

Comparison of DNA Extraction Methods for Soft Tissue Samples

Steve Cochancela Guzman*, Nathaniel Hill*, Mujtaba H. Shah, Jonathon Reynolds, Cole Farnsworth, Molly Henley, Alfred Amendolara, Laura Minor, John Jr. Dougherty, John A. Kriak, Kyle B. Bills, David W. Sant
¹Department of Biomedical Sciences, Noorda College of Osteopathic Medicine, Provo, UT
 * Authors Contributed Equally

Background

Testing of somatic mutations and epigenetic DNA modifications requires extracting DNA from tissues. However, several substances present in tissues are known to inhibit PCR, and commercial kits are traditionally not designed to work with all tissues. We will attempt to determine which commercial DNA extraction kits will yield high-molecular weight DNA that is free of PCR inhibitors.



Picture 1: Tissue homogenizer similar to the one used for homogenization of organs

PCR Inhibitors:

- Myoglobin
- Hemoglobin
- Bilirubin
- Antibodies
- Heparin
- Collagen
- Lactoferrin
- Polysaccharides
- Fats
- Calcium
- Magnesium
- Bile Salts
- Urea
- Melanin
- Hormones

Organs Tested:

- Heart
- Liver
- Spleen
- Adrenal Glands
- Testicles
- Bone Marrow
- Skeletal Muscle
- Skin
- Lungs
- Kidneys

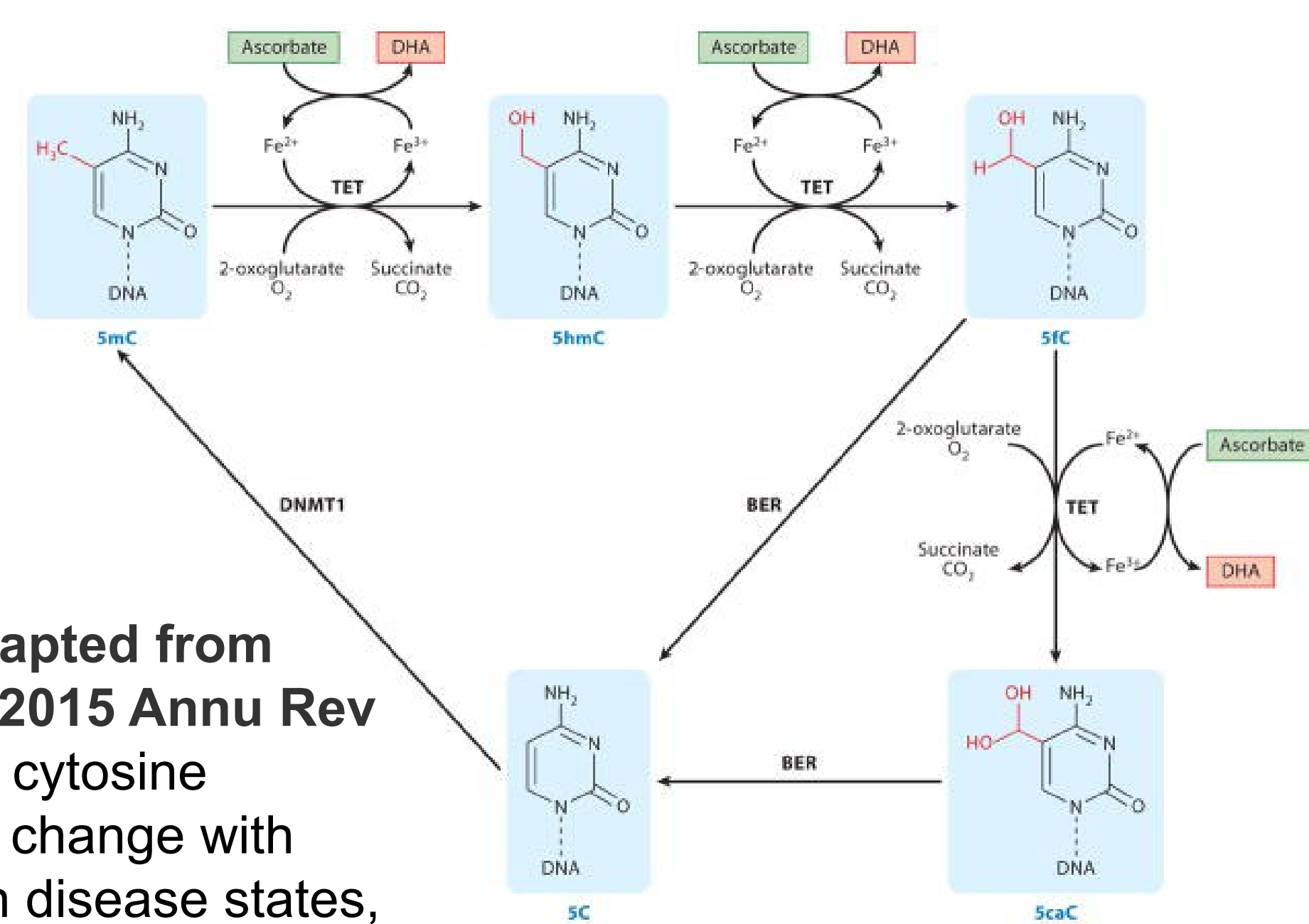
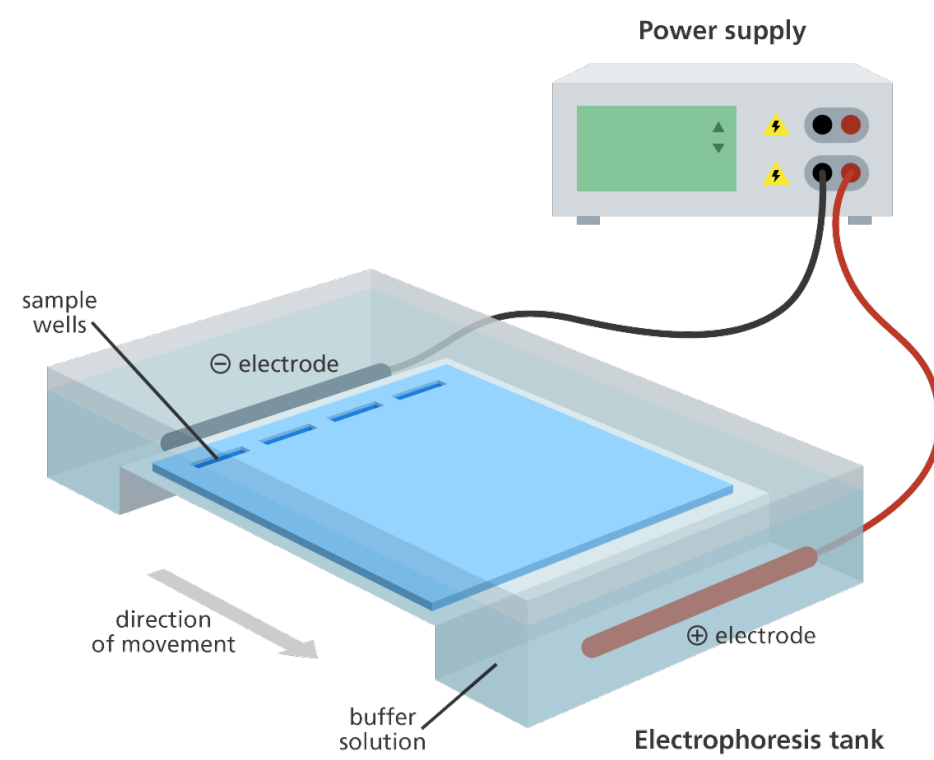


