

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 | |



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

Organs Tested:

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

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).

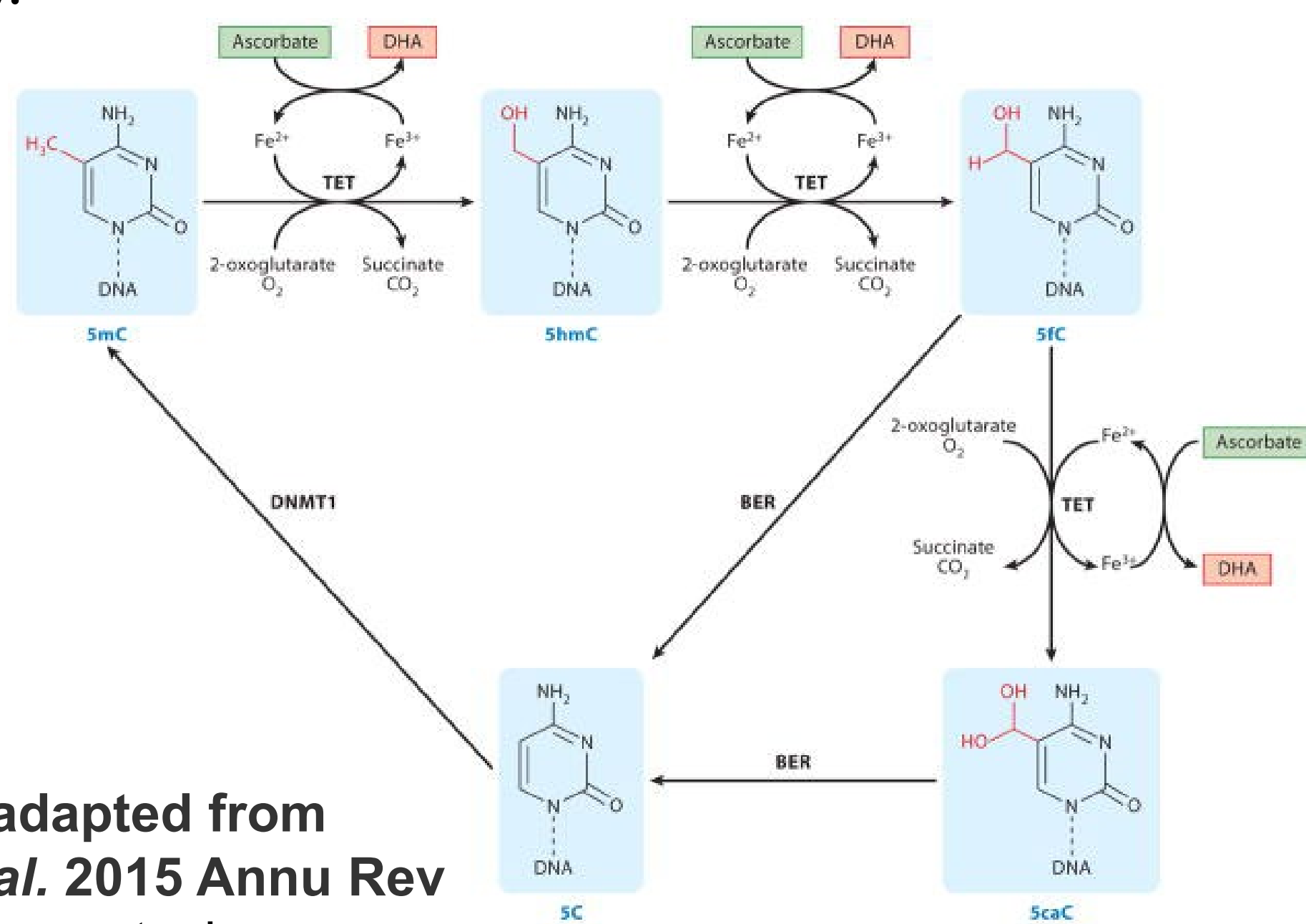


Figure 1. adapted from Young *et al.* 2015 *Annu Rev Nutr.* Various cytosine modifications change with tissues and in disease states, necessitating extraction of DNA directly from tissues.

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 | DNAAdvance | 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 System | 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.

