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White Paper

Relating Dietary Health to Accurate Quantification of the Gut Microbiome Using xMAP® Technology from the Molecular Diagnostic Company Genetic Analysis AS

By Dominic Andrada

Problem: The need to accurately measure DNA abundancies in microbiome research

The human gut microbiome has been a major topic of medical research. Bacteria residing in the gastrointestinal tract modulate many bodily functions from nutrient metabolism to immune system regulation.[1] Microbiome researchers generate these insights by comparing the abundance of DNA sequences mapped to different bacterial taxonomic groups between experimental samples. However, these abundance are typically based on changes in the proportions of mapped reads between samples. Conclusions drawn from these measurements can generate systemic biases that make comparing datasets and obtaining reproducibility challenging.[2] Hence, a means to quantify the actual amount of DNA belonging to a taxonomic group would help researchers examine the gut microbiome in human health.

Method: Identifying microbiome-based markers of human health with xMAP® Technology

To address the problem of accurate DNA quantification in microbiome research, scientists from Oslo Metropolitan University, Norway, utilized the GA-map™ Dysbiosis Test from the molecular diagnostic company Genetic Analysis AS (Oslo, Norway). This test which utilizes a fecal sample from its home collection kit is based on Luminex's xMAP Technology for multiplex molecular testing. The genomic test is composed of 48 single-stranded DNA probes coupled to xMAP MagPlex® Microspheres that can target ≥300 bacteria on different taxonomic levels. These sequences are complementary to different 16S rRNA sequences from the V3-V7 regions, providing higher taxonomic mapping resolution of selected bacteria in the intestinal microbiota. Specific 16S rRNA target sequences hybridize to their complementary DNA probe on the microspheres, after sample extraction, fluorescent labelling, and amplification by PCR.

For quantifying DNA belonging to a taxonomic group, prepared samples were read on the Luminex® 200™ instrument. The readout consisted of median fluorescent intensity of the labelled target sequences and the unique signature of the bead to which they were hybridized. Measured fluorescent intensity indicates bacterial abundance, while bead signature delineates the taxonomic group being measured. Measuring fluorescent signal intensities with xMAP Technology provides researchers with a standardized approach to quantify the abundance of many taxonomic groups within a sample beyond its proportions.

With diverse microbial taxa from Bacteroidetes to Verrucomicrobiota covered by probes in the GA-map® Dysbiosis Test, the researchers could now investigate the relationship between the gut microbiome and dietary markers of health with a cross-sectional study.[3]

Results: Generating robust correlations with human microbiome data

Using the GA-map® Dysbiosis Test allowed the researchers to identify a series of trends between the gut microbiome and dietary indicators of health. Here are just some of the correlations they observed through Pearson's correlation analyses (**Figure 1**):

- ***Streptococcus* spp.**: Elevated *Streptococcus* abundance were positively associated with whole-flour bread and trans-fat consumption in healthy participants (Pearson correlation ≥ 0.3) (**Figure 1**).
- ***Bacteroides stercoris***: The researchers observed increased *B. stercoris* abundance that were correlated with increased fiber consumption among healthy participants (Pearson correlation ≥ 0.3) (**Figure 1**). This feature aligns with observations that *Bacteroides* can degrade fibers and complex sugars with its array of carbohydrate-active enzymes (CAZymes).[4]
- **Bacilli**: The abundance of bacilli within Firmicutes was also significantly negatively correlated with systolic and diastolic BP values (Pearson correlation ≤ -0.3) (**Figure 2**).
- ***Lactobacillus* spp.**: Lower abundance of *Lactobacillus* spp. were significantly correlated with higher systolic and diastolic blood pressure ($P < 0.05$) after adjusting for age, sex, and body mass index (BMI) (**Table 1**). This result corroborates previous research suggesting that *Lactobacillus*-based probiotics can improve blood pressure control.[5]
- **Other bacterial taxa**: The researchers also observed the abundance of *Alistipes onderdonkii* and *Akkermansia muciniphila* being correlated with fat levels (%) (**Figure 2**). The latter is surprising given their correlation with reduced body fat after fecal microbiota transplantation.[6]

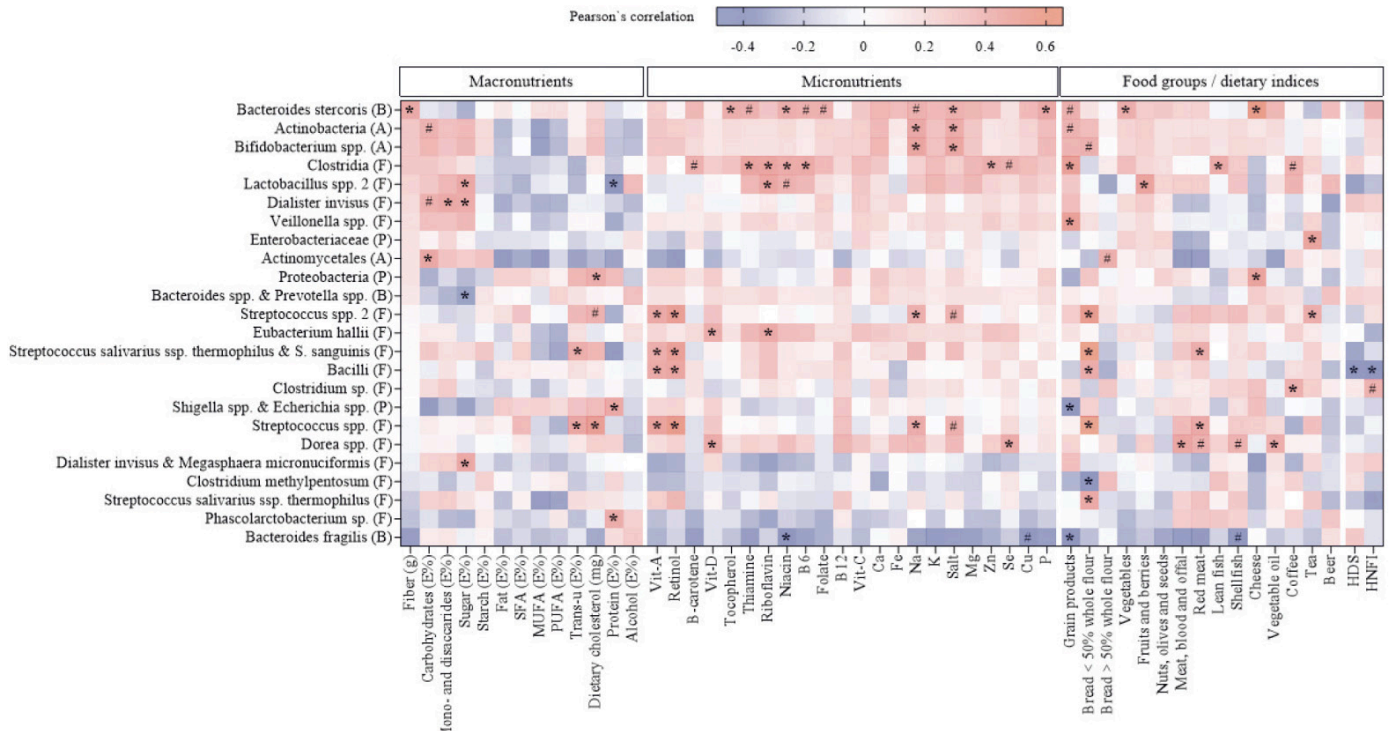

Figure 1. Heat map correlating the abundance of microbial taxa with nutrient and food group intake.[3]
Significant correlations (Pearson's correlation coefficient ≥ 0.3) are marked with an asterisk. The taxonomic level of each taxon is indicated beside their names in brackets. A, Actinobacteria; B, Bacteroidetes; F, Firmicutes; P, Proteobacteria.