Figure 12. adapted from Young *et al.* 2015 *Annu Rev Nutr.* Various cytosine modifications change with tissues and in disease states, necessitating extraction of DNA directly from tissues.

Methods

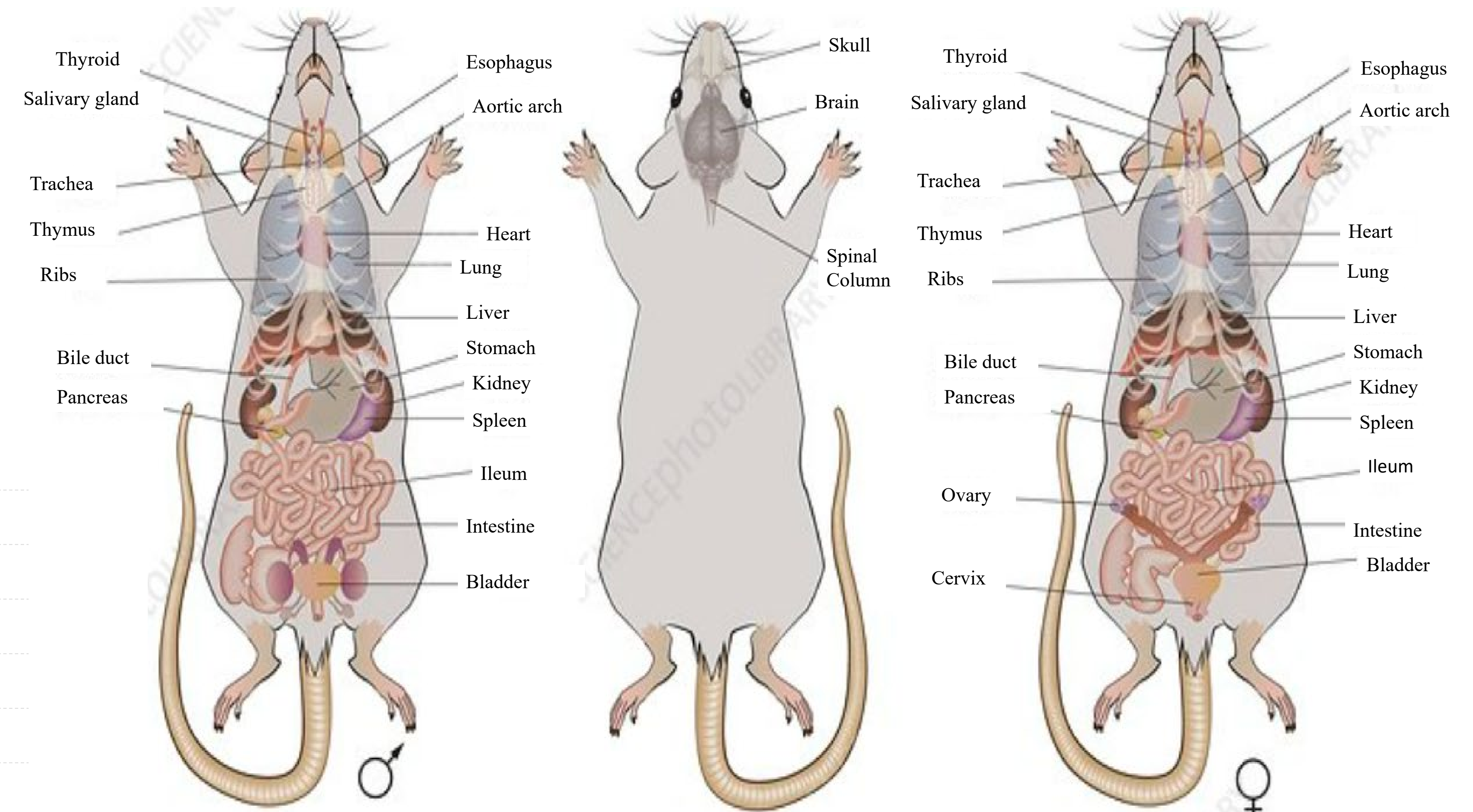
Tissues from 48 Sprague-Dawley rats were collected. Post-mortem rats from a morphine study were used to lessen animal burden. Homogenized organs were aliquoted at 10 mg. qPCR, Qubit fluorometer, and gel electrophoresis will be used to evaluate extracted DNA for inhibitors, amount, and fragment length.



Picture 2: Instrumentation used to test quality of DNA

Table 1: Kits ordered for extraction of DNA from various solid tissues

Company	Product	Cat. Number	Company	Product	Cat. Number
Beckman Coulter	GenFind V3 Readent Kit-50	C34880	Sigma-Aldrich	Extract-N-AMP Tissue PCR Kit	XNAT2-1KT
Beckman Coulter	DNAdvance	A48705	Takara	NucleoSpin Tissue	740952.5
Biomiga	Biomiga EZgene Tissue DNA Kit 50 Preps		Takara	NucleoMag Tissue	744300.1
cytiva life sciences	Tissue and cells genomicPrep Mini Spin Kit	28904275	Takara	NucleoSpin® 96 DNA RapidLyse	740110.1
cytiva life sciences	Nucleon BACC Genomic DNA Extraction Kits	RPN8501	Takara	NucleoBond HMW DNA	740160.2
cytiva life sciences	Sera-Xtracta HMW DNA kit	29429140	Takara	NucleoSpin DNA Lipid Tissue	740471.1
Fortis Life Sciences	Pure Tissue DNA Kit	EB-TDK-50	Takara	NucleoSpin Tissue XS	740901.5
New England BioLabs	Mondarch Genomic DNA Purification kit	T3010S	Thermo	DNA Extract All Reagents Kit	4403319
New England BioLabs	Monarch HMW DNA Extraction Kit for Tissue	T3060S	Thermo	MagMax DNA Multi-Sample Kit	4413020
Omega Bio Tek	E.Z.N.A. Tissue DNA Kit	D3396-01	Thermo	JetFlex Genomic DNA Purification Kit	A30700
Omega Bio Tek	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit	M6399-00	Thermo	GeneJet Genomic DNA Purification Kit	K0721
Omega Bio Tek	E.Z.N.A. MicroElute Genomic DNA Kit	D3096-00	Thermo	GeneJet Genomic DNA Purification Kit	K0721
Perkin Elmer	Chemagic DNA Cyte Pure Kit	CMG-196	Thermo	PureLink Genomic DNA Mini Kit	K182001
Promega	Wizard Genomic DNA Purification Kit	A1120	Thermo	PureLink Genomic DNA Mini Kit	K182001
Promega	Wizard SV Genomic DNA Purification System	A2360	Thermo	ChargeSwitch gDNA Mini Tissue Kit	CS11204
Promega	MagaZorb DNA Mini-Prep Kit	MB1004	Zymo	Quick-DNA Miniprep	D3024
Promega	ReliaPrep gDNA Tissue Miniprep System	A2051	Zymo	Quick-DNA miniprep plus	D4068
Promega	Wizard HMW DNA Extraction Kit	A2920	Zymo	Quick-DNA Magbead Plus Kit	D4081
Promega	ReliaPrep™ Blood gDNA Miniprep System	A5081	Zymo	Quick-DNA HMW MagBead Kit	D6060
Qiagen	DNEasy Blood and Tissue Kit	69504	Zymo	Quick-DNA Microprep Plus Kit	D4074



