HTBAA Homework #1

(1) What you would like to biomanufacture and why?

- **Describe the target product or output you would like to make.**
- **Why are you interested in making it? Your motivation might be utilitarian—for example, addressing a problem in health, sustainability, food, energy, or manufacturing—but it does not need to be. Your project might instead have a social, cultural, artistic, or personal motivation.**
- **Clearly articulate what you want to make, who or what it is for, and why you think it is interesting or important.**

I would like to develop a platform for producing **patient-derived intestinal organoids in a defined, reproducible, and cost-effective synthetic extracellular matrix (ECM)**. The long-term objective is to use this platform to study inflammatory bowel diseases (IBD), particularly Crohn's disease and ulcerative colitis, and eventually generate patient-specific functional profiles and treatment-response predictions.

This project is directly connected to my current PhD research. I am working on intestinal biology and organoids, and I see the development of an IBD-oriented platform as a potential direction for the second stage of my doctoral research. The project would therefore allow me to combine my current work with synthetic biology, tissue engineering, automation, and computational analysis.

IBD remains a major challenge because its underlying mechanisms are complex and heterogeneous, and patients with the same diagnosis can exhibit different biological phenotypes and responses to treatment. Although organoids provide a useful model of human intestinal tissue, their broader application is limited by factors such as the complexity and variability of extracellular matrices, the need for multiple growth factors and specialized culture media, cost, and difficulties in standardizing and scaling the system.

For this reason, I am particularly interested in developing a platform that addresses not only the biological model but also its **manufacturing requirements**. The goal would be to develop a synthetic or semi-synthetic ECM and, where economically and technically feasible, lower-cost sources of the media components and growth factors required for intestinal organoid culture. This could make the system more reproducible, accessible, and compatible with automated and high-throughput applications.

The immediate output would be standardized intestinal organoids derived from individual patients. These organoids could be exposed to controlled inflammatory conditions or therapeutic compounds and analyzed through imaging and molecular profiling. In the longer term, the resulting data could be used to identify disease-associated phenotypes and develop predictive models of therapeutic response.

Thus, the project is intended initially as a **research and translational platform for IBD**, rather than as a direct replacement for conventional clinical diagnosis. Its broader objective is to create a scalable system in which patient-derived cells, synthetic biomaterials, biological signals, automation, and computational analysis can be integrated to study intestinal disease and, eventually, support more personalized approaches to treatment.

(2) What living system would you use?

- **Propose at least one living-system manufacturing chassis that could produce your target—for example, a microbial, mammalian, or cell-free system.**
- **At a high level, describe how the system would make your target product. What goes into the system? What biological process or processes occur? What comes out?**
- **You do not need to design the complete biological system yet. Make reasonable assumptions where necessary and identify them.**

The product I would initially like to biomanufacture is a synthetic or semi-synthetic intestinal ECM capable of supporting the growth and organization of patient-derived intestinal organoids. Rather than trying to reproduce the entire natural intestinal ECM, I would aim to identify and reproduce the key physical and biochemical properties required for intestinal stem-cell maintenance, proliferation, differentiation, and three-dimensional organization. A defined matrix could potentially provide greater reproducibility, controlled composition, easier quality control, and better compatibility with automated culture systems. In addition, I would investigate whether some of the growth factors required for organoid culture, such as Wnt, R-spondin, Noggin, and EGF, could be produced using cell-free protein synthesis. These components could eventually form a modular culture system in which both the physical environment and the biological signals are more precisely controlled.

(3) How would you use automation?

- **Identify at least one automation technology that you would combine with your living system.**
- **This could include a large-scale Cloud Lab, a desktop liquid-handling robot such as an Opentrons, a programmable microfluidics platform such as Nuclera's, or another automation technology.**
- **Describe what part of the biomanufacturing workflow you would automate and why. For example, you might automate liquid handling, culturing, assembly, screening, sampling, measurement, optimization, or another step.**

According to the plan we have been developing, automation would not be an accessory element, but a central component for making organoid culture reproducible, scalable, and eventually useful for comparing multiple patients and experimental conditions.

