How to set up a wet lab for cell reprogramming experiments

Joshua Sodicoff

10/25/2022

Overview

- Intro
- Our wet lab
- Research directions
- The process
- Conclusion

Please ask questions whenever you want and answer discussion questions

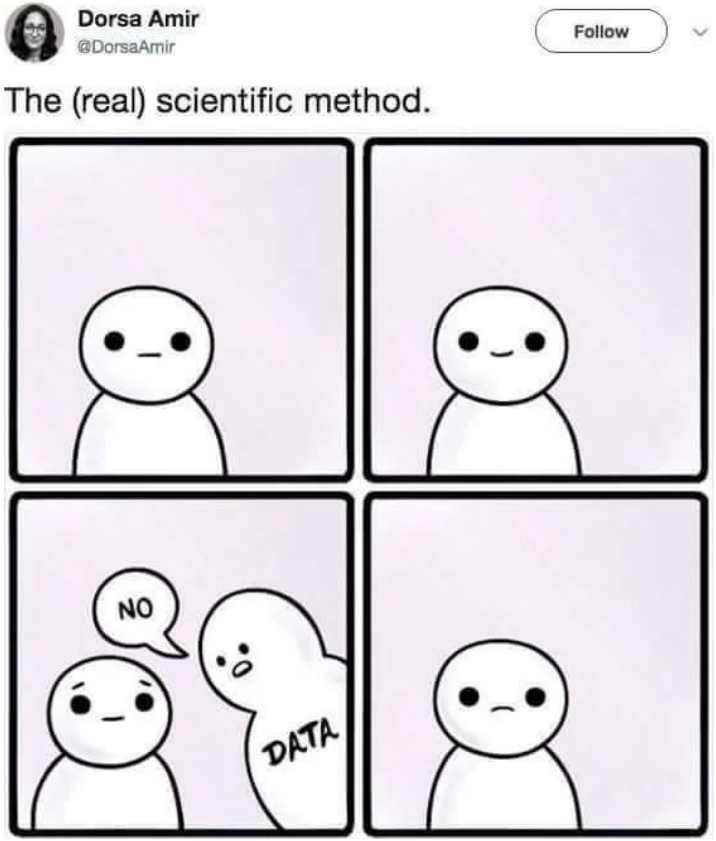
And apologies for structuring my presentation like this, my tech comm. professor would be disappointed

My job?

- Ordering lab lunch
- Charting out a path to having an experimental component to the lab
  - Setting up the lab space
  - Becoming well versed in equipment, university policies, the field we're moving into, etc.
  - Planning our first set of experiments
  - Developing a set of protocols and procedures for future lab operations and the next lab manager
  - Helping determine the direction of projects
  - Seeking out funding and collaborations
- Also helping manage and finish the BRAIN Initiative project

Motivation: Why run experiments?

- i.e. why not use data from collaborators?
- An independent wet lab will
  - Allow us to direct the course of our own research
  - Give us access to original data generated with the newest sequencing technologies
  - Provide opportunities to extend other sequencing methods and assays and develop our own
  - Give students more exposure to modern molecular biology/sequencing techniques



