Overview of DTT's Novel Tools and Approaches Program

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Outline

- DTT Organization
- Overview of Novel Tools and Approaches (NTA) Program
  - What it is and what we do
  - Portfolio
- Examples of projects in NTA-PMT portfolio
  - PSC-based cardiac and neural model systems
  - cfDNA in organoid models
  - Dual reporter line for neurological disorders: Parkinson's Disease

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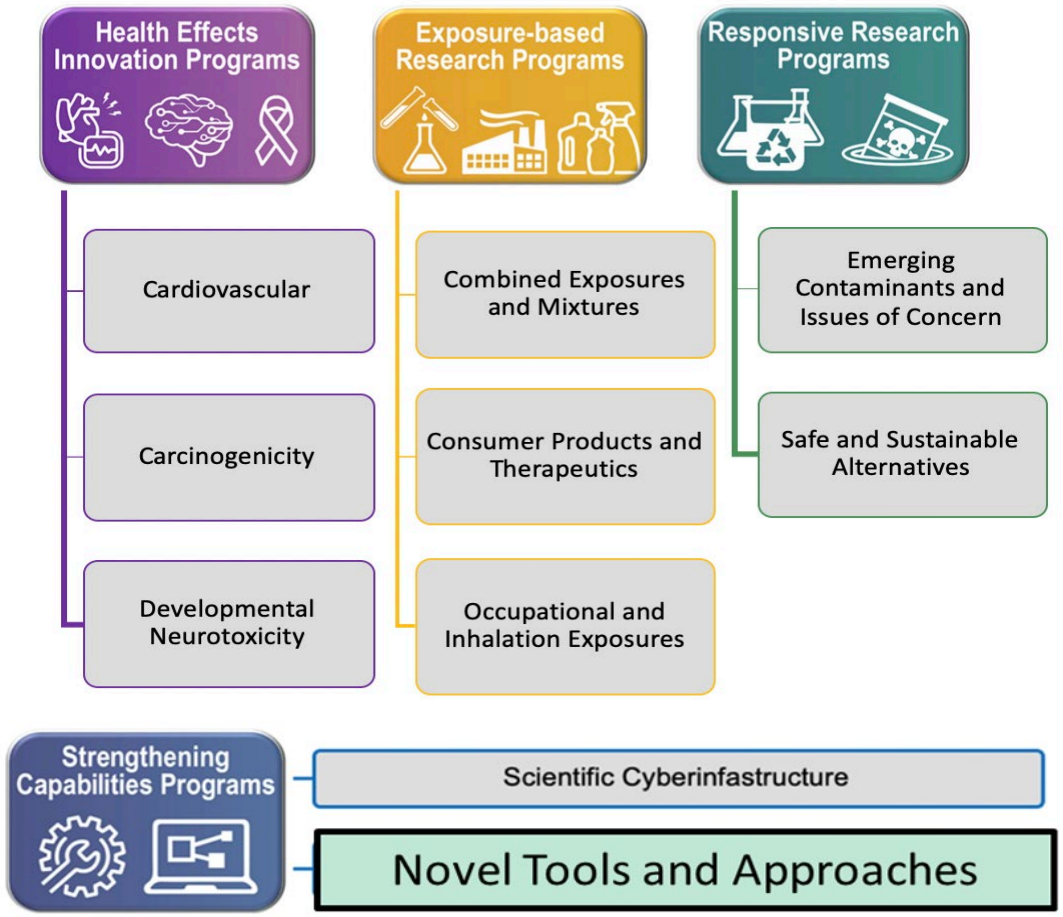
- Branches
  - Systems Toxicology
  - Predictive Toxicology
  - Cellular and Molecular Pathology
  - Integrated Health and Assessment
  - Mechanistic Toxicology
- Programs / Strategic Areas of Focus
  - Exposure-based
  - Health Effects Innovations
  - Responsive Research
  - Strengthening Capabilities
    - NTA is here

What is the NTA Program?

- Different than most other DTT programs:
  - NTA is not focused on a specific type of disease or exposure
  - NTA is 1 of 2 programs that provides special capabilities to DTT



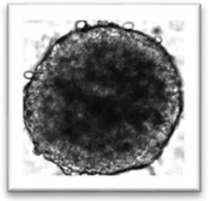
What Does the NTA Program Do?

- Identify new and novel approaches that may improve DTT science by:
  - Increasing testing throughput
  - Increasing speed of data acquisition from years to weeks
  - Increasing data accuracy and precision
  - Providing more in-depth analyses: molecular mode of action (MoA) and benchmark dose (BMD)
  - Enhancing human relevance of DTT studies