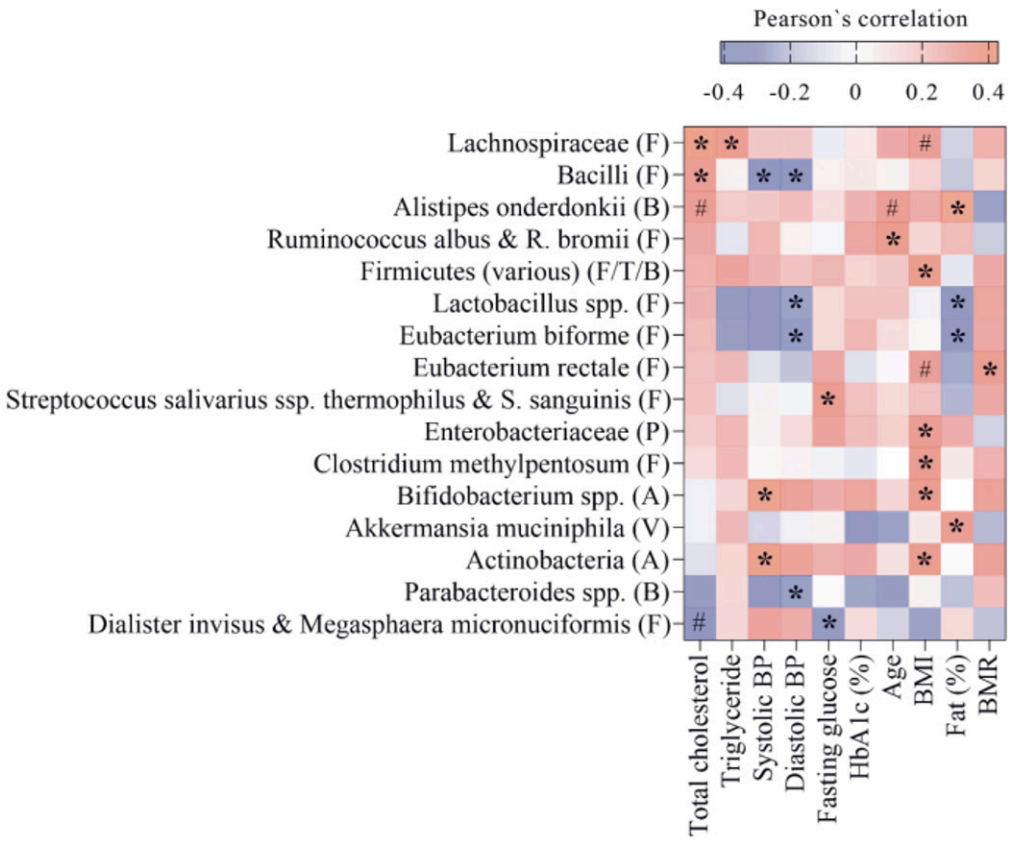

Figure 2. Heat map correlating the abundance of specific gut microbial taxa with metabolic and anthropometric markers.[3]
Significant correlations (Pearson's correlation coefficient ≥ 0.3) are marked with an asterisk. The taxonomic level of each taxon is indicated beside their names in brackets. A, Actinobacteria; B, Bacteroidetes; F, Firmicutes; P, Proteobacteria; T, Tenericutes; V, Verrucomicrobiota.

Table 1. Linear regression model for correlating gut bacteria abundance with blood pressure and total cholesterol levels.[3]

Gut bacteria	Unadjusted values		Adjusted values			
	B	95% CI	*B*	95% CI	*P*‡	*P*♯
Systolic BP (≥ 120 mmHg) (*n* = 24)						
(A) *Actinobacteria*	0.285	0.014, 2.028	0.238	−0.196, 1.903	**0.047**	0.108
(A) *Bifidobacterium*	0.305	0.097, 2.201	0.251	−0.152, 2.045	**0.033**	0.090
(F) *Lactobacillus* spp.	−0.229	−2.269, 0.251	−0.309	−2.583, −0.140	0.114	**0.030**
Diastolic BP (≥ 80 mmHg) (*n* = 10)						
(B) *Bacteroides stercoris*	−0.300	−2.570, −0.087	−0.320	−2.718, −0.123	**0.036**	**0.033**
(B) *Bacteroides* spp. and *Prevotella* spp.	−0.288	−1.259, −0.015	−0.225	−1.163, 0.166	**0.045**	0.138
(B) *Bacteroides* spp.	−0.279	−2.348, 0.010	−0.335	−2.635, −0.166	0.052	**0.027**
(F) *Bacilli*	−0.350	−1.286, −0.155	−0.387	−1.404, −0.189	**0.014**	**0.011**
(F) *Dialister invisus* and *Megasphaera micronuciformis*	0.330	0.363, 4.138	0.384	0.644, 4.584	**0.020**	**0.010**
(F) *Eubacterium biforme*	−0.345	−3.487, −0.389	−0.313	−3.361, −0.157	**0.015**	**0.032**
(F) *Eubacterium rectale*	−0.325	−2.661, −0.209	−0.357	−2.814, −0.343	**0.023**	**0.013**
(F) *Lactobacillus* spp.	−0.378	−3.553, −0.580	−0.348	−3.400, −0.407	**0.007**	**0.014**
(F) *Streptococcus* spp. 2	−0.294	−1.217, −0.029	−0.305	−1.284, −0.007	**0.040**	**0.048**
Total cholesterol (≥ 5.0 mmol/L) (*n* = 20)						
(F) *Ruminococcus albus* and *R. bromii*	0.374	0.433, 2.739	0.245	−0.579, 2.659	**0.008**	0.202

[1]Gut bacteria values were log-transformed before analysis.
‡*P* for unadjusted values assessed by a linear regression model.
♯*P* for values adjusted for age, sex, and BMI, assessed by a linear regression model.
Phyla are indicated within parentheses; A, Actinobacteria; B, Bacteroidetes; F, Firmicutes.
The level of significance was set at $P < 0.05$ and are indicated in bold italic.

Conclusion: Elucidating microbiome-based markers of human health

Together, the GA-map® Dysbiosis Test with xMAP Technology provided researchers with the platform to generate quantitative insights into the human gut microbiome. On one hand, the data agreed with the previous literature, strengthening possible connections between gut microbes and health status. However other correlations differed from the literature, providing room for further research to resolve the contrasting results. This standardized, multiplex test provides scientists with strong opportunities to complement existing microbiome research efforts with accurate, reproducible quantification of microbial taxa. Continued efforts with the GA-map® Dysbiosis Test will allow scientists to develop confident insights relating aberrant changes to the gut microbiome and its' association with disease.

xMAP Technology for easy quantitative, multiplex genetic analysis

In addition to the GA-map® Dysbiosis Test, xMAP Technology is utilized in other customized genetic tests. Various assay formats can be developed by your lab or chosen from ready-made kits from Partner vendors. Our LuminexPLORE Lab* provides technical expertise and assay services, as Luminex is an established leader in reliable and proven proteomic and genomic assays in medical research and clinical diagnostics.

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Change this statement to:

Genetic Analysis is an ISO 13485:2016 certified company. The GA-map® Dysbiosis Test Lx is CE-marked in Europe and the GA-map® is available for research in the US. For all other regions this product is for Research Use Only. Not for use in diagnostic procedures.

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TIERRA

Selecting a Cell-Free Protein Synthesis System

CELL-FREE PROTEIN SYNTHESIS

Navigate the cell-free system landscape and key influencing factors to optimize protein synthesis for your target proteins.

IN BRIEF

- Prokaryotic and eukaryotic systems have unique advantages and disadvantages for CFPS protocols.
  - » Prokaryotic systems are cost-effective with high growth rates but have limited post-translational modifications.
  - » Eukaryotic systems can perform complex post-translational modifications but are generally more expensive, difficult to genetically engineer, and slower in growth.
- Successful CFPS requires understanding protein types and factors affecting yields.
  - » Different proteins require specific reagents and conditions for successful synthesis.
  - » Yield influencers include the biochemical composition of lysates and DNA structures, impacting transcription efficiency.
- Advances in strain engineering enhance the production of biologics through CFPS.
  - » Engineered strains of *E. coli* and eukaryotic cells can produce proteins with non-canonical amino acids and industrial-scale protein quantities.
  - » Genetic modifications drive research and development by enabling detailed studies of biochemical pathways and protein interactions.