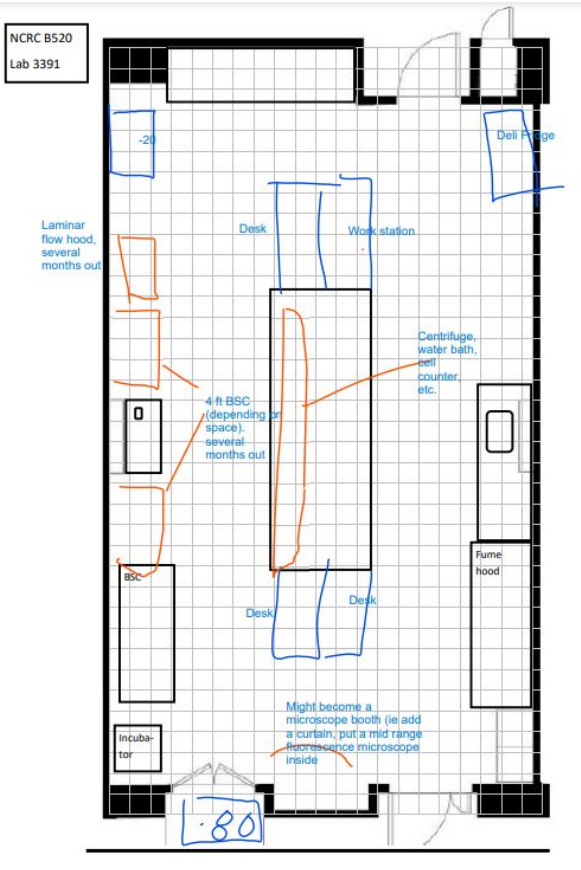
The Welch (Wet) Lab

Our wet lab

- Room 3391
- Our space
  - 714 square feet
  - Currently unfurnished
- Resources
  - Electrical, gas, vacuum
  - Chemical fume hood
  - Plenty of drawers and shelves

Our vision for the space (in progress)

OR



?

Our neighborhood

- NCRC Building 520
  - Former Pfizer research campus
- Our neighbors? Garmire and Rajapakse
  - Among other labs affiliated with DCMB, BME, pharmacology, etc.
- Our labspace-mate? Minji Kim, new faculty in DCMB
- Access to NCRC core facilities
  - Microscopy
  - Advanced genomics
  - Flow cytometry

Our lab rats

Justin Lee, Ph.D

2022 graduate of the MSU Microbiology and Molecular Genetics doctoral program

8 years pipetting

Enjoys prenatal infection models

Joshua Sodicoff, BSE

2022 graduate of the UMich Biomedical Engineering bachelors

3 years pipetting

Shewanella enthusiast

Who will be funding us?

University New Faculty Start-up Funds

"Integrative Single-Cell Analysis of Transcriptome, Epigenome, and Lineage in HIV Latency and Activation" R01 through the NIAID (in collaboration with the Collins Lab)

"Quantitative Definition of Cell Identity by Integrating Transcriptomic, Epigenomic, and Spatial Features of Individual Cells" R01 through the NHGRI

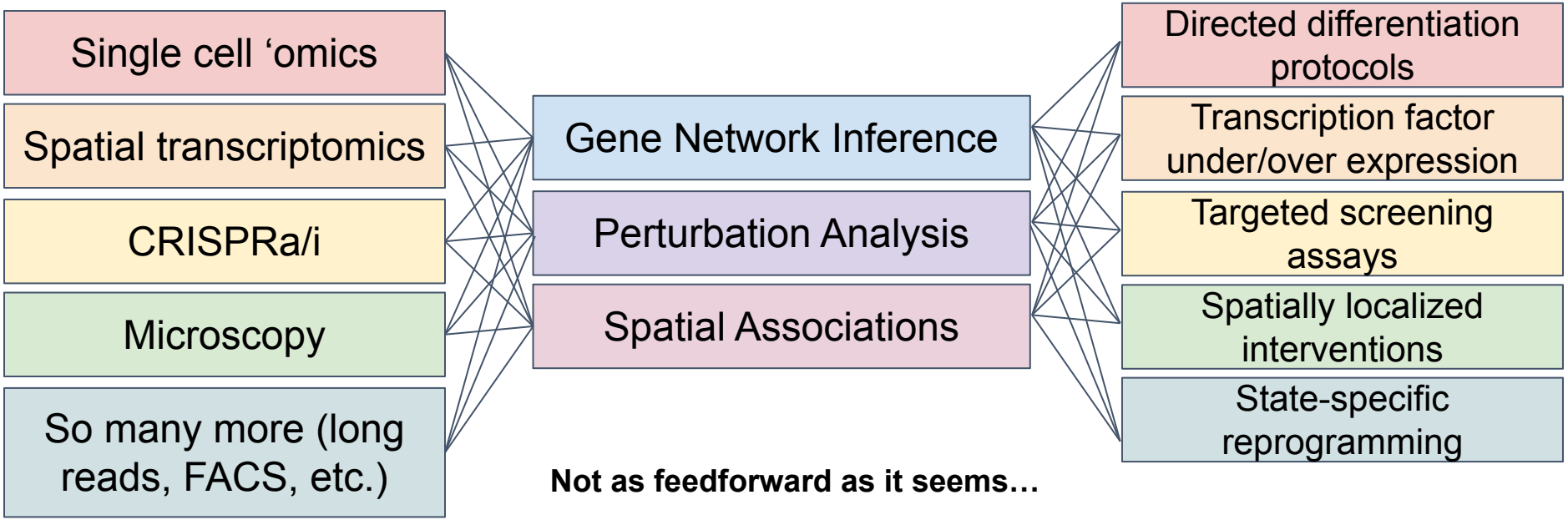
A new R01?