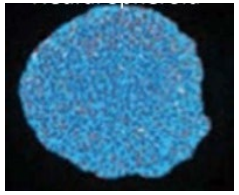
69 total projects in our portfolio

Bioassays and Biological Systems

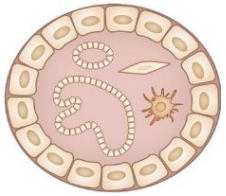
3D Liver Models



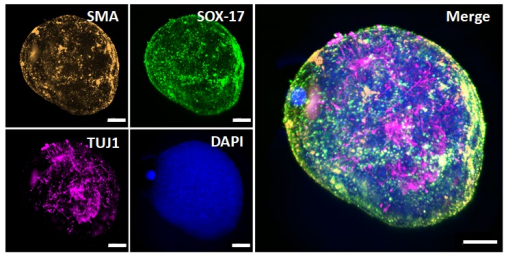
Neural Spheroids



Kidney Organoids



Embryoid Bodies



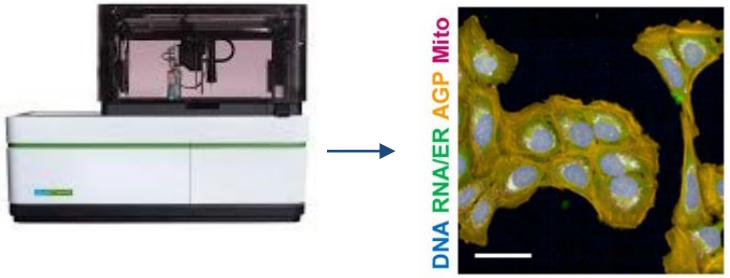
Zebrafish

5-Day Rat Assay

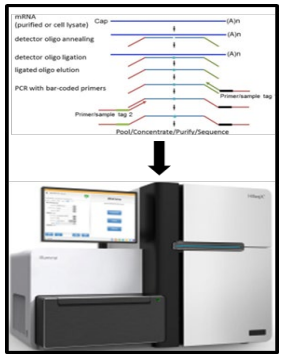
Novel Applications in Toxicology

Metabolomics

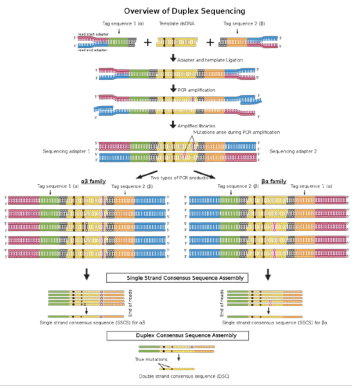
In Vitro Phenotype Screening



Transcriptomics



Duplex Sequencing



Examples of Projects in the NTA Portfolio

- **Pluripotent Stem Cell (PSC)-based Model Systems**

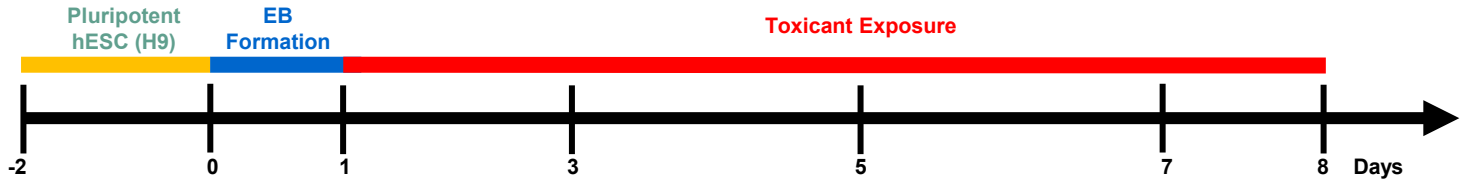
hPSCs for Studying Developmental Toxicity

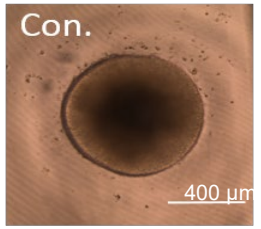
- Effects of environmental chemicals on developing embryos are relatively understudied
  - Lack of robust models that recapitulate human organogenesis
- Current *in vivo* modeling systems
  - Labor intensive, costly, time-consuming
- hPSCs offer an excellent opportunity to study developmental toxicity
  - Physiologically relevant, rapid, high-throughput
  - Mimic events in embryogenesis, organogenesis
  - Study defined stages of development → windows of susceptibility
  - Fewer animals