Picture a future where high-quality therapeutic proteins are manufactured in hours instead of days. Cell-free protein synthesis (CFPS) platforms enable reliable protein synthesis at any scale, driving biotherapeutic discovery and production. This protocol uses cellular lysates to extract the transcription-translation machinery and energy components, redirecting them to produce a target protein.

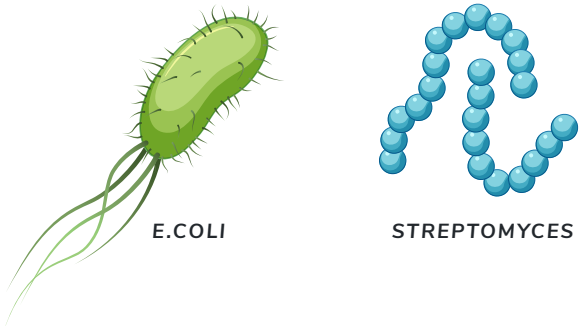
As CFPS becomes more affordable, various systems have emerged, necessitating careful selection to optimize protein yield and quality[1]. Here, we explore the current CFPS landscape, factors influencing system choice, and the role of strain engineering in the evolution of CFPS.

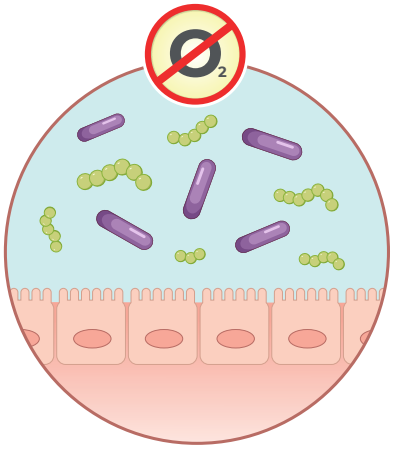
Prokaryotic CFPS systems

Prokaryotes couple transcription and translation through ribosomes associating with mRNA before transcription is completed[2]. This simultaneous process minimizes naked mRNA accumulation and resource depletion, enhancing efficiency during protein synthesis[3]. The potential was first realized over 60 years ago with an *E. coli*-based CFPS system, leading to the synthesis of various therapeutically relevant proteins[4].

Since then, multiple prokaryotic-based CFPS platforms have generated an array of biopharmaceutical proteins, including:

- ***E. coli*:** The first bacterial species used for CFPS, *E. coli*, is cost-effective due to its high growth rates, aiding in the discovery of SARS-CoV-2 antibodies and Shiga toxin characterization[5,6].
- ***Vibrio spp.*:** Fast-growing *Vibrio natriegens* synthesizes proteins efficiently, supported by 3-PGA as an energy source, making it ideal for producing nonribosomal peptides and natural products such as antibiotics[7-9].
- ***Streptomyces*:** *Streptomyces* has been a major source of natural products in medical care, from antibiotics to crop-protecting agents. Cells in this genus harbor enzyme-coding genes that confer strong capabilities to be a CFPS system[10]. Most notably, species in this genus can express phosphopantetheinyl transferases that activate carrier proteins involved in the biosynthesis of complex natural products like antibiotics and polyketides[11].
- **Anaerobes:** The emerging use of anaerobic bacteria in CFPS is opening new possibilities for sustainable production[12]. Recently, companies like LanzaTech have announced the development of commercial-scale bioreactors that use anaerobes to metabolize gas emissions to produce ethanol and other compounds.





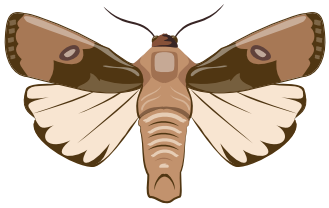
In addition to these species, multiple strains within each confer distinct advantages for CFPS. For example, *E. coli* DE3 strains eliminate the need to add polymerases by inducing sufficient T7 RNA polymerase expression[13].

Even so, most prokaryotes produce a limited number of post-translational modifications, making them less suitable for producing complex proteins with glycosylation or disulfide bonds, which are essential for their function and therapeutic efficacy[14].

Eukaryotic CFPS systems

The challenge of producing proteins with post-translational modifications in prokaryotic systems has led to the increased use of eukaryotic CFPS systems. Several eukaryotic systems have been developed and tailored for protein synthesis, including:

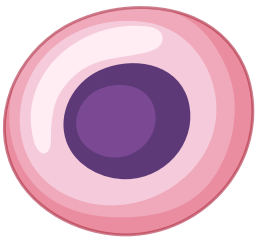
- ***Spodoptera frugiperda 21 (Sf21)*:** Despite being a pest, the fall armyworm's cell extracts are valuable for producing integral membrane proteins[15,16].
- **Wheat germ:** Wheat germ is used to produce proteins at the milligram scale. Although raw extracts are challenging to process, refined protocols minimize RNase activity and reduce yield variation. These improvements have enabled wheat germ systems to produce purified recombinant proteins and vaccine candidates, such as those for malaria[17-21].
- **Tobacco BY-2:** Tobacco Bright Yellow-2 (BY-2) lysates offer high translational activity, quick preparation, and scalability to milligram and gram levels of protein. BY-2 cell lysates were used to produce the first FDA-approved recombinant pharmaceutical protein, Taliglucerase alfa (Elelyso®)[22].
- **Human cell cultures:** Various human cell lines, such as HEK293, HeLa, and CHO cells, are used to express diverse proteins using an in vitro coupled transcription/translation system. Like Sf21, human-based systems have ER-derived microsomes that facilitate the production of integral membrane proteins[23].



SPODOPTERA FRUGIPERDA 21

WHEAT GERM

TOBACCO BY-2



HUMAN CELL LINES

Factors to consider when selecting a CFPS system

Like prokaryotic systems, eukaryotic CFPS systems have unique advantages and drawbacks that impact protein synthesis (Table 1). Each system's characteristics must be evaluated to select the best one for specific proteins. Here are key factors to consider when choosing a CFPS system:

Table 1. Advantages and disadvantages of the most common cell-free systems for protein synthesis.

Cell-free system	Protein expression yield	Application	Advantages	Disadvantages
Prokaryotic systems	Milligram scale (e.g. *E. coli* at 2300 µg/mL)	▪ Monoclonal antibodies ▪ Antimicrobial peptides ▪ Vaccines	▪ Low cost ▪ High protein yields ▪ Ease of preparation ▪ Highly optimized	Cannot produce PTMs
Wheat germ	Microgram scale	▪ Membrane proteins ▪ Large proteins (~200kDa)	Some PTM production capabilities	▪ Requires exosome microsome addition ▪ Expensive lysate preparation
Sf21	Microgram scale	▪ Biologics ▪ Membrane proteins	High microsome formation, encouraging PTM and membrane protein production	▪ Low protein yields ▪ High cultivation costs
Human cell lines	Microgram scale	▪ Enzymes produced by eukaryotes ▪ Membrane proteins	Can produce proteins with PTMs	▪ Sensitive to additives ▪ Expensive cultivation protocols
Tobacco BY-2	Microgram scale	Membrane proteins	▪ Can produce eukaryotic proteins ▪ Can produce proteins with PTMs ▪ Reactions can be completed in hours	Unoptimized for scaling up

- **Protein Yield:** Different CFPS systems produce varying yields based on the lysates' biochemical composition. Systems that couple transcription and translation typically generate higher yields. Additionally, DNA structures, like a 5'-UTR with an enhancer sequence, can boost transcription and protein synthesis[24,25].
- **Protein Type:** Different proteins require specific reagents and biomolecules. For example, integral membrane proteins need lipid bilayers or vesicles for purity and integrity[26]. Therapeutic proteins like onconases, which cleave tRNAs, require systems that pulse tRNA intake or maintain tRNA stability[27].
- **Post-Translational Modifications:** Some CFPS systems, particularly prokaryotic-based and wheat-germ systems, cannot perform post-translational modifications. For producing monoclonal antibodies with disulfide bridges or glycosylated proteins, eukaryotic CFPS systems are more suitable[28].