If Justin and I can nab it

What are we going to do with the lab?

The goal: effective cell reprogramming, informed by high throughput sequencing/screening assay data

The path:



How we go about this is crucial

UNIVERSITY OF MICHIGAN

Quality of differentiated cells

- Accuracy
- Maturity
- Yield

Feasibility of reprogramming

- Length
- Stability
- Simplicity

Biological relevance

- Translational need
- Availability of original cell type
- Viability of application

This is a well-tread path but our approach is novel

- Comprehensive atlas generation as the primary
  - Limited manual iteration
  - Biological insight through the process
- Unique computational tools
  - LIRIpipe/DUMFound for single cell/spatial atlas generation
  - MultiVelo and VeloVAE for RNA velocity
  - PerturbNet for inference of unmeasured perturbations
  - What will you contribute?
- Innovative wet lab methodology
  - Single cell multiome readouts on CRISPRa/i screens
  - High MOI infection for multiplexed perturbations
  - Tools for state specific overexpression/knockdown
  - Localized interventions
  - Willingness to develop new protocols

How are we going to start?

- Skills to master
  - iPSC culture
  - Directed differentiation
  - Histology/microscopy
- Experiments
  - Time course, multi-ome sequencing of enteroids paired with Visium/Xenium
  - Perturb-seq on iPSCs with multiome readout
  - High MOI Perturb-seq of all transcription factors on iPSCs
  - CRISPR screens of full enteroids paired with Visium/Xenium
- Additional modalities
  - Fluorescence microscopy and cell painting
  - Flow cytometry
  - qPCR

Want to read a little more?

Check these papers out

- Good reviews
  - “Direct cell reprogramming: approaches, mechanisms and progress”(2021)
- Church Lab “cell fate engineering”
  - “A comprehensive library of human transcription factors for cell fate engineering” (2020)
  - “Machine-guided cell-fate engineering”(2022)
- Spence Lab directed differentiation
  - “Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro” (2010)
- CRISPRa/i
  - “Perturb-seq: Dissecting molecular circuits with scalable single cell RNA profiling of pooled genetic screens” (2017)
  - "A Genome-wide Framework for Mapping Gene Regulation via Cellular Genetic Screens" (2019)

What is your dream dataset?

Discussion Question

What have we done so far?

1. Regulatory
   a. IBC
   b. HPSCRO
   c. EHS Standards
2. Procurement
   a. Large equipment
   b. Small equipment
   c. Cell lines
3. Facilities
   a. Cleaning & Renovations
4. Training
   a. iPSCs
   b. Organoids
   c. Misc.

Regulations

IBC

About the Committee

As mandated by the NIH Guidelines, U-M's Institutional Biosafety Committee is responsible for assessing the biosafety containment level for research involving recombinant DNA and synthetic nucleic acid molecules. The U-M Institutional Biosafety Committee (IBC) also oversees research with other potentially hazardous biologics. The U-M IBC currently oversees:

- Recombinant DNA and synthetic nucleic acid molecules (this includes **human gene transfer studies**)
- Infectious agents
- Biological toxins
- Human-derived tissues, fluids, cells
- Certain animal-derived tissues, fluids, cells (i.e., from non-human primates, ruminants, swine, chickens or other fowl, or wild vertebrate animals)
- Federally-regulated Select Agents, experiments with Dual Use Research of Concern potential, and research requiring BSL3 containment

- Currently, only requiring info on our experiments/cell lines/mitigation techniques
- Will require a serious revision as soon as perturbation experiments are in sight
- One revision away from **APPROVAL**

HPSCRO

- Short for Human Pluripotent Stem Cell Research Oversight
- Monitors potential ethics concerns given controversy
- **APPROVED** on first submission

