LET'S BE A SCIENTIST!

AMGEN® Biotech Experience
Scientific Discovery for the Classroom
Italy

6 students, **10 days**, many **experiments**, lots of **scientific discussions** and a generous amount of fun! These are the ingredients of "Let's be a Scientist", a project of **Amgen Biotech Experience Italy** in collaboration with TIGEM (Telethon Institute of Genetics and Medicine) and SSM (Scuola Superiore Meridionale).

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Giuseppe Betta
Liceo Scientifico E. Fermi, Ragusa

For about 10 days in July 2023, thanks to Amgen Biotech Experience, I had the opportunity to join this project called "Let's be a scientist" in which me and other 5 guys who finished high school last June participated in different research projects tutored by the scientists of TIGEM (Telethon Institute of Genetics and Medicine).

I had the opportunity to work with lots of scientists who studied different things, in particular I was tutored by the biologist Mariagrazia Monaco who works in the neuroscience area guided by Elvira De Leonibus. The group works on the identification of these early behavioural and synaptic alterations, through sensitive behavioural tasks for rodents, with the aim of discover novel therapeutical approaches to ameliorate the neurological symptoms of the disease. In particular in this period we focused on MPS-IIIA better known as "Sanfilippo syndrome". We worked in parallel on more field. As concern laboratory activities we made 2 PCR to amplify mice's genes that we studied. PCRs were followed by southern blot, a method to separate the amplified genes. We made also a western blot, a method to separate proteins based on their molecular weight. We saw important differences in genetic expression between knock out mice and wild type ones. We also worked on RNA extraction and Real Time PCR. As concern the studying activities my PI E. De Leonibus proposed me to make a journal club, to learn how to give a speech about a scientific article. The one I chose was about the first phase of the disease and about different possible pharmacological approaches to solve the disease. I also learnt lots of things about other aspects of a centre of research as concern organisation, bureaucracy, searching for founding, animal management.

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Luigi A. Di Pietro
Istituto Omnicomprensivo Mattioli
D'Acquisto, San Salvo (CH)

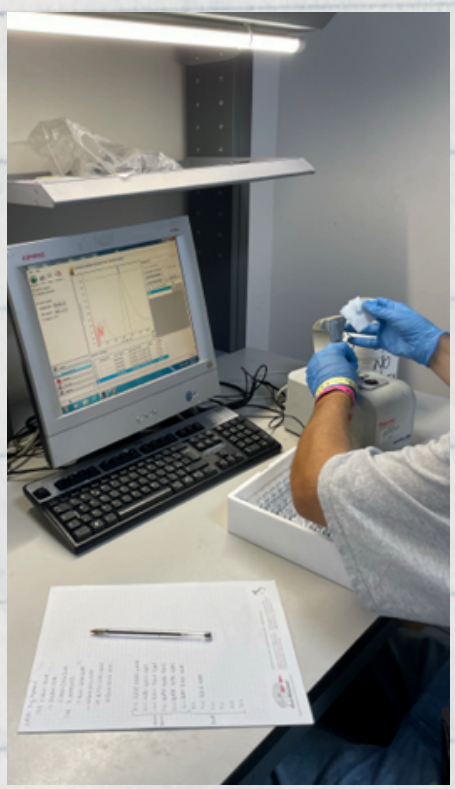
Amgen Biotech Experience (ABE) is an innovative science education program that introduces students to the excitement of scientific discovery and build bridges between school and real-life biosciences. ABE prepares the next generation of scientist and innovators. Thanks to this project me and other five students had the opportunity to attend for 10 days the TIGEM's research labs where various genetic disorders are studied.

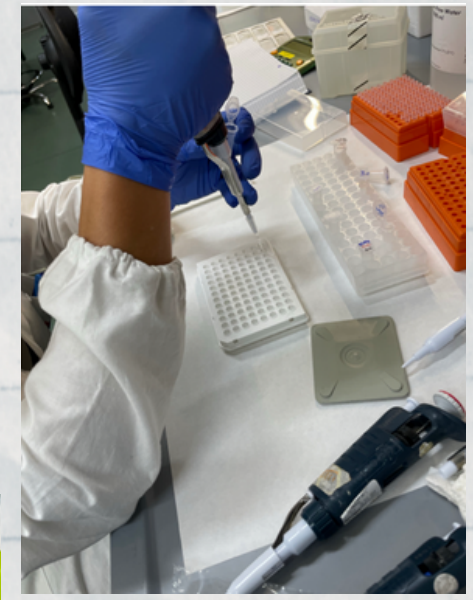
During this period, I had the opportunity to work in Nicola Brunetti's research group at the molecular therapy program at TIGEM, who mainly investigate novel therapies for inborn errors of metabolism (IEMs). Among several IEMs, they are investigating how the regulation of urea cycle enzymes could impact on the development of therapies for urea cycle disorders (UCDs), a group of genetic disorders leading to hyperammonemia. The team is also interested in organic acidaemias and glycogen storage disease besides the UCDs. To be more precise, they have an active research program on the liver disease caused by mutant Argininosuccinate lyase (ASL), the second most frequent UCD.
The two main goals are:

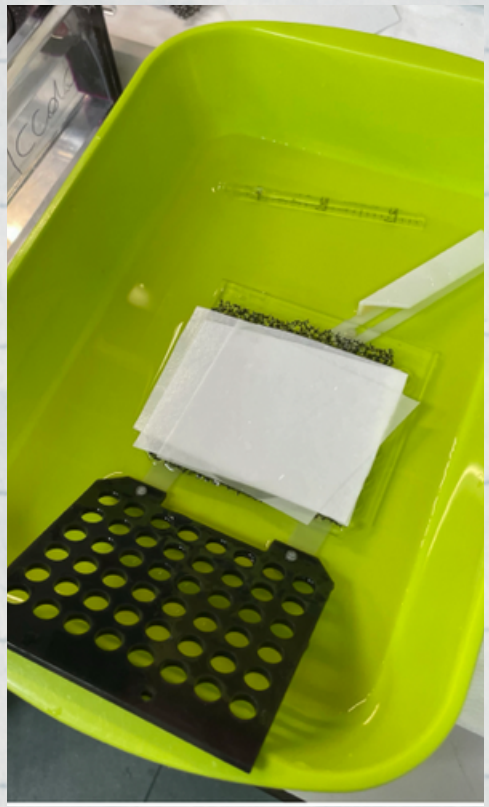
- start research in the clinical area in order to treat these conditions through directed liver gene therapy
- finding small-molecule drugs to improve the disease phenotype.

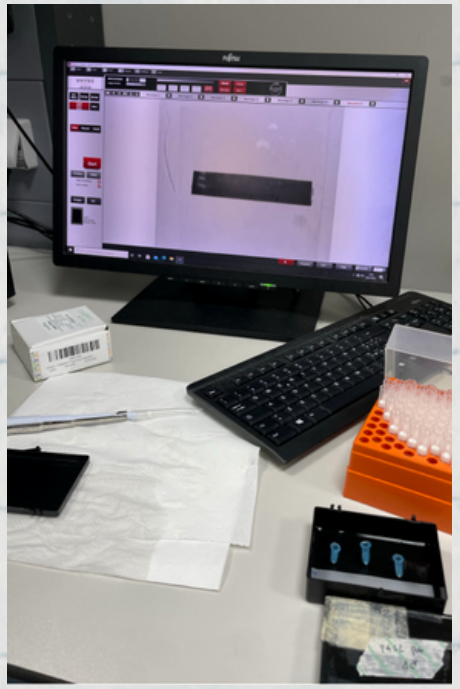
In this experience I had some really well-prepared tutors, who gave me perfectly clear explanation on a novel topic regarding genetic disorders. They allow me to co-work with them and let me perform different experiments on my own and explain to me the scientific reasons behind the experiments. Elisabetta is a PhD who graduated in medical biotechnology in the University of Federico II at Naples. The other tutor that I had was Alfonso, who graduated in Medicine in Naples and decided to spend his knowledge in the research field.

Another important figure in my group is Leandro who showed me the place and made me approach for the first time to the biotechnological word. I perform several techniques from cell and molecular biology. With Alfonso to detect liver enzymes, that we are interested, we firstly separated the nuclear fraction from the cytosolic one through a long protocol of which I performed a step: the sonication, that uses ultrasounds to destroy the nuclear membrane. Secondly, I quantified the fractions by measuring the absorbance of the samples and based on their concentration we prepared and loaded our nuclear fractions in the polyacrylamide gel. After the electrophoretic run, I made the sandwich blotting in order to transfer the proteins from the gel to a membrane. We incubated overnight our membrane with a specific antibody for the protein of interest. The following morning, after the secondary antibody incubation, we detected the proteins bands on the membrane comparing nuclear wild-type to mutant samples. Instead, with Elisabetta I had the opportunity to perform the gene silencing by siRNA transfection and to design the oligo-primers for Real- time PCR in order to be sure that the depletion occurred.