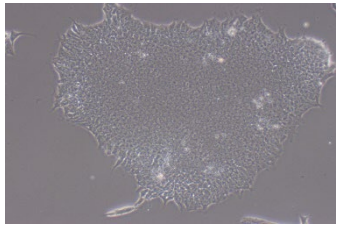
Components of Our Model Systems

Cardiac model shown

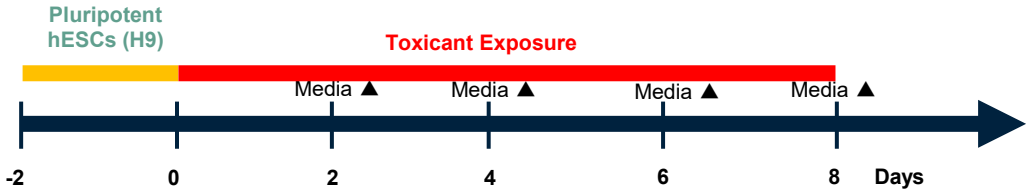
Embryoid body formation

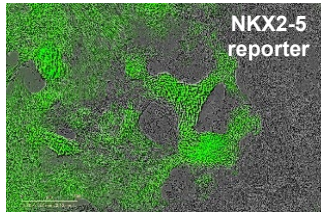




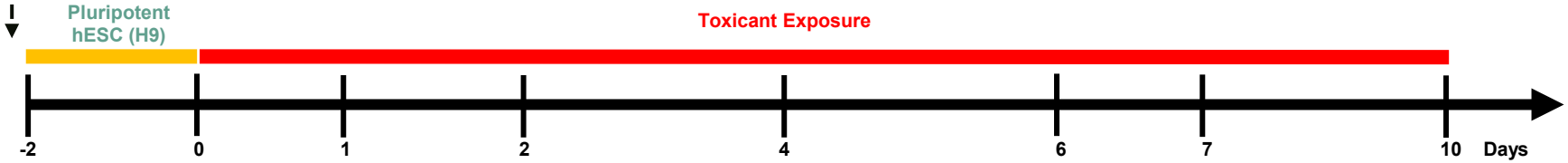


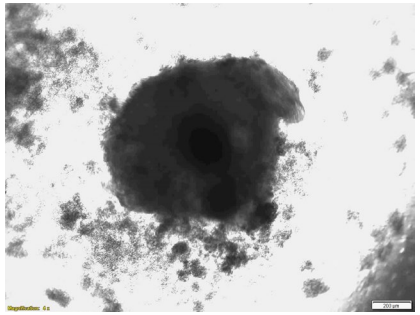
2D directed differentiation





Organoid formation





Embryoid Body (EB) Model

- **Screening for teratogens**

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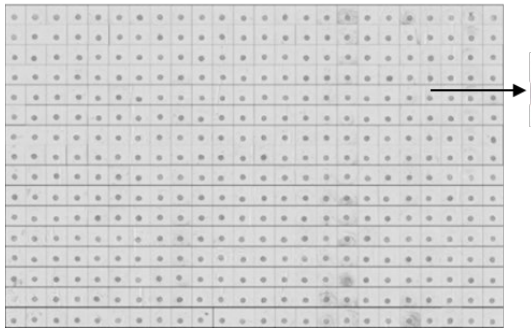
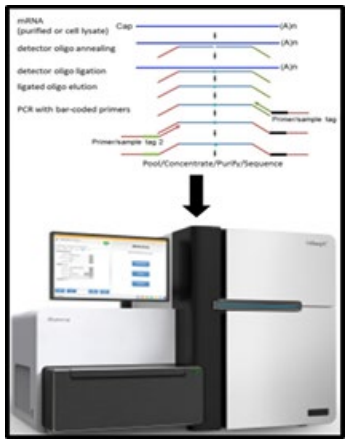
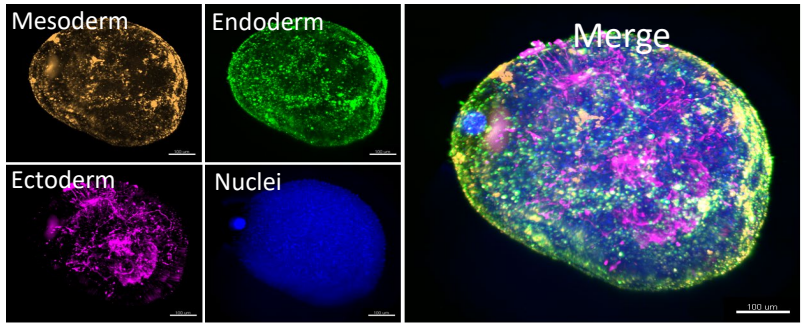
Embryoid Bodies (EBs) for Developmental Toxicity

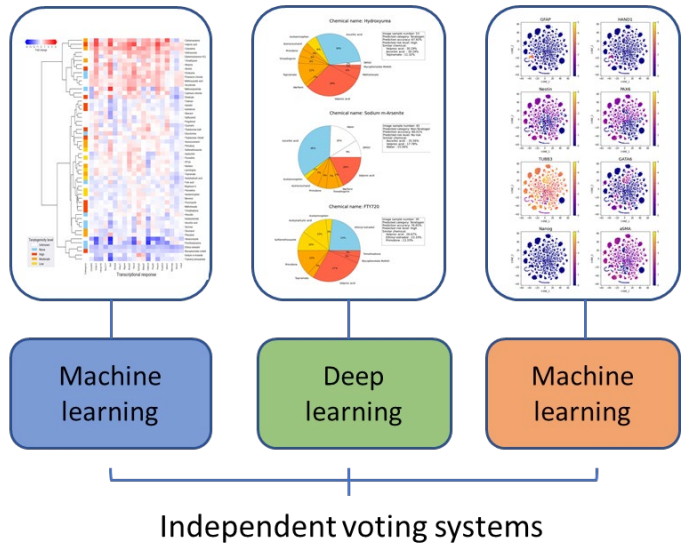
3D aggregates of PSCs that will spontaneously differentiate into cells of the 3 germ layers

