Catholic Health Initiatives
Center for Translational Research

A Survey of Tissue Specimen Collection Techniques: Impact on Biomarker Data Quality

Ann Allen, MS

BRN Symposium

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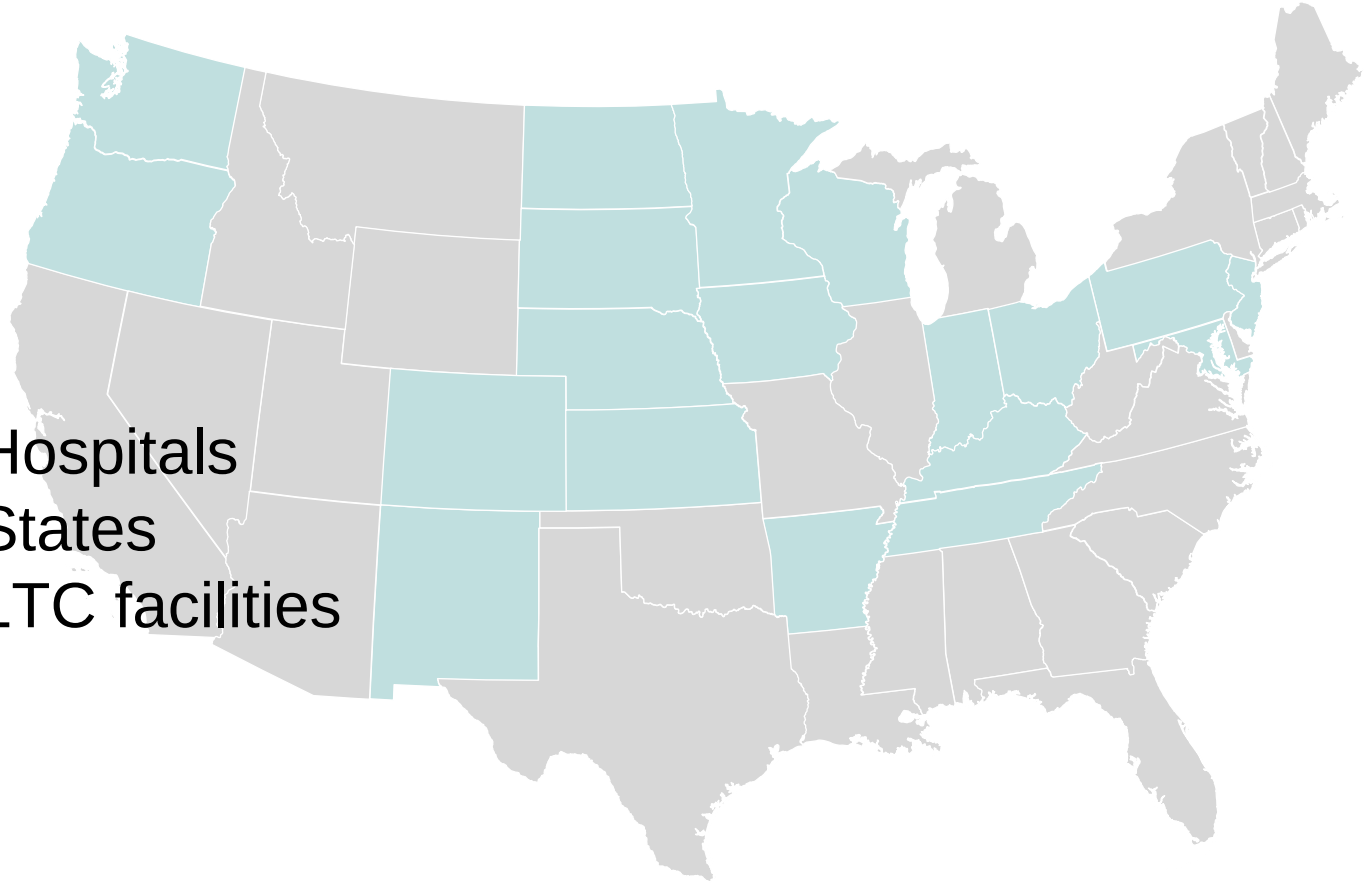
Catholic Health Initiatives (CHI)