The first technology I would use would be a benchtop liquid-handling robot, such as Opentrons, integrated with 96- or 384-well plates. In the first stage, I would automate the preparation and dispensing of the synthetic extracellular matrix, the addition of biopsy-derived cells, the addition of media and growth factors, and periodic media changes. This would reduce variability introduced by manual handling and standardize culture conditions across patients and experiments.

A second level of automation would involve controlling culture conditions and experimental perturbations. Once the organoids are established, the robot could programmatically administer different concentrations of growth factors, signaling pathway modulators, inflammatory stimuli, or drug treatments. This would make it possible to perform dose-response experiments and systematically compare how organoids derived from different patients respond. In the context of IBD, this step would be particularly important because the goal would not simply be to culture organoids, but to generate an individual functional profile for each patient.

The next step would be to incorporate automated microscopy and imaging. After each experimental condition, an imaging system could automatically record organoid size, number, morphology, structure formation, viability, and other quantifiable phenotypes. These data could then be analyzed using computer vision or machine learning to detect differences that may not be easily identified through manual observation. In this way, the system would move beyond simply automating culture and would also automate phenotypic measurement.

A subsequent step would be to automate sampling and molecular analysis. Depending on the scale, the collection of cells or culture supernatants and the preparation of samples for transcriptomics, proteomics, or other analyses could be automated. At a later stage, these data could be integrated with imaging data and experimental conditions to build computational models capable of identifying patterns associated with different IBD phenotypes or treatment responses.

The complete workflow could therefore evolve as follows:

Patient biopsy → isolation of intestinal cells → incorporation into synthetic ECM → automated culture → automated addition of factors/media → induction of inflammatory conditions or treatments → automated imaging → molecular sampling → computational analysis → patient functional profile.

For the first version of the project, I would not try to automate everything. The most parsimonious strategy would be to begin with Opentrons, multiwell plates, and automated imaging. Once the biological system is reproducible, a more advanced automation infrastructure, such as a Cloud Lab or an integrated robotic platform, could be incorporated to greatly increase the number of patients, conditions, and treatments that can be analyzed.

There is also an important step that I would add to the original plan: automation should also be used to optimize the culture system itself. For example, the composition of the ECM, concentration of growth factors, cell density, and culture conditions could be systematically varied. An automated experimental design could identify which combination produces the most reproducible and robust organoids. This would be particularly relevant for developing a synthetic ECM and lower-cost media and growth factor formulations.

Thus, automation would have four main functions: manufacturing and maintaining the cultures, performing controlled perturbations, measuring the resulting phenotypes, and optimizing the system conditions. This would transform the project from a simple organoid culture into a standardized and scalable experimental platform.

(4) How much would you need to make, and how long would it take?

- **Perform a back-of-the-envelope calculation estimating what it would take to manufacture your target at a meaningful scale.**

(a) How much?

- **Define a scale relevant to your project and calculate approximately how much of your target product you would need to manufacture.**

- **For example, if you are producing a medicine, you might estimate the amount required to treat 1,000 people, everyone in Massachusetts, or everyone in the world. If you are producing a building material, you might calculate the amount required for one structure or an entire neighborhood. If you are producing an art or design artifact, define an appropriate scale yourself.**
- **Clearly show your calculation, including units, assumptions, and sources for important numbers.**

(b) How long?

- **Using your proposed living system and automation technology, estimate how long it would take to manufacture that amount.**
- **Consider quantities such as production rate, yield, volume, number of parallel experiments or reactors, automation throughput, or whatever parameters are most appropriate for your system.**
- **You may discover that the automation technology you would use to prototype your system is very different from what you would use to manufacture at scale. If so, describe both.**
- **Again, we are looking for a reasonable engineering estimate. Show your assumptions and calculations.**

How much would I need to produce, and how long would it take?

I would approach scalability in two stages: first, establishing and validating the complete biological and manufacturing workflow, and then scaling it to a meaningful number of patients.