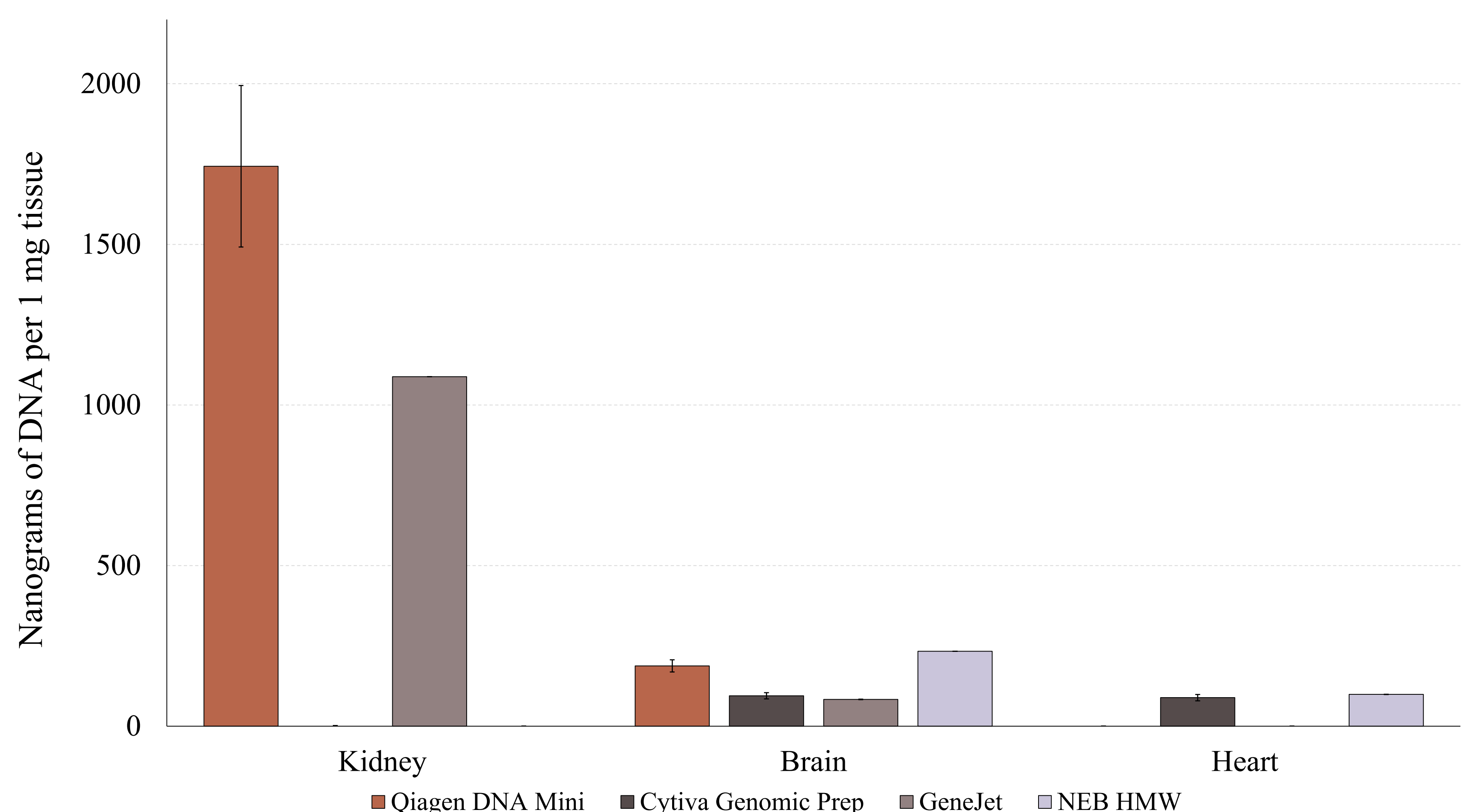


Figure 2. Yield of DNA from different extractions (ng of DNA from 1 mg of tissue). DNA was extracted from tissues using four different DNA extraction kits: Qiagen DNA Mini, Cytiva Genomic Prep, Thermo Fisher GeneJet, N.E.B. High Molecular Weight Tissue Kit. Zero yields are shown in place of untested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

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.