Evolution of CFPS systems

Currently available prokaryotic CFPS systems can produce a wide variety of biopharmaceutical proteins. However, each species has unique strains that can further enhance protein yields and quality. Prokaryotic systems, particularly *E. coli*, can be engineered to synthesize non-canonical amino acids using strains like C321.DA[29].

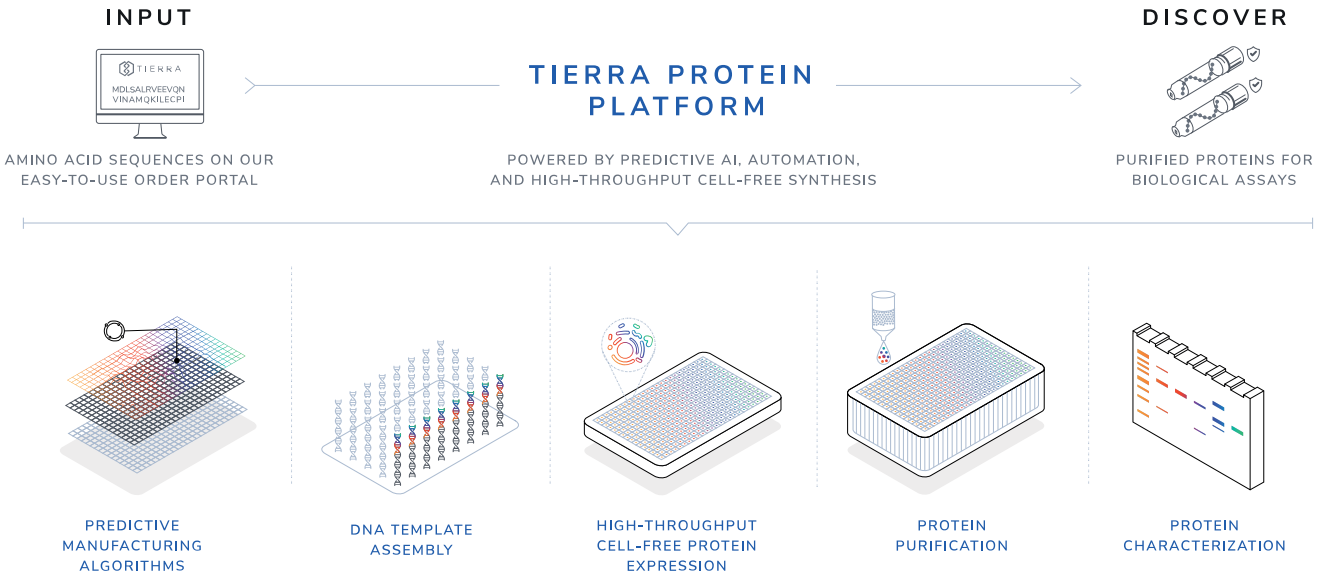
Eukaryotic CFPS systems can also be cloned to produce specific proteins at an industrial scale. Additionally, genomes from both prokaryotic and eukaryotic systems can be engineered to study biochemical pathways and assess protein interactions, driving R&D efforts[30,31].

CFPS systems offer diverse and versatile formats for synthesizing specific proteins, each with unique advantages and considerations. Understanding the landscape of prokaryotic and eukaryotic systems, as well as the key influencing factors such as protein type, yield, and post-translational modifications, is essential for optimizing protein synthesis.

The future of advanced CFPS systems looks bright, with genetic engineering addressing shortcomings of today's systems by enhancing protein yields, quality, and functionality. Moreover, the integration of automation, artificial intelligence, and machine learning is further streamlining protein production, enabling precise control and high-throughput screening at unprecedented scales.

Work With Us

Accelerate your scientific discovery with the Tierra Protein Platform. Our platform integrates automation, computation, and high-throughput screening allowing you to synthesize custom proteins from diverse sources at the click of a button. Visit our ordering portal or contact Tierra Biosciences today to learn how our platform and expert guidance can drive the success of your programs.



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Using PCR to Fight Antimicrobial Resistance

Last Updated on July 31, 2023

By Excedr

The World Health Organization has named antimicrobial resistance (AMR) as one of the most pressing threats to global health. Over 1.27 million deaths worldwide have been attributed to

Current AMR testing results need 2 to 3 days to be produced. This represents a critical time delay that increases treatment costs and elevates the risk of death. Such a delay also makes AMR monitoring in the environment harder. The biotech industry has made efforts to develop new antibiotics. Nonetheless, a holistic approach to combating AMR also needs a means to track its spread.

Having tools that can quickly identify antimicrobial-resistant microbes is essential for addressing the AMR crisis. That's where the polymerase chain reaction (PCR) comes in. For one, PCR can detect AMR genes in the environment. PCR can also help inform clinicians of the antibiotics that a microbe is resistant to. Most importantly, PCR assays can be done within a single day, cutting the time needed to begin antibiotic treatment.

But how does PCR produce these results? To better understand PCR's role in fighting the AMR crisis, we first need to discuss how AMR arises in microbes. We'll then discuss how PCR works and how it can detect resistant microbes in the environment. We will then end by highlighting a series of companies developing innovative PCR kits tailored for detecting AMR.

How Does Antimicrobial Resistance Work?

AMR arises when infecting microbes continue to survive even after doctors administer antibiotics. Microbes can gain resistance through several mechanisms.

- **Intrinsic Resistance:** The way a bacterium is structured may confer on it an innate ability to survive antibiotic treatment. Here, the cells don't have to be changed to survive after antibiotic exposure. Cells can be naturally resistant to an antibiotic in many ways. For example, the cells of some microbial species have thick cell walls that prevent antibiotics from entering them.
- **Acquired resistance - mutations:** Microbes can also acquire resistance through genetic mutations. Mutations alter a kind of biomolecule called proteins that affect how cells behave. Genes whose mutations make a microbe more resistant to an antibiotic are called AMR genes. Below are some examples of proteins where gene mutations can affect antibiotic resistance:

- **Efflux pumps: These** are proteins that force toxins such as antibiotics out of the cell. Any antibiotic that must enter the cell will not work as well when the efflux pumps are working. Mutations can increase their activity, contributing to AMR.
- **Beta-lactamases: These proteins** break open what's called a beta-lactam ring. These rings provide the foundation of beta-lactam antibiotics like penicillin. Beta-lactam

- **Proteins involved with DNA replication:** Some antibiotics bind to enzymes that mediate DNA replication, such as DNA gyrase. DNA replication allows genomes, an organism's genetic material, to be copied to the next generation. Stopping this process with antibiotics leads to death. However, mutations to the genes encoding these enzymes can prevent antibiotics from binding.

- **Acquired resistance - taking in foreign DNA:** Microbes can also take genetic material from other bacteria or free-floating DNA in the environment. This occurs through horizontal gene transfer (HGT), where genetic information is moved between organisms from divergent evolutionary lineages. This information can be picked up from the environment or through mobile genetic elements (MGEs) that can jump around within a genome. MGEs can also be transferred to another organism's genome through HGT.

Doctors must be careful not to administer antibiotics that microbes are naturally resistant to. Nevertheless, knowing whether a microbe acquired resistance is a much harder problem to solve. That's why techniques that help people know when and how AMR arises and spreads will help curb the AMR crisis.

What Is PCR?