The first stage would begin with optimization of the synthetic extracellular matrix (ECM). This would be followed by development of the cell-free system for producing selected growth factors, with the goal of reducing dependence on expensive commercial recombinant factors and improving scalability. The protocol for biopsy processing and the tissue bank are already established. However, before moving to patient-derived organoids, I would first establish the organoid culture protocol using mouse colonic tissue. Published murine colonoid protocols generally require approximately 5–7 days to obtain growing organoids after seeding, with subsequent passages every 4–7 days.

I would therefore allocate approximately 2–3 months for the initial development stage: about 3–4 weeks for ECM formulation and optimization, 3–4 weeks for establishing the mouse colonic organoid workflow, and an additional 4–6 weeks for integrating the ECM, culture conditions, and growth-factor system. This would be followed by approximately 2–3 months of sustained testing to demonstrate reproducibility across independent cultures and experimental runs. Thus, a realistic estimate for establishing a functional prototype of the complete circuit would be approximately 4–6 months. This is intentionally conservative because the objective is not simply to obtain organoids, but to establish a reproducible system suitable for later automation and patient comparison.

For the population-scale estimate, I would use Rosario as an initial geographical target. Assuming a population of approximately 1.3 million inhabitants and an estimated IBD prevalence of 134 cases per 100,000 inhabitants:

1,300,000 × 134 / 100,000 ≈ 1,742 patients with IBD.

This represents the potential population of patients with IBD in the Rosario metropolitan area. However, the initial platform would not need to process all 1,742 patients. A more realistic first meaningful scale would be approximately 50 patient-derived organoid profiles per year. This corresponds approximately to the estimated 35–50 new IBD diagnoses per year in Rosario based on the incidence range provided, while also allowing recruitment of some previously diagnosed patients. This would represent roughly 3% of the estimated prevalent IBD population and would already provide a substantial initial dataset.

Assuming that one patient requires approximately 8 experimental wells for the core assay—including biological replicates, controls, and treatment conditions—a 96-well plate could accommodate approximately 12 patients:

96 wells / 8 wells per patient = 12 patients per plate.

Therefore, processing 50 patients per year would require approximately:

50 / 12 ≈ 4.2 → 5 plates per year.

A 384-well format could theoretically increase this to approximately 48 patient-equivalents per plate under the same eight-well-per-patient assumption, although the 96-well format would be more appropriate during initial validation. Opentrons Flex is compatible with both 96- and 384-well formats and has a 96-channel pipette specifically designed for high-throughput liquid handling.

The relevant production unit, therefore, would not simply be one organoid. It would be one standardized patient-derived organoid assay containing multiple biological replicates and experimental conditions. At a scale of 50 patients per year, the system would generate approximately 400 patient-assay wells annually, before including controls and additional optimization experiments.

In terms of time, the biological expansion of a patient-derived intestinal organoid culture is the main constraint rather than liquid handling. Published human intestinal organoid protocols report approximately 2–4 weeks from initial biopsy establishment to sufficient biomass for experiments and cryopreservation, although previously established cultures can expand more rapidly. Therefore, I would initially plan approximately 3–4 weeks from patient biopsy to a standardized experimental assay, while recognizing that this could decrease as the protocol becomes optimized and the tissue bank provides established lines.

At 50 patients per year, the average throughput required would be:

50 patients / 12 months ≈ 4.2 patients/month,

or approximately one new patient every 6–7 days.

This is compatible with a small automated workflow because patient cultures would be staggered rather than processed simultaneously. The Opentrons system could perform liquid handling, medium changes, growth-factor addition, treatment administration, and plate preparation while different patient-derived cultures are at different stages of expansion. Its 96-channel configuration allows entire 96-well plates to be manipulated in parallel, reducing the manual component of the workflow.

At the prototype stage, I would therefore use a benchtop automation system such as Opentrons together with 96-well plates and automated imaging. At larger scale, the same biological workflow could be transferred to a more integrated robotic or Cloud Lab infrastructure, with automated plate handling, imaging, sampling, and molecular analysis. The transition to 384-well plates would further increase experimental density and reduce reagent consumption.