This process starts with the preparation and the positioning of the cDNA in a specific multiplate where we put the siRNA-treated samples and the control one in order to detect if any changes happened. Once we set up the plate, the thermocycler machine started to analyze the data and we saw that the genes were not modified in a correct way because the analysis showed that also the silenced gene was amplified like the normal one. After it we tried to find a new oligo-primer in order to understand the reasons why the last one was not successful trough specific sites and different articles.

I understood that this medicine's field works mainly thanks to the collaboration of different scientists that help each other in order to resolve genetic disease. To sum up I will say that I am really lucky because I had the chance to work some really important researchers and see how this world works.

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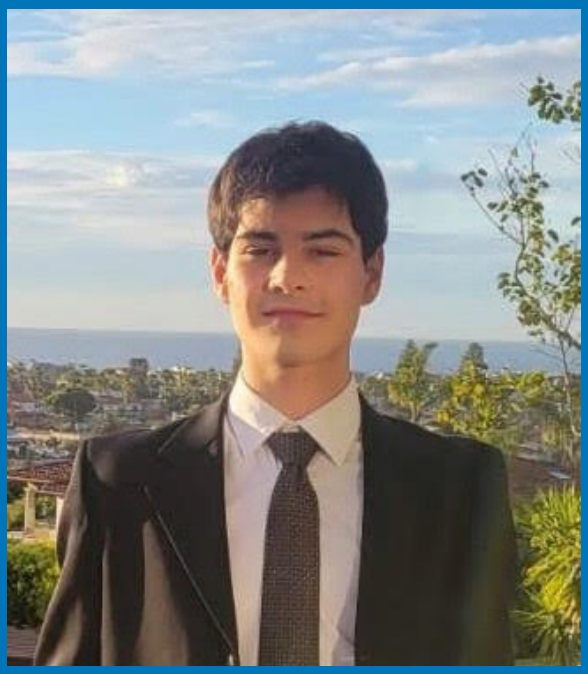


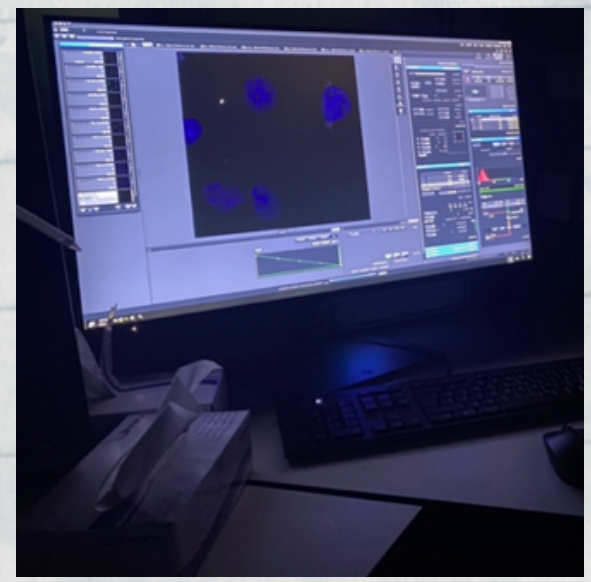
Jacopo Ferrara
Liceo Statale R. Caccioppoli,
Scafati (SA)

In the last days I have taken part, together with other 5 peers, in the 10-day research internship program "Let's Be a Scientist", organized by Amgen Biotech Experience Italy, and intended for high school students fond of biological sciences, genetics and medicine. This program gave us the opportunity to familiarise with laboratory and research activities and took place at TIGEM, the Telethon Institute of Genetics and Medicine, in Pozzuoli (NA).