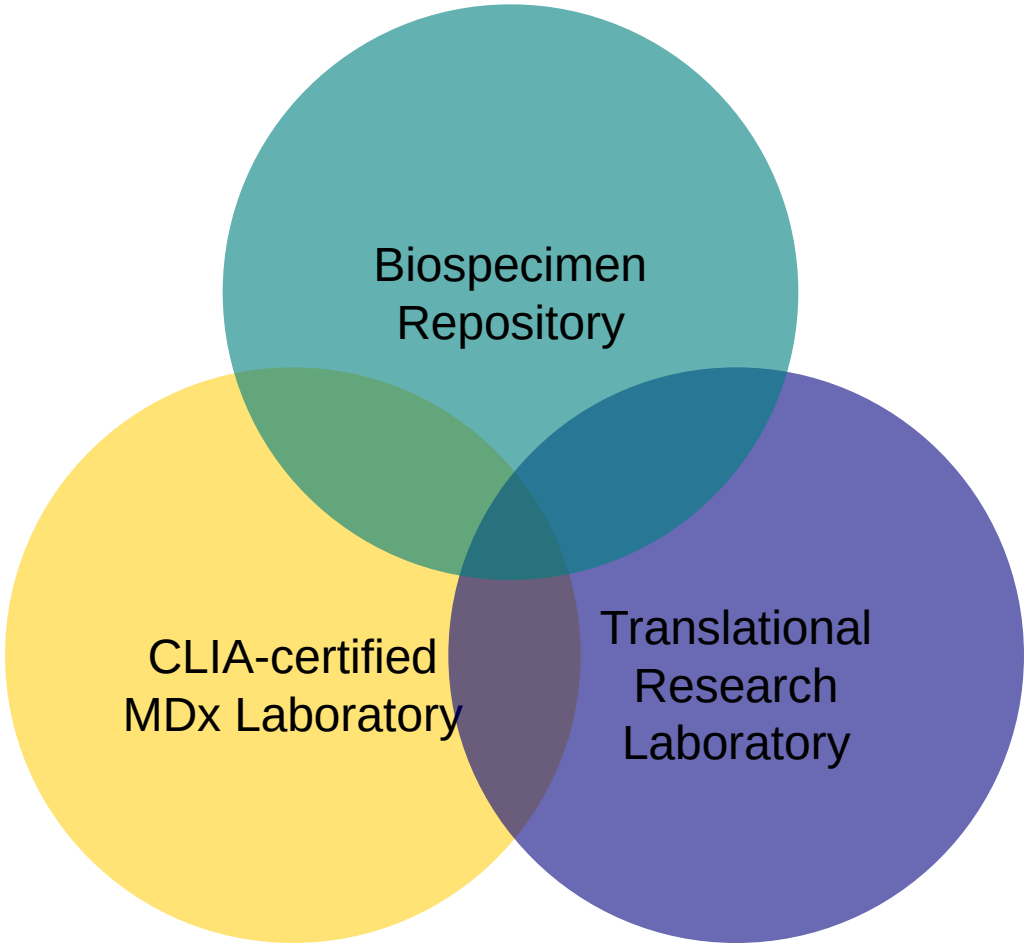
- 73 Hospitals
- 19 States
- 42 LTC facilities

CATHOLIC HEALTH INITIATIVES®

A spirit of innovation, a legacy of care.

Center for Translational Research

Center for Translational Research (CTR)



