DNA Extraction Method Development for Solid Tissues

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Background

- Studying somatic mutations and epigenetic modifications requires DNA to be extracted from specific tissues.
- DNA extraction kits are traditionally designed for liquid tissues, but several exist for solid tissues.
- Several enzymatic inhibitors are present in tissues, and different kits are good at removing different inhibitors.
- We are testing 30 different extraction kits to determine which kits work for which tissues.

PCR Inhibitors:

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

Organs Tested:

Heart	Skin
Liver	Lungs
Spleen	Kidneys
Pancreas	Brain
Testicles	Bone
Adrenal Glands	
Bone Marrow	
Skeletal Muscle	
Adipose Tissue	

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

Methods

Extractions have been performed using the following kits: DNEasy Blood and Tissue Kit (Qiagen), GeneJET Genomic DNA Purification Kit (ThermoFisher Scientific). Quantity has been tested using a Qubit Fluorometer (Thermo Fisher Scientific). Several more kits are currently being tested. Extracted DNA will be tested for inhibitors using quantitative polymerase chain reaction (qPCR).

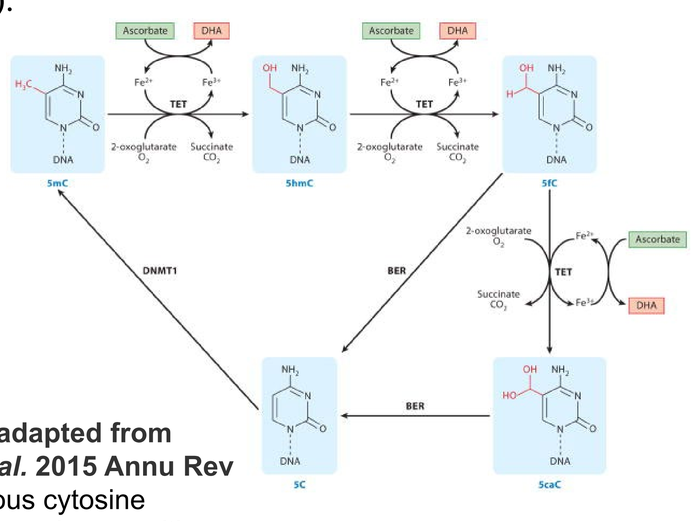


Figure 1. adapted from Young *et al.* 2015 Annu Rev Nutr. Various cytosine modifications change with tissues and in disease states, necessitating extraction of DNA directly from tissues.

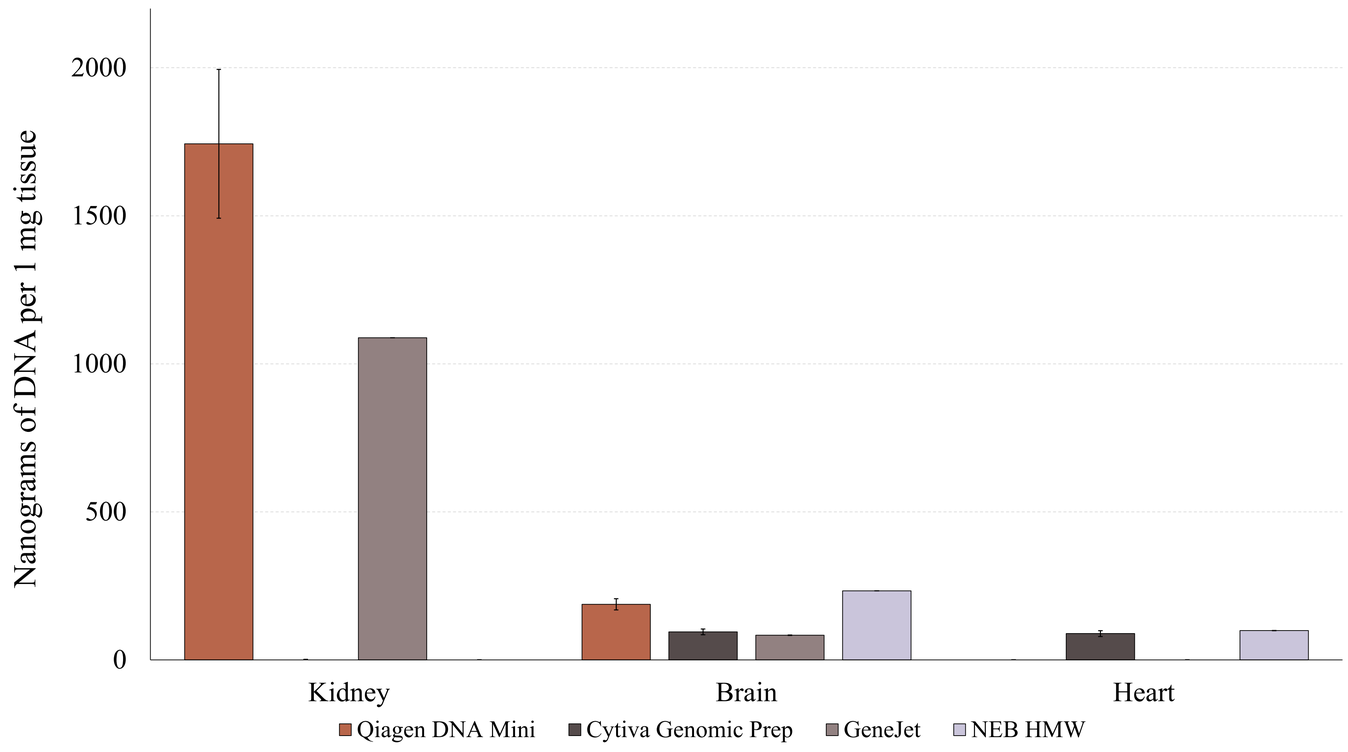
Several kits can be used to obtain high molecular weight DNA from tissues