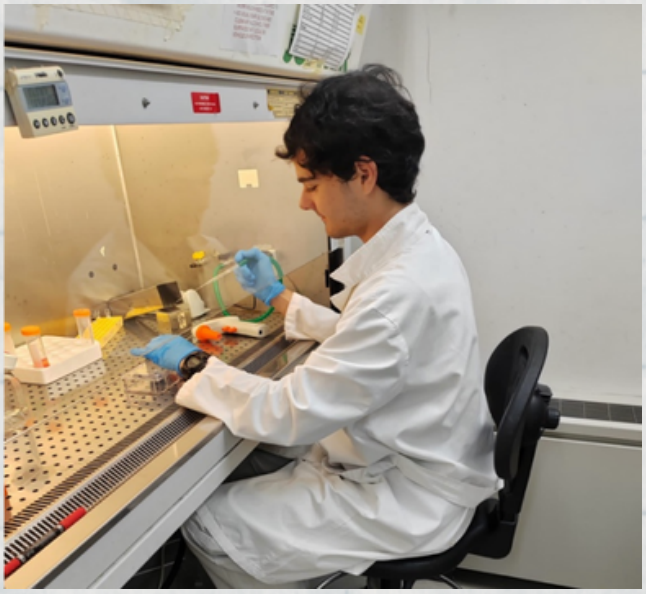
At the beginning of the TIGEM experience, each student was assigned to a researcher, tutor and temporary mentor, belonging to a different research project, to observe the activities carried out by the team and to perform some experiments under supervision. This formula (one student-one project) was chosen to differentiate the experiences of the entire group of interns as much as possible. In fact, we felt the need to share what we had learned during our internship. This allowed us to understand what each one of us had seen and done, according to the natural and fundamental attitude to science: sharing. The research team to which I was assigned, led by the Principal Investigator (PI) B. Franco, is studying and characterizing the effects of three different pathogenic mutations found in patients with HNF1B gene variants and responsible for kidney cysts and/or diabetes (including a rare form called MODY5 characterized by an early onset, typically before the age of 25, and a progressive dysfunction of the pancreatic beta cells). The hepatocyte transcription factor, HNF1B, is a protein involved in the development and proper functioning of various organs, such as the kidneys, pancreas, and genitourinary tract. HNF1B, as a transcription factor, is therefore involved in the processes of proliferation, differentiation, and signal transduction in cells, and is able, depending on the cellular contexts, to activate or repress the transcription of target genes.

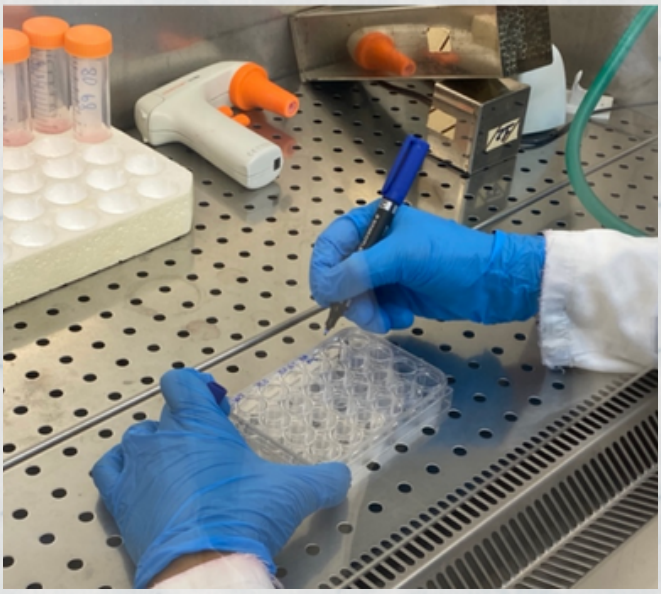
The group, coordinated by the project manager to whom I was assigned, Dr. F. Massaro, specifically studies the effects of these mutations on the kidneys using HK2 (Human kidney 2) cells as a cellular model, which are adult kidney cells that have been engineered to express these three mutations in the HNF1B gene.

The goal is to understand the molecular mechanisms underlying the same clinical manifestations in patients with mutations in different points of the gene. Dr. Massaro explained to me the different approaches used to modify these cells, from the production of lentiviruses that were used to introduce these mutations into HK2 in the gene of interest, to the use of a new generation system represented by Crispr/Cas based on the use of the Cas protein, a kind of molecular scissors used in this case to modify the HNF1B DNA. Francesco explained to me the method they used to understand whether the Cas protein has correctly inserted the mutations of interest: after extracting the genomic DNA from these same cells subjected to genetic engineering, under his supervision, I amplified the HNF1B gene with the PCR (polymerase chain reaction) method, which was then subjected to enzymatic digestion with restriction enzymes, proteins capable of cutting the same DNA only if the desired mutation has been correctly inserted. Finally, the same DNA, if recognized and cut by these enzymes, will be checked by sequencing the HNF1B gene. The next phases of the research will consist of a first characterization with Western Blotting, Immunofluoresence, and Real time (experiments with which you can understand the molecular weight, expression, cellular location, and some of the HNF1B gene targets) to understand what happens to the HNF1B protein after these sequence variants (mutations). Finally, through a more targeted analysis with proteomics and transcriptomics experiments (in order to establish all the molecular interactions of the mutated protein), it will be possible to have a more complete picture of what happens from the molecular point of view, laying the foundations for possible therapeutic strategies.