Type: **hESC** **iPSC**	No. of lines	Action: Obtain (O) Derive (D)	Provider / vendor / repository (e.g., patient sample, WiCell, etc.)	Line/source material: • NIH Registry No. or Name (e.g., H9) • Vendor No. • Cell source: embryo or tissue	U-M IRB No. (HUM), U-M Repository No. (REP), or external IRB name/number	MTA Number
iPSC	1	O	NIGMS HUMAN GENETIC CELL REPOSITORY	GM25256		23-UFA00676 (Coriell, processing)
iPSC	1	O	Heinz C. Prechter Bipolar Genetics Repository	C8WW C9G C10V	HUM00043228	

- University lab safety watchdog
- Involved in
  - Decommissioning (**APPROVED**), approving a lab space as vacated
  - Precommissioning (**APPROVED**), approving a lab space to be set up
  - Commissioning, approval for research activities
  - Regular, self inspections (first one **APPROVED**)
  - Yearly inspections
- Specialized interventions for
  - General biosafety (**APPROVED**)
  - BSC/incubator placement (guidance provided and taken into account)
- They have been very kind to us so far!

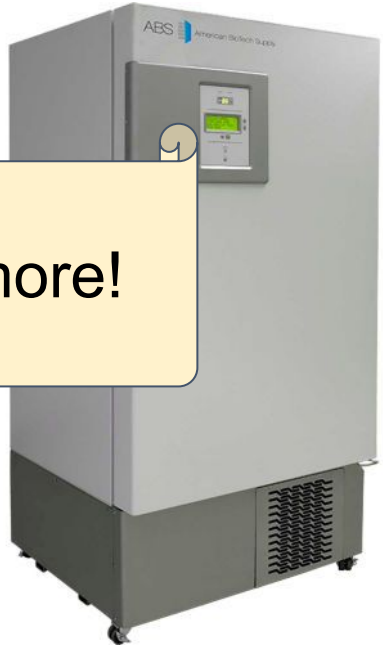
What are our next regulatory steps?

- IBC approval (almost certain)
- IBC revisions to include synthetic nucleic acids/virus work
- Grant revisions
  - Ensure funding for wet lab given grant originally included an experimentalist as a collaborator
- EHS Commissioning
  - Requiring
    - Training records
    - SOPs and chemical safety sheets
    - Inspection/testing of most equipment
- Write a new grant to fund exactly what we'll be doing
  - Unfortunately, needing preliminary data to get funded also applies in experimental biology


Procurement

We have a lab to fill

- Without anything set up in the room, procurement has been challenging
- What do we need?



… and more!

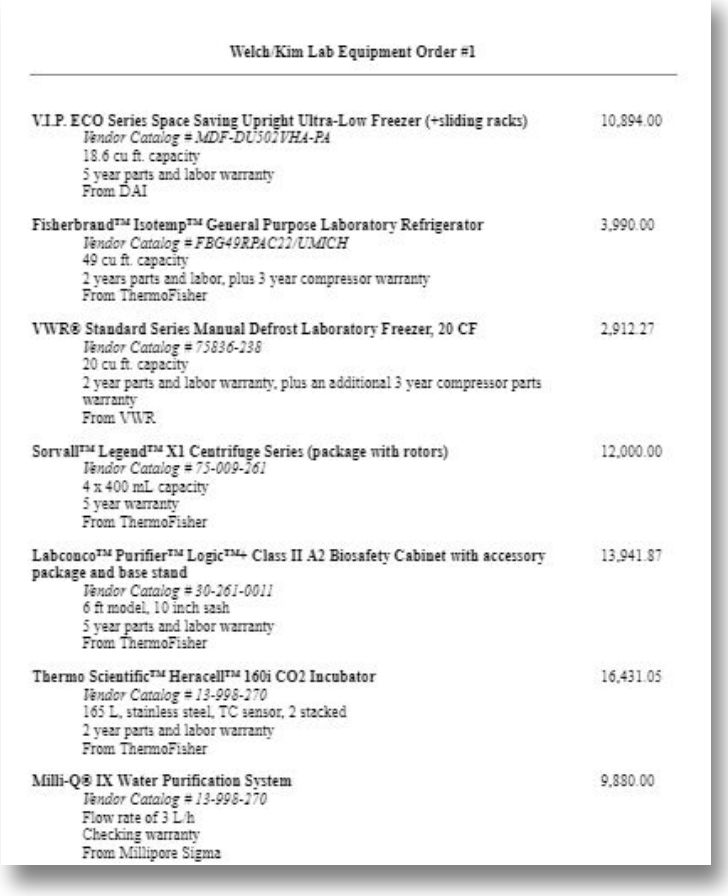
How to fill it?

