Primer Design and Efficiency for Various Equine Tissues

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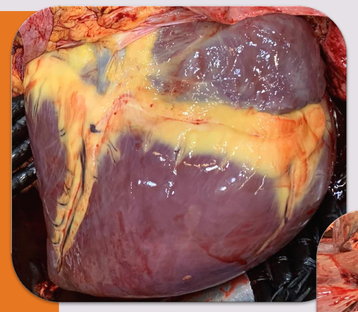
Internship Overview

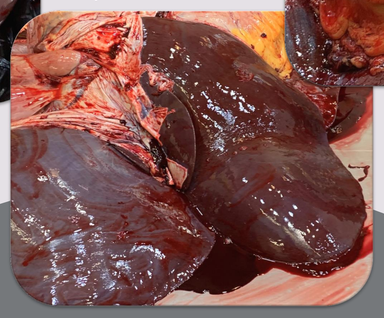
Where – University of Tennessee, Knoxville Animal Science Department
When – May 2022-August 2022 (20-30 hours each week)
What – Equine Nutrition and Genetics Research
Goal – To design and identify desirable gene primers for equine tissues

UT Animal Science Building

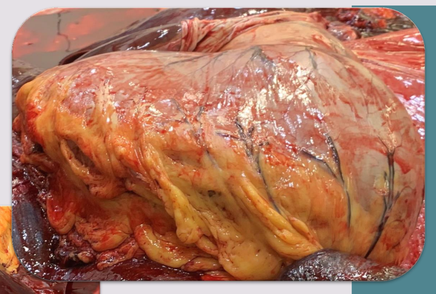
qPCR Machine



Equine Heart



Equine Liver



Equine Stomach

My Responsibilities

1. Tissue Collection

2. RNA Isolation

After tissue collection, RNA was isolated for 8 horses with 10-14 tissues each using TRIZOL reagent protocol.

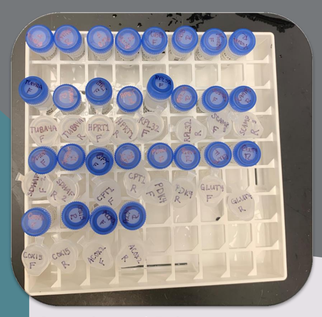
3. cDNA Synthesis

From the RNA, cDNA was synthesized using iScript transcriptase protocol for all 105 samples.

Pooled cDNA qPCR

4. Primer Design

Primers were originally selected and designed based on other mammalian research and equine trials.



Constituted Primers

5. Primer Efficiency

Once selected, primers were tested for adequate efficiency using pooled cDNA samples of various concentrations via RT-qPCR.

Results

Reference Gene	Ct Average	Stdev %
GAPDH	23.08	2.07
YWHAZ	1.79	0.40
ActB	23.08	2.48
TUBA1	26.49	1.94

- Standard deviation (Stdev) - ideal at 3% and below.
- Cycle threshold (Ct) - number of cycles required for the fluorescent signal to cross the threshold
  - Below 29 = strong positive reaction and indicates abundant target nucleic acid in the sample
- Reference genes - similar across all tissues
  - Ct average is formed using randomized samples from two horses' individual tissue cDNA

- The table below shows efficiencies for the target genes chosen for individual tissue RT-qPCR processing
  - Low efficiencies were thrown out of the experiment early
  - 10 genes had good efficiencies
- The selected genes were based on function related to metabolism and tissues included in this experiment

Target Gene	Primer Efficiency	Gene Function
RPL32	91%	• RPL32 - encodes L32 ribosomal protein (cytoplasm) • Ribosomes catalyze protein synthesis; some ribosomal proteins are expressed in tissue-specific patterns • Affects RNA processing and regulation of translation, affecting the overall expression of RPL32 across each tissue
GLUT4	75%	• GLUT4 - insulin-regulated glucose transporter • responsible for insulin-regulated glucose uptake into fat and muscle cells • Absence of insulin - GLUT4 found in intracellular vesicles referred to as GLUT4 storage vesicles (GSVs)
PDK4 Set 3	97%	• PDK4 - increased in hibernation; helps decrease metabolism and conserve glucose • Decreases conversion to acetyl-CoA, which enters the citric acid cycle and is converted to ATP
CPT1 Set 5	92%	• CPT1- increases when "carnitine palmitoyl transferase" (enzyme) is missing or not working • Breaks down fats from food and storage and converts to energy

Why Is This Important?

The issue of starved horses has become increasingly prevalent across the United States.

This research is aimed at understanding what happens during metabolic processes on the cellular level throughout starvation to help educators and owners know the best method to refeed and rehabilitate emaciated horses.

BCS 5

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BCS 1

What Are Primers?

- **Primers** are short sections of single stranded DNA that are made to compliment amplified strands of cDNA in order to target specific genes
- **Reference Genes** – Primers that show low variability across tissues and other experimental factors. For this project, GAPDH, TUBA4A, YWHAZ, and ActB were reference genes chosen for further efficiency testing
- **Target Genes** – Primers that target a precise section of cDNA to show expression of a specific trait. For this project, we focused on metabolic related target genes and tested 55 for adequate efficiency

Conclusion: This internship expanded my laboratory skills and further educated me on advanced equine nutrition and genetics through research and hands-on experience.

Acknowledgements

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