PCR is one such technique used to monitor AMR spread. It is a foundational technique that allows DNA sequences to be found and amplified. Every PCR assay requires the following reagents and components for it to work:

- **Nucleotides:** The building blocks of DNA. Every nucleotide is composed of a phosphate-sugar backbone with a nitrogen-containing base attached to it. Four types of nucleotides in equal abundances are required to replicate DNA and run the PCR assay: adenine, guanine, thymine, and cytosine.
- **Template DNA:** This is the DNA extracted from a clinical or environmental sample where AMR is being surveyed. Care must be taken to ensure that the target DNA sequences are present for amplification.
- **DNA polymerase:** This is the enzyme that drives DNA replication in the PCR assay. Without this enzyme, no DNA amplification can take place. The polymerase must also have high fidelity to ensure that the replicated DNA contains the correct sequence.
- **Primers:** These short, synthesized DNA sequences, or oligonucleotidesprovide the boundaries for DNA polymerase to replicate DNA. These sequences are typically 18-30 bases and anneal to the ends of the DNA target. Primers can cover DNA sequences as short as 100 base pairs (bp) and as long as thousands of bp in length. Primer design

- **PCR buffers:** These solutions contain the molecules and ions necessary for the DNA polymerase to operate optimally. These include molecules that stabilize the reaction's pH, ions that activate DNA polymerase, and other ions that allow primers to anneal to the target sequence. By definition, buffers also prevent changes to acidity, or pH, that impacts how well the reactions work.
- **Thermal cyclers:** These machines carry out all PCR assays. They contain metal blocks that allow tight temperature control while running the PCRs. These machines also have software that allows users to input the thermal cycling conditions.

Once these reagents are present, the PCR assay proceeds in a series of up to 35 cycles. Each cycle contains a series of three steps that enable DNA targets to be doubled:

- Denaturation: DNA starts as two strands linked together in a helix. In this step, the strands are separated by heating at 94°C.
- Annealing: In this step, the single-stranded DNA molecules are cooled to the annealing temperature (~50-65°C). At this temperature, the primers can bind to the regions flanking the DNA target.
- Extension: This step features the DNA polymerase amplifying the DNA from one end of the primer (the 3' end) to the other (the 5' end).

PCR has many extensions and modifications that have been used for a wide range of applications. Two of these include:

- **qPCR:** In a quantitative PCR (qPCR), assay, fluorescent dyes or DNA probes are added to a typical PCR assay. The binding of the dye or probe sequence to the DNA target allows not only the gene target to be amplified, but its abundance to be measured as well. This can help researchers assess how much and how quickly antimicrobial resistance could be spreading in diverse environments such as wastewater, urban aquatic environments, and soil.
- **Multiplex PCR:** Multiplex PCR introduces multiple primer sets in a single PCR to search for multiple gene targets at the same time. This allows health monitors and scientists to identify MDR bacteria and produce a comprehensive AMR survey for clinical treatment.

Irrespective of the PCR extension employed, PCR assays proceed with a series of common ingredients that must be prepared in the right amounts. Additionally, it's the primers that allow researchers to detect AMR genes in the clinic and environment.

Amplified Global AMR Efforts

PCR assays can produce AMR data more quickly than conventional assays. Multiple biotech companies have sought to develop kits for detecting AMR. Many of these kits can test AMR spread or help doctors diagnose resistant infections.

- Resistomap and Takara Bio: These companies have teamed up to prepare an integrated qPCR workflow to monitor AMR over time. The pipeline begins with Takara Bio's Smartchip Real-time PCR system performing the qPCRs. The qPCRs feature two sets of primers designed to target up to 384 AMR genes at the same time. Then, Resistomap's ResistApp digitally analyzes the generated qPCR data. The app determines the extent of AMR spread by measuring the amount of an AMR gene present relative to a bacterial control gene called 16S rRNA. Absolute quantification of an AMR gene is also possible provided standards containing known amounts of DNA are also prepared.
- Cepheid: The fight against AMR also depends on knowing which microbes are causing the disease. The Xpert Xpress Strep A test provides a way to screen for a specific type of microbe called Group A Streptococcus (GAS). These bacteria cause a wide range of diseases such as Strep throat, rheumatic fever, and necrotizing fasciitis. The assay allows researchers to diagnose GAS more easily without needing to wait for days by growing the infecting microbes in the lab.
- Streck: The Nebraska-based company has developed a series of qPCR kits specifically designed to detect different sets of AMR genes. Their specialty lies in detecting different kinds of ampC genes, each of which encodes a type of b-lactamase such as OXA. The kits can also help find multiple variants of the same ampC gene.
- Hologic: The Novodiag System is an innovative platform that uses multiplex qPCR assays to detect AMR microbes in clinical settings. With this system, patients can obtain results within an hour after sample processing. The Novodiag CarbaR+ panel assay also searches for resistance to two types of antibiotics: carbapenems (eg. Imipenem) and Colistin.

Need a PCR Machine for Your Research?

Diagnostic companies are taking the AMR fight to the environment and the clinic. You too can join the fight. Maybe you're ready to do the PCR assays, but don't know what thermal cycler to use.

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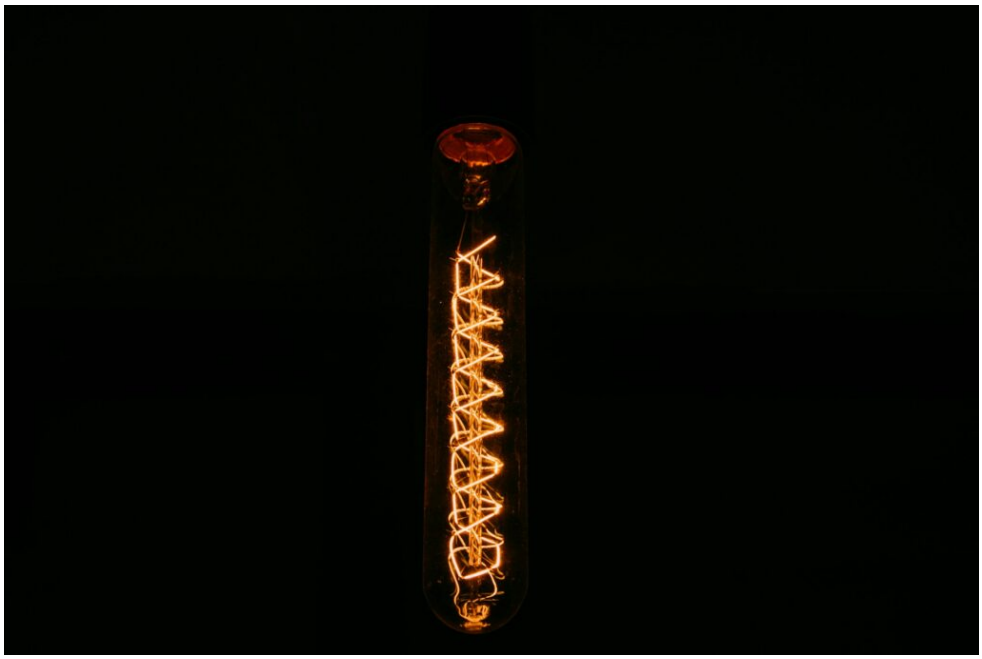
EPIWRITE - MOLECULAR BIOLOGY / 28 MAY 2024

Hazel Jones: The Power of 3D Genomics in Medicine



The human genome encodes human life as we know it. Its DNA sequences encode almost 20000 proteins that affect what we look like, how we behave, and how well our bodies operate. Yet in the process of characterizing the human genome, scientists have also uncovered a plethora of other elements. Although these regions in the human genome do not encode a functional protein, they affect how genes are expressed and impact disease severity and outcomes.

That's not to mention how DNA is structured.

Too often we reduce DNA to strings of four letters: A, T, G, and C. Yet behind these letters are chemical compounds that are bound by specific proteins and generate 3D shapes. In this geometric space, we can learn how our DNA sequences affect who we are. We may also find new ways to treat disease in these structures.

That's why I'm delighted to interview the new CEO of Enhanc3D Genomics, Hazel Jones. Her previous experience with business planning and operations at AstraZeneca leaves her well-suited to continue pushing the company at the forefront of studying the 3D structures of the human genome.