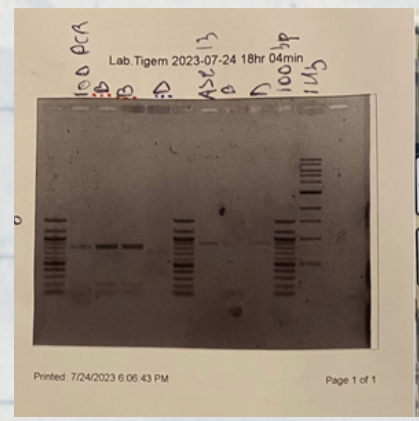
This experience has enriched me a lot! It will be useful for me to clarify my ideas about my future: now I can say that I have lived in the laboratory first-hand participating in the maintenance of these cell cultures and in their screening. My tutor dedicated a lot of his time and lab resources to let me execute some of the experiments that he himself had performed. In particular, I carried out, under his supervision, but by myself: the amplification through PCR of two mutant HNF 1B genes; the digestion of the latter with specific restriction enzymes and a final electrophoretic run to verify that I had been clean and precise in my laboratory activity. It was exciting to discover that at least half of my experiments actually worked.

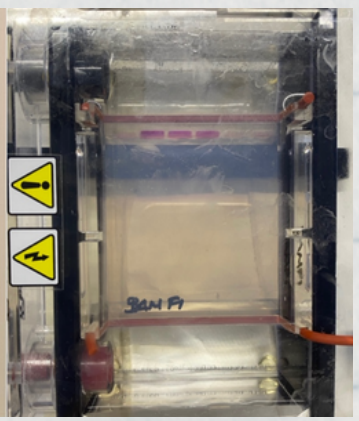




The mere thought of having worked correctly and effectively with such microscopic instruments (with "instruments" I refer to for example the Q5 DNA Polymerase), made me feel a cascade of new emotions that researchers surely feel all the time. My activity at TIGEM also included several operations performed on cells (the HK2 mentioned earlier), knock-out for the HNF 1B gene. Francesco guided me in the activities of changing the medium (the liquid in which the cells live in culture), splitting (to go from the maximum confluence, i.e., the situation in which the cells no longer have the means to continue to proliferate, to lower confluences) and pelleting (useful for being able to store large quantities of cells). **It has been a fantastic experience, some of the most intense days of my life**; I therefore feel like reiterating the thanks that I have already made in the video that accompanies this text.

Thank you for your attention!





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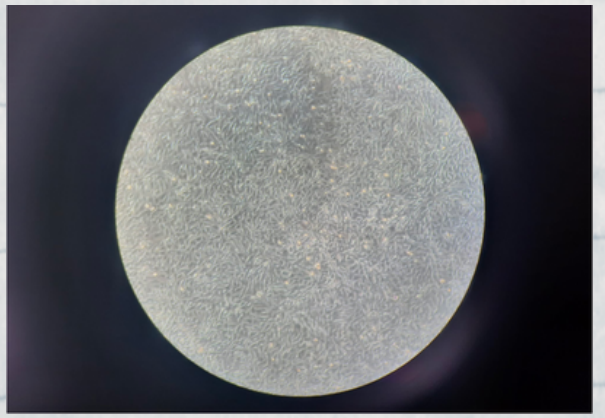
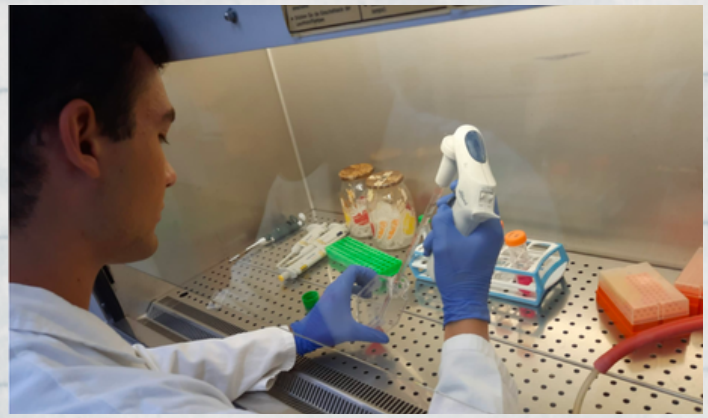
Giulio Gentile
Liceo Scientifico Einaudi, Siracusa

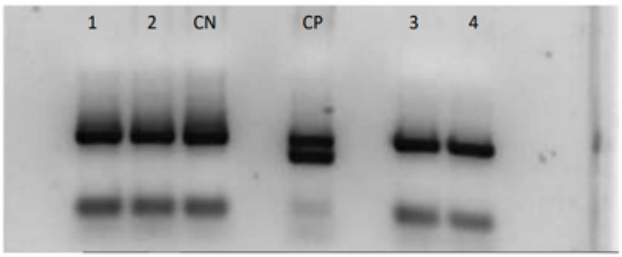
From 19 to 28 July I had the opportunity to visit TIGEM labs thanks to "Amgen Biotech Experience" with other High School Graduate students. Each of us was matched with a different research group. I was paired with di Bernardo's group, which is made up of members coming from different backgrounds such as Biology, Biomedical and Automation Engineering, and Computer Science.

