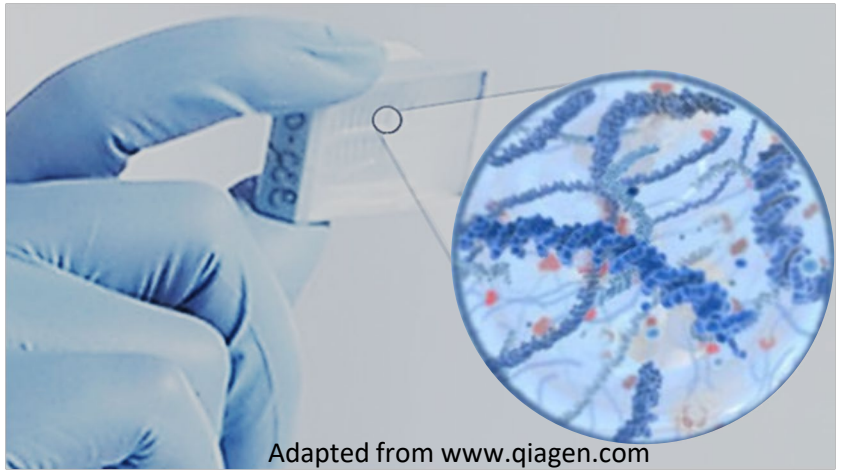
Genomics in formalin-fixed paraffin embedded tissue (FFPE) samples for quantitative risk assessment



Leah C. Wehmas, Ph.D.
Genomics Scientist
ORD USEPA

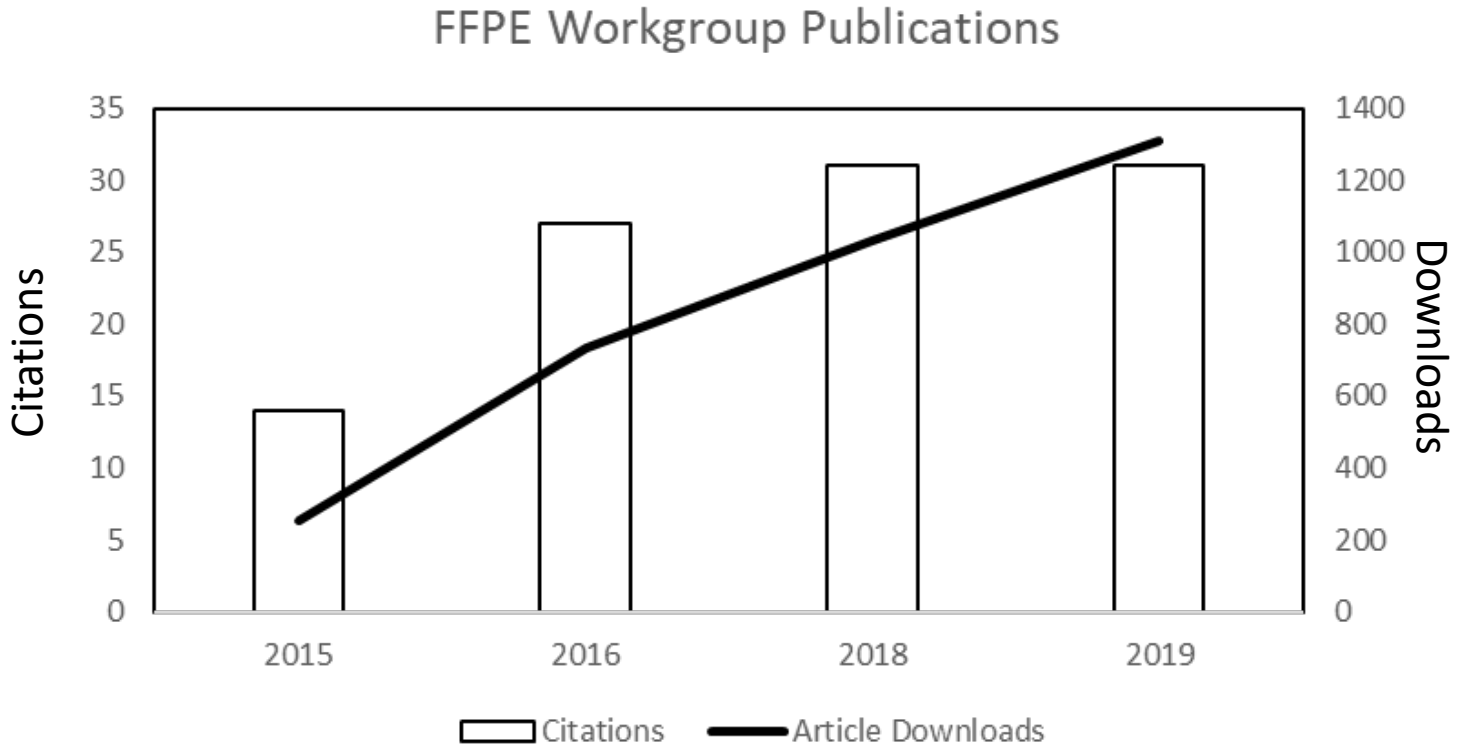
HESI eSTAR Webinar
May 26, 2020

EPA United States Environmental Protection Agency

Disclaimer

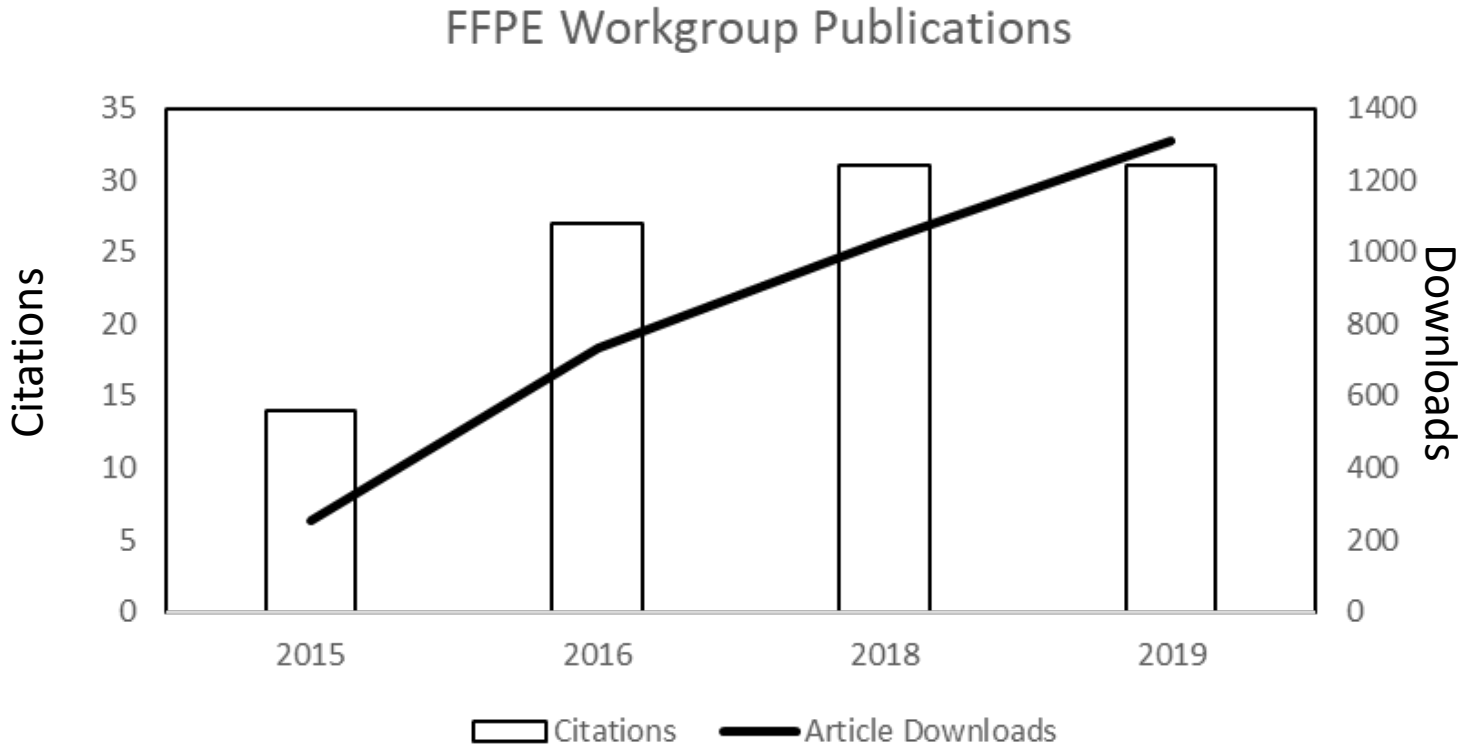
The views presented in this webinar are mine and do not represent the US EPA. Any mention of products does not constitute endorsement.

eSTAR FFPE Workgroup Impact



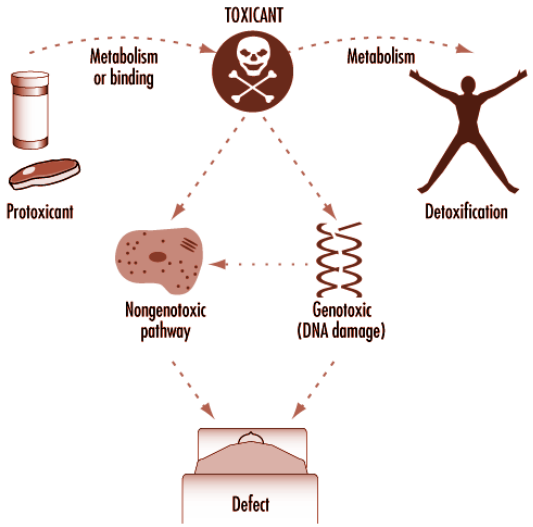
- *Mining the Archives: A Cross-Platform Analysis of Gene Expression Profiles in Archival Formalin-Fixed Paraffin-Embedded Tissues*. Webster *et al.* 2015
- Editor's Highlight: *Dose-Response Analysis of RNA-Seq Profiles in Archival Formalin-Fixed Paraffin-Embedded Samples*. Hester *et al.* 2016
- *Demodifying RNA for Transcriptomic Analyses of Archival Formalin-Fixed Paraffin-Embedded Samples.* Wehmas *et al.* 2018
- *Enhanced Quality Metrics for Assessing RNA Derived From Archival Formalin-Fixed Paraffin-Embedded Tissue Samples.* Wehmas *et al.* 2019
- *Improving DNA-sequencing Analysis from Formalin-Fixed Paraffin-Embedded Tissue Samples.* Wehmas *et al.* in progress 2020

Major findings

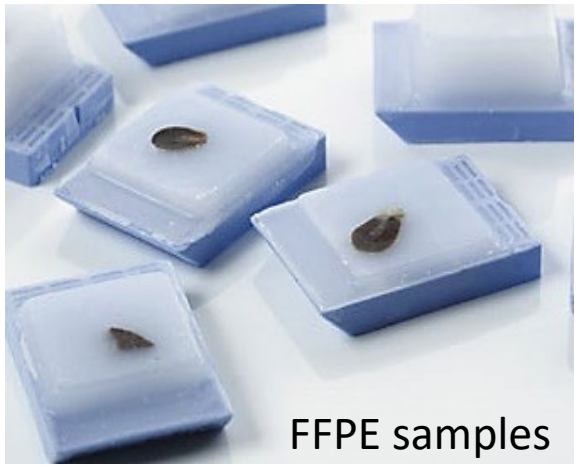


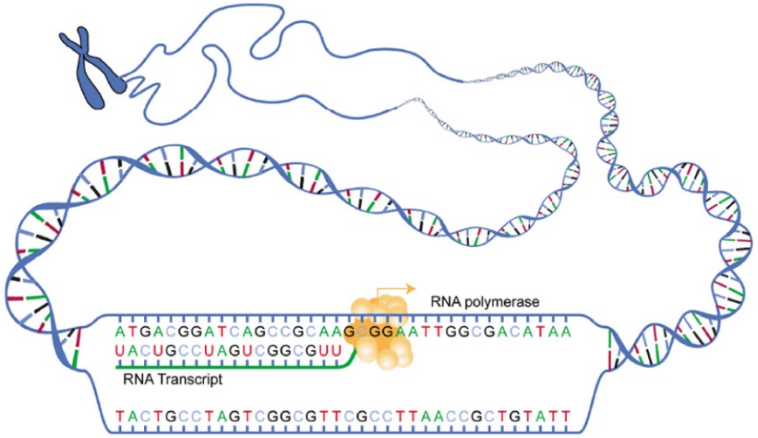
- Applied new technologies
- Characterized major factors impacting FFPE quality
- Identified methods to improve gene expression data
- Developed better metrics for quality assessment
- Translated results to clinical FFPE and improved SNP detection

Archival tissue samples can be repurposed to:

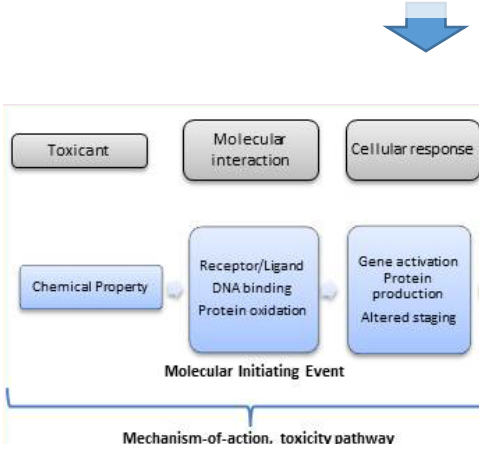


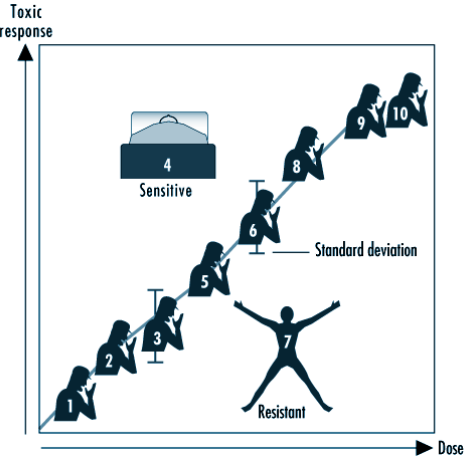
Understand mutations





Define modes of action and toxicity pathways

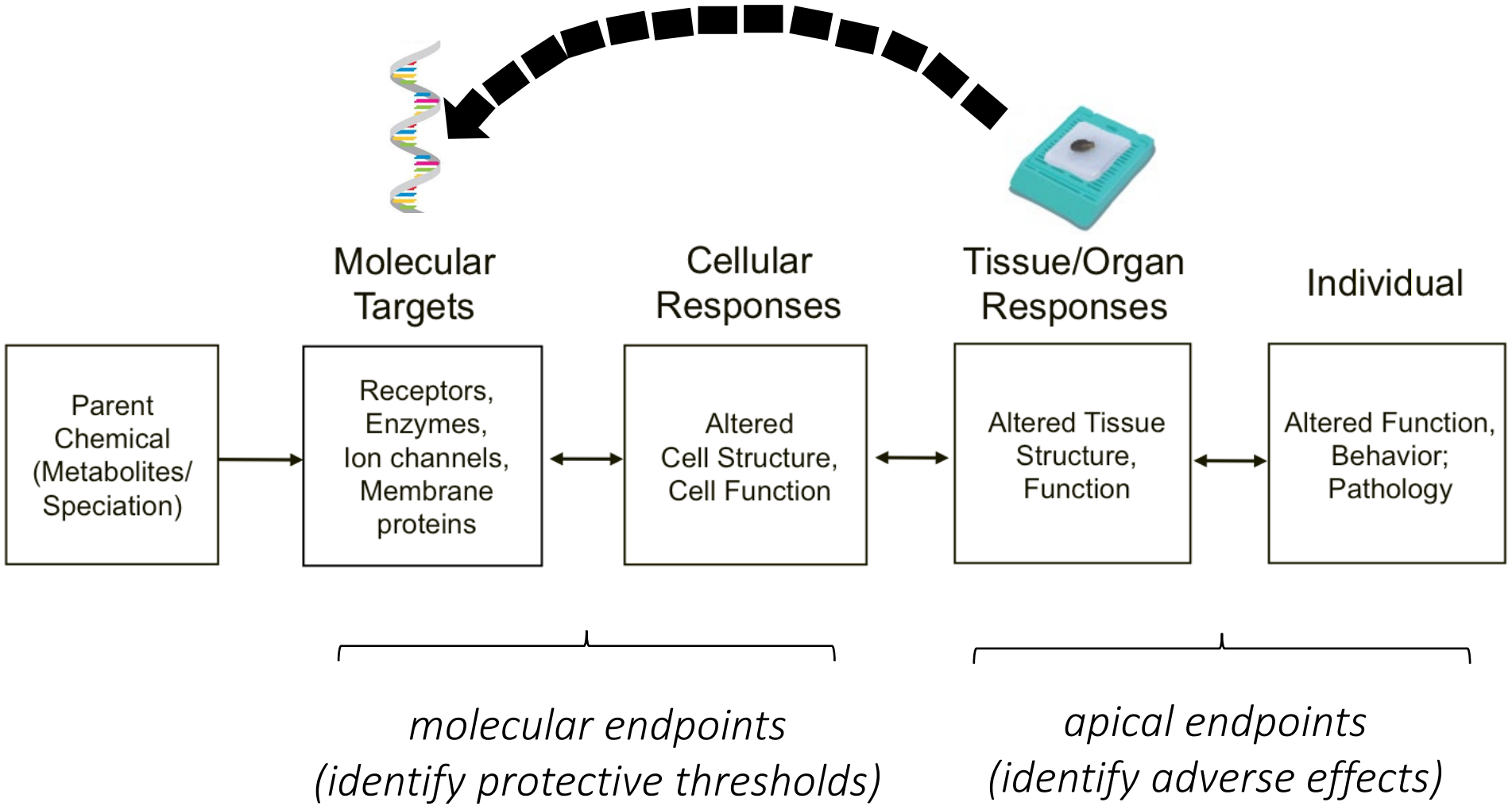




Quantify and model gene expression

https://opentextbc.ca/anatomyandphysiology/chapter/3-4-protein-synthesis/
http://topcapteam.org/
https://humantoxicologyproject.org/tox-101/pathway-based-toxicology/
http://www.ilocis.org/documents/chpt33e.htm

Bridging pathology and pathways



http://www.oecd.org/chemicalsafety

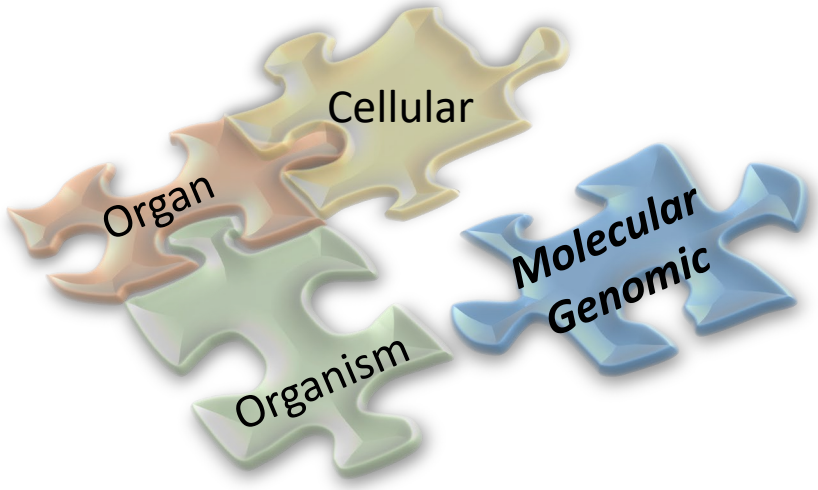
Great potential for adverse outcome pathways

- Billions of archival samples
- Well-characterized pathological data
- Clinically annotated
- Enable rapid assessment of target pathways, dose response, and human health relevance

FFPE samples

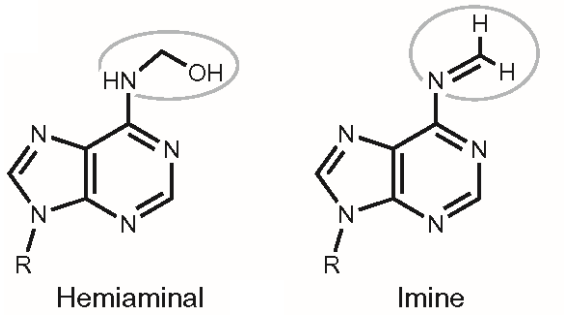
http://topcapteam.org/

AOP development

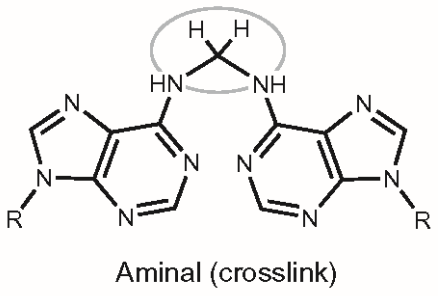


Important to identify candidate signals of toxicity risk resulting from chemical exposure