Results

Company	Product	Cat. Number
Beckman Coulter	GenFind V3 Readent Kit-50	C34880
Beckman Coulter	DNAdvance	A48705
Biomiga	Biomiga EZgene Tissue DNA Kit 50 Preps	
cytiva life sciences	Tissue and cells genomicPrep Mini Spin Kit	28904275
cytiva life sciences	Nucleon BACC Genomic DNA Extraction Kits	RPN8501
cytiva life sciences	Sera-Xtracta HMW DNA kit	29429140
Fortis Life Sciences	Pure Tissue DNA Kit	EB-TDK-50
New England BioLabs	Mondarch Genomic DNA Purification kit	T3010S
New England BioLabs	Monarch HMW DNA Extraction Kit for Tissue	T3060S
Omega Bio Tek	E.Z.N.A. Tissue DNA Kit	D3396-01
Omega Bio Tek	Mag-Bind® Blood & Tissue DNA HDQ 96 Kit	M6399-00
Omega Bio Tek	E.Z.N.A. MicroElute Genomic DNA Kit	D3096-00
Perkin Elmer	Chemagic DNA Cyte Pure Kit	CMG-196
Promega	Wizard Genomic DNA Purification Kit	A1120
Promega	Wizard SV Genomic DNA Purification Systen	A2360
Promega	MagaZorb DNA Mini-Prep Kit	MB1004
Promega	ReliaPrep gDNA Tissue Miniprep System	A2051
Promega	Wizard HMW DNA Extraction Kit	A2920
Promega	ReliaPrep™ Blood gDNA Miniprep System	A5081
Qiagen	DNEasy Blood and Tissue Kit	69504

Company	Product	Cat. Number
Sigma-Aldrich	Extract-N-AMP Tissue PCR Kit	XNAT2-1KT
Takara	NucleoSpin Tissue	740952.5
Takara	NucleoMag Tissue	744300.1
Takara	NucleoSpin® 96 DNA RapidLyse	740110.1
Takara	NucleoBond HMW DNA	740160.2
Takara	NucleoSpin DNA Lipid Tissue	740471.1
Takara	NucleoSpin Tissue XS	740901.5
Thermo	DNA Extract All Reagents Kit	4403319
Thermo	MagMax DNA Multi-Sample Kit	4413020
Thermo	JetFlex Genomic DNA Purification Kit	A30700
Thermo	GeneJet Genomic DNA Purification Kit	K0721
Thermo	GeneJet Genomic DNA Purification Kit	K0721
Thermo	PureLink Genomic DNA Mini Kit	K182001
Thermo	PureLink Genomic DNA Mini Kit	K182001
Thermo	ChargeSwitch gDNA Mini Tissue Kit	CS11204
Zymo	Quick-DNA Miniprep	D3024
Zymo	Quick-DNA miniprep plus	D4068
Zymo	Quick-DNA Magbead Plus Kit	D4081
Zymo	Quick-DNA HMW MagBead Kit	D6060
Zymo	Quick-DNA Microprep Plus Kit	D4074

Figure 2. Yield of DNA from different extractions (ng of DNA from 1 mg of tissue). DNA was extracted from tissues using four different DNA extraction kits: Qiagen DNA Mini, Cytiva Genomic Prep, Thermo Fisher GeneJet, N.E.B. High Molecular Weight Tissue Kit. Zero yields are shown in place of untested experiments.



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Conclusion

Kidney tissue yields exceptionally high quantities of DNA in comparison to brain and heart. Yields from all kits tested were sufficient, indicating that several kits will work for these tissues. Future work will determine the presence of enzyme inhibitors and average molecular weight of the DNA extracted. Other tissues will be tested, including compact bone and adipose tissue, which may require modified protocols for extraction.

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