Now read on to learn more about the intricacies of genome structure and how harnessing

GenoWrite

The Interview

PN: When we sequenced the human genome, we thought that we could cure all diseases by that point. But even after that, we still have a long way to go. What information are we still missing?

HJ: When the first sequence of the human genome was released in 2003, we believed we could identify the genes that caused every disease we knew. But since then, we've come to realise that the race to sequence the human genome was not a race to the finishing line; it was a race to the starting line. Only 2% of the human genome encodes a functional protein. In the other 98%, we encounter gene regulatory elements including so-called enhancers that control which genes are expressed where and when in the human body, and their magnitude. These elements do not encode a protein but rather act as 'molecular switches' that control where and when protein-coding genes are expressed. From a disease perspective, enhancers contain most disease-associated gene variants, determined through genome-wide association studies (GWAS).

PN: Tell us a bit more about these disease variants. How do they have such a big role in disease?

HJ: I think it would be best to discuss a specific example: the Sonic Hedgehog (Shh) gene in mice. For video game enthusiasts, the gene is named after the SEGA character Sonic the Hedgehog. In developing limbs, an enhancer sequence located almost a million base pairs away 'jumps over' its neighbouring genes to contact the *Shh* gene and activate its expression. When this enhancer was deleted in a mouse model, the mice are born without limbs. Fascinatingly, single nucleotide changes (i.e. variants) in the human Shh limb bud enhancer cause limb malformations known as polydactyly, as a consequence of altered *Shh* expression. This is a key example of a disease-associated long-range enhancer with evolutionarily conserved function. Identifying the target genes of these long-range enhancers is impossible without profiling the folding of the genome in 3D.

PN: I can't imagine this being the only example of 3D genome structures affecting disease onset.

HJ: I wholeheartedly agree. In fact, there are numerous examples of mutations in enhancers and enhancer dysregulation that cause of human disease – and this class of 'enhanceropathies' is constantly growing. We are probably still only scratching the surface at the moment, and we aren't close to unpacking all of these complex relationships. The challenge in linking changes in 3D structure to various disease states lies in determining which target gene a specific non-coding disease-associated region regulates, because like the first example I gave, the majority of enhancers regulate genes further away than the nearest gene via 3D-genome folding events, and thus cannot be predicted without performing a physical 3D-genome mapping exercise.

PN: I believe that's where your GenLink3D™ platform comes in.

HJ: Yes, that's right. Our platform uses what's called Hi-C sequencing to profile a DNA's

loops and then determines which two distant DNA-regions are brought into close physical proximity.

Our GenLink3D pipeline, called "promoter-capture Hi-C (PCHi-C)" however, distinguishes itself by providing 3D-data across all genes in the human genome and their enhancers in a single experiment. This gives us the unique ability to unlock the therapeutic potential of non-coding variants and their target genes.

PN: It's amazing how we're now learning about the ways the DNA structure affects who we are. What new insights have you gleaned with the GenLink3D platform?

HJ: Our platform plays a new important role in preclinical drug discovery. More specifically, we revealed that different cell types possess unique 3D genome shapes that fundamentally drive their core identity and function. In a ground-breaking 2016 study we mapped the genome-wide 3D contacts across 17 different cell types of the immune system, creating the first comprehensive immune cell 3D genome atlas.

This atlas then allowed us to finally understand which genes are the long-range targets for 100s of previously known GWAS "risk variants" in ulcerative colitis (UC), opening new doors to the diagnosis and treatment of this condition. Moreover, target and enhancer gene interactions in activated memory T cells, which mediate adaptive immune responses in our bodies, were found to be particularly important in causing their overactivity, which is key to causing gut inflammation.

Interestingly, different cell-types and mechanisms seem to be at play in different patient populations. Our work has also helped to identify a specific single nucleotide polymorphism (SNP) among East Asians associated with genes expressed in monocytes, CD4+ cells, erythroblasts, and B cells, highlighting the power of our technology to pave the way for treatments that are tailored for specific ethnicities or individuals – the key aim of personalised medicine.

Now, we're looking into obtaining 3D genome folding information from as many human cell types as possible. This effort will give us a complete 3D human genome atlas that we can use to interrogate any gene function in any human cell type.

PN: I can already begin to see the power behind your GenLink3D platform, especially given how high cell and DNA inputs are required in typical Hi-C sequencing protocols.

HJ: Exactly. We have put a lot of effort into developing an advanced version of PCHi-C that allows us to profile rare cell types. Most Hi-C and capture Hi-C protocols require millions of cells to start with; we can now go down to about 50 thousand cells. This substantially increases the range and sensitivity of our sequencing pipelines. Both, in turn, reduce the costs of generating and analyzing the data through bioinformatics.

PN: So now that you're the new CEO of Enhanc3D Genomics, where do you see your

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HJ: So we're currently in a growth-transition phase. We're moving away from being a small biotech platform company towards applying our knowledge of 3D genomics to important biological questions and unmet medical needs. To that end, we have identified three main areas:

- **Disease knowledge:** We believe that understanding disease pathophysiology at the molecular and genetic level is essential to treating diseases. Given that the vast majority of non-coding human genome has yet to be "de-orphaned" for which disease variants regulate which target genes, in which cell-type, our 3D platform has the abilityto uncover hundreds of new disease causation mechanisms for therapeutic exploitation..
- **Target identification:** Here, we will define which enhancer-target interactions represent effective nodes for novel therapeutic development, by performing key "target-validation" experiments using functional genome editing "knock-out" studies.
- **Biomarker prioritization:** Through these functional experiments, we can also define the strongest biomarkers for disease onset, improvement and worsening. With this model, we can therefore also impact the field of diagnostics, as well as accelerate drug discovery efforts, thus facilitating true personalised patient care.
- **Technology development:** we are constantly developing and advancing our cutting-edge technology platforms to remain at the forefront of 3D genomics. Currently, our efforts in this space are focussed on single-cell and long-read sequencing applications, which will expand the range of tissue cell-types that can be interrogated.

To build upon these four areas, we are establishing collaborations with various biopharmaceutical companies across the UK, Europe, and the globe. All of them are dedicated to harnessing the 3D genomics data we provide to identify clearer endpoints in clinical trials and ultimately tailor treatments to patients. We are convinced that a deeper understanding of 3D genomics will catalyse step changes in precision medicine.

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The story of Fanny Angelina Hesse, a pioneer in microbiology, with Corrado Nai

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BY PAUL NAPHTALI JULY 15, 2024 BACTERIA

"History is written by the victors." Or so the saying goes.

I personally prefer, "The truth shall set you free".

The true story behind Robert Koch's discovery of bacteria brings this truth to light. For over a century, we have attributed the culturing of bacteria to Robert Koch, who eventually coined his famous postulates. Those postulates became the cornerstone of microbiology research, spurring the development of myriad antibiotics to treat infectious diseases. Yet the truth is, his culturing method was refined by a rarely remembered woman: Fanny Angelina Hesse (1850-1934). It was she who came up with the idea to grow bacteria in a special kind of jelly called agar.

I had never heard of her world-changing breakthrough, having always assumed that Koch was the first to use agar. That was until Corrado Nai posted about his Kickstarter project. He has brought together a team of skilled visual artists, science writers, and scientists to create a graphic novel to tell this story. Once it gets funded, they hope to complete the story and publish it in the summer of 2025. Upon hearing the amazing tale, I pitched into the project. Now, I have the delight of interviewing him about it.

Read on if you want to hear more about the tale and his plans to deposit newly emerged historical materials at the Museum of the Robert Koch Institute! You can also pitch into the Kickstarter campaign by clicking on the banner below. And with that, I hope you enjoy!

The interview

PN: Congratulations on your journey to write about Fanny Angelina Hesse! What inspired you to begin such an effort?