Overall, the proposed development timeline would be approximately 4–6 months to establish the integrated prototype, followed by approximately 2–3 months of sustained reproducibility testing. Once validated, a target throughput of approximately 50 patient profiles per year would be a realistic first population-scale implementation, with the possibility of increasing capacity substantially through 384-well plates, parallel cultures, automated imaging, and more advanced robotic infrastructure.

Ultimately, this project would be conceived not simply as a diagnostic system for IBD, but as a scalable synthetic-biology platform for producing standardized human intestinal models and generating functional disease-response data. IBD would represent the initial application, allowing the platform to be developed and validated using patient-derived organoids, but the underlying infrastructure could later support hundreds or thousands of patient samples, automated drug screening, treatment-response testing, and the study of other intestinal diseases such as colorectal cancer or celiac disease. In parallel, the development of a defined synthetic ECM and potentially scalable production of organoid growth factors could become independent technological branches applicable to other organoid and tissue-engineering systems. This broader scalability is what would justify the investment in automation and infrastructure: rather than supporting a laboratory that processes only a limited number of patients per year, the objective would be to establish a reusable biological manufacturing platform capable of generating models, experiments, and data at progressively larger scales.

5. What don't you know yet?

- **After completing your analysis, identify the 2–3 biggest technical unknowns, bottlenecks, or assumptions in your proposed biomanufacturing system.**
- **What would you need to learn, measure, or experimentally determine to know whether your idea could actually work?**

The first major uncertainty is whether the proposed synthetic extracellular matrix (ECM) can effectively replace Matrigel for intestinal organoid culture. Although I have not yet established the composition or formulation of the proposed ECM, alternative and defined matrices for organoid culture have already been explored, providing evidence that Matrigel replacement is technically plausible. The main challenge is therefore to determine whether a new matrix can provide the appropriate biochemical and mechanical environment for intestinal stem-cell survival, proliferation, and differentiation while remaining reproducible, stable, and

substantially less expensive. I would need to experimentally determine its composition, physical properties, organoid formation efficiency, growth, viability, differentiation, and long-term expansion compared with Matrigel.

The second major uncertainty is whether a cell-free protein synthesis system would be technically and economically suitable for producing the growth factors required for organoid culture. I currently know cell-free systems mainly at a theoretical level, so I cannot assume that they will efficiently produce functional proteins such as Wnt, R-spondin, Noggin, or EGF at the required yield, stability, and cost. This would need to be experimentally determined by evaluating protein production, biological activity, stability, and cost per culture. If this approach is not feasible for some factors, commercial recombinant proteins would remain an alternative. Importantly, the development of a successful cell-free production strategy for organoid growth factors could itself become a scalable technological branch of the project, beyond its use in the IBD platform.

A third uncertainty is the overall cost and reproducibility of the complete system. Even if the ECM and selected growth factors could be produced internally, some expensive specialized components would still need to be purchased. In particular, supplements such as B-27 and potentially other specialized organoid-culture components may remain significant cost contributors and may not be practical targets for the same cell-free manufacturing strategy. I would therefore need to determine the real cost per culture after replacing the two major cost drivers -Matrigel and recombinant growth factors- and identify whether the resulting system provides a meaningful economic advantage.

To determine whether the concept is viable, I would first compare the synthetic ECM with Matrigel using standardized mouse colonic organoids. I would measure organoid formation, growth, morphology, viability, differentiation, and expansion capacity. Once the matrix is validated, I would test whether selected growth factors can be produced using a cell-free system and whether the resulting proteins retain biological activity. Finally, I would integrate the validated components and determine whether the complete system produces reproducible organoids at lower cost and with sufficient scalability for patient-derived samples.

The key unknown is therefore not whether these technologies exist individually, but whether they can be combined into a robust, reproducible, biologically functional, and economically viable workflow. If successful, however, the development of a defined intestinal ECM and potentially scalable production of organoid growth factors would represent advantages not only for the IBD platform itself, but also potential independent technological and commercial branches that could be adapted to other organoid and tissue-engineering applications.