EB formation in 384-well ULA plate









- Predicting at ~80-90% accuracy

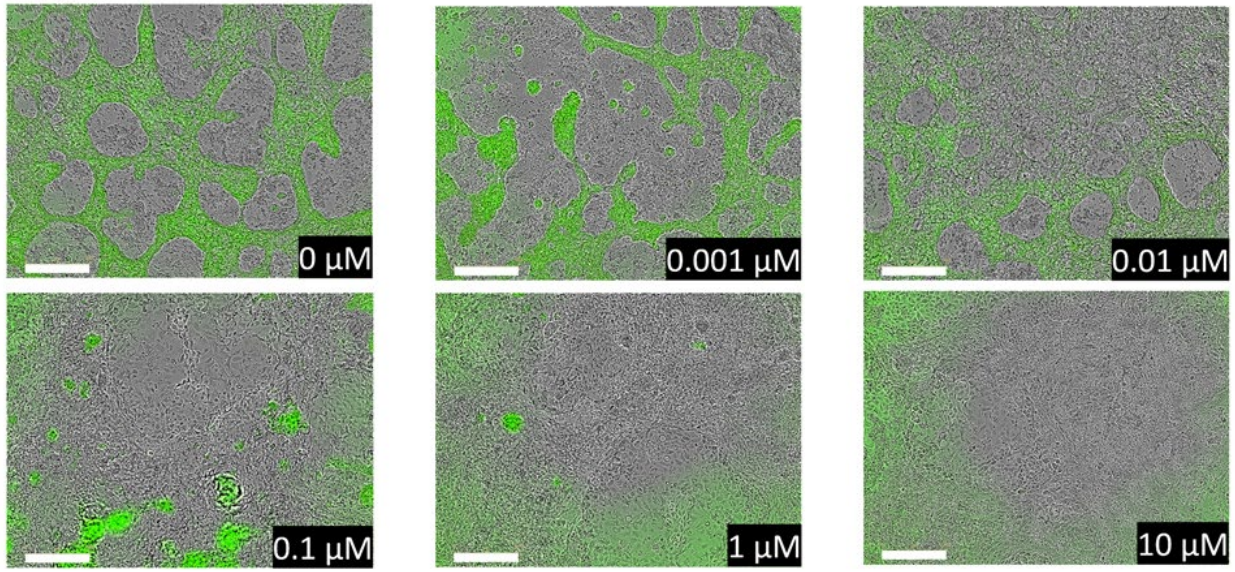
Ian Chen, PhD

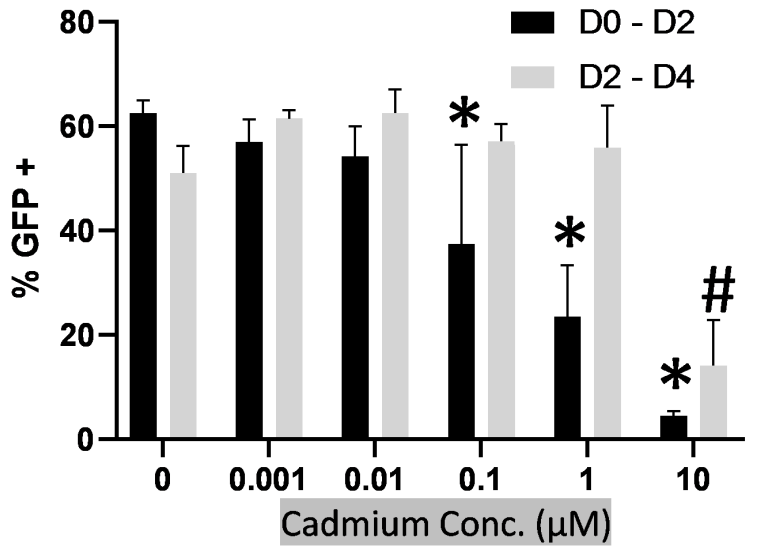
PSC-based Cardiac Model System

- **Early-life Development in Presence of Toxicants**

Cadmium Inhibits Cardiomyocyte Formation

- Mesoderm induction (Day 0-2) stage:
  - Inhibition as low as 0.1 μM
- Cardiac induction (Day 2-4) stage:
  - No inhibition
- As had different window of susceptibility
  - Similar endpoints with Cd
  - Inhibition during cardiac but not mesoderm induction stage