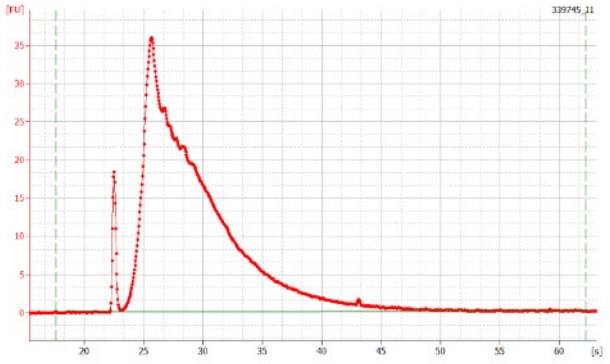
Challenges with FFPE



Adducts



Crosslinks

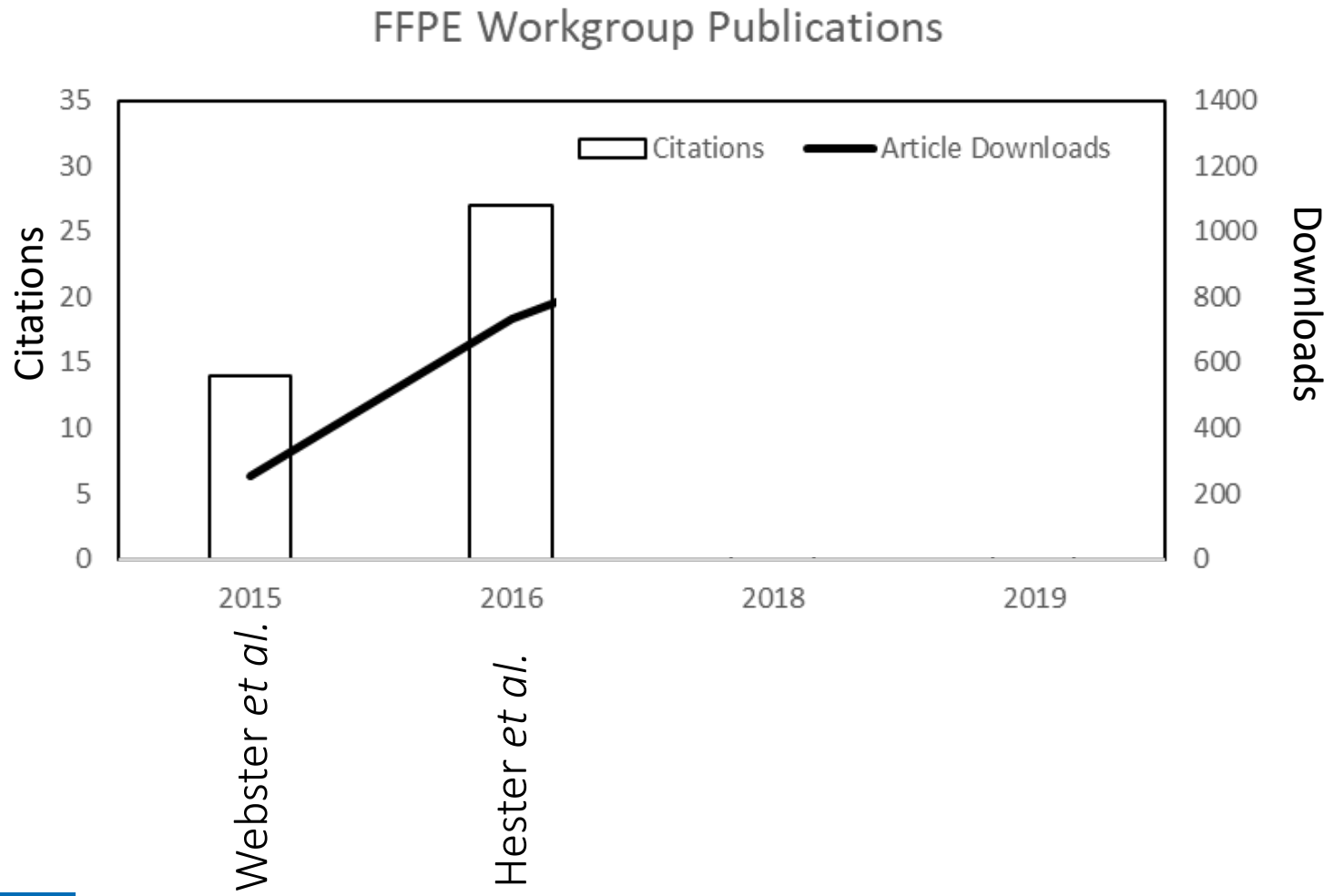


Fragmentation

Variability in quality — Inadequate assessment methods — Potential artifacts

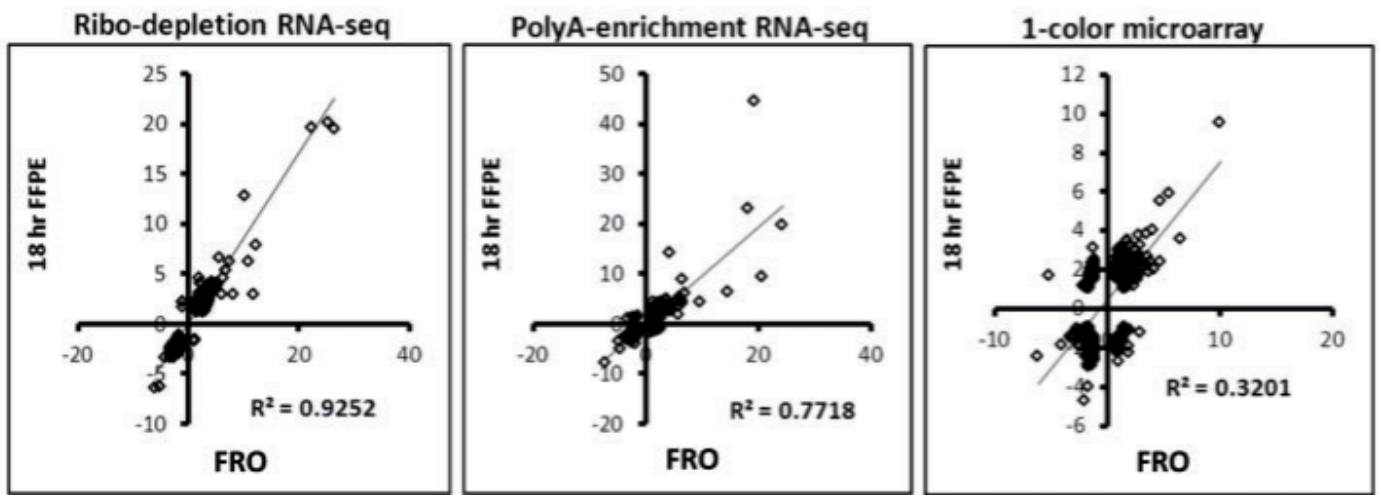
Inconsistent results

Objectives



- Apply new technologies
- Characterized major factors impacting FFPE quality

Ribo-depletion RNA-sequencing improves gene expression analysis in FFPE



OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 148(2), 2015, 460–472

doi: 10.1093/toxsci/kfv195
Advance Access Publication Date: September 10, 2015
Research Article

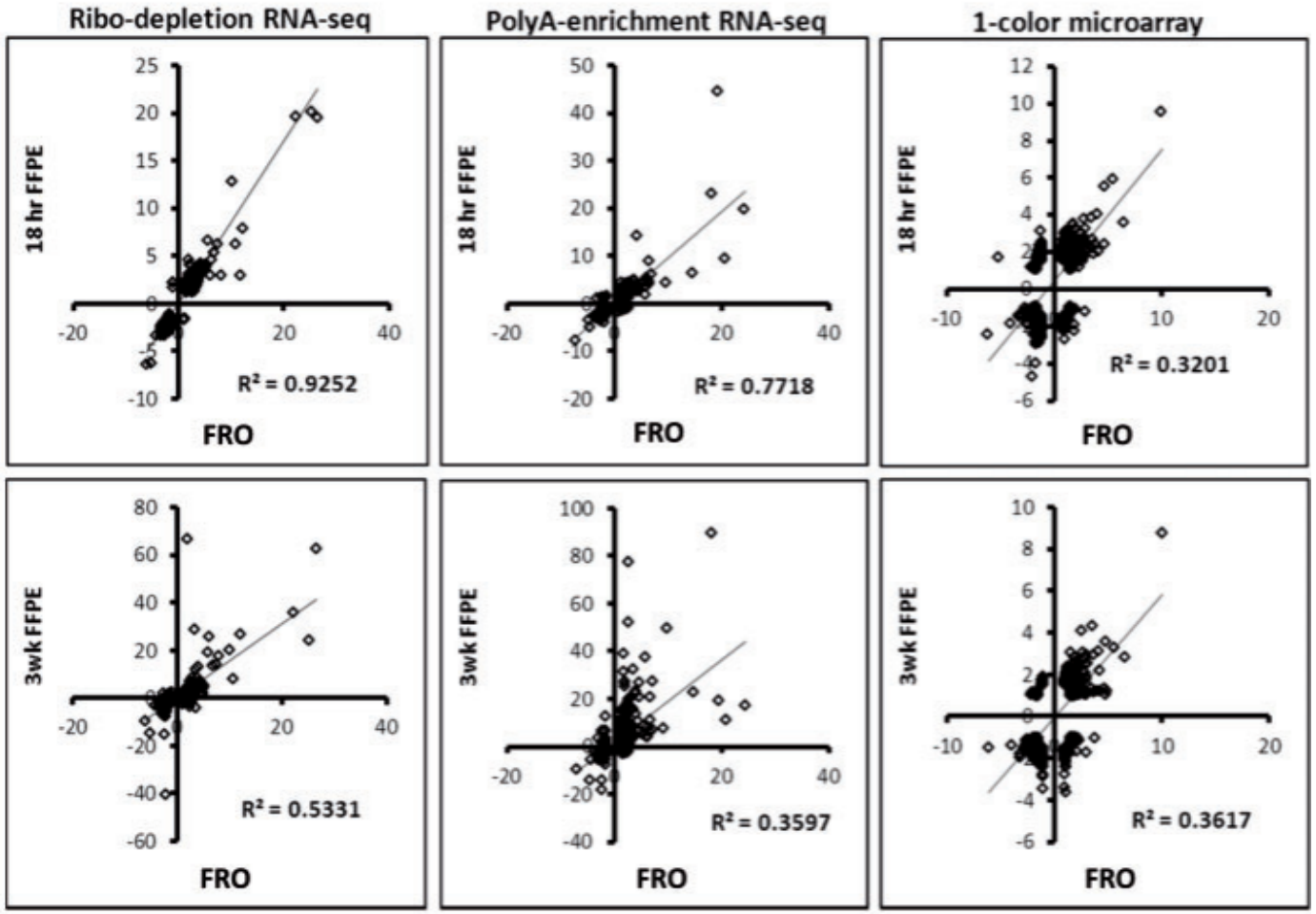
Mining the Archives: A Cross-Platform Analysis of Gene Expression Profiles in Archival Formalin-Fixed Paraffin-Embedded Tissues

A. Francina Webster*,†, Paul Zumbo‡, Jennifer Fostel§, Jorge Gandara‡, Susan D. Hester¶, Leslie Recio||, Andrew Williams*, Charles E. Wood¶, Carole L. Yauk*,1,2, and Christopher E. Mason‡,|||,||||,1,2

Paired liver

- Fresh frozen
- 18 h. FFPE
- 3 wk. FFPE

Time in formalin reduces gene counts



OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 148(2), 2015, 460–472

doi: 10.1093/toxsci/kfv195
Advance Access Publication Date: September 10, 2015
Research Article

Mining the Archives: A Cross-Platform Analysis of Gene Expression Profiles in Archival Formalin-Fixed Paraffin-Embedded Tissues

A. Francina Webster*,†, Paul Zumbo‡, Jennifer Fostel§, Jorge Gandara‡, Susan D. Hester¶, Leslie Recio||, Andrew Williams*, Charles E. Wood¶, Carole L. Yauk*,1,2, and Christopher E. Mason‡,|||,||||,1,2

Paired liver

- Fresh frozen
- 18 h. FFPE
- 3 wk. FFPE

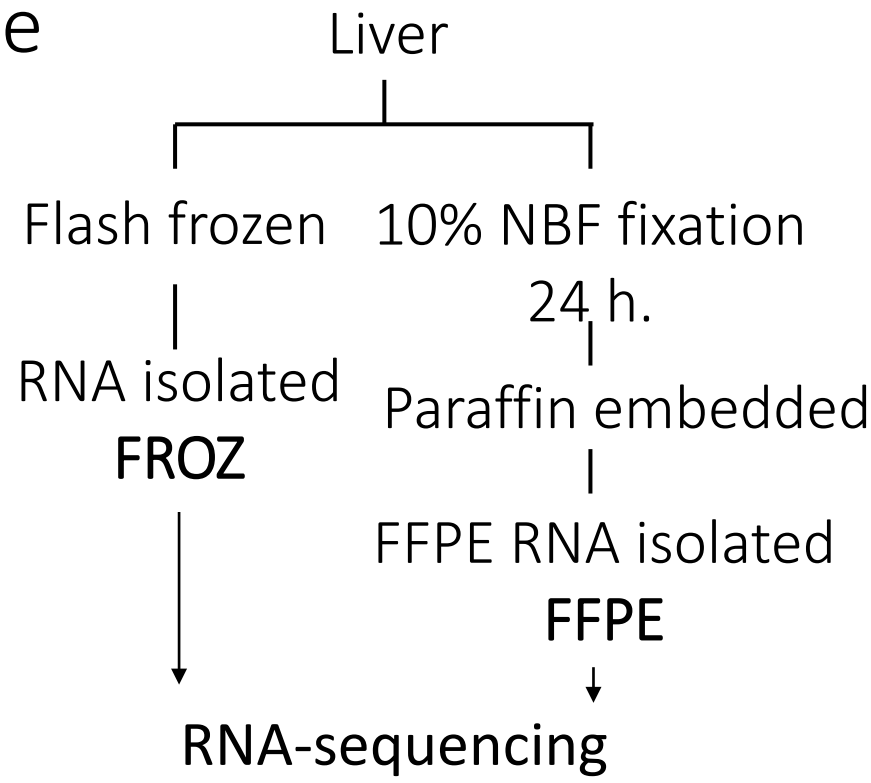
Age in paraffin block influences detection of chemical treatment induced gene response

< 2 yrs. Di-2-ethylhexyl phthalate (DEHP)

- 4 week-old male B6C3F1 mice
- Doses 0, 1500, 3000 & 6000 ppm in diet
- N=4/dose
- 7 day exposure

>21 yrs. Dichloroacetic acid (DCA)

- 4 week-old male B6C3F1 mice
- Doses 0, 1, 2 & 3.5 g/L in drinking H_2O
- N-6/dose
- 6 day exposure



Volume 154, Issue 2
December 2016

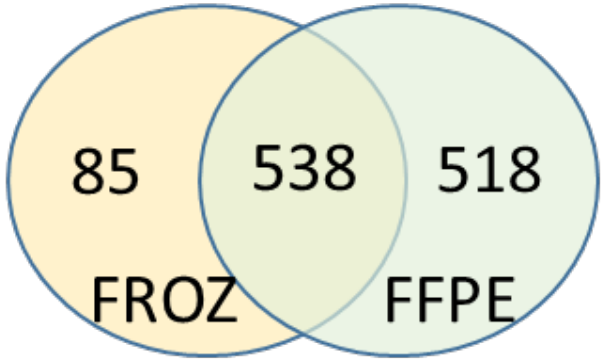
Editor's Highlight: Dose–Response Analysis of RNA-Seq Profiles in Archival Formalin-Fixed Paraffin-Embedded Samples

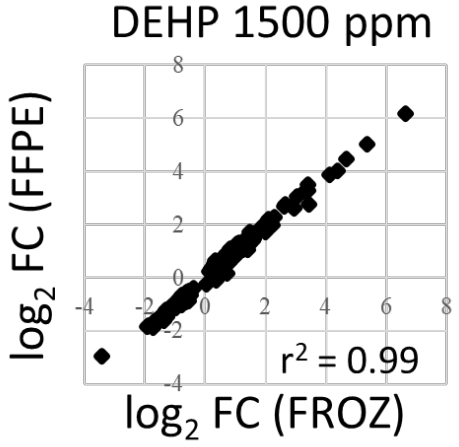
Susan D. Hester, Virunya Bhat, Brian N. Chorley, Gleta Carswell, Wendell Jones, Leah C. Wehmas, Charles E. Wood

Toxicol Sci (2016) 154 (2): 202-213.
DOI: https://doi.org/10.1093/toxsci/kfw161

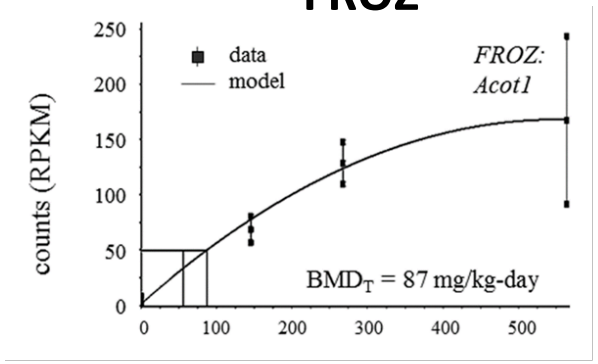
High level of concordance in DEGs across 2 yr. old FFPE samples

DEHP 1500 ppm



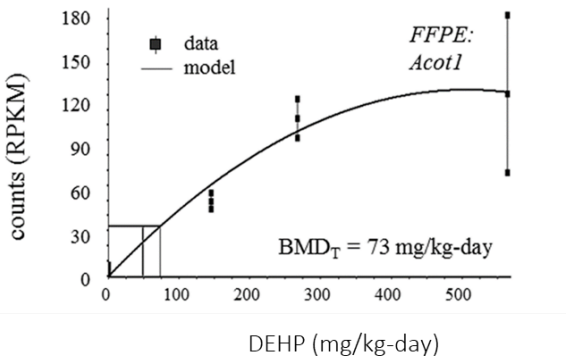


FROZ



Cyp4a10 (BMD_T=44mg/kg-day)

FFPE



Cyp4a10 (BMD_T =47 mg/kg-day)

97% reduction in gene counts across >21 yr. FFPE samples

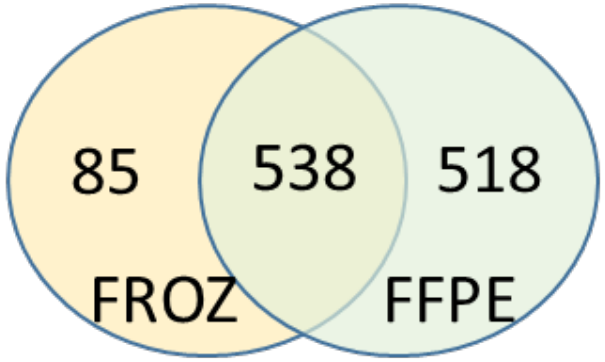
DCA (all doses)

FROZ

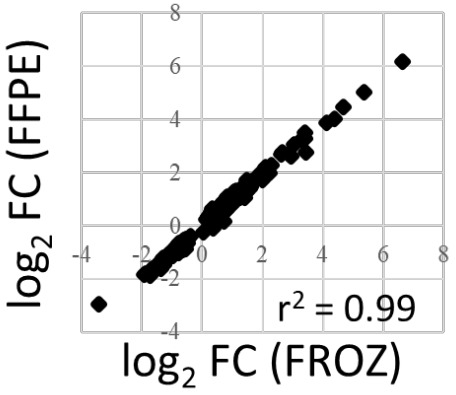
FFPE

High level of concordance in DEGs across 2 yr. old FFPE samples

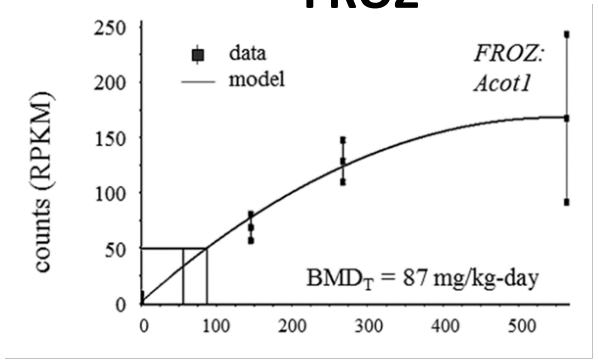
DEHP 1500 ppm



DEHP 1500 ppm

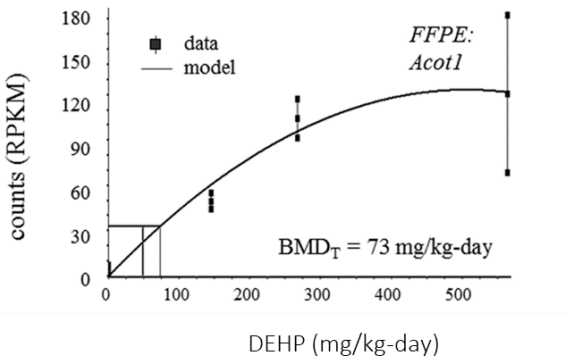


FROZ



Cyp4a10 (BMD_T=44mg/kg-day)

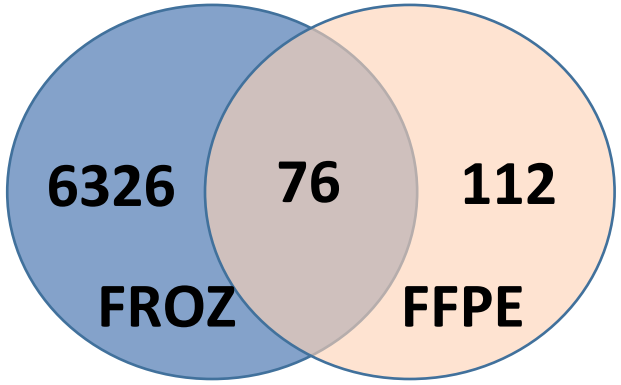
FFPE



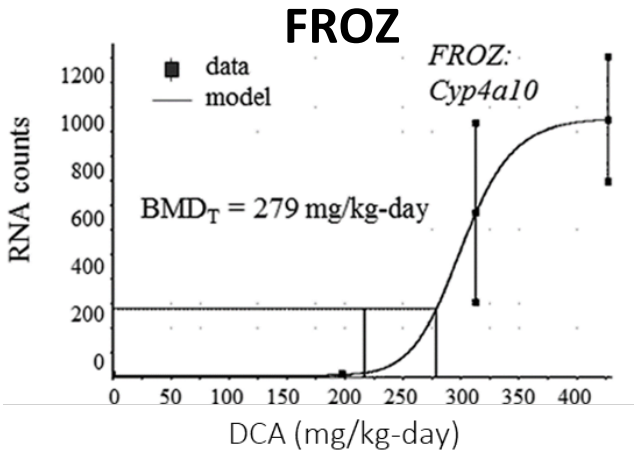
Cyp4a10 (BMD_T =47 mg/kg-day)