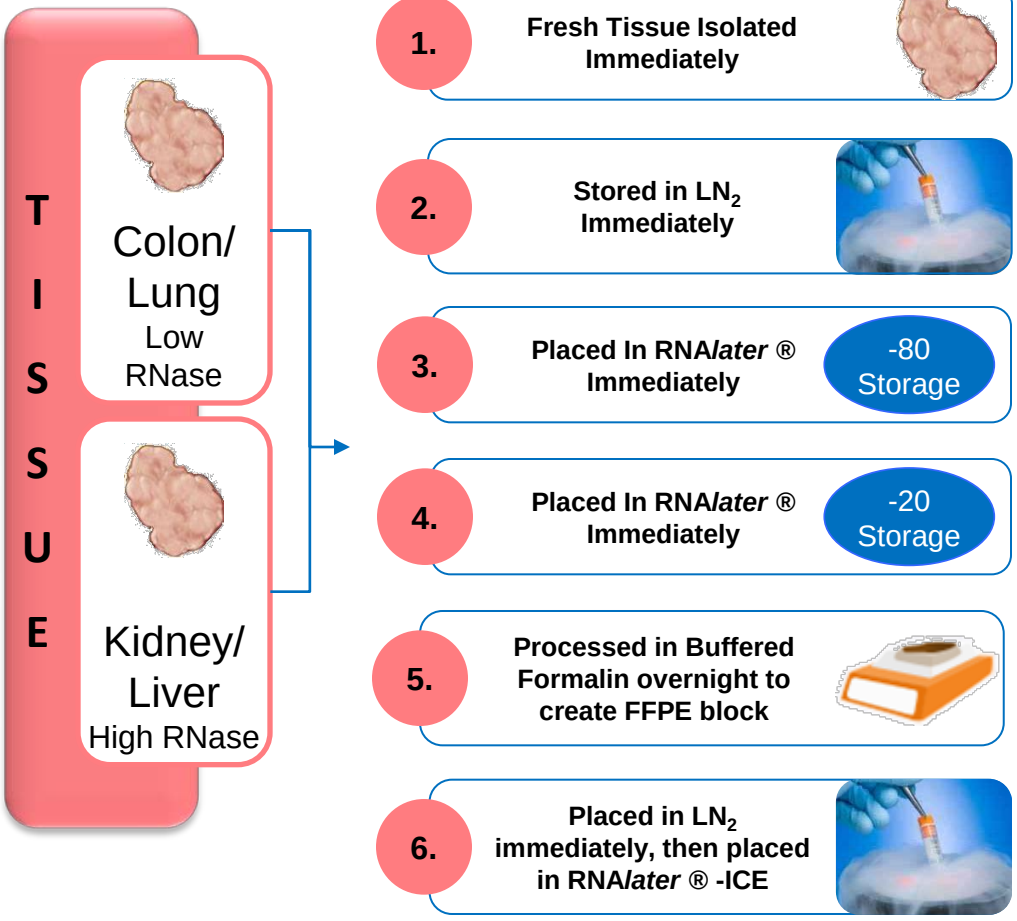
Factors Impacting the Advancement of Personalized Medicine

- Biospecimen collection and storage
- Well-annotated clinical & longitudinal data
- Adoption of tests and technologies

Current Collection and Storage Methods

Method	Pros	Cons
Fresh	High RNA quality and yields	Requires immediate extraction of tissue
Liquid Nitrogen (LN_2)	High RNA quality and yields	Requires pulverization of tissue and rapid homogenization to minimize RNA degradation
FFPE	Preserves tissue morphology, widely available with clinically annotated data	Crosslinking and chemical modification degrades and fragments nucleic acids, resulting in compromised quality of molecular data
RNA*later*®	Suitable for both short term and long term storage at different temperatures	Passive diffusion of aqueous sulfate salts requires small sample size. Yields denatured proteins only
RNA*later*®-ICE	Stabilizes RNA in tissues during the transition of tissue from frozen to a non-frozen state	Alcohol based solution requires overnight storage at -20°C prior to RNA isolation. Yields denatured proteins only
AllProtect Tissue Reagent	Stabilizes DNA, RNA, and protein for both short term and long term storage at different temperatures	Proteins are denatured thus, requiring separate tissue for the isolation of native proteins
PAXgene Tissue System	Stabilizes DNA , RNA and histomorphology for both short term and long term storage at different temperatures	Does not yield protein products