CN: It wasn't my original plan to write about Fanny Angelina Hesse. Neither did I plan to write a graphic novel! Rather, it began when I pitched a short news story for a popular magazine. The story centered on a study that featured years of experiments and multiple microbial species in a single culture. I pitched the idea but was rejected because, according to the editor, the story was too niche. Although I disagreed with the assessment, I returned to the writing desk.

While brainstorming more ideas, I decided to take a different angle and explore the breeding ground of the experiments themselves: agar. I found the jelly fascinating because microbiologists have been using it for over a century without fail. From there, I thought of Fanny Angelina Hesse. Despite her importance, she has remained largely forgotten in the annals of history. I thought that this angle would convince an editor about the universality of this story.

PN: Aside from raising awareness, what about Angelina Hesse made her so special to write about? And what about her do you find most inspiring for scientists today?

CN: Because of her historical time and place, she was what people at the time called a "Deutsche Hausfrau", a German Housewife. You can see a picture of her with her husband through the image below.

A bactograph of Fanny Angelina Hesse and her husband, Walther Hesse. Both bactographs in this blog were kindly prepared by David J Westenberg and Jeff Tabor at www.bactograph.com

She didn't find her role as uncomfortable or degrading. From the documents and articles that I encountered about her, I formed the image of a humble and modest person. Despite the enormity of her innovations, she never spoke of her achievements in her lifetime.

Imagine that!

I found her humility so antithetical to today's times, where attention is a very rare and coveted commodity. She was content with her role as a family woman, and supported her husband Walther with dedication, archiving his results over the years. This is not to say that she was passive, either! In fact, she left behind stunning scientific illustrations on top of her incredible achievement of bringing agar to the life sciences.

PN: Such a story would be appealing to a wide audience, I bet.

CN: Exactly! Although our Kickstarter campaign focuses on connecting with microbiologists and others in the life sciences, this story is relevant for anyone who has benefitted from microbiology. It's not an exaggeration to say that I mean *everyone* with that.

That's not to mention how unaware the Indonesian communities are of the role that agar plays in microbiology research. Fanny Angelina knew about agar because of her Dutch heritage. Her mother knew about a dessert called agar-agar from her Dutch neighbours. You see, The Dutch encountered the delicacy when they colonized the Dutch East Indies, which is now known as Indonesia.

PN: I'm amazed that the stroke of genius to use agar remains mostly untold! But what about agar made it so appealing to grow microbes compared with other media?

CN: Before agar, microbiologists were using gelatine to grow bacterial cultures. However, gelatine melts easily at 37ºC, the most common temperature to grow pathogenic bacteria. Conversely, agar remains firm at the same temperatures needed to grow them. Agar also sets without refrigeration, which is quite handy for preparing agar plates. Lastly, the substance is not degraded by microbial digestive enzymes and so remains stable in the presence of bacteria.

PN: We've discussed the interest that scientists would have in this story. But let's pamper the science historians in our discussion, for your novel is based on historical documents related to Angelina. How did you obtain the documents about her, and how did you react when you saw them for yourself?

CN: Luck and perseverance from my side, along with intense care in documenting and archiving historical documents from the Hesse family, played big roles in my obtaining the documents. I began by reading through one of only two published papers about Fanny Angelina Hesse. In that document, there was a sentence written by her grandson Wolfgang that read, "The author is proud to possess his grandmother's original drawings."

The paper had been published over 30 years ago, but I thought I should try and see if I could find Wolfgang Hesse or his descendants in Germany and see if they still had these scientific illustrations from her. The search wasn't easy! There are so many Wolfgang Hesse in Germany, and Google could not help. Finally, I learned that Wolfgang had three children! A personnel register in Karlsruhe helped me establish contact with them, who have been very generous and gracious in supporting me with historical documents and have shown a lot of trust in me. I am grateful to them!

It was a thrilling journey that led me to the historical treasure I searched for, and I can't wait for all the materials to be available for everyone to see. Upon my suggestion and those from one of my informal mentors in science communication, Regine Hengge, the family will deposit the historical documents at the Museum of the Robert Koch Institute in Berlin. We also plan to write a peer-reviewed paper and put the documents in the scientific record for posterity. All these plans filled me with joy; it meant that this story would live on!

By the time I completed my search, a reputable magazine became interested in this story! More information about this story can be read in a recent article I wrote for *Smithsonian Magazine*. Along with scientific illustrations from Fanny Angelina from 1906, the family shared never-before-seen family portraits and an unpublished biography written by Wolfgang with many personal and touching memories about his grandparents. It was from these memories that I got the idea to present the story as a graphic novel! Reading Wolfgang's memories about Lina, as the family called Fanny Angelina, made me think about my own grandmother Emilia, whom I loved dearly.

PN: Let's jump into the graphic novel now. What kind of writing and art style are you aiming for with your novel? How will it help your audience appreciate and love microbiology?

CN: So far, we have concluded the concept art and we still conceptualizing the writing. Last week I just pitched the book to a publisher – fingers crossed! As we put the story onto paper, many aspects of the storytelling can change. Nonetheless, I want our story to raise curiosity in the reader. I plan to accomplish this with our team of artists by presenting snapshots of history that will eventually converge into the discovery of agar as a gelling agent to grow microbes. From there, we will show the significance of agar in our lives today.

We want readers young and old to be fascinated with science and to learn from it. We also want to inspire the next generation of scientists to explore the microbial world and learn new things, one agar plate at a time.

Through our graphic novel, we also want to raise discussions and ask, "Why is Fanny Angelina Hesse not well-known today? What can we do today to change that? And how do we ensure that the truth comes out from the work we do?"

At the end of the day, Fanny Angelina's story is a very personal one that will be told from the perspective of Wolfgang. His unpublished biography about her grandmother contains many touching and personal memories. As such, we want the graphic novel to show how important it is to keep memories alive, especially of persons who are dear to us. The memory of Fanny Angelina Hesse should be dear to microbiologists and everyone who has ever benefitted from agar. But at the end of the day, what is important to me is that people know her name and her achievements. And I think we can achieve that by encouraging the reader to think about someone they hold a dear memory of, just like Wolfgang did with her grandma Lina.

PN: That's an admirable goal, one that every author strives to achieve. On that note, let's round off the interview with this: why is it important to tell the true stories behind historical discoveries such as the one you're sharing?

CN: The stories of many people have been marginalized and ignored throughout history. Being able to tell these stories is the starting point to rectify many of the wrongs done to others and the discrimination that runs rampant throughout time. With the difficulties that come with raising interest in telling Fanny Angelina's story, I am grateful for the excitement and interest I have witnessed from people who want to know more about her. That includes the people who are helping tell her story, those who are backing this project, and those who are sharing the news, including GenoWrite. Thank you for your support!

Author

Paul Naphtali

Paul Naphtali is the founder of GenoWrite, a life sciences communications company. He holds an MSc in Biology and went through the PhD program in Biochemistry and Biomedical Sciences, both at McMaster University. Before GenoWrite, he created Microbe Musings out of a passion for communicating microbiology research to diverse audiences around the globe and from all walks of life.

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Jared Kehe: Unlock the microbial sound of music

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BY PAUL NAPHTALI APRIL 29, 2024 HUMAN MICROBIOME

Let me begin by saying I love classical music. Brahms's and Dvorak's symphonies (here and here respectively) are to die for. I have also played piano and violin for almost two decades. And I performed full concerts on the piano, both as a soloist and with orchestras.

In my experience, any successful orchestral performance comprises talented musicians, a great conductor, and excellent acoustics. But what truly makes them produce beautiful music? Any group of individuals can produce sounds on their own. But an orchestral group can only succeed when a certain component brings them all together: teamwork.

The same applies to microbial communities. Microbes, like us, rarely live alone. How they interact with each other has broad-ranging effects on microbial behavior, environmental conditions, and health outcomes.