97% reduction in gene counts across >21 yr. FFPE samples

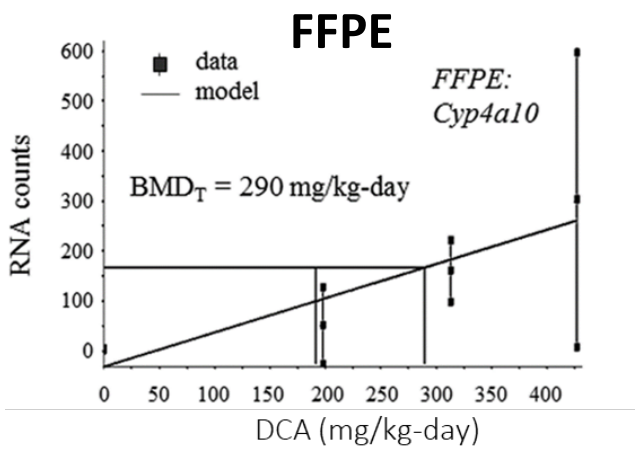
DCA (all doses)



FROZ



FFPE



Major findings

Apply new technologies

- Total RNA-seq outperforms microarray
- Ribodepletion improves gene detection

Characterized major factors impacting FFPE quality

- Time in formalin
- Age in block

OXFORD SOT Society of Toxicology
www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 148(2), 2015, 460–472
doi: 10.1093/toxsci/kfv195
Advance Access Publication Date: September 10, 2015
Research Article

Mining the Archives: A Cross-Platform Analysis of Gene Expression Profiles in Archival Formalin-Fixed Paraffin-Embedded Tissues

A. Francina Webster*,†, Paul Zumbo‡, Jennifer Fostel§, Jorge Gandara‡, Susan D. Hester¶, Leslie Recio||, Andrew Williams*, Charles E. Wood¶, Carole L. Yauk*,1,2, and Christopher E. Mason‡,|||,||||,1,2

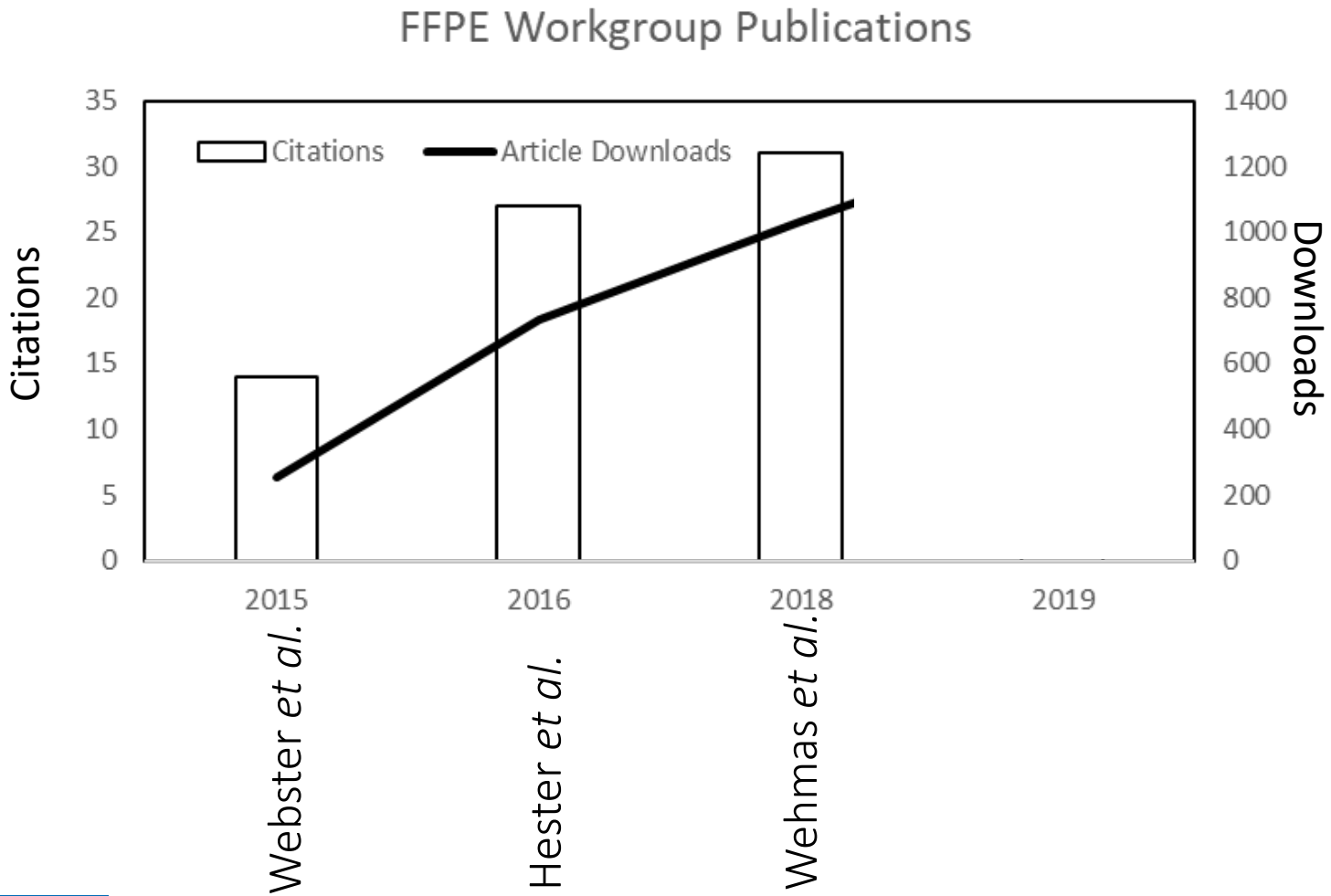
Volume 154, Issue 2
December 2016

Editor's Highlight: Dose–Response Analysis of RNA-Seq Profiles in Archival Formalin-Fixed Paraffin-Embedded Samples

Susan D. Hester, Virunya Bhat, Brian N. Chorley, Gleta Carswell, Wendell Jones, Leah C. Wehmas, Charles E. Wood

Toxicol Sci (2016) 154 (2): 202-213.
DOI: https://doi.org/10.1093/toxsci/kfw161

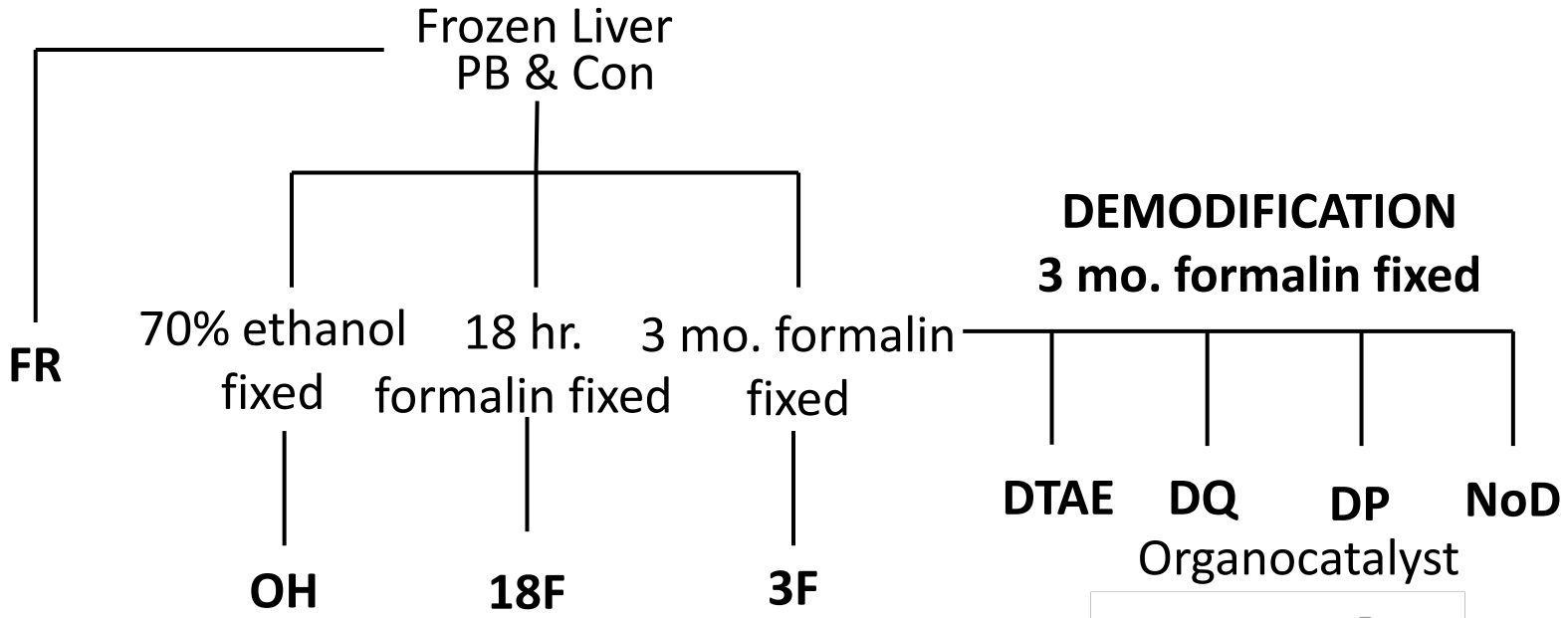
Objectives



- Apply new technologies
- Characterized major factors impacting FFPE quality
- **Identified methods to improve gene expression data**
- **Developed better metrics for quality assessment**

Improving quality of FFPE RNA

Karmakar *et al*. Nat Chem. 2015 Sep; 7(9): 752–758.

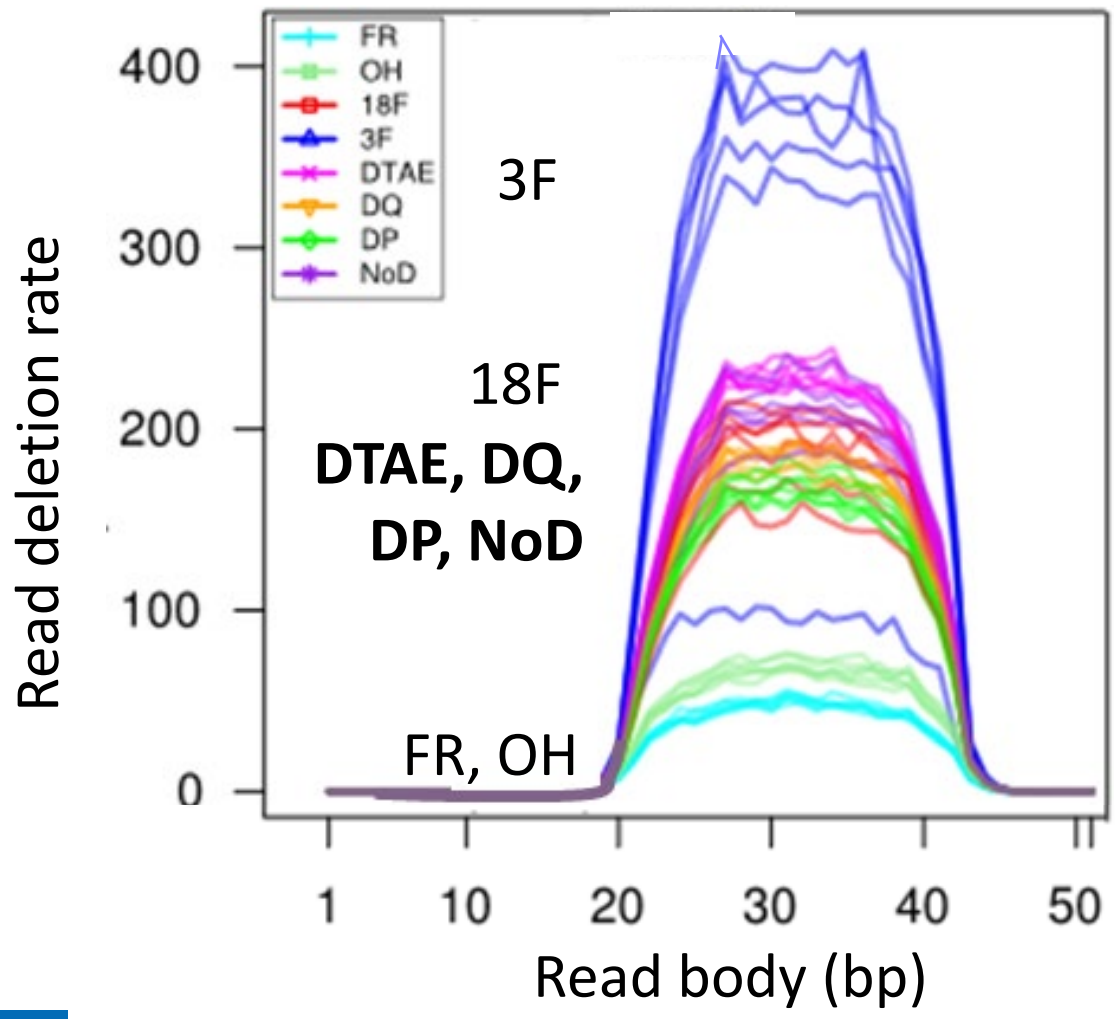


ILSI Health and Environmental Sciences Institute

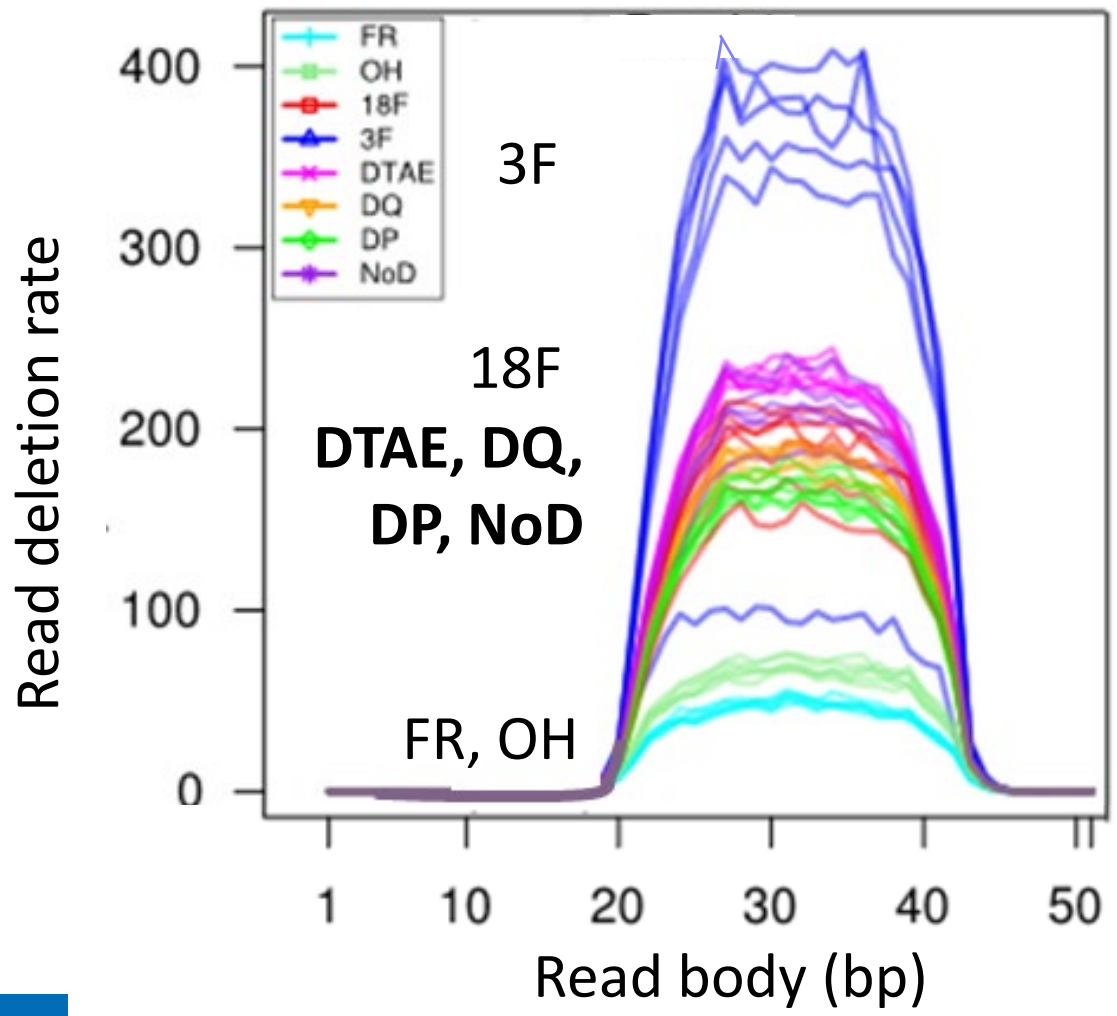
Supporting CSS AOPDD Task 1.1g

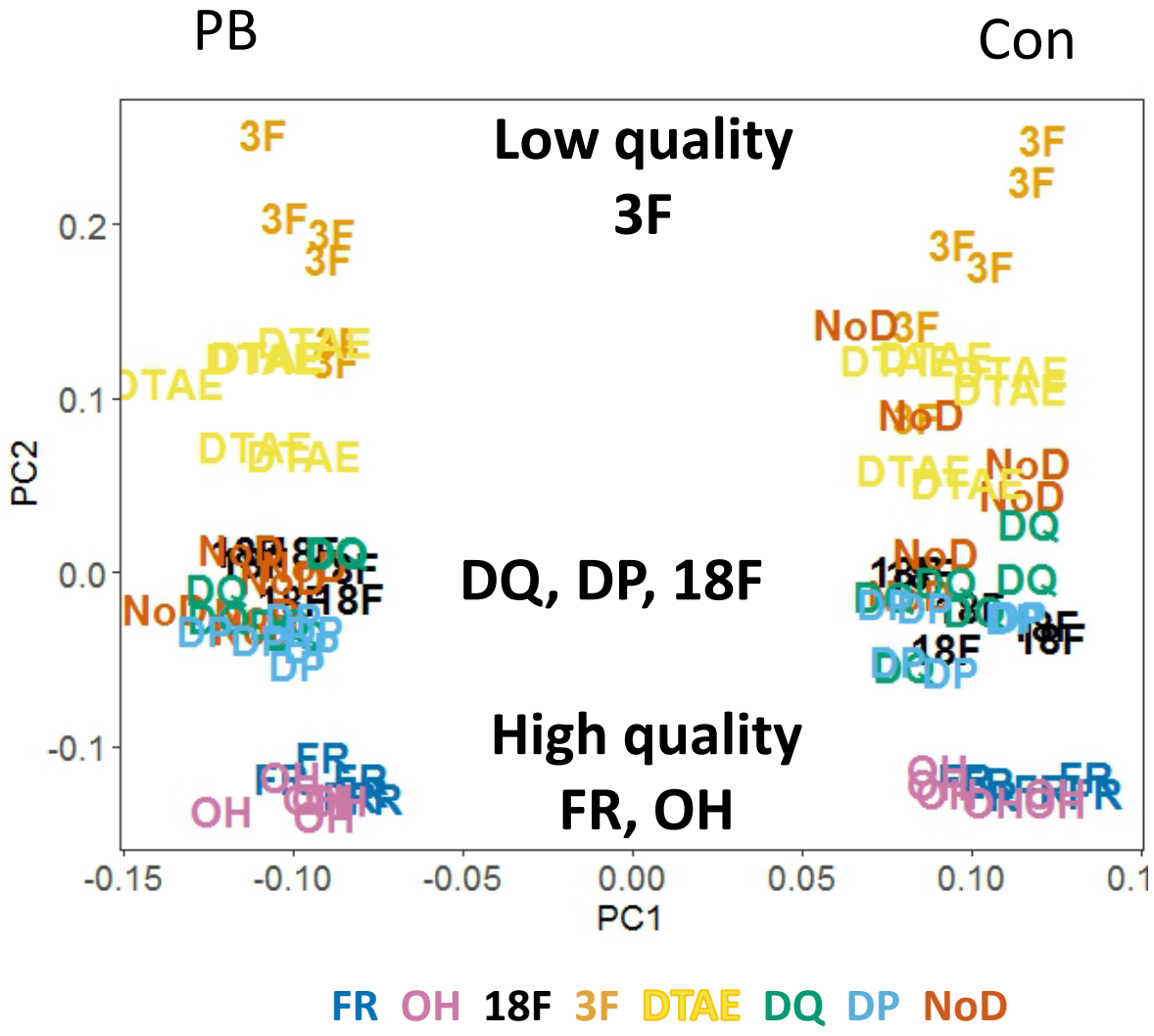
Wehmas *et al*. Toxicol Sci. 2018 Apr 1;162(2):535-547.