Their aim is to develop and apply innovative experimental protocols and computational approaches to observe, model and manipulate cell behavior for biomedical and biotechnological applications. I was tutored by Barbara Tumaini, lab manager of the group, and she made it possible for me to have a wide overview of the projects carried out by different members of the group. She showed me the research which is involved in studying the behavior and the physiological response to several stimuli of Breast Cancer Organoids. **I was also able to carry out some experiments by myself**, supervisioned by Barbara and another member of the group, Lorena Postiglione. I splitted a cell culture of engineered HeLa cells. At first, we had to verify that the culture was not contaminated by Mycoplasma using PCR and Electrophoresis techniques. Having found no traces of mycoplasma, we extracted genomic DNA and designed primers for a new PCR, so as to verify that our gene of interest was under the control of viral promoter CMV. After having performed PCR, I prepared an agarose gel for a new Electrophoresis. The result we obtained confirmed the correct position of the gene and promoter, because the DNA strand amplified by PCR had a length in accordance with our expectations and was very similar to the positive control.

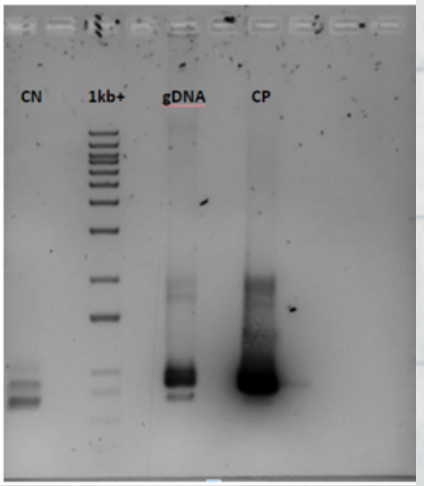


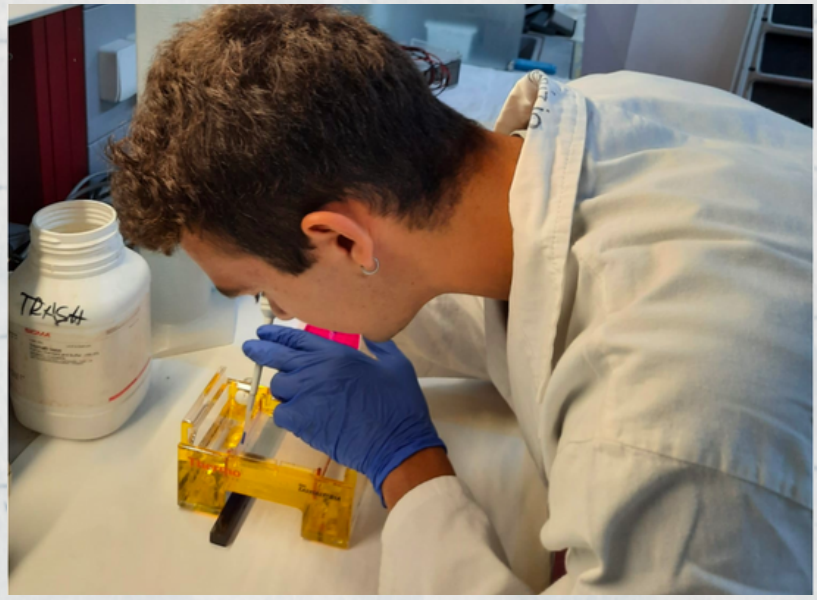


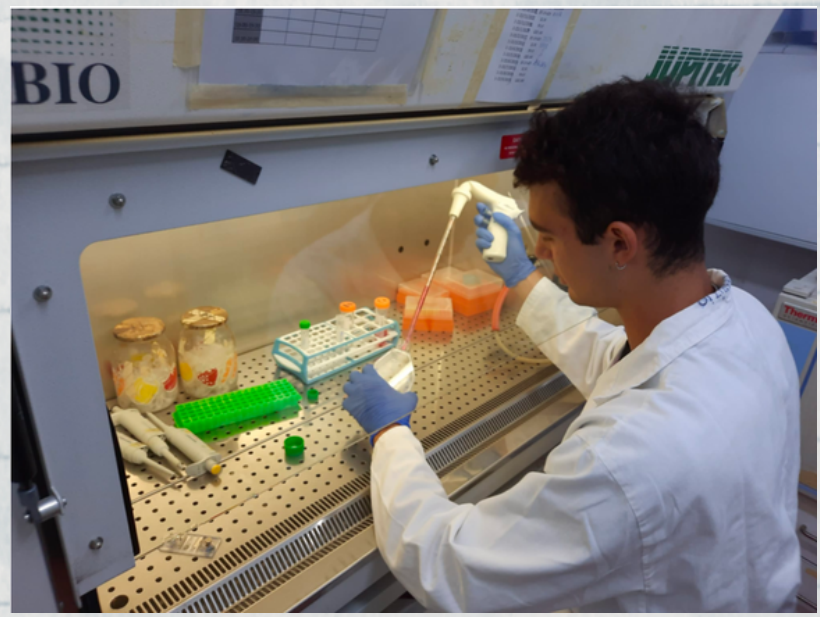
Myc test on HeLA_TFEB:GFP#B2 infected with LV carrying VHP or KRAB 21/7/23



1. VHP_BT
2. KRAB_BT
3. VHP_GG
4. KRAB_GG







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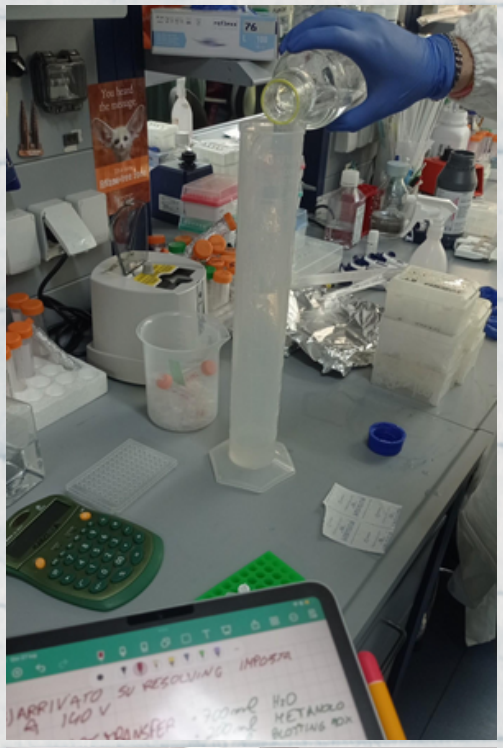
Omar Khaled
Liceo Torricelli, Somma Vesuviana (NA)

The German philosopher Nietzsche stated in his work 'The gay science' that the scientists never should be pedantic laboratory men, but it's necessary to have an open minded, flexible and creative behavior, basically like an artist. And me, a high school student who is ready to engage the universitary path of medicine, I've been given an opportunity to be a scientist as Nietzsche intended, by approaching the microscopic world, whose scary complexity is hidden to our eyes.

