nitrogen has an extremely low temperature (boiling point = -195˚C). Personal hazards include frostbite and tissue damage resulting from contact with LN_2 or contact with extremely cold surfaces associated with LN_2. ALWAYS WEAR INSULATED GLOVES. Injury can also result from the fact that other materials, such as plastics, become brittle in LN_2 and might crack or splinter. There is also a risk associated with rapid expansion when changing from liquid to gas (1L LN_2 = 700L gaseous nitrogen) causing the explosion of tubes containing samples, as a consequence lids of tubes should be only loosely secured and EYE PROTECTION should be worn to prevent injury. If LN_2 is used in an unventilated area this can lead to oxygen displacement and a dangerous reduction in the amount of oxygen available for breathing (1 breath of pure nitrogen can be enough to asphyxiate), always ensure there is fresh air ventilation to the room and operate a buddy system.