Demodification improves FFPE RNA



Organocatalyst improves FFPE RNA and gene detection

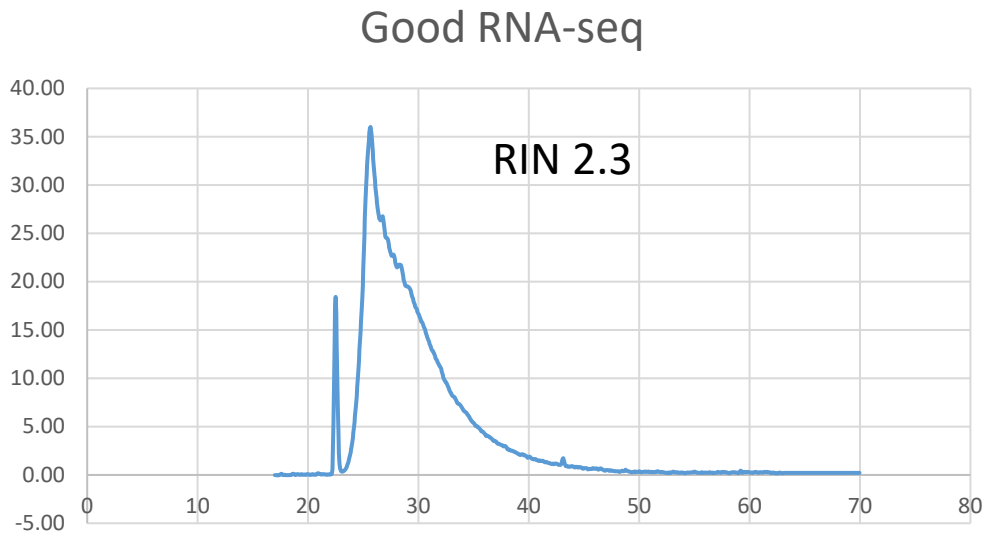


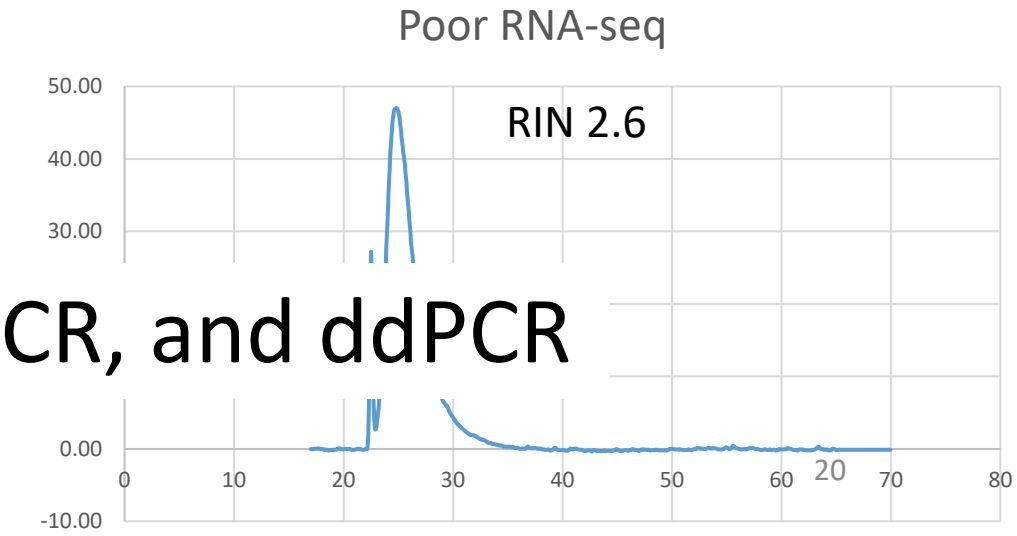


Identifying methods to better evaluate FFPE RNA quality

Before Sequencing

- Age and time greatly impact quality
- Better characterize FFPE RNA quality
- Better indicate sequencing success
- Adequately reflect experiment dependent response



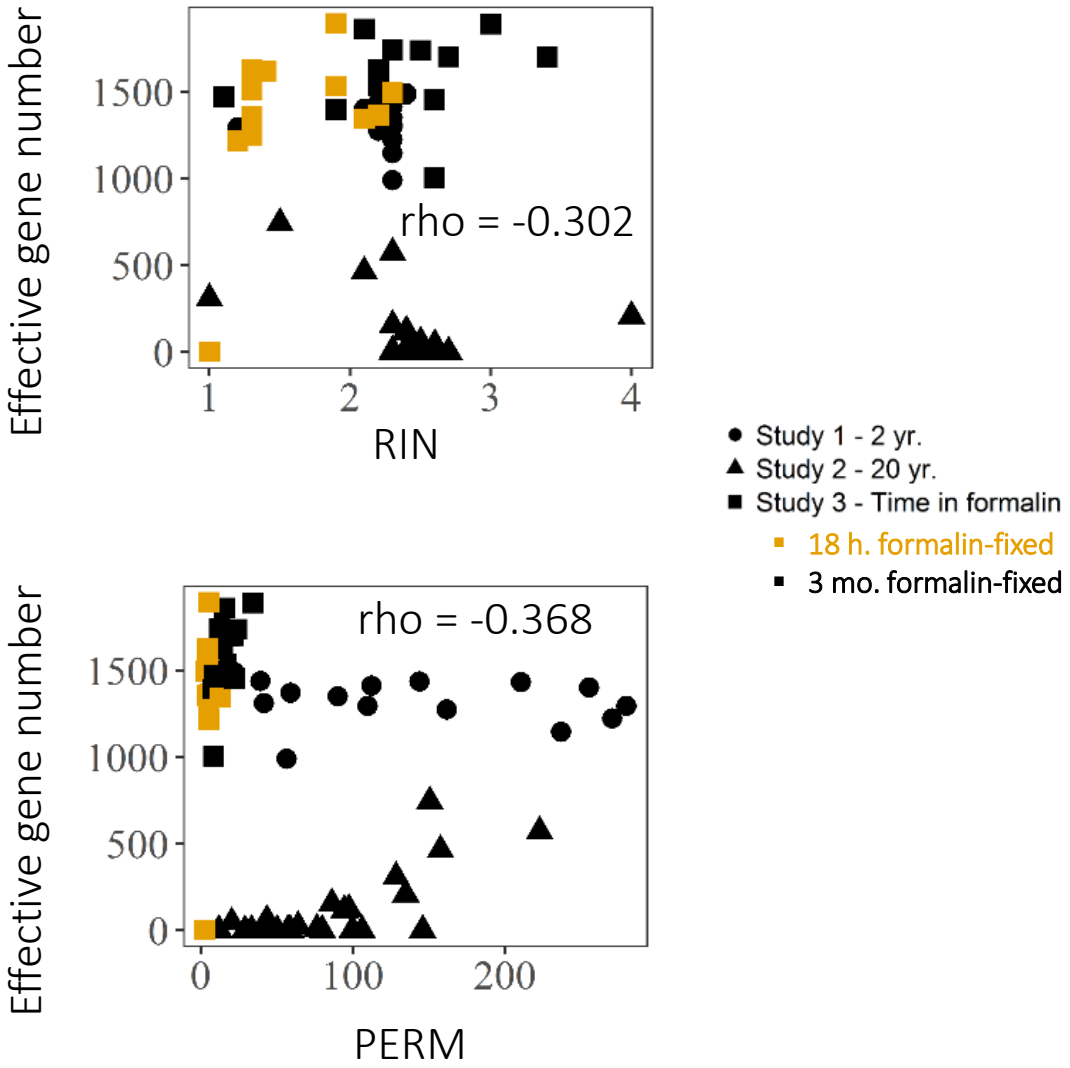


Assess RIN, DV, PERM, Real time qPCR, and ddPCR

RIN and PERM perform poorly with FFPE

Age in block and time in formalin

- RIN performs poorly
- PERM performs poorly
- DV200 shows improvement
- DV100 and ddPCR perform best

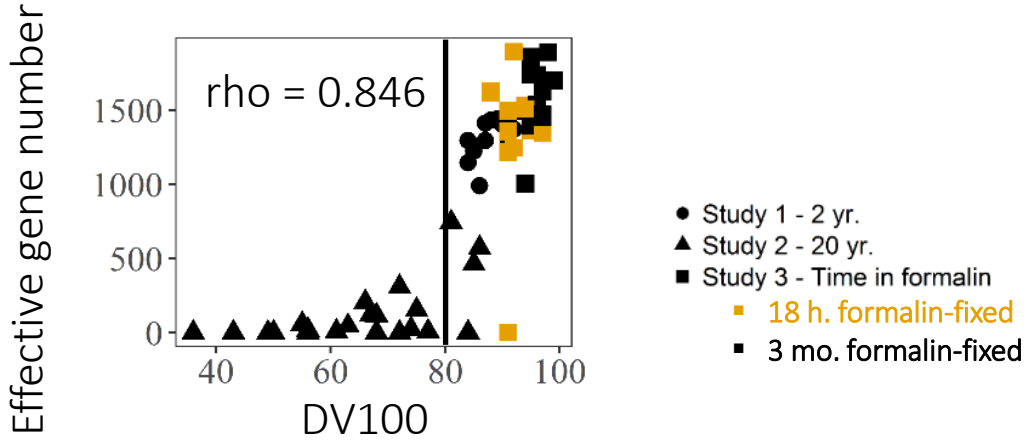


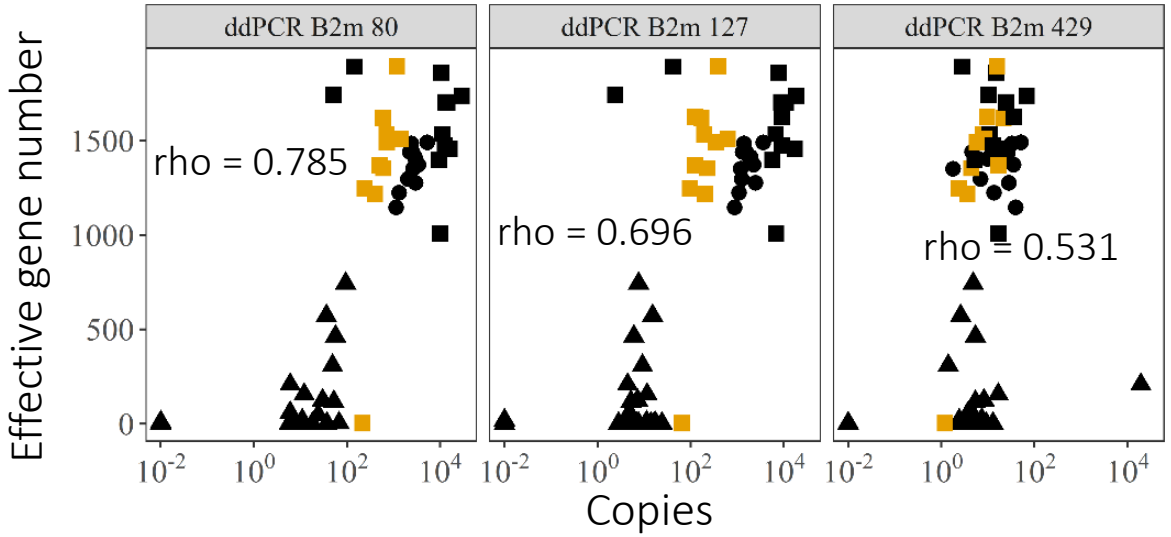
Wehmas *et al.* 2019. Toxicol Sci. 2019 Aug 1;170(2):357-373. doi: 10.1093/toxsci/kfz113.

DV100 and ddPCR best distinguishes FFPE RNA quality

Age in block and time in formalin

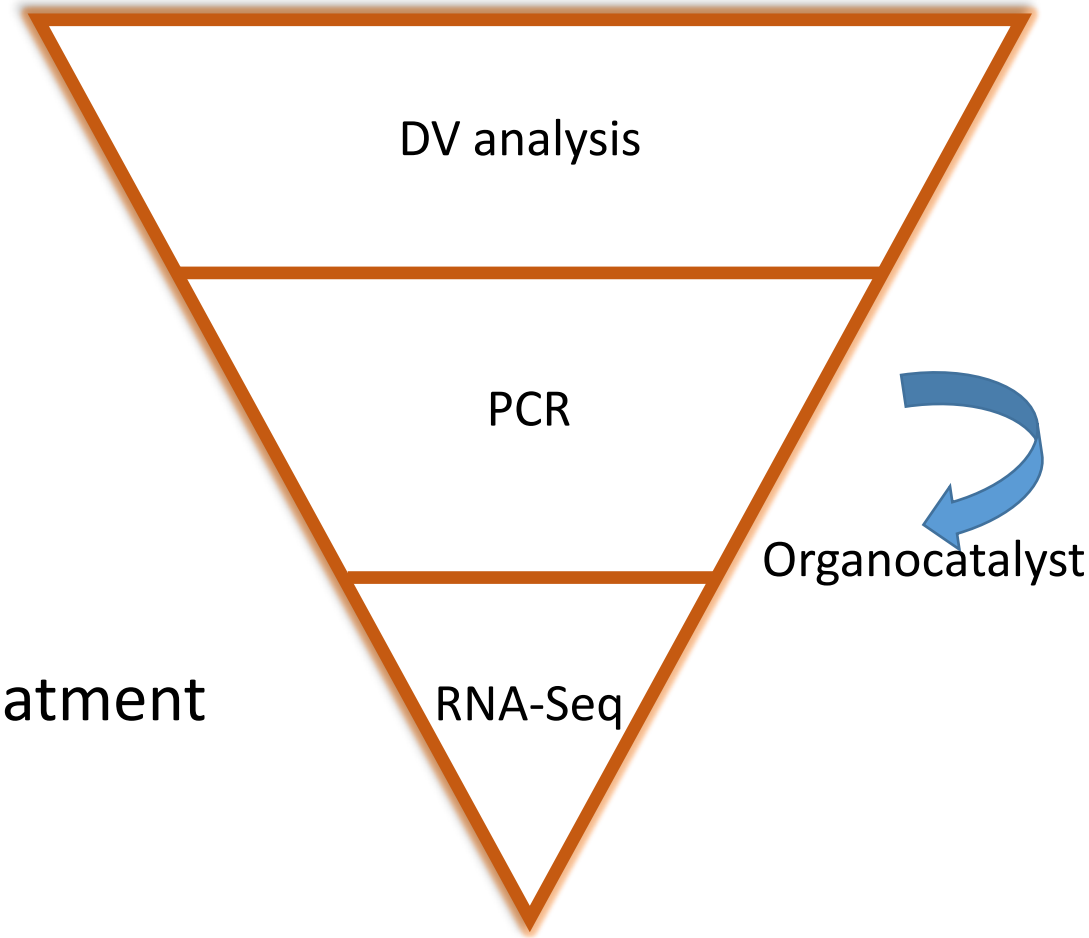
- RIN performs poorly
- PERM performs poorly
- DV200 shows improvement
- DV100 and ddPCR perform best
- DV100>80 distinguishes high and low quality FFPE



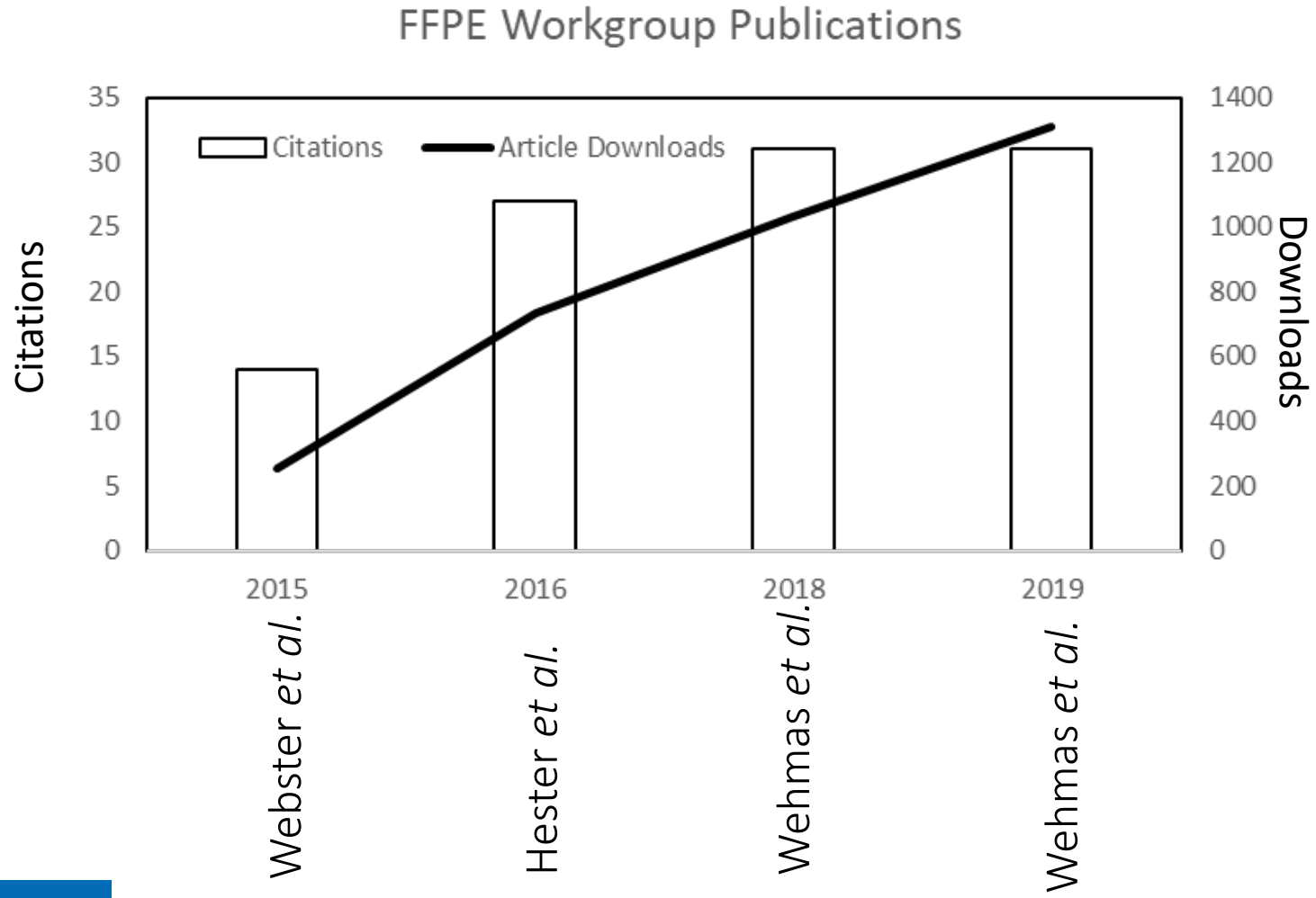


Major findings: Strategy for sequencing FFPE samples

1. Evaluate DV100
2. If DV100 > 80%, sequence
3. If DV100 is near 80%, run qRT-PCR
4. Use 80-100 nt amplicon size
5. If >500 copies of housekeeping gene detected, sequence
6. If <500 copies, try organocatalyst treatment