Picture 2: Anatomy of a rat demonstrating vital organs adapted from Science Photo Library

Organ Weights

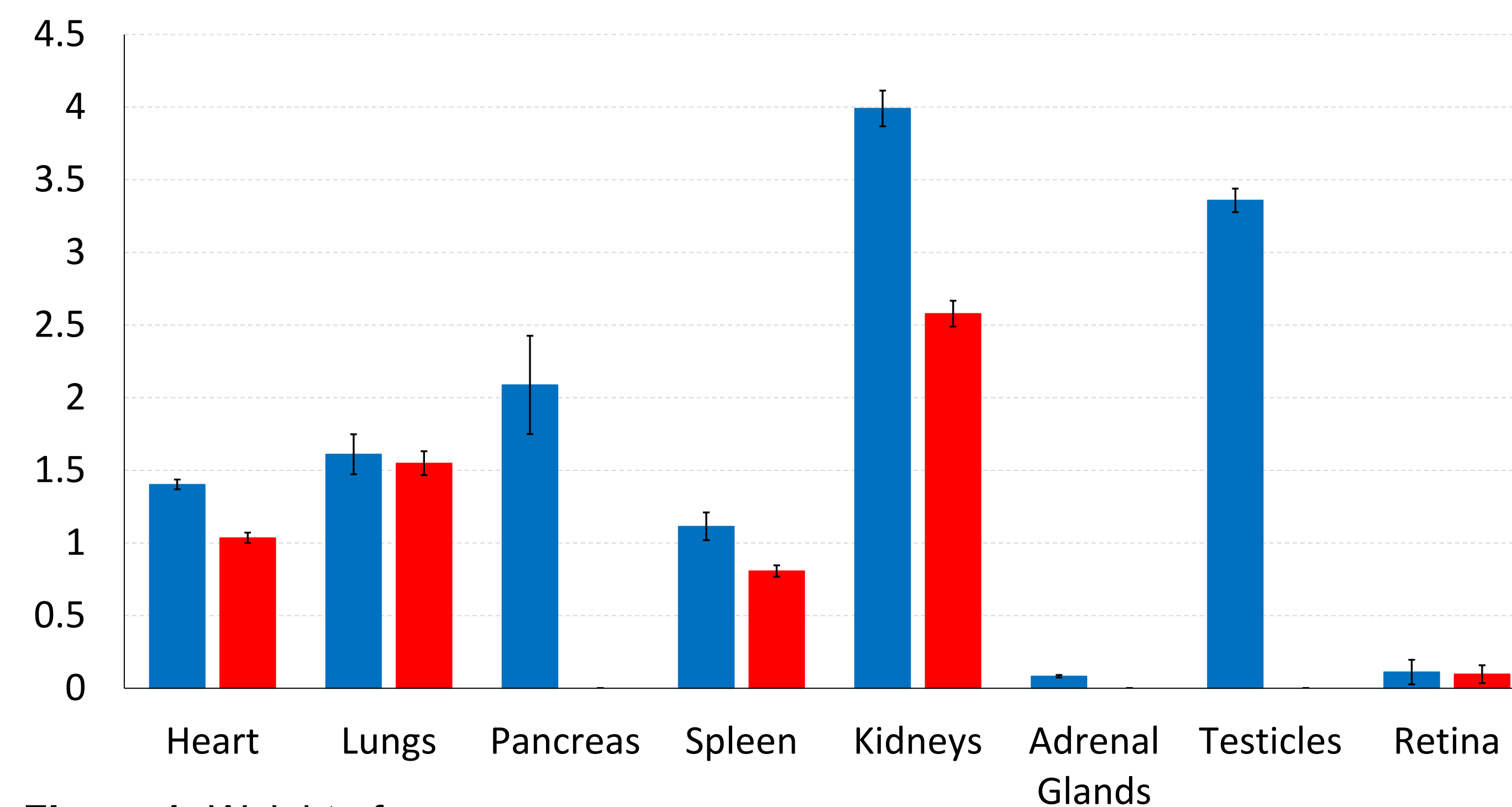


Figure 1: Weight of organs gathered from rats (grams) Male Female