1. Construct a list of items
   a. Minji Kim's list + review of protocols for our experiments + discussion with collaborators
2. Shop around to sales reps

   a. Theoretically, this should instead be done via a competitive bidding process if over 10K, unless the manufacturer is a "strategic supplier"
3. Get quotes, compare
   a. Specs, lead times, costs, bundling/deals
4. Negotiate
5. Place a PO

Where we are

- We have a “dynamic” list of items
  - Major, necessary products with limited means of comparison
  - Smaller devices with a large number of specifications to compare
  - Unique products that would be cool to have
- We have met with so many sales reps
  - If you see a posting for a product showcase you should go for the food
- Our first set of items is **PROCESSING THROUGH PROCUREMENT**!



Welch/Kim Lab Equipment Order #1

V.I.P. ECO Series Space Saving Upright Ultra-Low Freezer (+sliding racks) — 10,894.00
Vendor Catalog # MDF-DU502VHA-PA
18.6 cu ft. capacity
5 year parts and labor warranty
From DAI

Fisherbrand™ Isotemp™ General Purpose Laboratory Refrigerator — 3,990.00
Vendor Catalog # FBG49RPAC22/UMICH
49 cu ft. capacity
2 years parts and labor, plus 3 year compressor warranty
From ThermoFisher

VWR® Standard Series Manual Defrost Laboratory Freezer, 20 CF — 2,912.27
Vendor Catalog # 75836-238
20 cu ft. capacity
2 year parts and labor warranty, plus an additional 3 year compressor parts warranty
From VWR

Sorvall™ Legend™ X1 Centrifuge Series (package with rotors) — 12,000.00
Vendor Catalog # 75-009-361
4 x 400 mL capacity
5 year warranty
From ThermoFisher

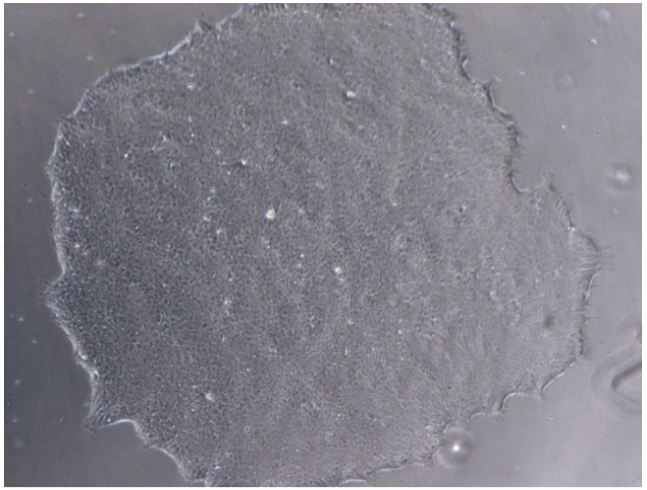
Labconco™ Purifier™ Logic™+ Class II A2 Biosafety Cabinet with accessory package and base stand — 13,941.87
Vendor Catalog # 30-261-0011
6 ft model, 10 inch sash
5 year parts and labor warranty
From ThermoFisher

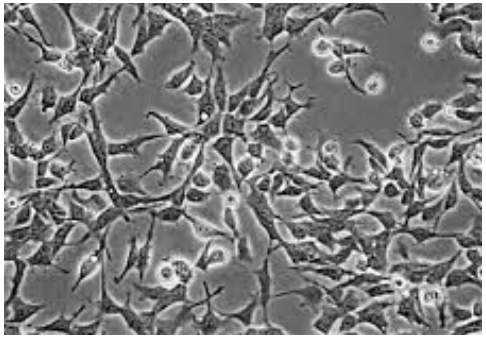
Thermo Scientific™ Heracell™ 160i CO2 Incubator — 16,431.05
Vendor Catalog # 13-998-270
165 L, stainless steel, TC sensor, 2 stacked
2 year parts and labor warranty
From ThermoFisher

Milli-Q® IX Water Purification System — 9,880.00
Vendor Catalog # 13-998-270
Flow rate of 3 L/h
Checking warranty
From Millipore Sigma

… we need cells, too.