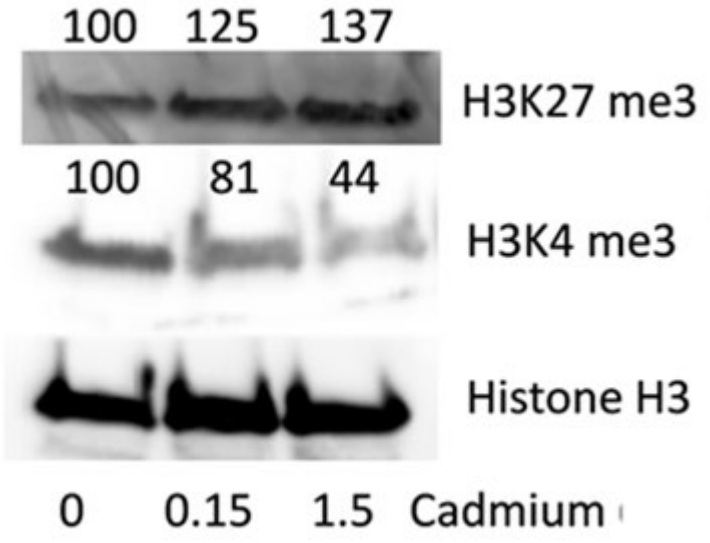


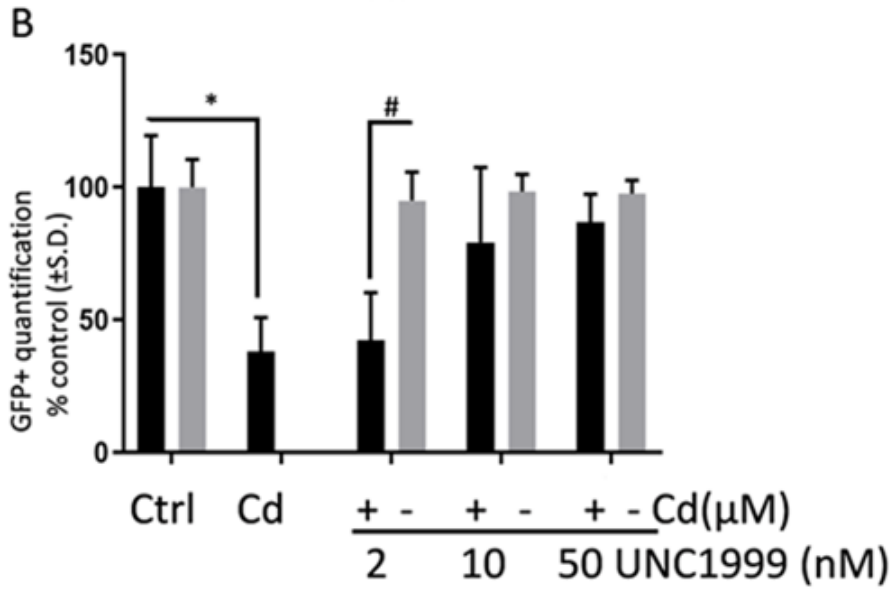
Xian Wu, PhD

Wu X … Hu, G, Tokar EJ, *Environ Health Perspect* (2022) 130(11):117002

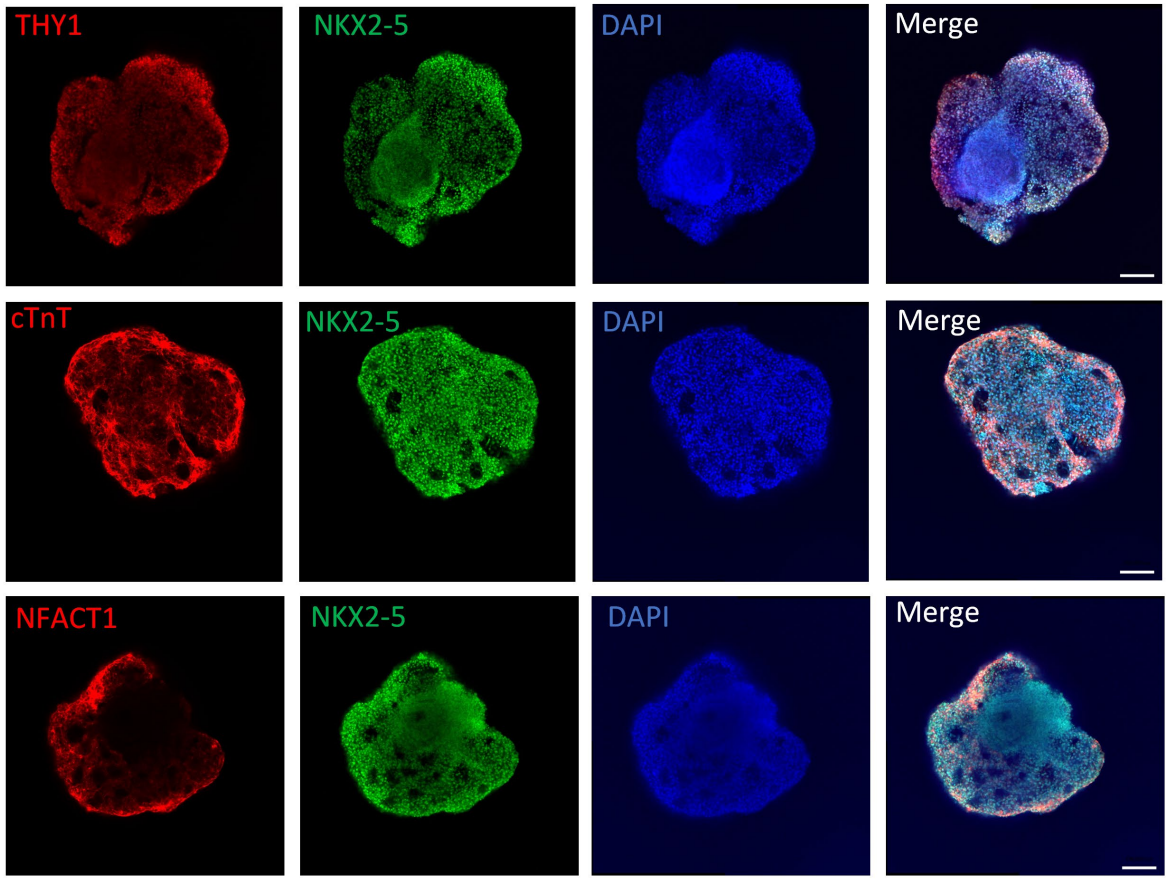
Role of Histone Methylation During Cd Inhibition

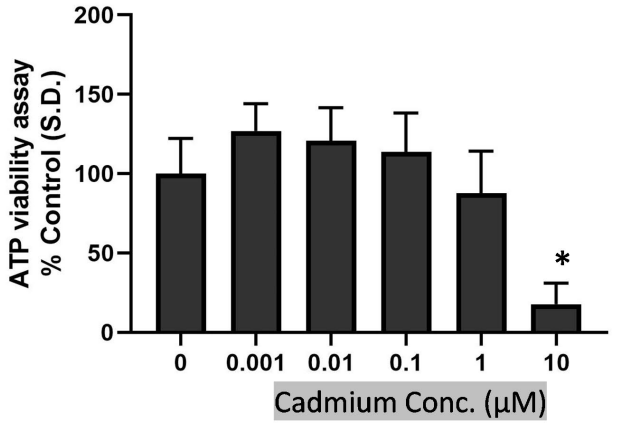
- Genes, TFs associated with mesoderm and cardiomyocytes downregulated (not shown)
- H3K27me3: marker of inactive/repressive chromatin
- H3K4me3: marker of active promoters
- UNC1999 targets EZH2, specifically suppresses H3K27me3/2
  - Removed Cd ability to inhibit differentiation to cardiomyocytes