Experimental Setup



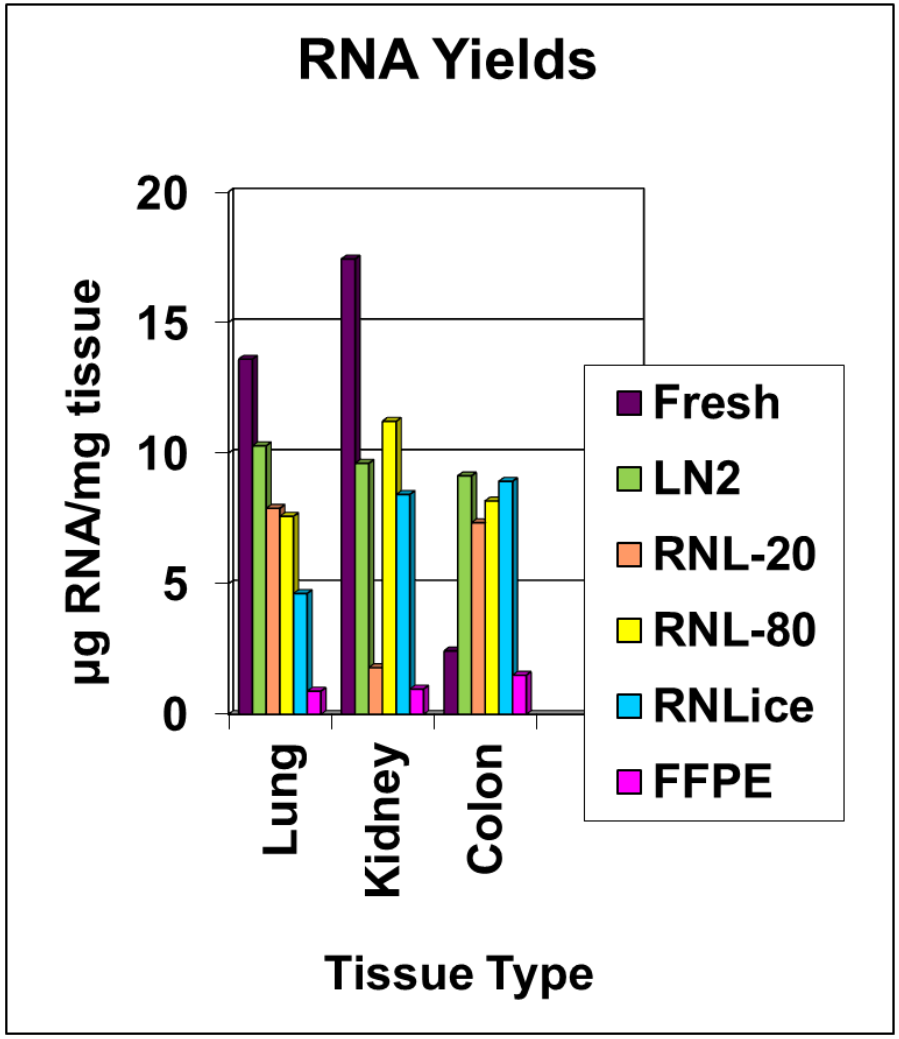
Time (months)				
Sample Type	0	6	12	24
Fresh	+	N/A	N/A	N/A
LN_2	+	+	+	+
RNA*later* ® -80°	+	+	+	+
RNA*later* ® -20°	+	+	+	+
FFPE	+	+	+	+
RNA*later* ® ICE	+	+	+	+

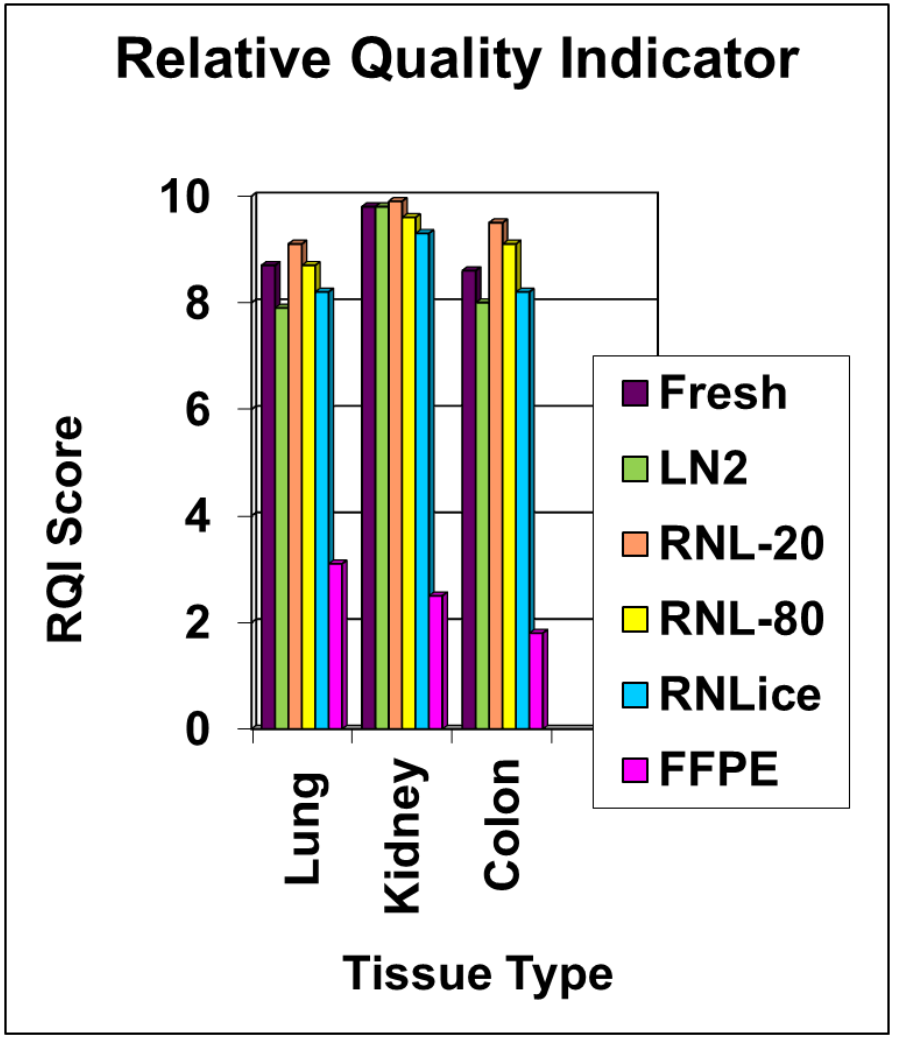
RNA Isolation → QC: Sample Assessment → QC: Performance Assessment