- iPSCs
  - Allen Cell Line, well-characterized fluorescently tagged iPSCs
    - Allowing for straightforward visualization of cellular structures
  - WTC-11 (GM25256), the parental line for Allen Cell Line
    - **DELIVERED** (and stored in the Lama Lab)
  - Control lines for comparison
    - **TRANSFER ARRANGED** from o'Shea Lab





- HEK293s
  - Well-known immortalized "embryonic kidney" cell line
    - Likely adrenal cells
  - Used for virus generation and media conditions

What are our next purchasing steps?

- Bargain with sales reps for bundles of mid-sized equipment
- Put together Equipment Orders 2,3,... etc.
  - Next will include mid-sized equipment
- Continue to assess the market for specialized equipment, namely
  - Fluorescence microscope
  - qPCR machine
  - Sequencing-related tools
- Meet with core facilities to determine needs and material requirements
- Getting deliveries and doing setup (albeit likely not much because purchases are full service)
- Think about consumables

Are there any new technologies/equipment you think we should look into?

Discussion Question

Facilities

We have a room. Is it ready?

- Not really…yet
- When we showed up, the room was filled with old papers
- Through efforts primarily by Walter (Indika's lab manager) and Justin, the room was mostly cleaned
  - Aside from a decrepit -80* freezer, a water purifier, and still many papers
- That being said, the room is well equipped, just not set up

FACILITIES & OPERATIONS UNIVERSITY OF MICHIGAN is here to help!

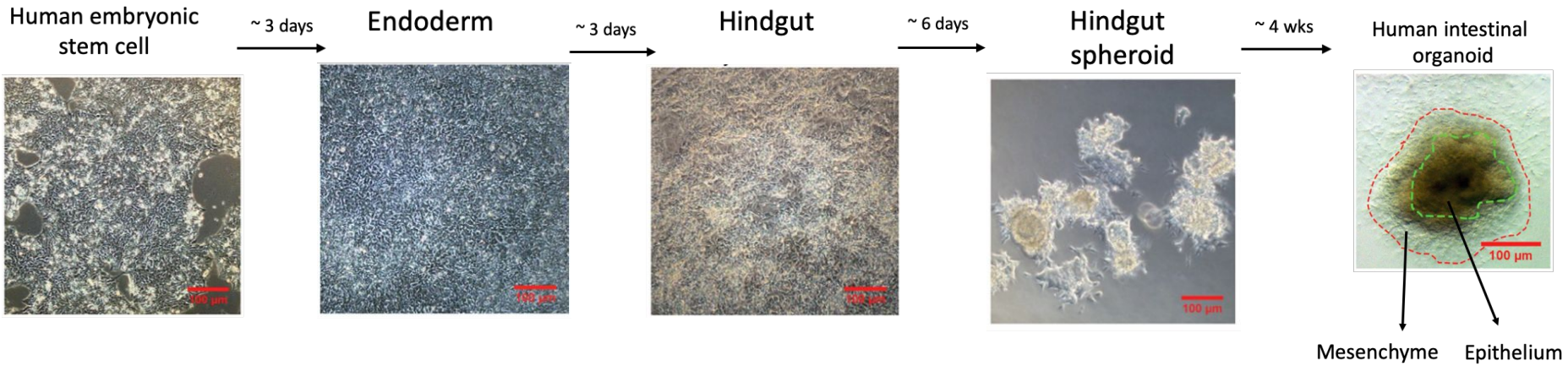
- Removal of extra equipment
  - -80, water purifier, even more papers
- Modification of power/supply lines
  - Seemingly pretty extensive
- Mounting/raising of desks
- Deep clean of space
- Delivery from loading dock/setup for some equipment
  - “White coat” services from large companies
- Free of charge!