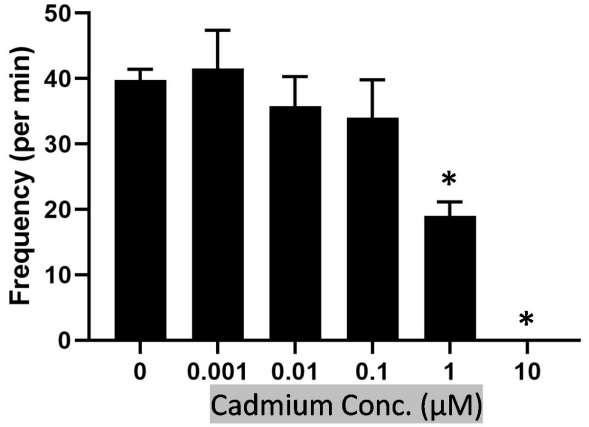


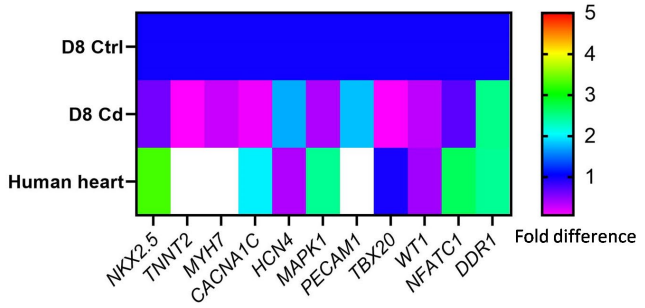


Cd Represses Cardiac Organoid Function









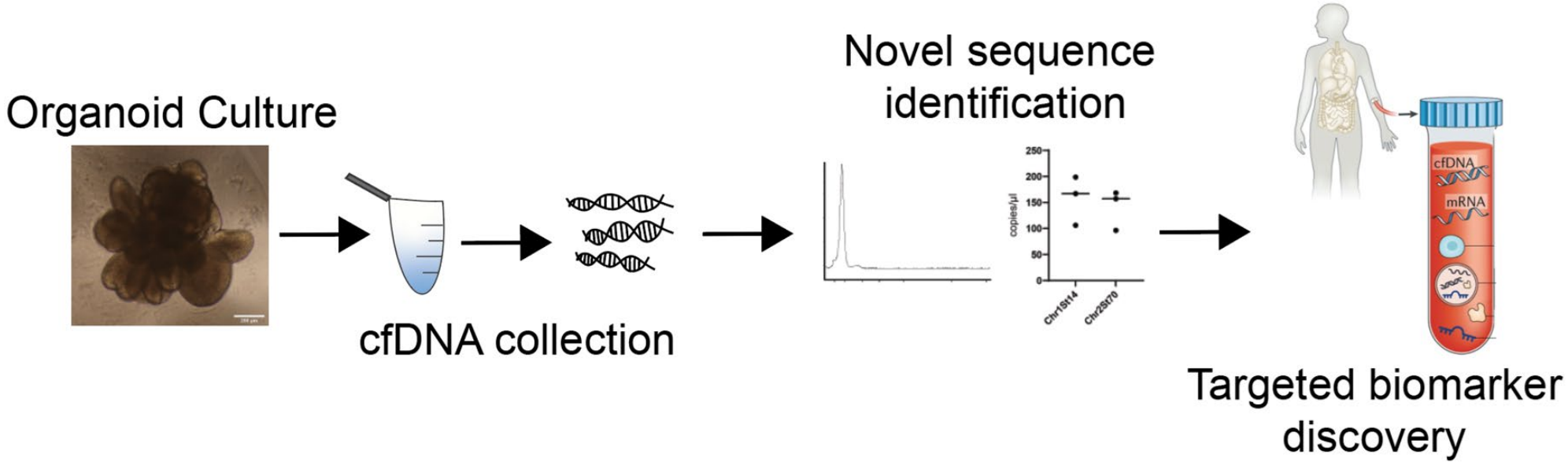
Wu X … Hu, G, Tokar EJ, *Environ Health Perspect* (2022) 130(11):117002

Cell-free DNA (cfDNA) in Organoid Models

- **Neural model**

Cell-free DNA (cfDNA)

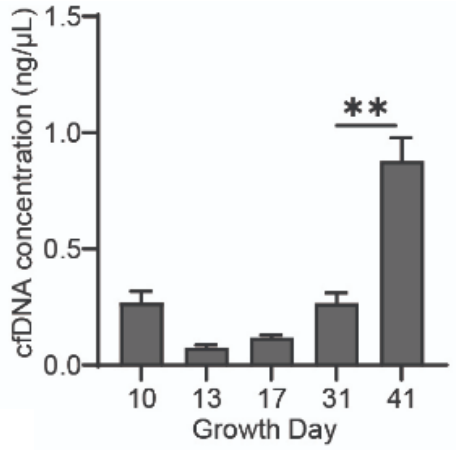
- Cell-free DNA (cfDNA)
  - Much recent interest as a clinical biomarker
    - Prenatal screening, fetal gender, cancer screening, etc.
  - Limited understanding of changes during differentiation, organogenesis, toxic insult