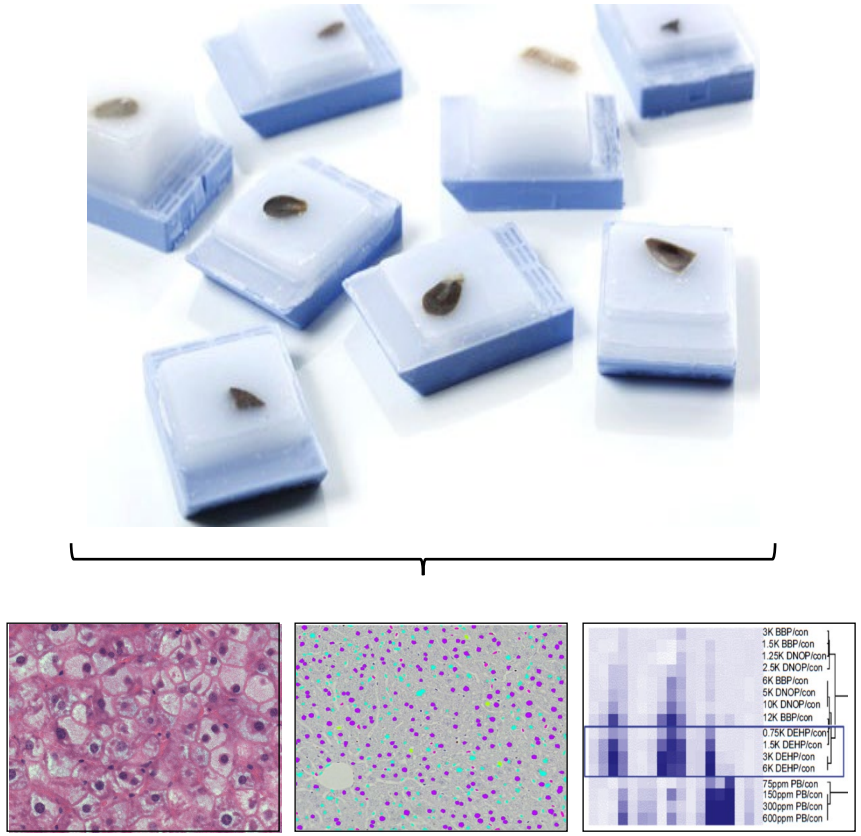


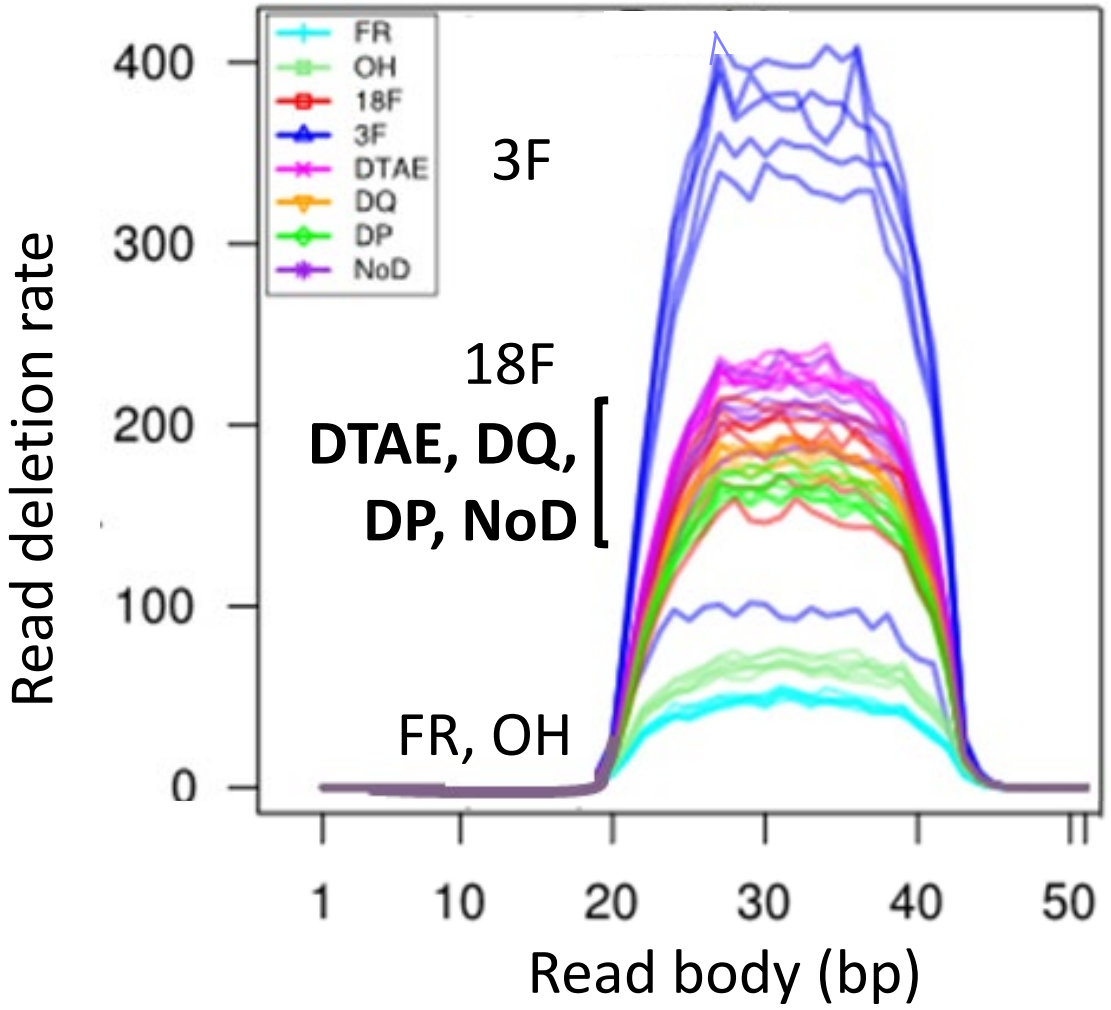
Objectives



- Applied new technologies
- Characterized major factors impacting FFPE quality
- **Identified methods to improve gene expression data**
- Developed better metrics for quality assessment;
- **Translated results to clinical FFPE and improved SNP detection**

Organocatalyst reduces RNA deletion rates, what about DNA?





Organocatalyst increases confidence in variant calls

Human tumor resections

Frozen Tumor samples

FFPE Tumor samples

Tumor FR

RNA-seq + DNA-seq

(standard protocol)

ILSI Health and Environmental Sciences Institute

Tumor FFPE

DNA-seq

Organocatalyst Tumor FFPE

RNA-seq + DNA-seq

NIH NATIONAL CANCER INSTITUTE

Wehmas *et al*. 2020 in preparation

Organocatalyst increases confidence in variant calls

Human tumor resections

Frozen Tumor samples

FFPE Tumor samples

Tumor FR

RNA-seq + DNA-seq

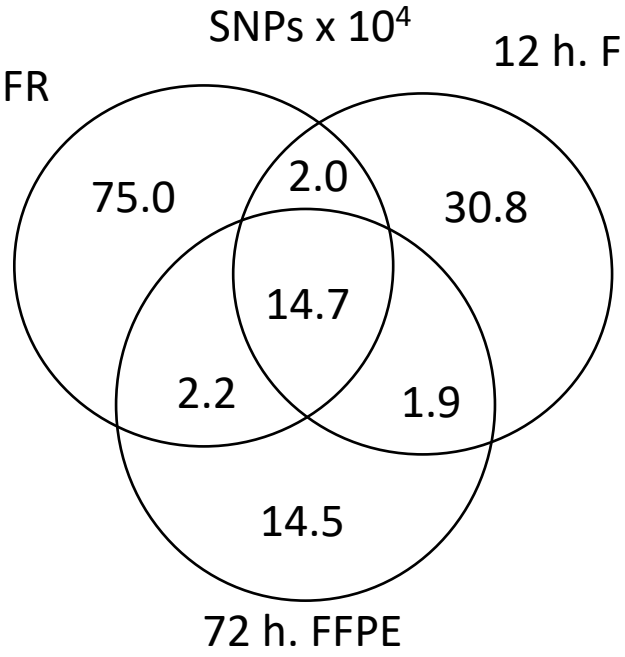
(standard protocol)

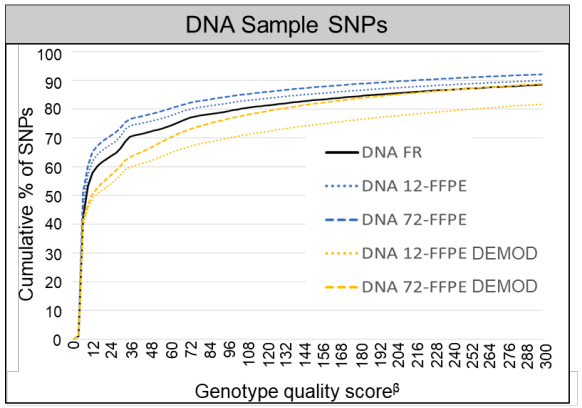
Tumor FFPE

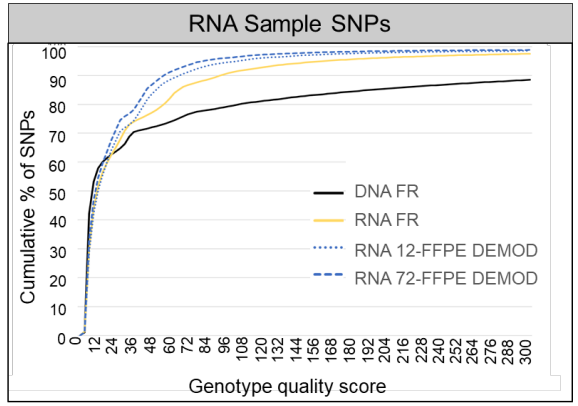
DNA-seq

Organocatalyst Tumor FFPE

RNA-seq + DNA-seq

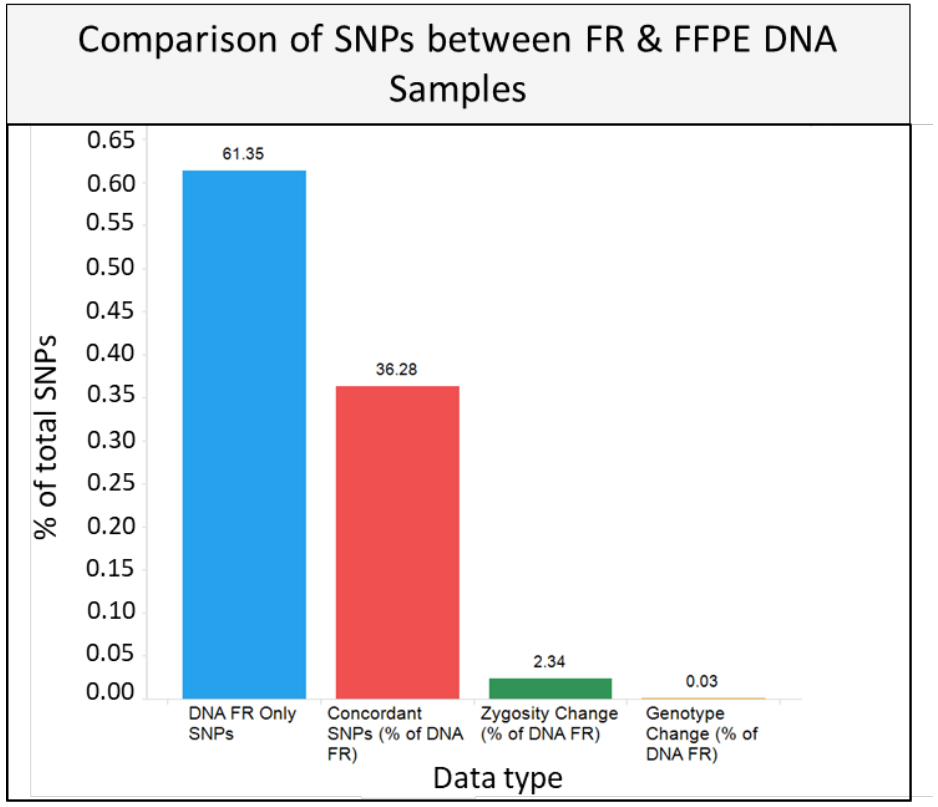


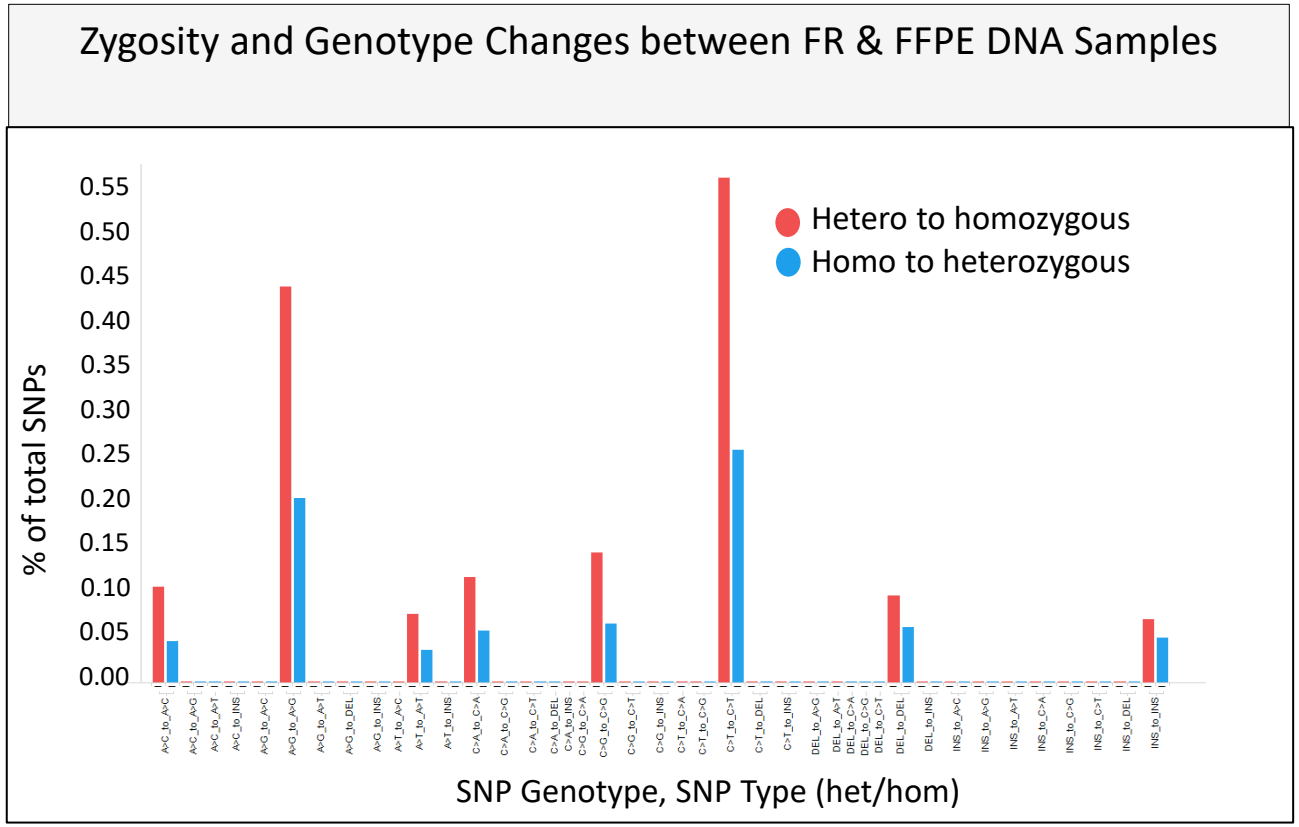




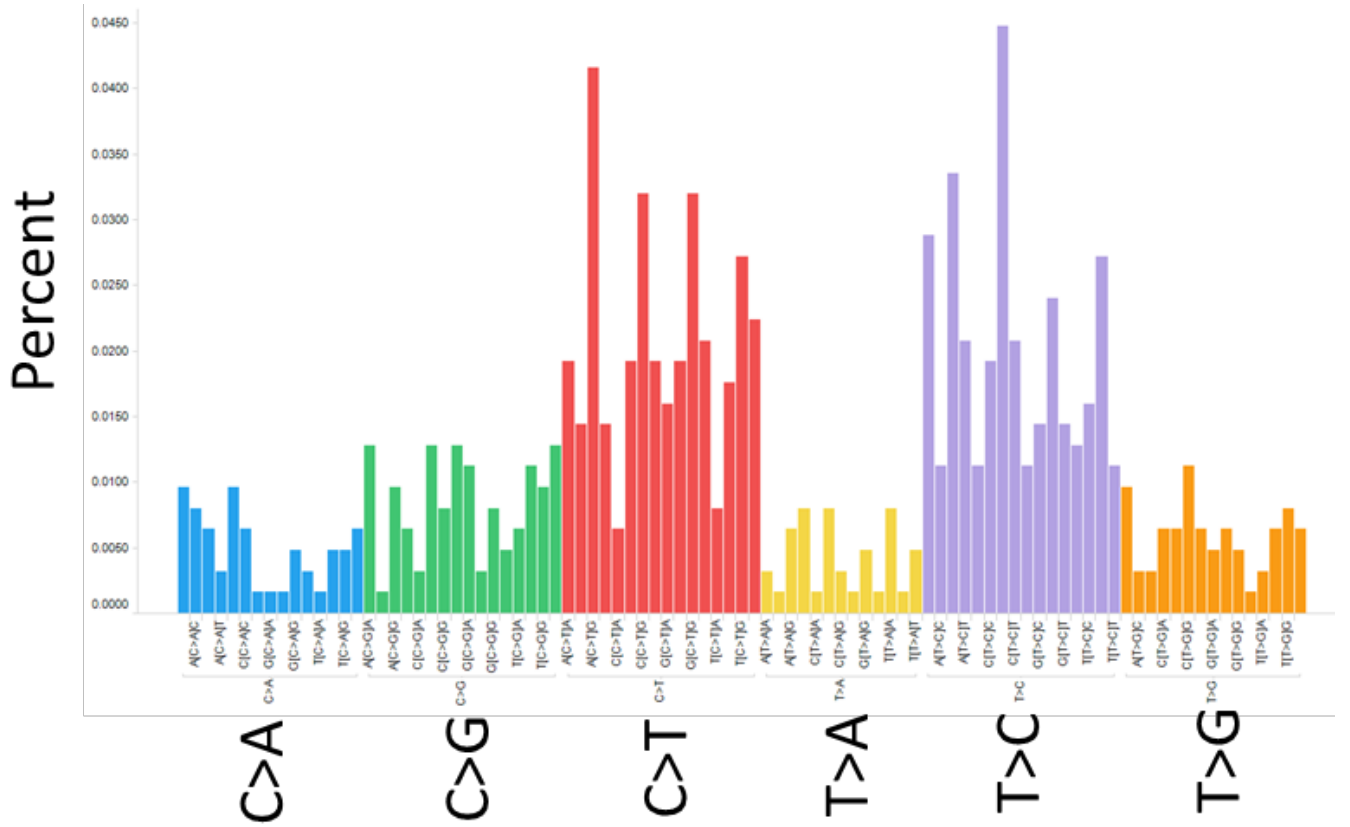
β The Genotype quality score represents the Phred-scaled confidence that the genotype assignment is correct

Fixation results mainly in zygosity changes for variant calls

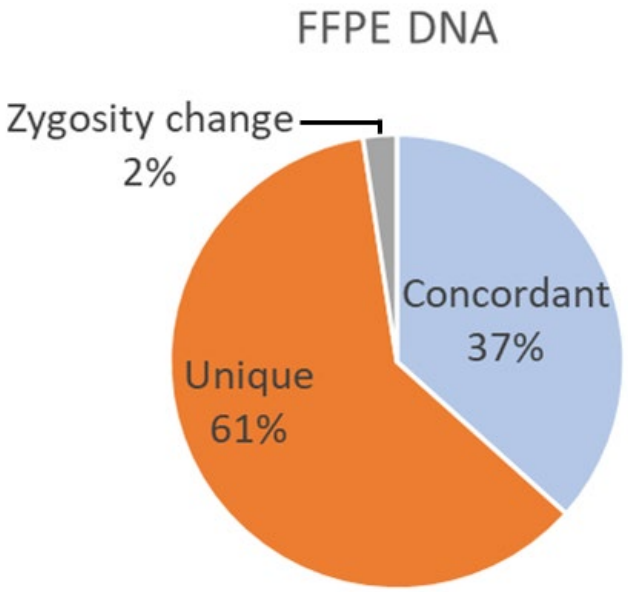


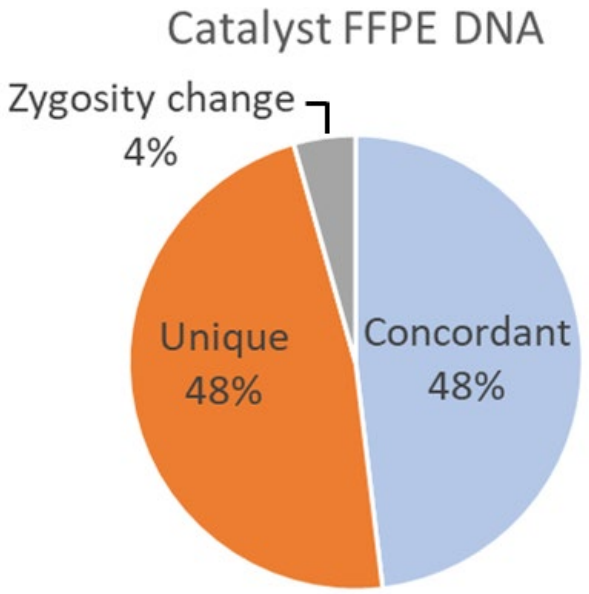


FFPE damage more likely to cause changes of C>T and T>C with < 1% = “Genotype Changes”