A Study in Progress

- Data is presented as zero timepoint and is preliminary
- Three tissues collected to date (one each lung, kidney, and colon)
- A total of 5 tissues from each of the two classifications (high and low RNase activity) will be collected for the study

Total RNA Isolation & Quality

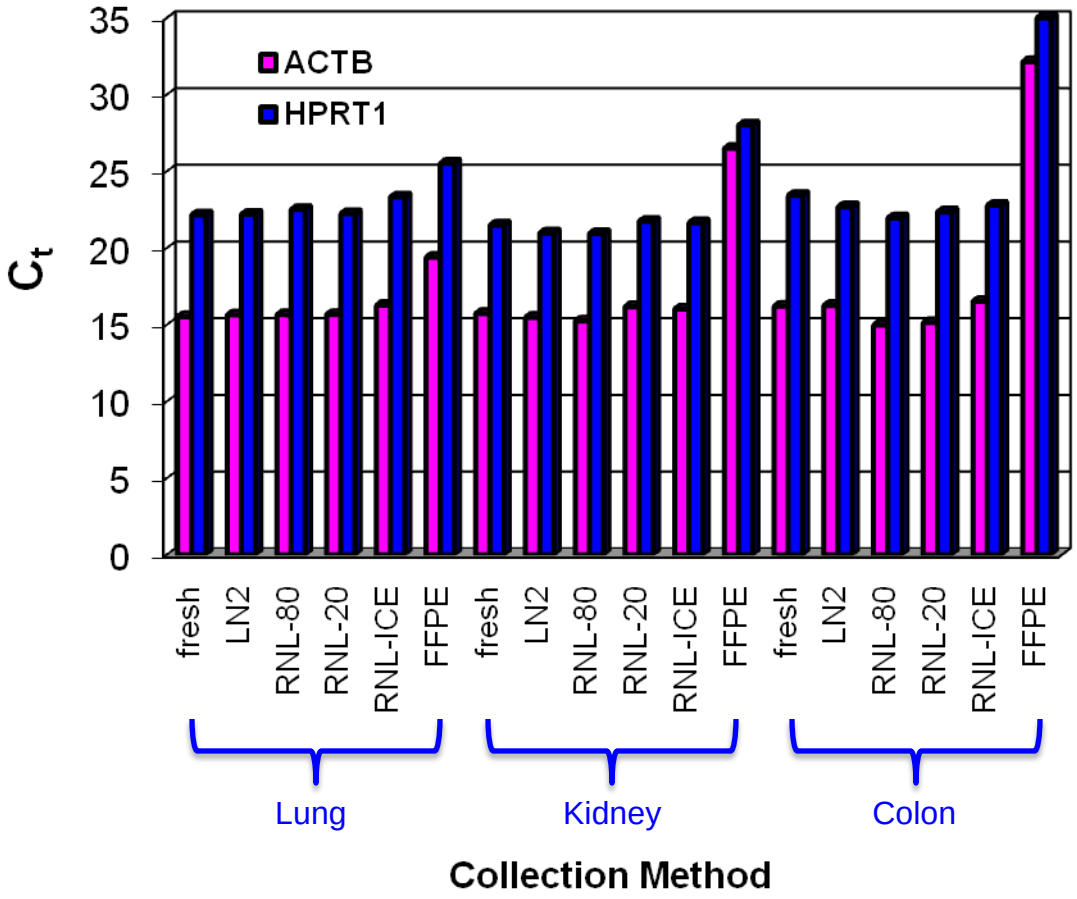




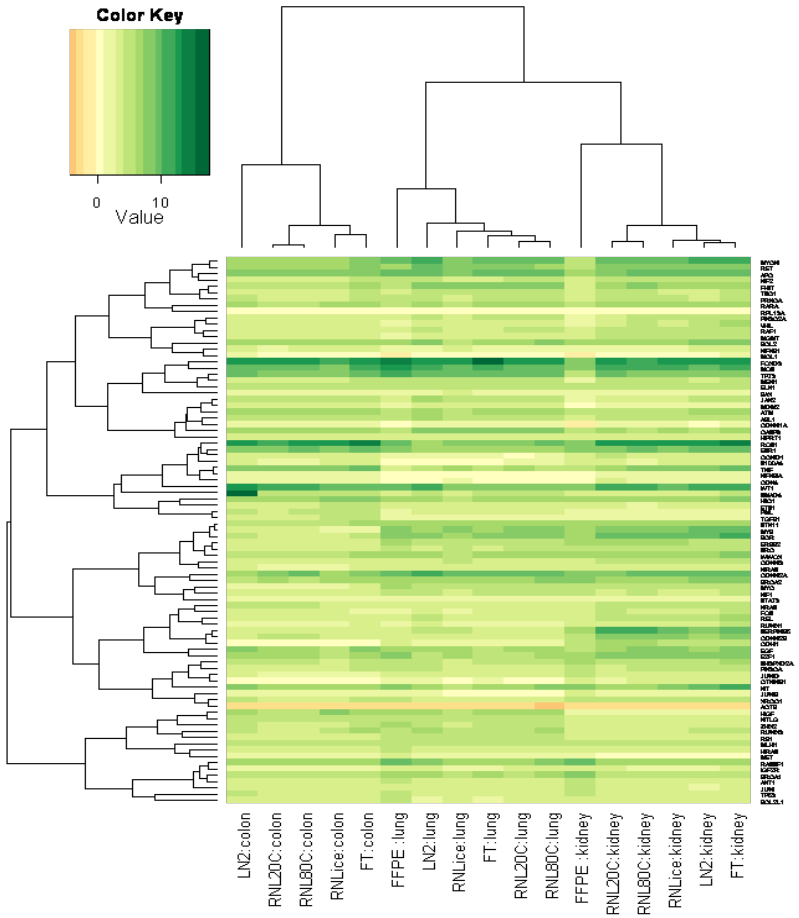
QC Arrays Assess Total RNA Quality

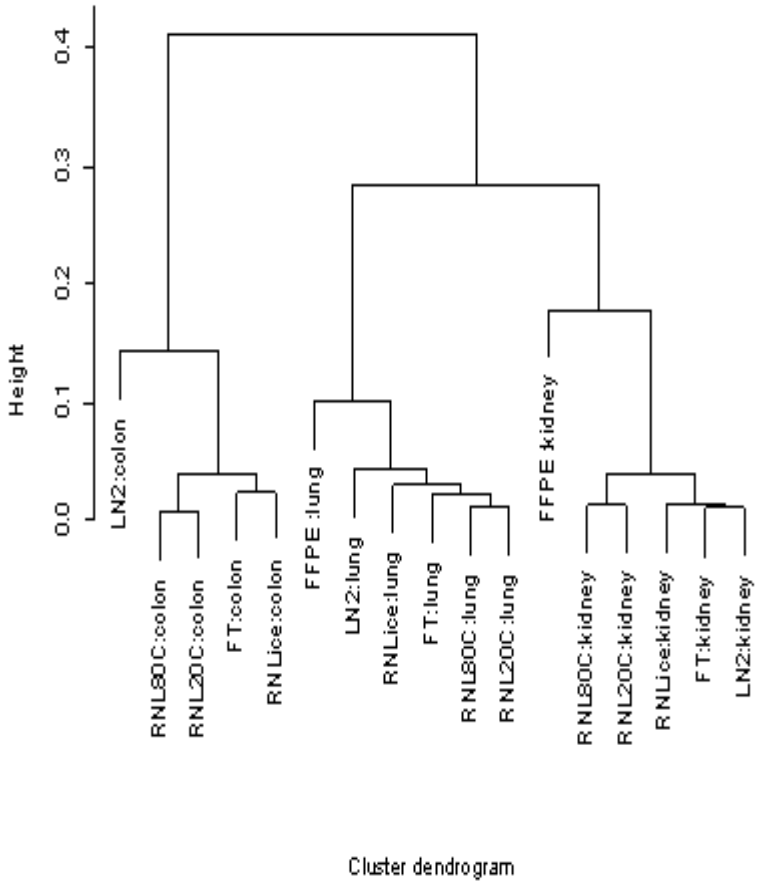
Array contains elements that interrogate:

- PCR inhibitors
- Non-transcribed genomic DNA contamination
- General DNA contamination

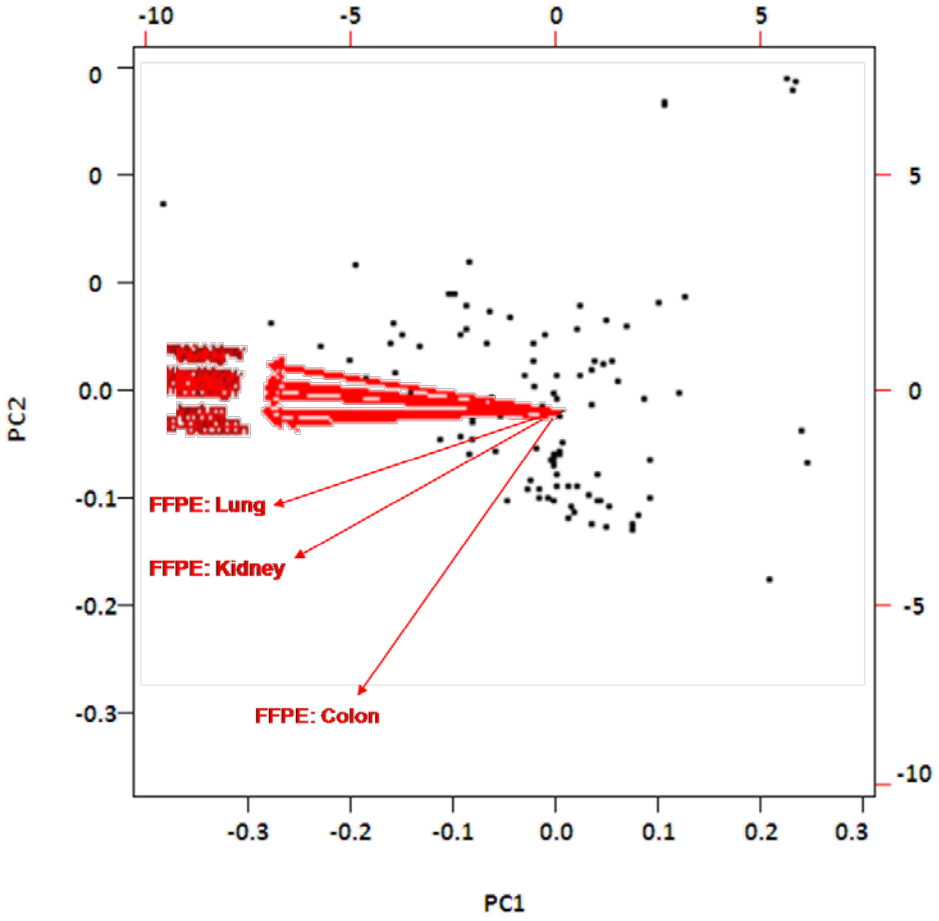


Hierarchical Clustering of Genes, Samples, & Collection Methods

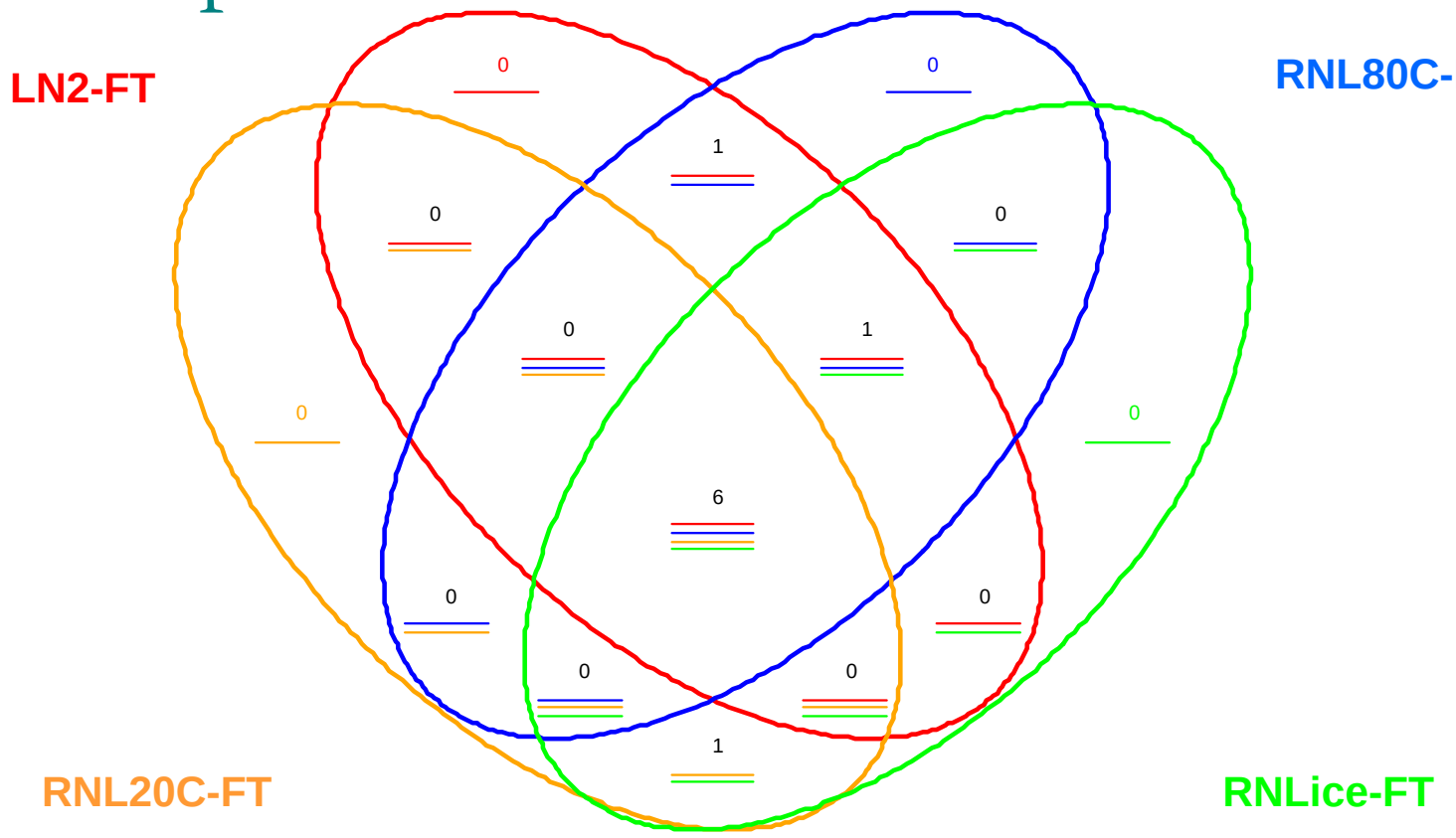




Principle Component Analysis (PCA)



Venn Diagram of Differentially Expressed Transcripts



Preliminary Results

- No major differences in quality of RNA due to stabilization methods, with the exception of FFPE
- All collection methods demonstrated good RNA integrity by QC arrays, with exception of FFPE
- Hierarchical clustering suggested three distinct groups: 1) Fresh, LN2 and RNA*later*®-ICE, 2) RNA*later*® and 3) FFPE suggesting that RNA stabilization treatments may influence gene expression patterns.

Ongoing Studies

- Collection of additional samples to perform both targeted and whole genome transcriptome analysis to identify changes in gene expression profiles included by tissue stabilization media over a period of two (2) years.

- Evaluation of new tissue and nucleic acid stabilization media such as AllProtect and PAXGene on downstream molecular analysis.

Study Benefits

Completion of our studies is expected to illustrate the influence of stabilization media on gene expression patterns, and to identify the most effective stabilization media thus enabling the standardization of biorepository specimen collection and handling

Acknowledgements

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Thank You