Jared Kehe, co-founder and Chief Scientific Officer at Concerto Biosciences, is fully aware of this paradigm. Inspired by classical music, Jared began Concerto Biosciences alongside Cheri Ackerman and Bernardo Cervantes to examine the microbial world with unmatched experimental depth. This effort, in turn, aims to discover breakthrough microbe-based products that harness microbial interactions for human benefit. Their work yielded the kChip, a discovery engine that physically constructs millions of miniature, defined microbial communities simultaneously.

If you love music, microbiology, and teamwork, this interview is for you! Read on to learn why studying microbial interactions will help you develop products and therapeutics that best enhance human health.

The Interview

PN: I first heard of your love of the piano after speaking with your fellow co-founder, Cheri. Tell me more about your love of music and how it oriented you towards Concerto Biosciences.

JK: My musical background informs my work and perspective to my core. And I'm not the only scientist who thinks this way. Music provides an analog; it is both deeply technical—rooted in theory and rules and structures that can be learned and creatively applied—and deeply emotional, a profoundly beautiful language of its own.

Biology is the same. Think about it in this way: The microbial world is one huge musical performance. For 150 years we've tried to understand this music — learn the theory behind it — by plucking out each instrument one at a time and listening to how it sounds. We learned the solo music of microbes such as *E. coli, S. cerevisiae*, and *B. subtilis*. We developed an impressive understanding of these "instruments"— how to amplify them, modify them, and silence them.

If we're to compose the music of nature, we haven't heard nearly enough of it yet to understand all its richness and complexity. We have no idea how these instruments sound together. I want us to be able to compose the microbial world like Beethoven, Mahler, Rachmaninoff composed complex, expressive musical ensembles. Each instrument plays its role, reacting to the others, informed by and informing the others, and synergizing with the others.

PN: My heart sparkled when you mentioned Beethoven and Rachmaninoff. Their piano concerti are must-hear quality, borderline divine even. Was that where you got your company's name?

JK: Yes, you are right. It's a reference to a specific type of musical piece where there is one primary instrument, such as a piano, accompanied by an ensemble of supporting instruments. We often think about product design this way: Can we find a supportive combination of microbes that brings out desirable behaviors from a primary target microbe? More broadly, human hosts are much like the primary instrument of their own private concerto. We're each supported by a massive assemblage of symbiotic microbes that draw out our better health.

PN: We've started out this interview talking about microbes in community with one another. Yet historically we've focused on single pathogens in our microbiology research. What did we miss from this approach?

JK: Historically, we've characterized bacteria with laboratory cultures in a Petri dish. Yet amid those efforts, we have left out many bacteria that could be profiled. Most of them, in fact. That's because we could only culture a small fraction of the microbial strains we know could occupy an ecosystem. Microbiologists call this "The Great Plate Count Anomaly."

The phrase is a striking misnomer to me. We were clearly missing something. For me, the true anomaly is the fact that any microbe can grow by itself. Evolution has selected for microbes to be interdependent. Only in the rarest cases do we see the environment provide all the conditions needed for a single microbe to grow. Those bacteria are the anomalies in our world. And not just anomalies — typically those ones are the pathogens that wreak havoc!

PN: What's intriguing to me is that even after knowing this, we've spent a lot of time searching for the one microbe that will give us human health or cause disease directly.

JK: Right! 'Omics technologies have been an established mainstay of microbiome research. But while it can tell us which microbes are present, which genes are expressed, and which byproducts are secreted, none of this information tells us how microbes behave relative to each other. You wouldn't treat a human in isolation from everyone else; they may behave differently depending on who they hang out with. It's the same with bacteria. Just because we find a microbe consistently present among those with a skin condition, doesn't mean they're the direct cause. For all we know, they may enable disease by affecting how other skin-dwelling microbes behave. Gaining these kinds of insights with our technologies represents the primary goal for Concerto.

PN: That's a perfect segue into the core of Concerto Biosciences: the kChip. I'm impressed that it can monitor thousands of microbial interactions at once. How does it work?

JK: Imagine rolling a bunch of colored marbles over a huge table. A bunch of divots have been carved into the table surface. Each divot is designed to accommodate a precise number, say 3, marbles that randomly fall into the divot. Very quickly all your divots will be filled with 3 random marbles each. You know the identity of the marbles within each because you can see their colors. Now shrink this setup down to the size of my hand, and replace marbles with microbial cultures.

That's a kChip.

Every divot is a tiny "microwell", each on the order of hundreds of microns across. Instead of marbles, we roll "droplets", single-nanoliter liquid compartments each containing a microbial culture. Every droplet is color-coded with dyes, and each color maps to a specific microbe within the droplet. Say you generate a grouping of a red, blue, and orange droplets in a microwell; well, we know that represents strains #52, #317, and #642. We do this tens of thousands of times per kChip; and, over the course of running many kChips in a screen, come to generate millions of these combinations.

After all the microwells are filled, we expose a kChip to an electric field that merges all the individual droplet groupings. We monitor the behavior of all of these combinations over time to understand how each combination of microbes interacts and identify those that are performing desirable behaviors.

If you want to read more about the technical aspects of the kChip, you can check out the paper we published in PNAS.

PN: The dyes are used to identify the microbes, but what kinds of behaviors do you care about tracking?

JK: We can track all kinds of behaviors or targets of interest on the kChip. We focus on those most relevant to a given disease. Using optical methods, we can monitor bacterial growth, gene expression, and protein production using our kChip setup for every constructed combination.

PN: I'm just picturing the many aspects of microbial interactions we can monitor with the kChip, and it's amazing! What aspects of the skin microbiome have you already examined with the kChip?

JK: We were interested in tracking *S. aureus* behavior within millions of different combinations of skin-dwelling microbes. The thing about *S. aureus*, like all other microbes, is that each kind of *S. aureus*, or strain, has differences at the gene level that affect how they behave (See this interview to learn more about strains). Some *S. aureus* strains possess genes that encode skin-degrading proteases and inflammation-activating toxins, making them more dangerous than others. Even so, just having these microbes doesn't mean that you will get ill. Those more dangerous strains may encounter other microbes that inhibit those genes and pacify them.

To learn about how skin microbes affect *S. aureus*, we generated millions of microbe combinations on kChips and spiked in a fluorescently engineered *S. aureus* into every combination. The amount of fluorescence corresponds to the amount of activity of a specific *S. aureus* gene, like one that encodes an inflammatory toxin. By doing so, we identified the microbial combinations that most potently suppressed toxin production. That's just one example—we measured all kinds of *S. aureus* activities across these combinations.

PN: I believe your efforts have already produced your first microbial product.

JK: Yes, we nominated our first product for restoring *S. aureus* protection to a deficient skin microbiome based on insights from kChip screening. We call it "Ensemble No. 2" (ENS-002). We composed ENS-002 after

generating over 6 million data points on how *S. aureus* behavior changes in the presence of different skin-dwelling microbial combinations and nutrients. Through these screens, we selected a combination of microbes that inhibited a panel of undesirable *S. aureus* activities in a manner that was robust to the presence of any other microbes.

PN: The odds of you finding such a combination must be so low. Which makes your discovery even more impressive!

JK: Thank you! It was incredibly brute force: We looked at a tremendous number of miniature skin communities to paint a picture of the interaction network governing the behavior of the skin microbiome. That's the real vision — to generate enough data to understand how these complex microbial ecosystems behave so we can shepherd them to healthier compositions. ENS-002 is our first attempt at this — and we're excited to try it out on human beings this summer — but there's a ton of discovery work to be done if we're to learn how to engineer these complex systems.

PN: Congratulations on such a major milestone. What's the best way we can keep in touch about your work?

JK: We love visitors! There is so much edgy, exploratory, and paradigm-shifting science happening in our lab — so much new microbial music to be heard! We invite you to come listen with us.
Check our website (www.concertobio.com) and our LinkedIn (www.linkedin.com/company/concerto-biosciences) as well for updates on our ENS-002 clinical work and discovery initiatives in other areas. We have new projects coming down the pipeline in women's health and other areas where microbial products are poised to make a real impact.

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