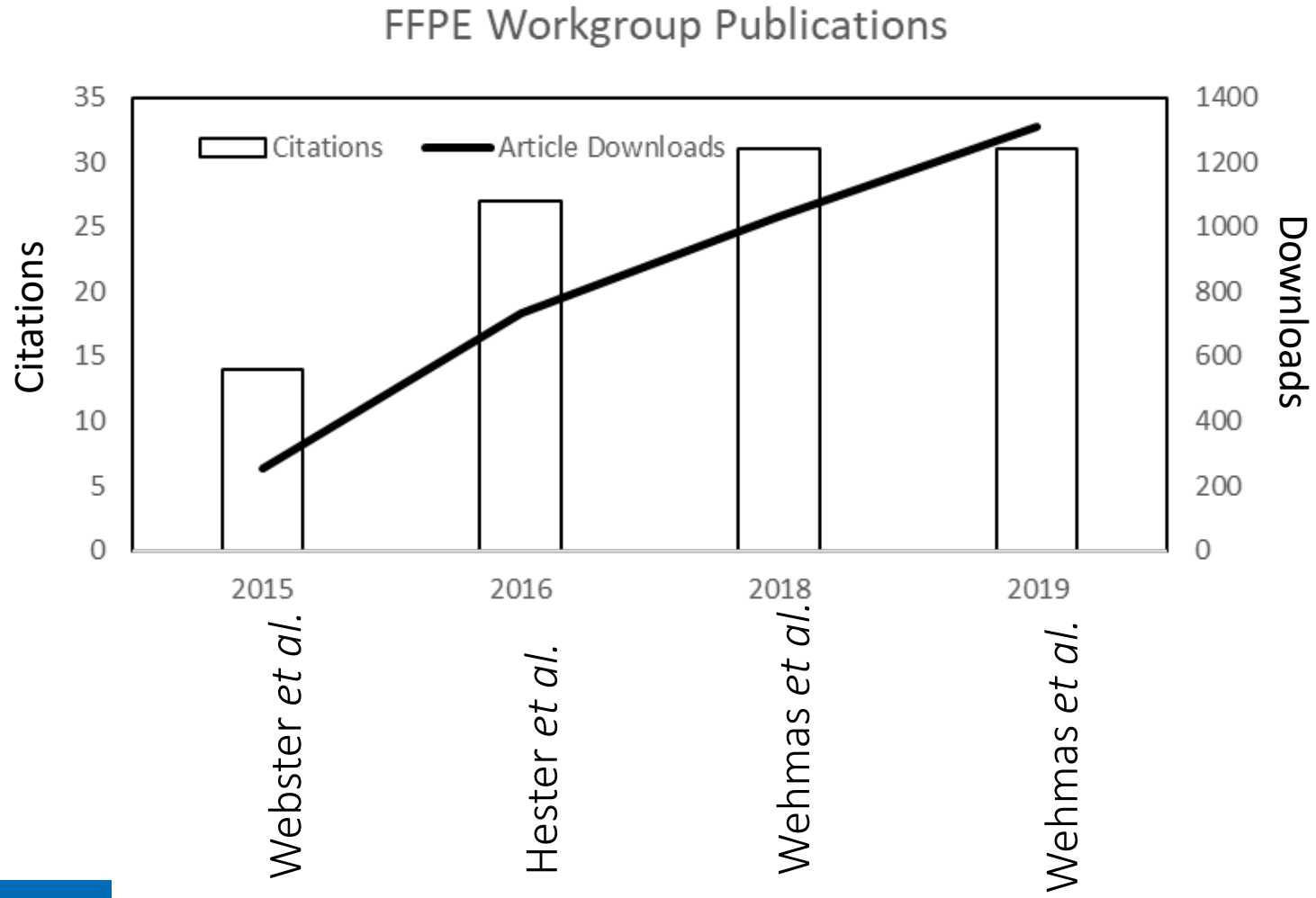


Organocatalyst treatment improves concordance in mutation calls by 11.5%





Major findings and unanswered questions

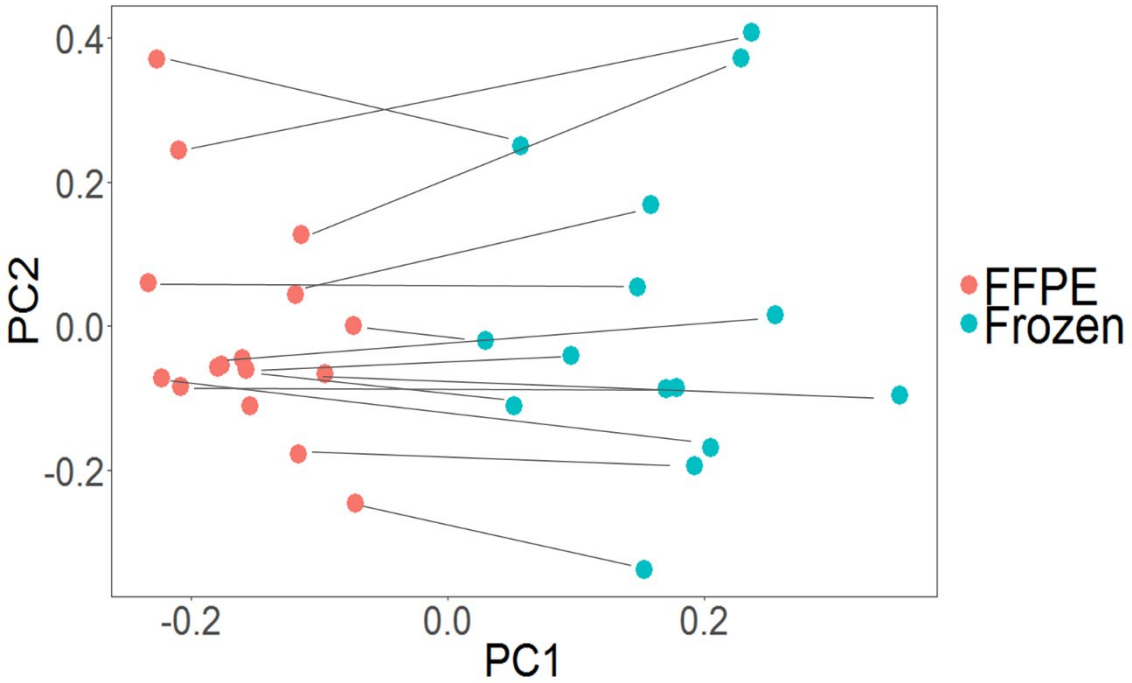


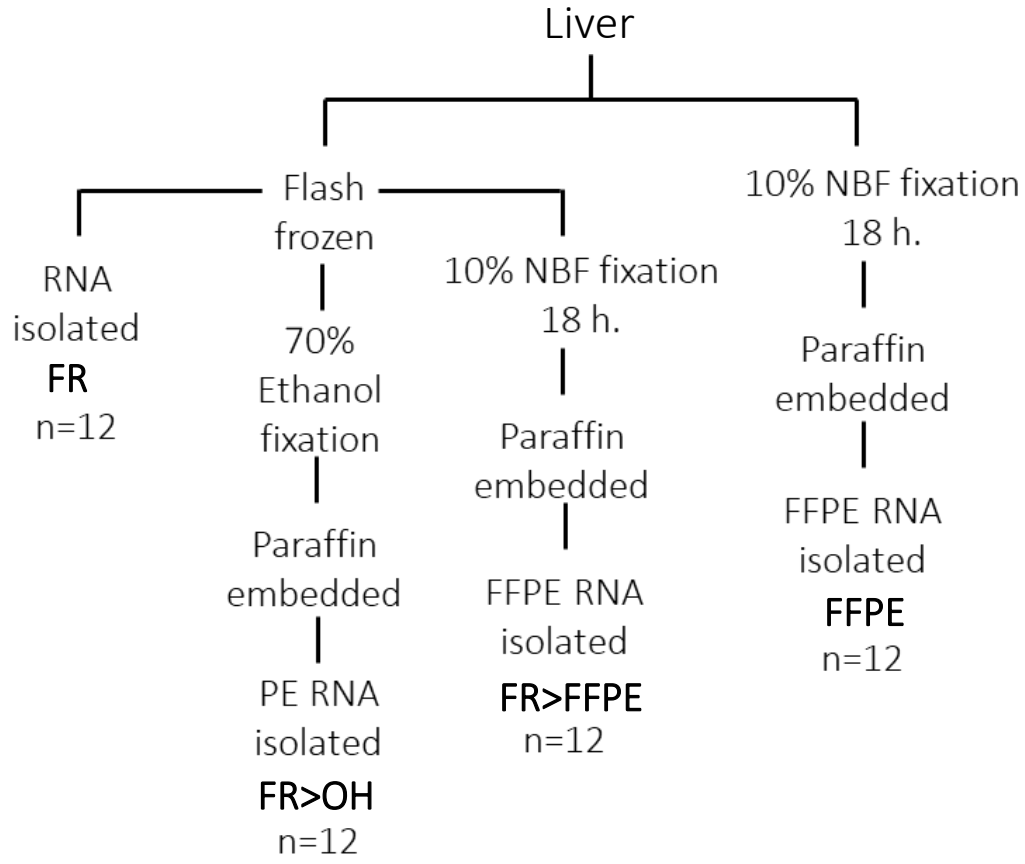
- Applied new technologies
- Characterized major factors impacting FFPE quality
- Identified methods to improve gene expression data
- Developed better metrics for quality assessment;
- Translated results to clinical FFPE and improved SNP detection
- **FFPE artifacts?**
- **Age limitations?**

Formalin fixation causes shift in gene response

- Male B6C3F1 mice
- PB exposed 0 or 600 ppm
- n =6/dose

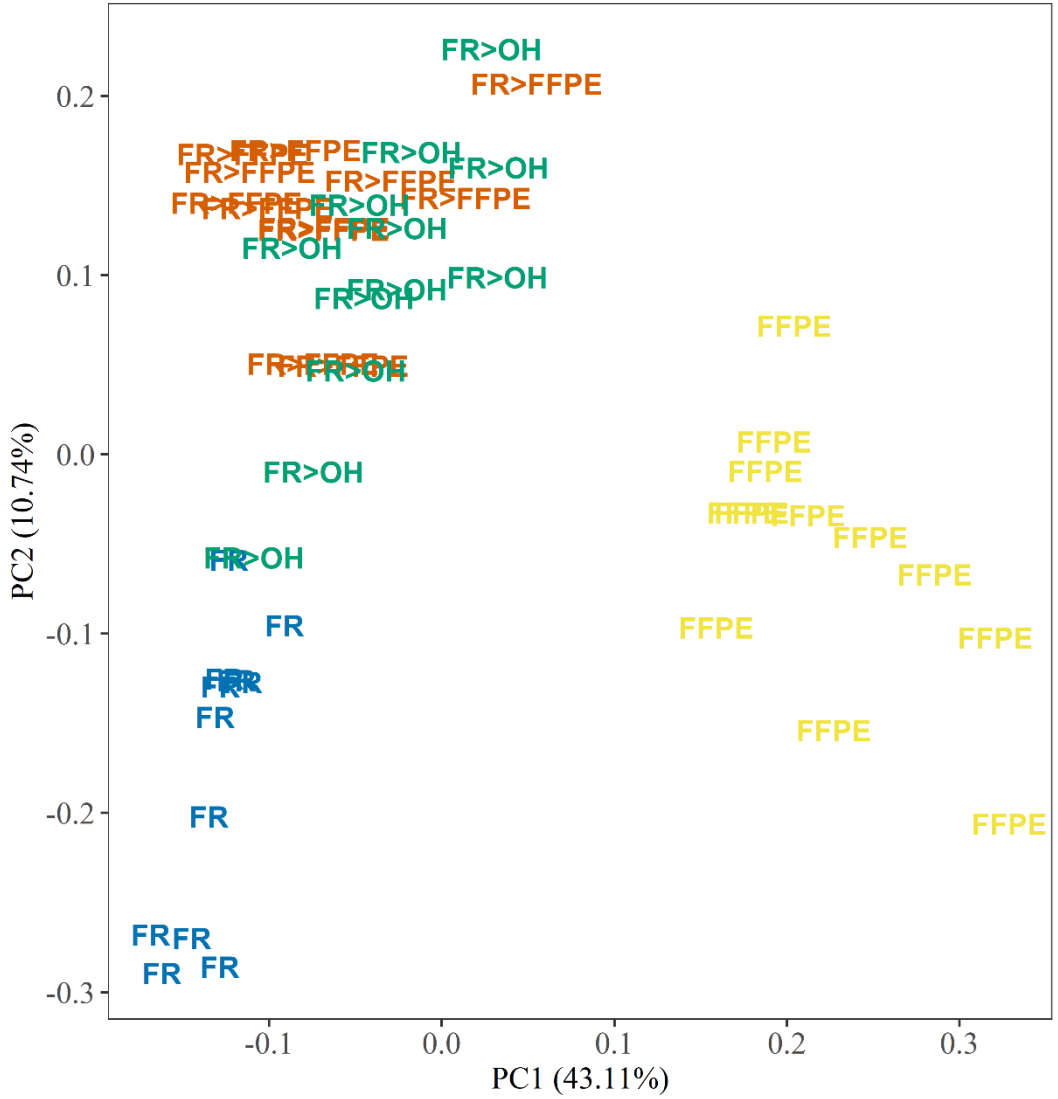
Previous observation

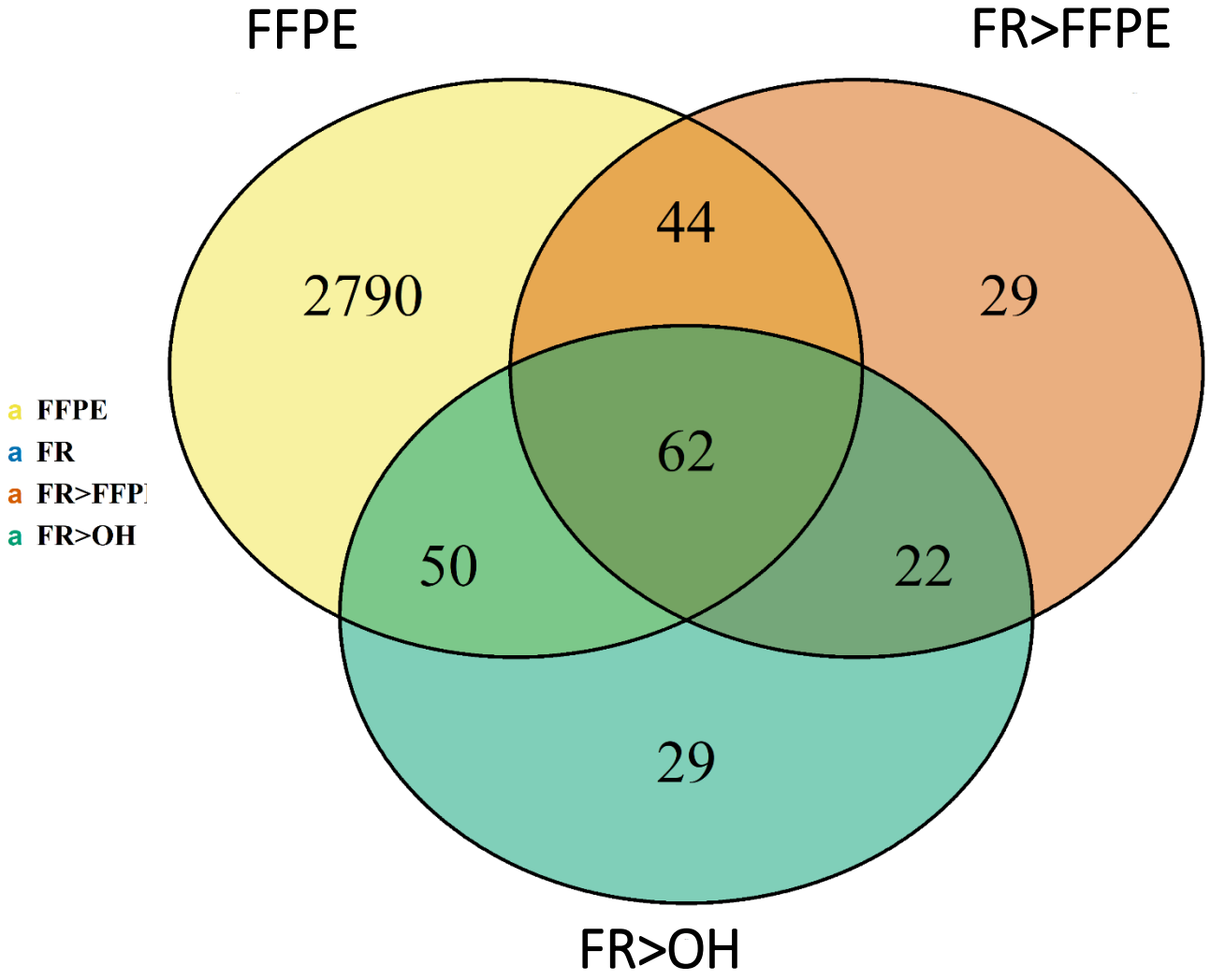




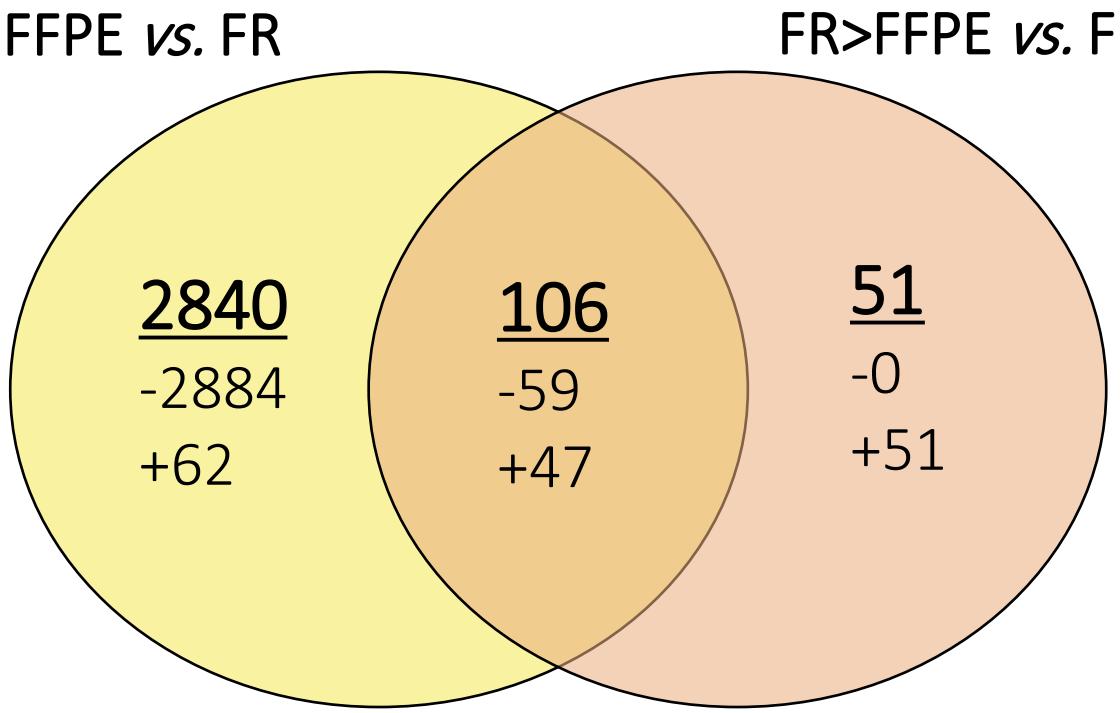
Wehmas, L. C., et al. (2020). "Formalin fixation has wide spread genomic effects in preserved tissue samples". Submitted.

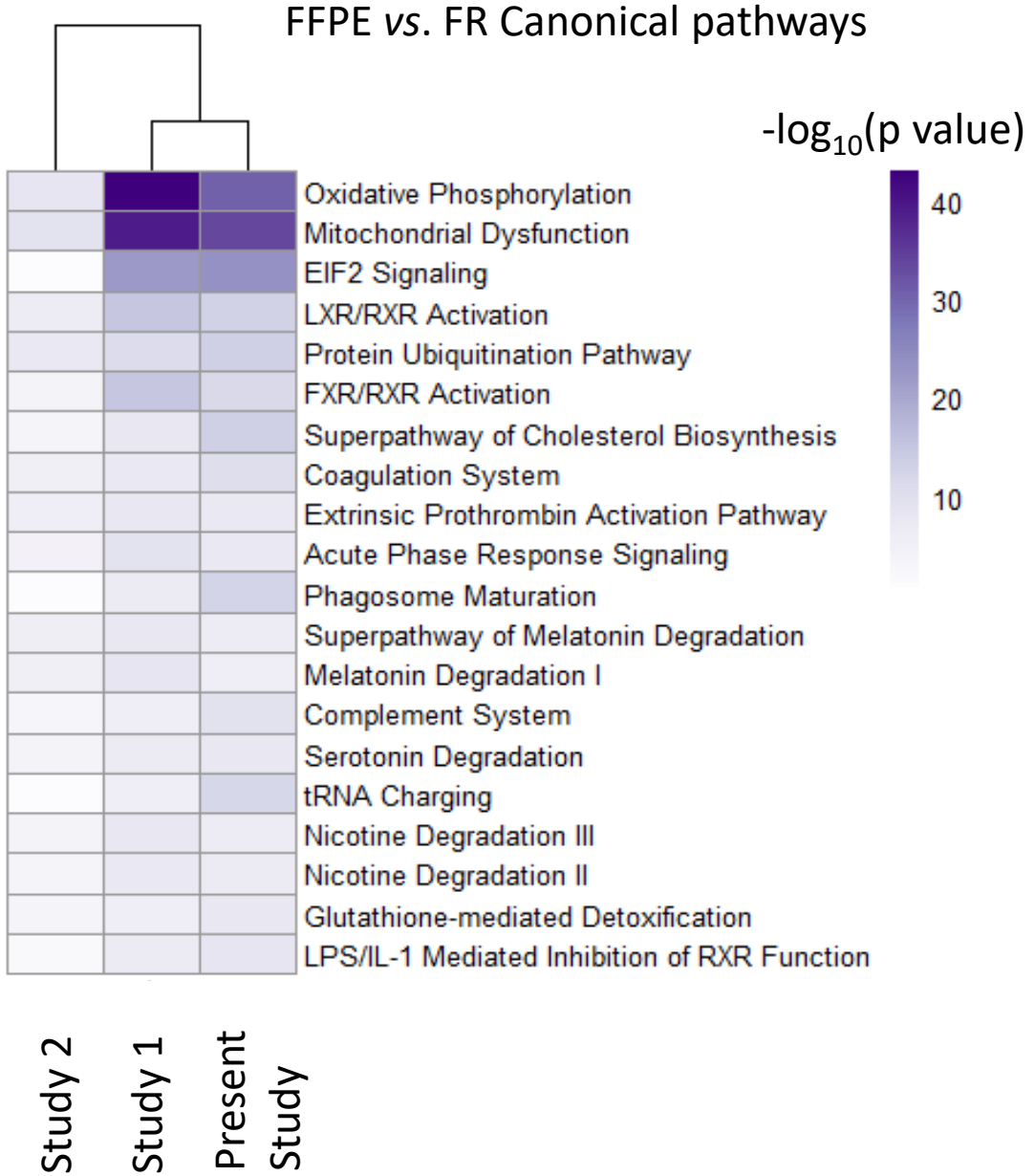
Direct formalin fixation significantly impacts gene expression