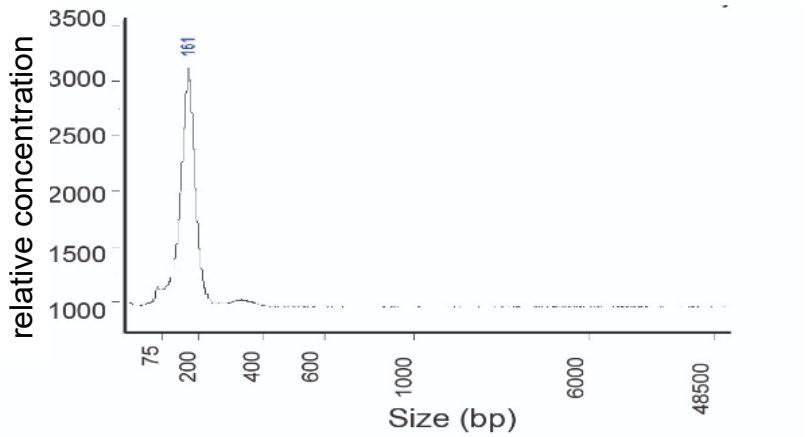


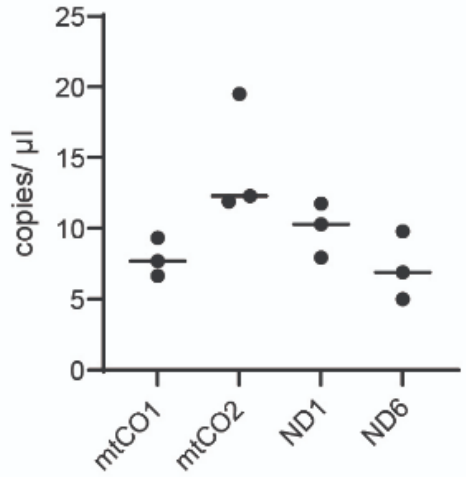
Brian Silver, PhD (co-mentored with Kevin Gerrish (MGC))

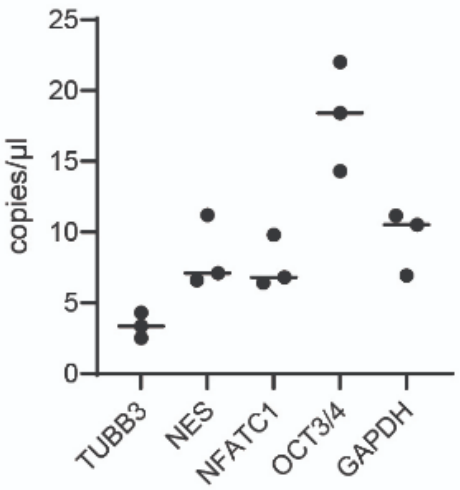
Isolation of cfDNA from Cerebral Organoids

- Low concentrations
  - ≤ 1 ng/ul
- Fragment analysis
  - Majority are ~160 bp
- Mitochondrial and nuclear genes
  - ddPCR
- cfDNA of both mitochondrial and nuclear origin is released in quantities sufficient for downstream analyses