Trainings

Specialized trainings will be crucial to successful operations

- Fairly stringent requirements for completing biological research from the university
  - Most on MyLINC, some in person through EHS
  - Administration, purchasing, operations and safety procedures
- Stem cell/organoid studies are well-tread, but are difficult to get right
  - iPSCs require highly specialized care to ensure pluripotency
    - Frequent passaging
    - Checks on morphology
    - Specialized media
  - Organoids
    - Many unique steps over a long period (>1 month for maturity)
    - Likewise, many opportunities for failure along the way
- Core facilities generally have trainings

Justin will be skilled in growing enteroids



- Media must be changed every day (up to hindgut)
- Specific growth factor(s) is required at different stage of the differentiation
- If the plate gets contaminated, you have to start over..!
- Many have failed thus far to replicate Spence lab's intestinal organoids protocol…!
- We will both be learning iPSC culture

We will be learning a lot from the Cores

- iPSC cell culture training from the Pluripotent Stem Cell Core
  - A week+ of intensive training growing and maintaining our own iPSCs
- Project planning and consultation with the Advanced Genomic Core and Epigenomics core
- Microscope-specific trainings for the Microscopy Core
  - Interested in the fluorescence light sheet Zeiss for 3D cell painting
- Basics of Flow Cytometry

What skills would you like to practice in the wet lab?

Discussion Question

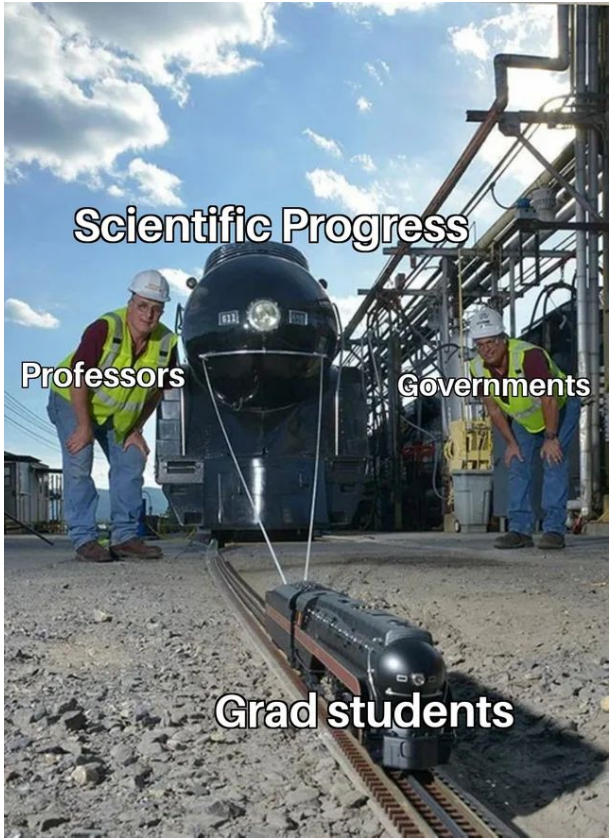
Conclusion

Who has helped us so far?

- Spence Lab (Sha in particular)
  - Support in planning lab setup, offers for consumables group ordering and transfer of media-conditioning HEK cells, training Justin to grow enteroids
- O'Shea Lab/Pluripotent Stem Cell Core
  - Free transfer of multiple iPSC control lines, paid extensive training in iPSC culture/research
- Lama Lab
  - Storage of our iPSCs that arrived way too early
- Walter from the Rajapakse Lab
  - Lab manager guidance, support in cleaning our lab space
- Drs. Aaron Morris and Sasha Cai Lesher-Perez
  - Guidance on lab setup as new faculty
- Shannon and Franchesca from EHS
- Adam from Facilities
- … and many more

Opportunities

- The cells aren't going to pipette themselves!
  - Students interested in any element of cell culture work, molecular biology techniques, or designing sequencing experiments should reach out to me
  - We can be flexible – so much of this is about creating well rounded bioinformaticians
- Think about integrated methods design
  - You choose the data and the analysis methodology
  - Innovate where you see fit



Questions?

Slack me or email sodicoff@umich.edu