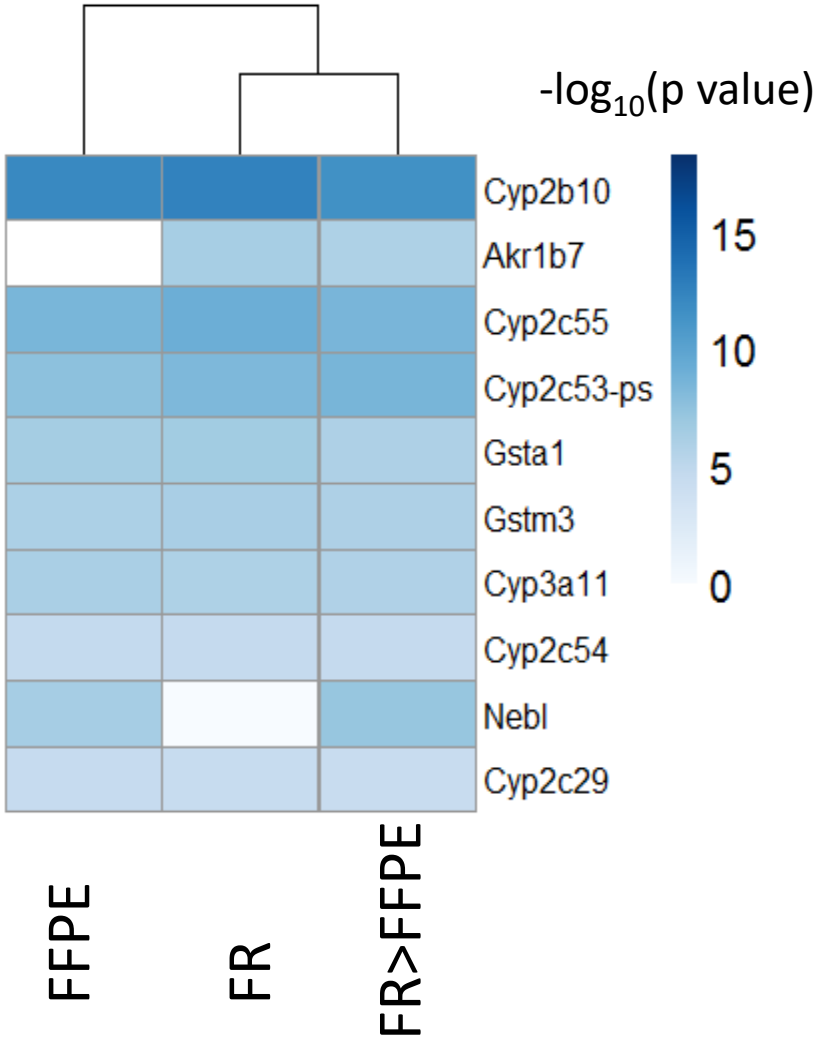


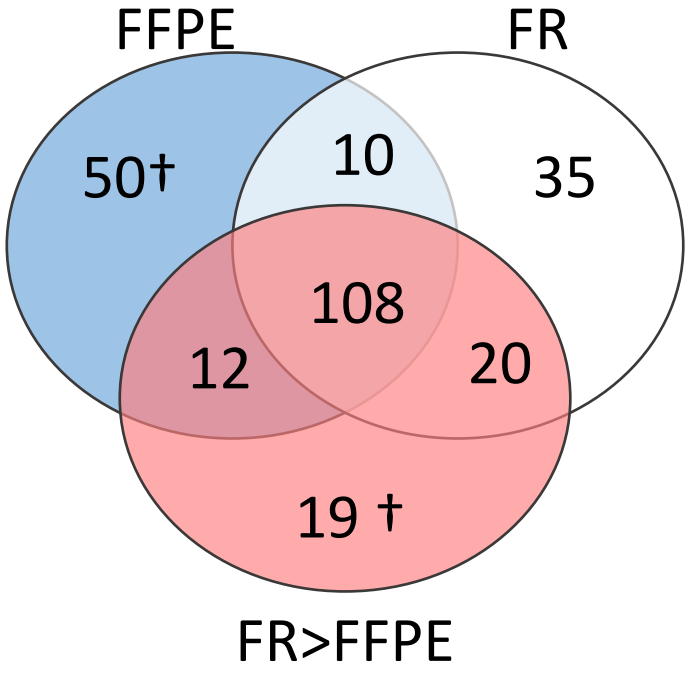
Direct formalin fixation impacts cell metabolism and transcription





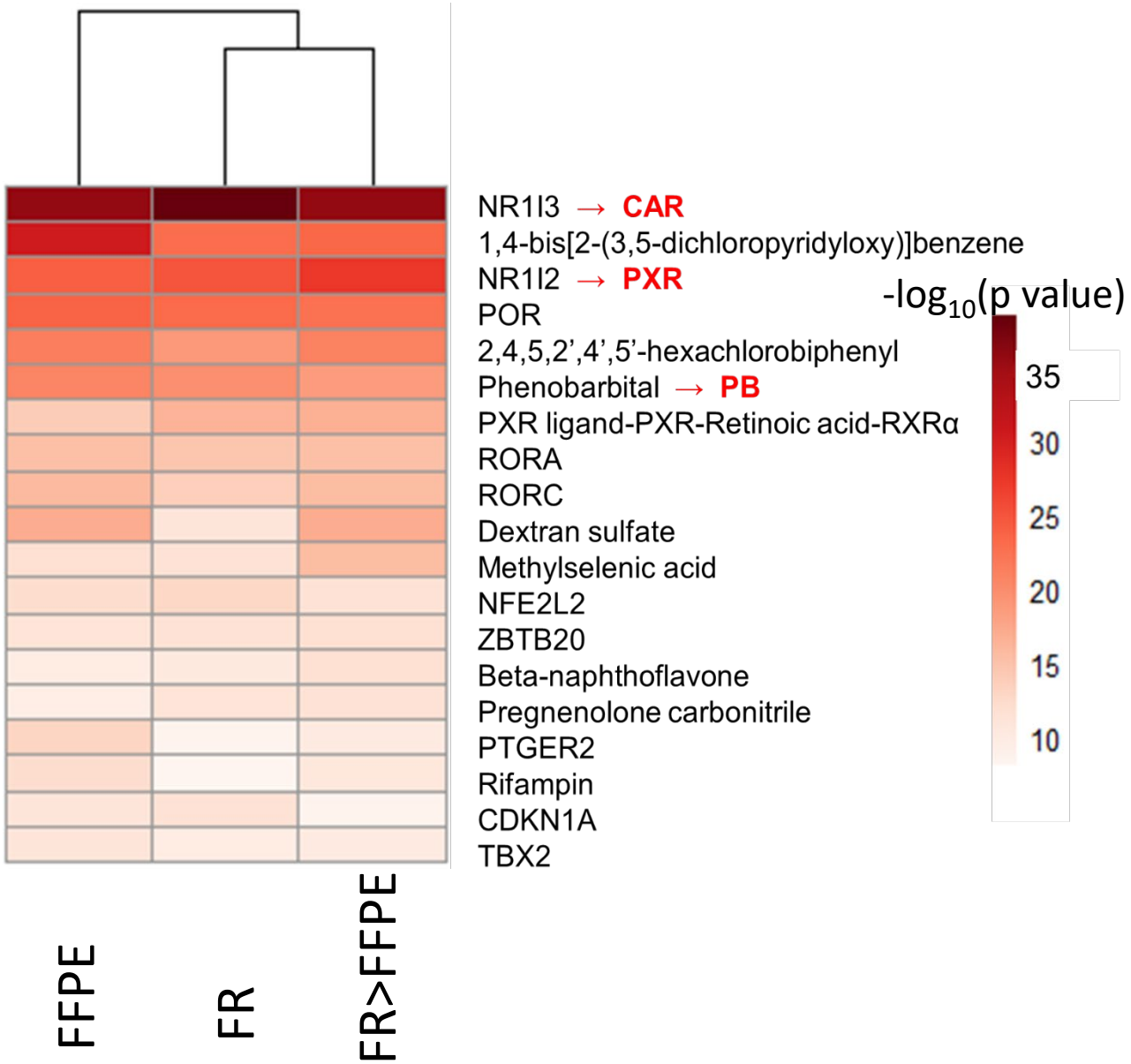
Biomarkers of PB exposure are detected across all groups

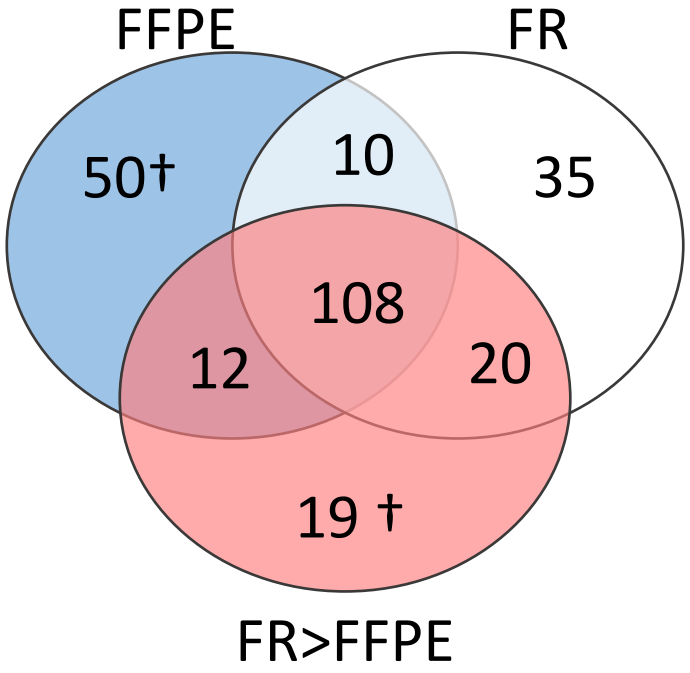




† chi squared p value < 0.005
50/180 for FFPE
19/159 for FR>FFPE

Chemical (PB) response remains detectable





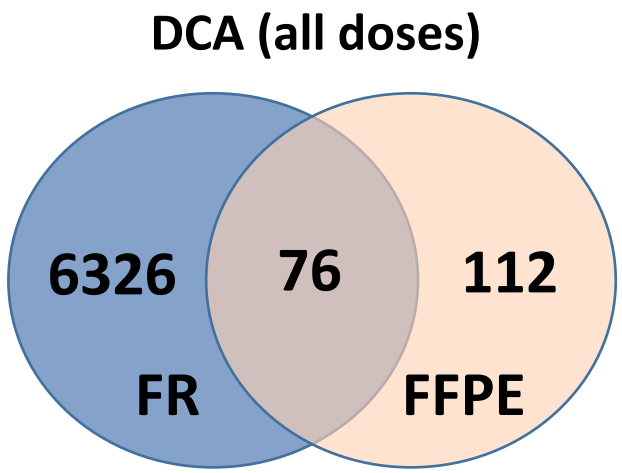
† chi squared p value < 0.005
50/180 for FFPE
19/159 for FR>FFPE

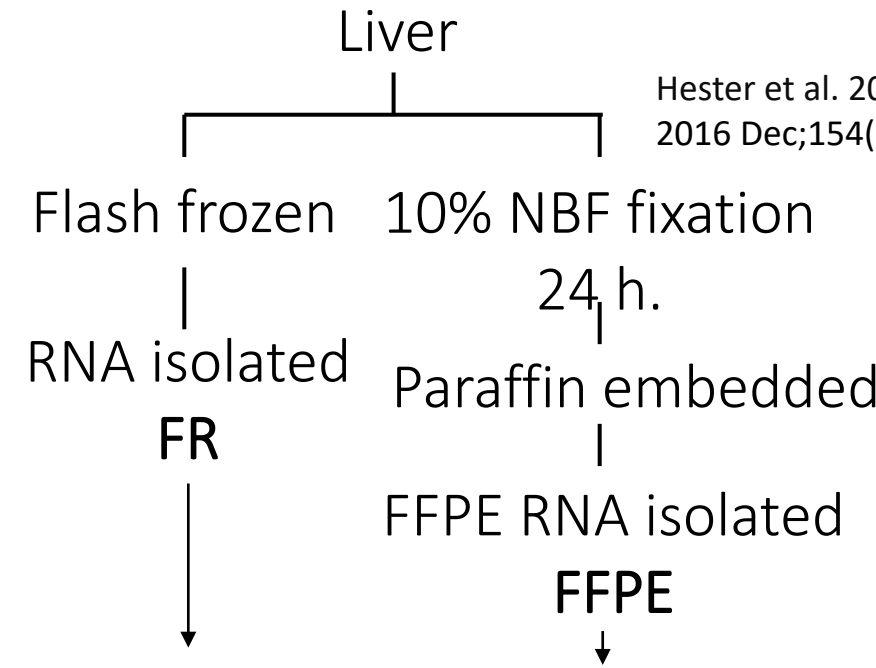
>21 yrs. FFPE results in 97% reduction in gene counts

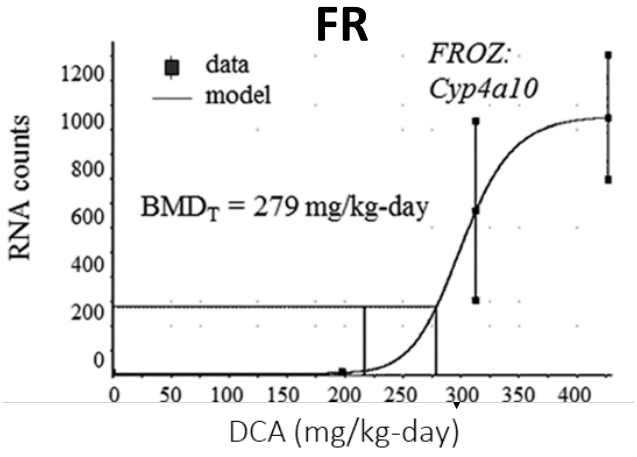
Hester et al. 2016. Toxicol Sci. 2016 Dec;154(2):202-213

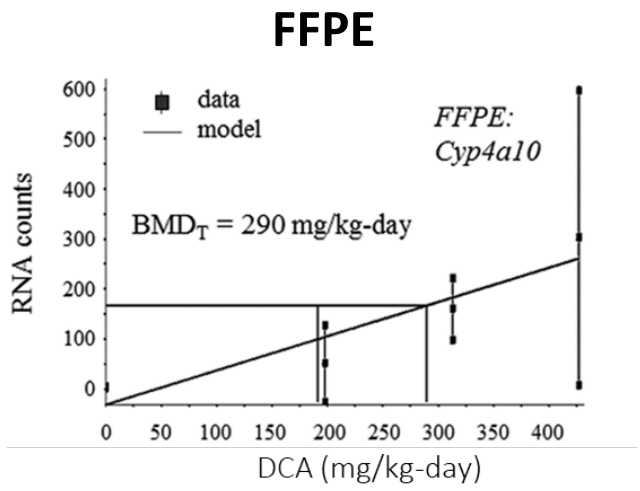
>21 yrs. Dichloroacetic acid (DCA)

- 4 week-old male B6C3F1 mice
- Doses 0, 1, 2 & 3.5 g/L in drinking H_2O
- N-6/dose
- 6 day exposure









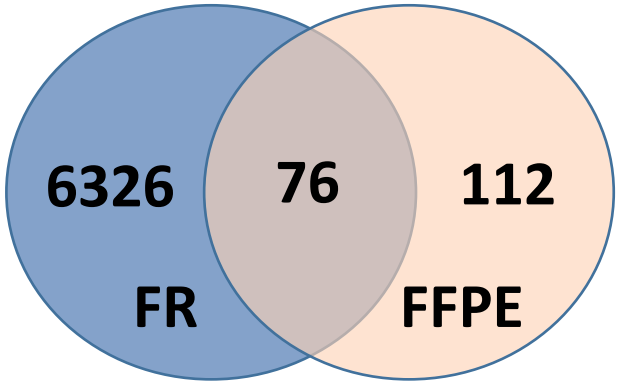
ILSI Health and Environme Sciences Institute

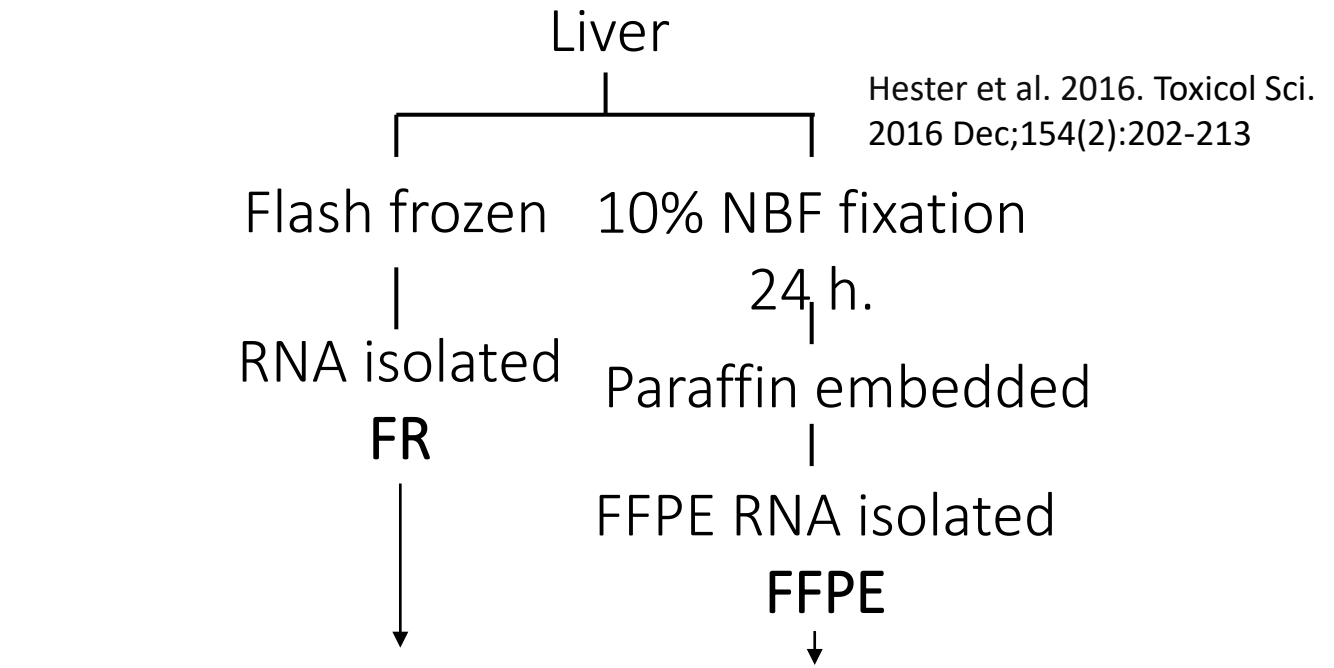
>21 yrs. FFPE results in 97% reduction in gene counts

>21 yrs. Dichloroacetic acid (DCA)

- 4 week-old male B6C3F1 mice
- Doses 0, 1, 2 & 3.5 g/L in drinking H_2O
- N-6/dose
- 6 day exposure

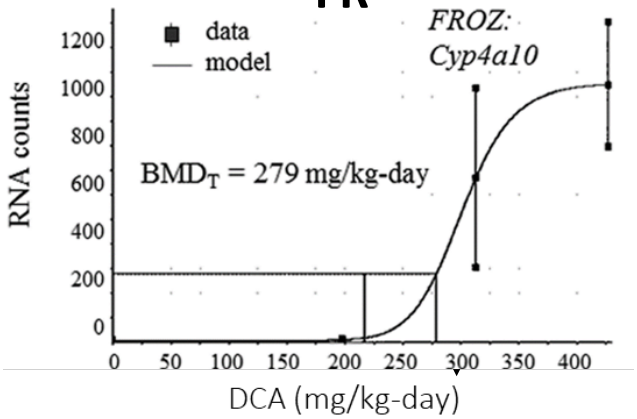
DCA (all doses)