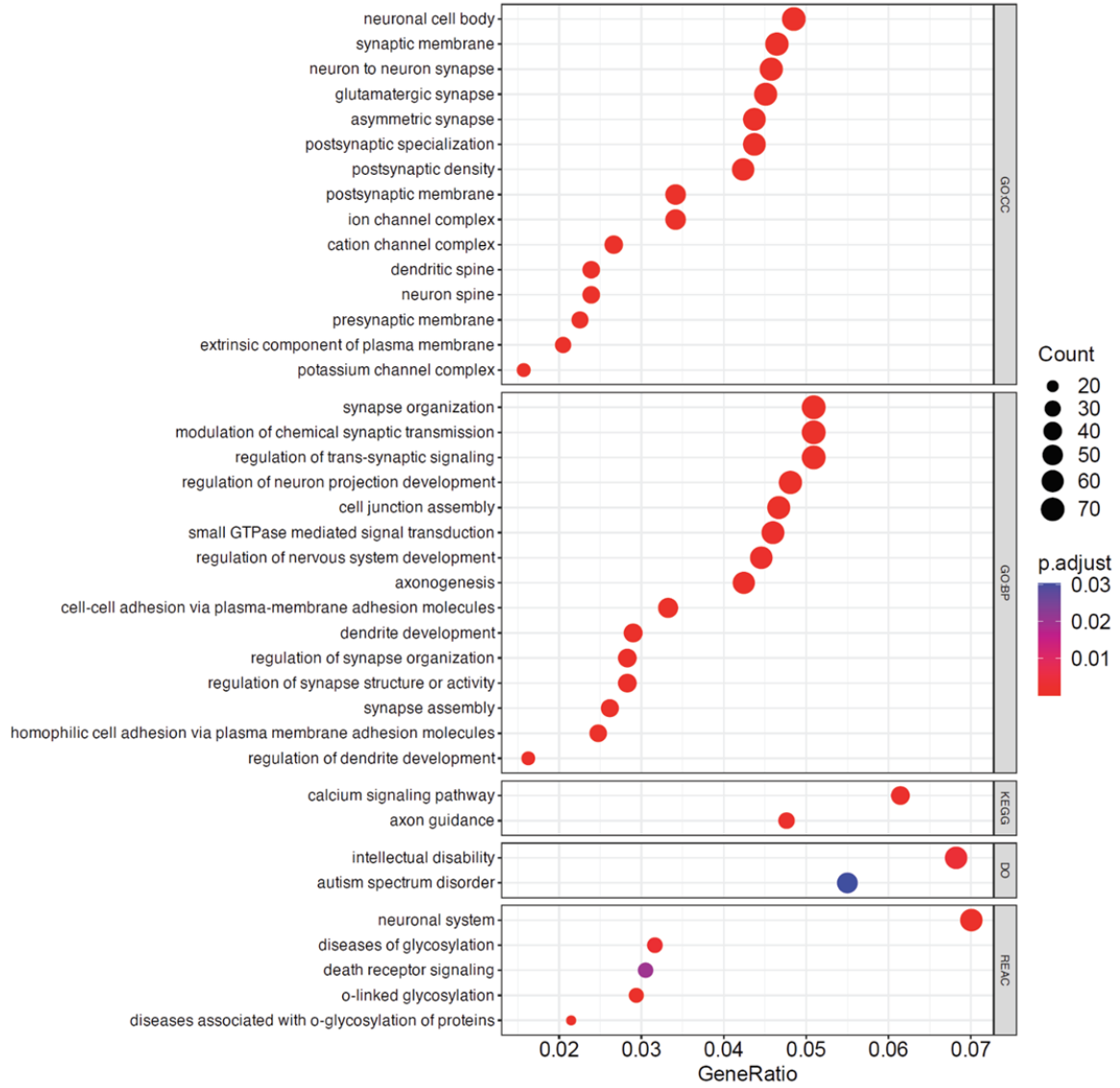




Silver B, … Gerrish K. and Tokar EJ. *Int J Mol Sci* (2024) 25: 5522.

WGS to Characterize cfDNA from Cerebral Organoids

- Gene Ontology analysis
  - Neurodevelopment and brain structure/organization
- Disease Ontology analysis
  - Intellectual disability and autism
- cfDNA that is relevant to brain tissue and human neurological disorders or development
- Future:
  - Human plasma/CSF
  - Toxicant-exposed organoids
  - Patient-derived iPSC lines/models



Silver B, … Gerrish K. and Tokar EJ. *Int J Mol Sci* (2024) 25: 5522.

Dual Reporter Model System for Neurological Disorders

- **Parkinson's Disease**

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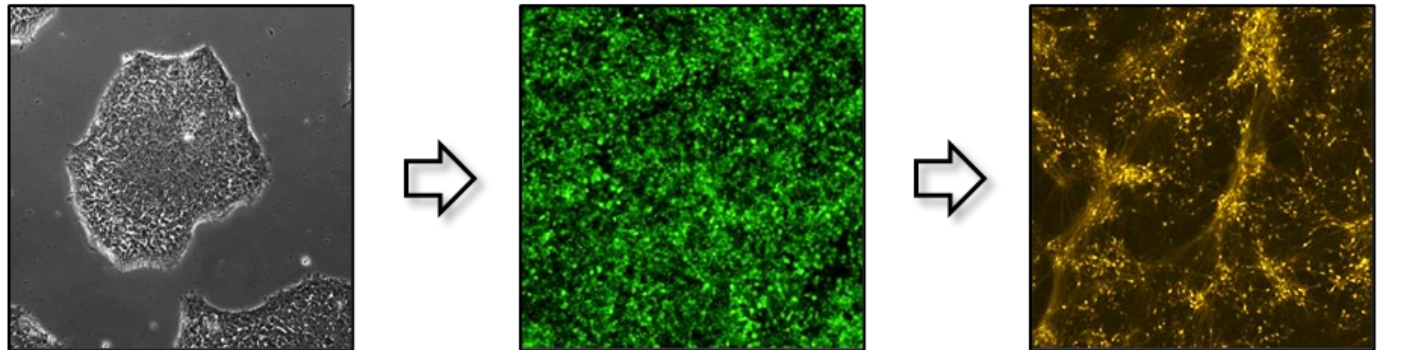
ESC Reporter for Dopaminergic Neuron Development

- Dopaminergic (DA) neurons play key roles in neurological disorders (e.g. Parkinson's Disease)
- CRISPR gene-editing technique used to insert fluorescent markers
  - GFP for Nestin – neural stem cells
  - mScarlett for Tyrosine Hydroxylase – DA neurons
- Easy & rapid imaging of different stages of differentiation/development
- Monitor formation of neural stem cells and dopaminergic neurons



Embryonic Stem Cells *Neural Stem Cells* *Dopaminergic Neurons*

Anna Kreutz, PhD (co-mentored with Guang Hu (DIR))

Kreutz, A; Hu, G; Tokar, EJ, 2023, Stem Cell Reviews and Reports, PMID: 37273171

Summary and Conclusions

- Several useful model systems for improving DTT science (developmental toxicity)
  - EB, cardiac, neural, reporter line for monitoring DA neurons
- Address tasks assigned to NTA
  - High(er)-throughput screening
  - Rapid data acquisition
  - Investigations into mechanisms
  - Enhance human relevance
  - Fewer animals
- These model systems offer great opportunity for studying effects of early-life exposures on development and later-life disease

Acknowledgements

Current NTA Program Members

- David Crizer, PhD (PMT Lead)
- Rachel Frawley
- Georgia Roberts, PhD
- Kristine Witt, PhD
- Vesna Chapppell, PhD
- Erik Tokar, PhD
- Darlene Dixon, PhD (ExMT Liaison)

Stem Cell Tox Group

- Brian Silver, PhD
- Carri Murphy
- Xian Wu, PhD (now at ECU)
- Ian Chen, PhD (now at EPA DC)
- Anna Kreutz, PhD (now at Inotiv)
- Justin Gutkowski (now at NICHD)

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Thank You!