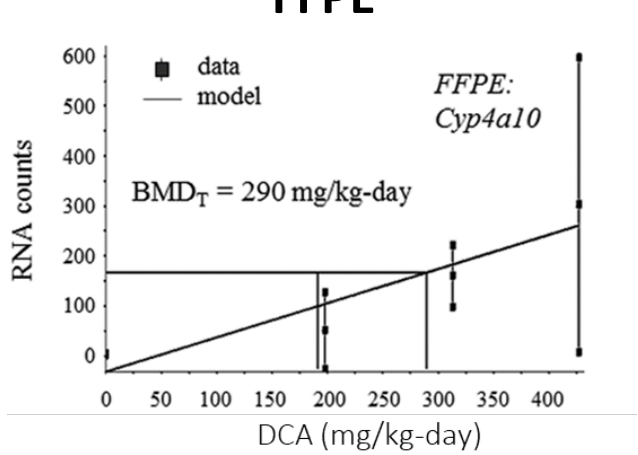


Hester et al. 2016. Toxicol Sci. 2016 Dec;154(2):202-213

FR

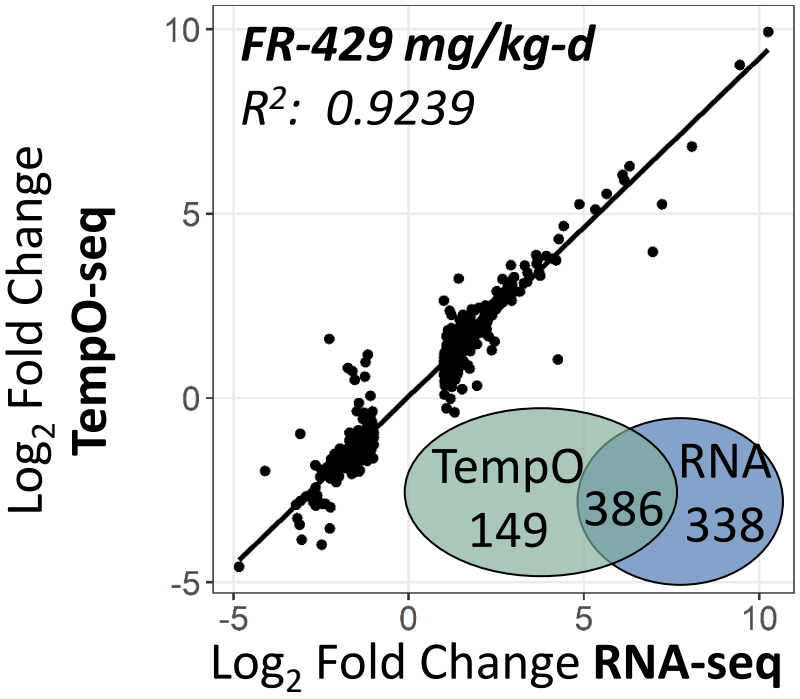


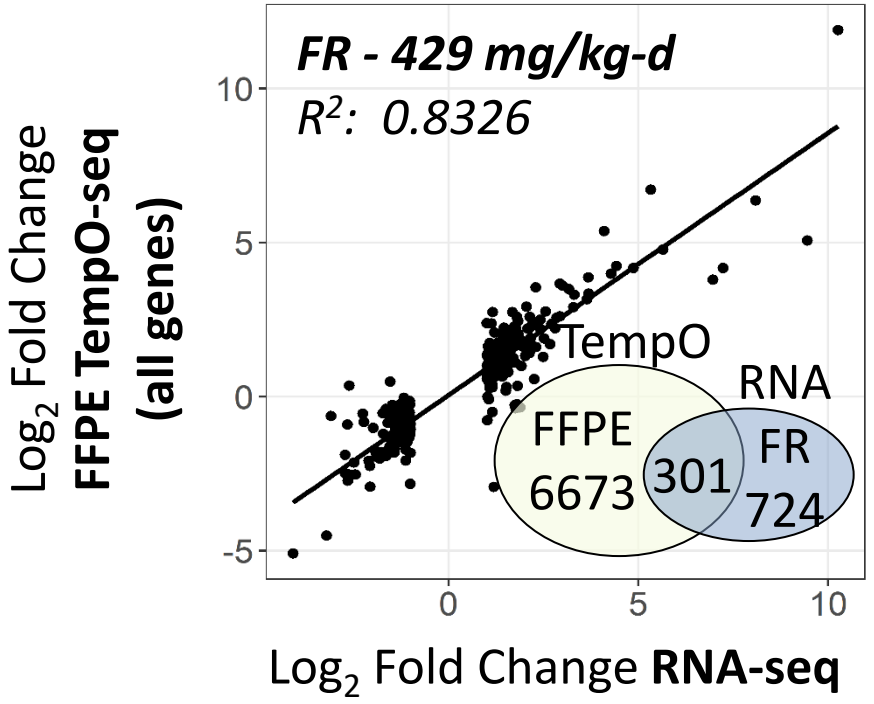
FFPE



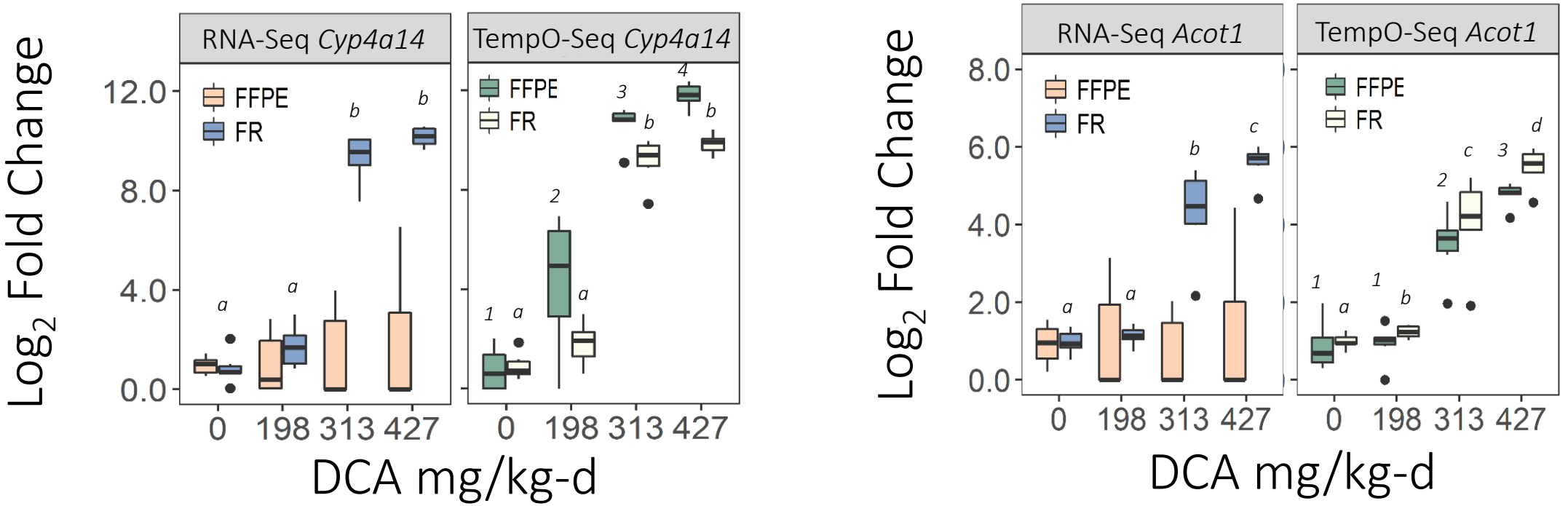
ILSI Health and Environme Sciences Institute

TempO-seq significant genes concordant with RNA-seq



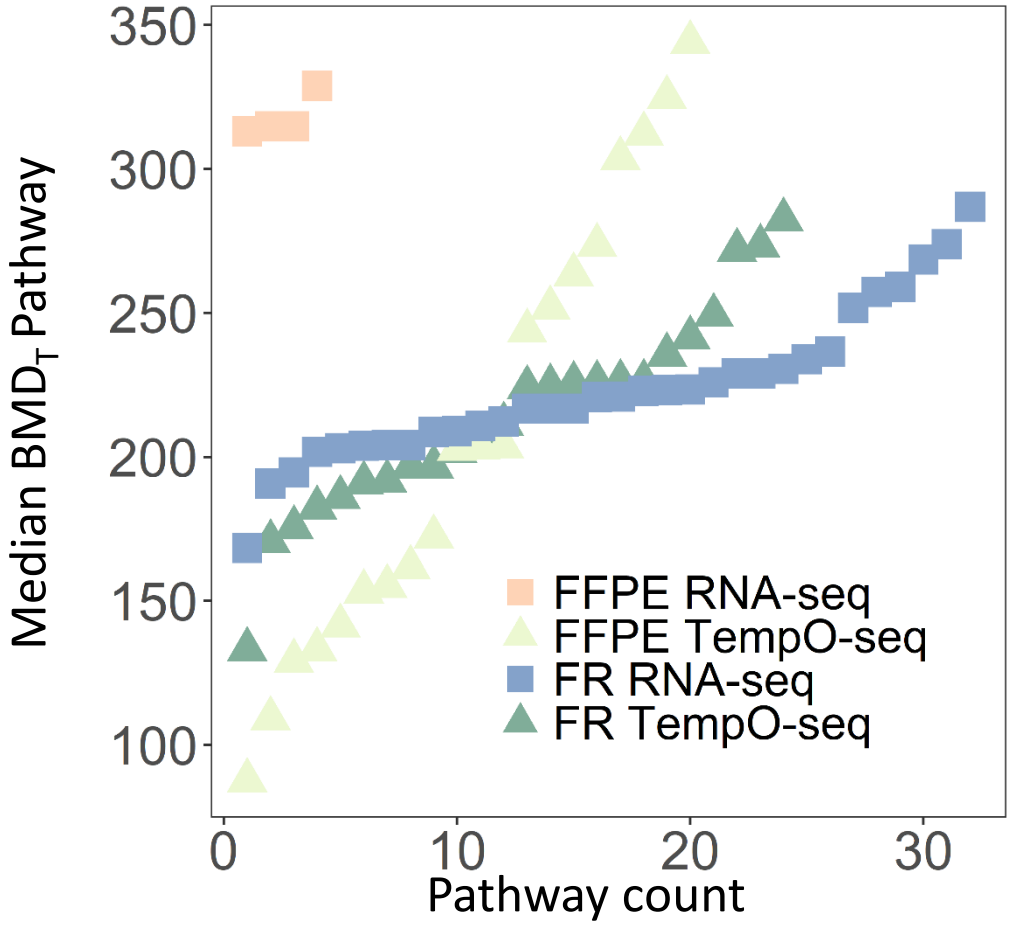


TempO-seq FFPE more consistent with frozen marker genes



a, b, c, and d: FR significant difference (p-value <0.05) between dose groups. 1, 2, 3, and 4: FFPE significant difference (p-value <0.05) between dose groups.

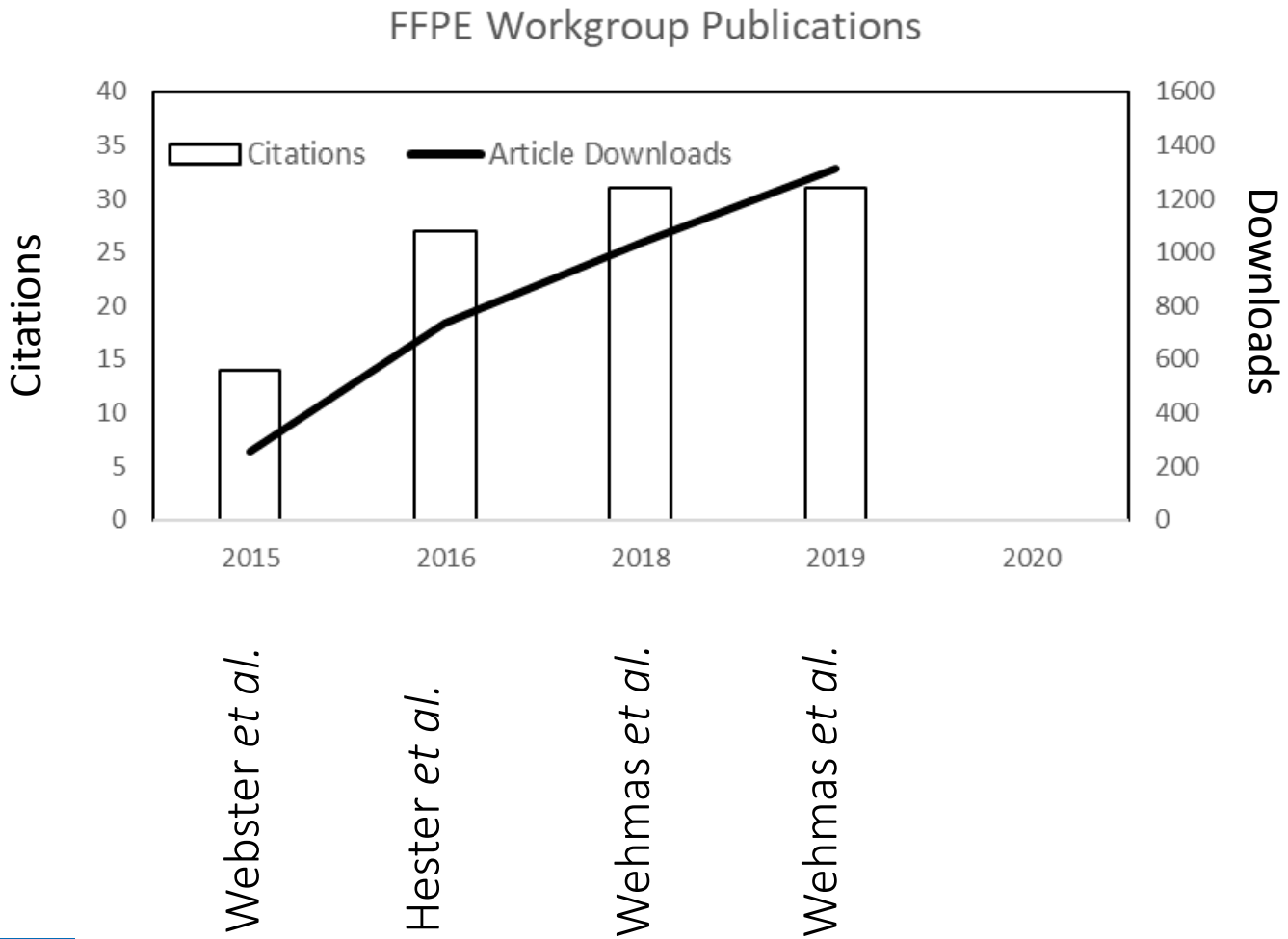
TempO-seq gene response BMD within 2.1-3.1 fold of traditional value



	Lowest Median BMD (mg/kg-d)	Median BMDL (mg/kg-d)	BMD_T: BMD_A*
FFPE TempO-seq	87.7	55.1	2.1
FR TempO-seq	133.2	101	3.1
FR RNA-seq	168.4	127.8	4.0
FFPE RNA-seq	313.1	202.0	7.3

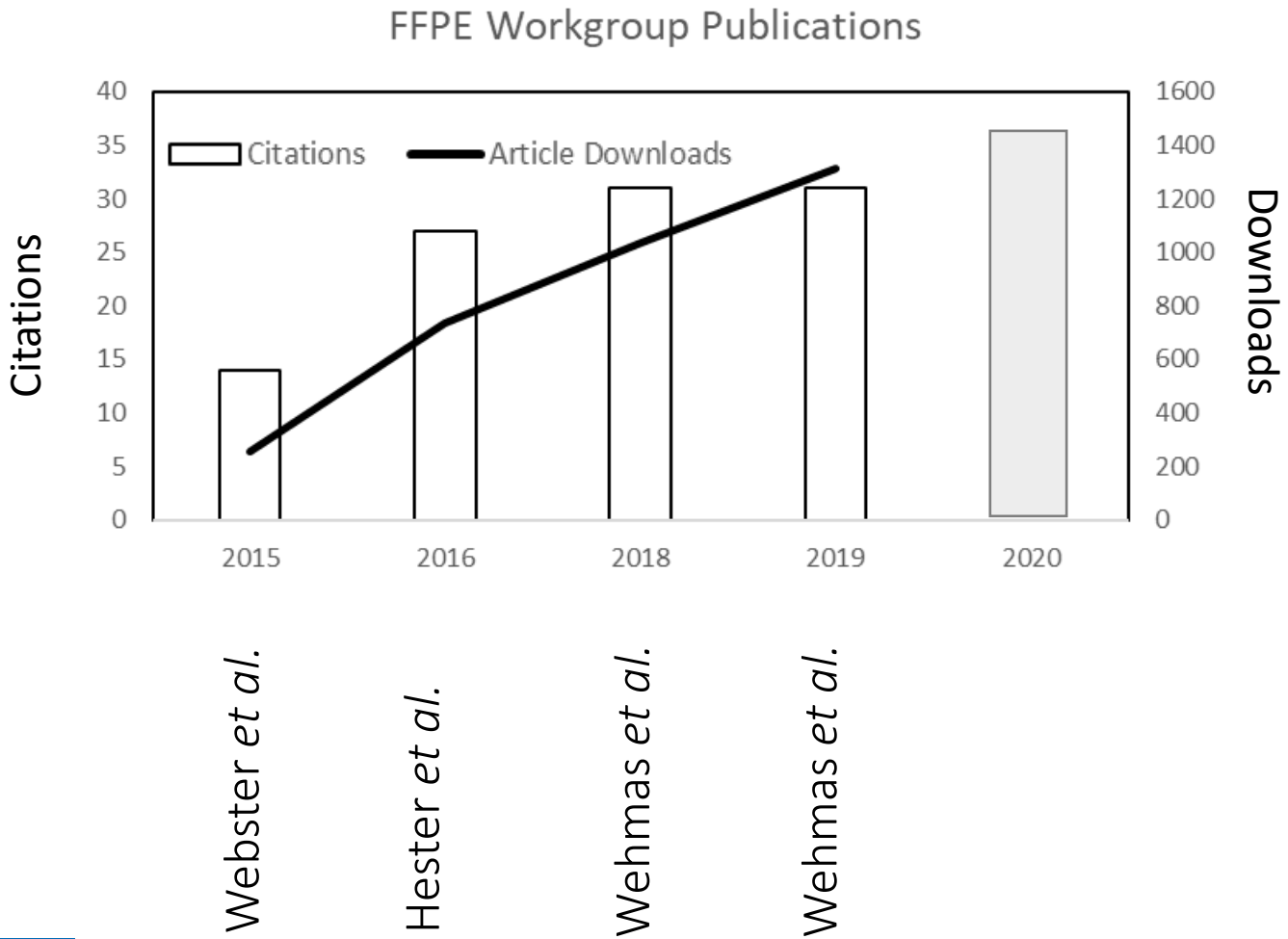
*BMD_A: IRIS BMD for mouse liver adenoma and carcinoma = 42.6 mg/kg-d

Major findings



- Applied new technologies
- Characterized major factors impacting FFPE quality
- **Identified methods to improve gene expression data**
- Developed better metrics for quality assessment;
- Translated results to clinical FFPE and improved SNP detection
- Identified artifacts and importance of controls
- Improved transcriptomic benchmark dose analysis for >21 yr. old FFPE

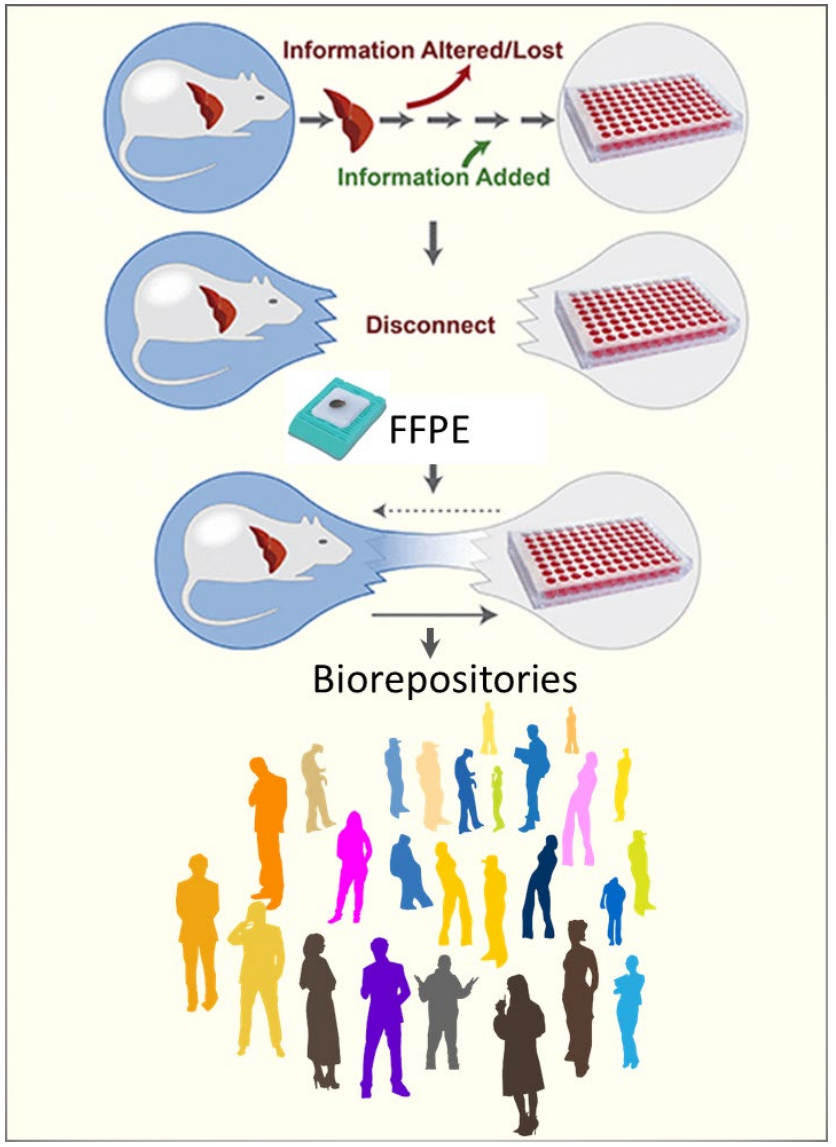
Major findings



- Applied new technologies
- Characterized major factors impacting FFPE quality
- **Identified methods to improve gene expression data**
- Developed better metrics for quality assessment;
- Translated results to clinical FFPE and improved SNP detection
- Identified artifacts and importance of controls
- Improved transcriptomic benchmark dose analysis for >21 yr. old FFPE

Archival tissue samples can be repurposed to:

- Quantify gene expression
- Understand chemical mode of action
- Expand toxicity pathways
- Bridge molecular and traditional toxicology data
- Save resources and time
- Aid translation to humans



https://doi.org/10.1124/jpet.117.245019

Biorepository resources

- Specimen Resource Locator - https://specimens.cancer.gov/
- Biorepositories & Biospecimen Research Branch (BBRB) - https://biospecimens.cancer.gov/default.asp
- Mayo Clinic Biorepositories program - https://www.mayo.edu/research/centers-programs/mayo-clinic-biobank/overview
- Specialized Programs of Research Excellence (SPORE)- https://www.cancer.gov/about-nci/budget/fact-book/extramural-programs/spores
- National Toxicology Program Archives- https://ntp.niehs.nih.gov/data/archives/index.html
- National Gene Vector Biorespository - https://www.ngvbcc.org/Home.action

Acknowledgments

EPA
Charles Wood, DVM, PhD (now BI)
Susan Hester, PhD
Gail Nelson
Judy Schmidt
AOP task members

NCI
Ping Guan
Helen Moore

Quintiles
(Q2-Solutions, Durham, NC)
RNA-Seq processing

HESI eSTAR Committee

HESI Staff
Syril Pettit, PhD, Director
Carolina Morell-Perez, MS Scientific Program Manager
Raegan O'Lone, PhD, Associate Director (Former)
Lauren Peel, Scientific Program Associate (Former)

Health Canada
Carole Yauk, PhD
Remi Gagne
Andrew Williams, PhD

Pfizer
Mark Gosink, PhD

Funding: US EPA CSS Program

Supplemental-Direct formalin fixation effect does not confound chemical treatment response