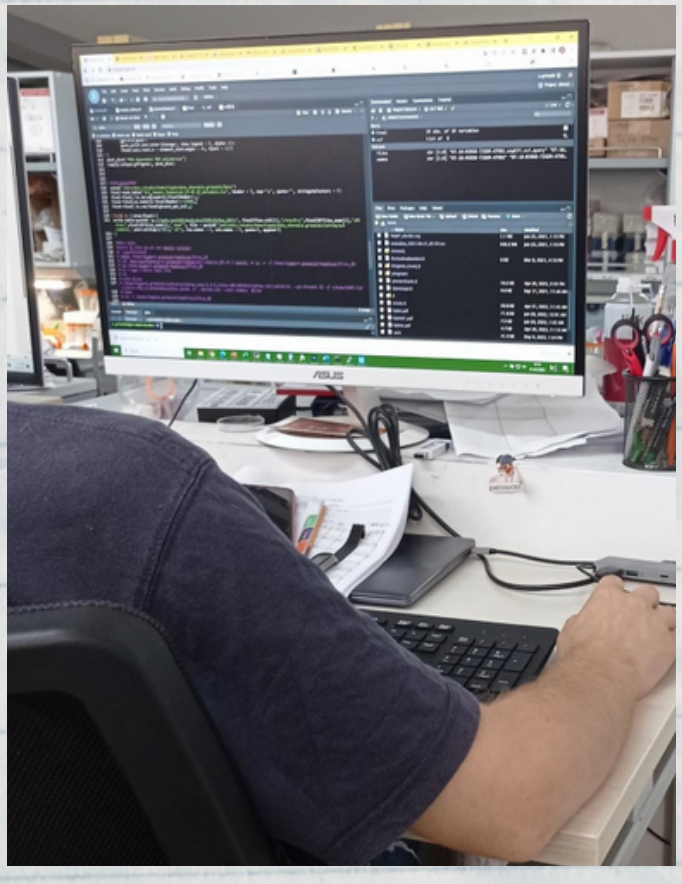
However, this is made easy and comprehensible by the scientific research, which is not a magic or esoteric knowledge, but is accessible to everybody, educational and aspiring to the publicity of the results: projects and their results should be made available to the whole community in order for them to be verified as a starting point for new discoveries. That opportunity was conceded to me by the Amgen Biotech Experience (ABE) program, which has decided to bet on a group of young students, by giving them a glimpse of the life of the researchers. ABE is a didactic initiative promoted by the ANISN (associazione nazionale insegnante di scienze naturali) in collaboration with the Federico II university of Naples, the CNR and others, which offers to schools and teachers the materials, the content and the preparation to enable students to approach molecular biology and biotechnologies.'Let's be a scientist!' Is the name of the project that allowed me and my selected (throughout a test) colleagues to experience a 10 days stage in the TIGEM center of research, located in Pozzuoli, Naples, where the passion unites biologists, biotechnologists, engineers and medical doctors, who cooperate to reach the common objective of turning the basic research in pragmatic medical treatments in order to enhance the health conditions of people affected by genetic diseases.



The working environment is avant-gardist and flexible, based on the collaboration between several groups of research involved in different fields, whose projects cross and assist each other to reach the common aim. In order to have an all-round experience, each one of us students was assigned to a different working group. Personally, I've been working with the group belonging to the PI (Project Investigator) Davide Cacchiarelli, which focuses on researching new diagnostic approaches for the screening of germinal variants in hereditary genetic diseases. In our lab, we carried out a multifaceted approach that combines a wide range of genomic strategies to significantly advance our understanding of the regulatory logic driving cell fate during human reprogramming. Our ultimate goal is to improve the quality and fidelity of such strategies to unlock the full potential of induced cell fate reprogramming in regenerative medicine therapies.

I was particularly amazed by accessing this work environment, because I was thoroughly inserted in the group of research: I was immediately involved in the daily activities, such as the processes of extraction and purification of DNA using magnetic beads, the amplification through PCR, quantization using the fluorometer Qubit-4, and sequencing using a 2nd generation sequencer. The main source of the DNA we worked with was the SARS-CoV-2 virus, whose genome sequencing would eventually allow us to identify potential mutations, determining the generation of the so-called 'variants'. The analysis of the sequences are then carried out in parallel with other centers of research, in order to determine whether they were already discovered and to study their diffusion in the population. However, if a specific variant was found to be unknown, then it would've been subject to further studies for its identification. Moreover, our studies focused also on the mutations of the genes BRCA-1 and BRCA-2, which surge the risk of being affected by mammarian and ovarian cancer by 57% and 40% respectively. The goal is to perform a mass screening of women affected by breast cancer with NGS (next generation sequencing) and computational techniques, which lower the costs and the time taken, and enhance the sensibility of diagnoses.

However, before being actually allowed to follow and collaborate in the activities of the researchers, I've been firstly introduced to the work environment of the lab, learning how to use the specific technologies, such as the micropipettes and the various devices. Then, I was provided with a clear explanation of the processes I was going to carry out, in order to have clear in mind what I would've done and widening my knowledge in the fields of biotechnology and genetic engineering, comprehending the functioning of techniques and procedures such as electrophoretic runs, western blots or the aforementioned PCR, in a theoretical way. Furthermore, I've been notified about all the proper behavioral norms to keep in a laboratory, especially if near dangerous reactives such as viruses and similar: for example, when I've been introduced in a cell culture to withdraw cells transfected with a virus, I was provided with all the necessary precautions not to endanger my health.



In conclusion, I want to remind you that **not every time the experiments carried out were successful after the first try. But this is absolutely normal in the work of a researcher, whose proactive and trustworthy behavior, granted by a solid knowledge of the principles, always ensured the success of the following endeavors**. There are not enough words I can use to express my gratitude towards Mrs. Anna Pascucci for allowing us to live such an incredible experience, Mr. Lorenzo Vaccaro and Mr. Antonio Grimaldi, and the whole group of researchers that have followed me during my activities.

It has been an honor,

Khaled Omar

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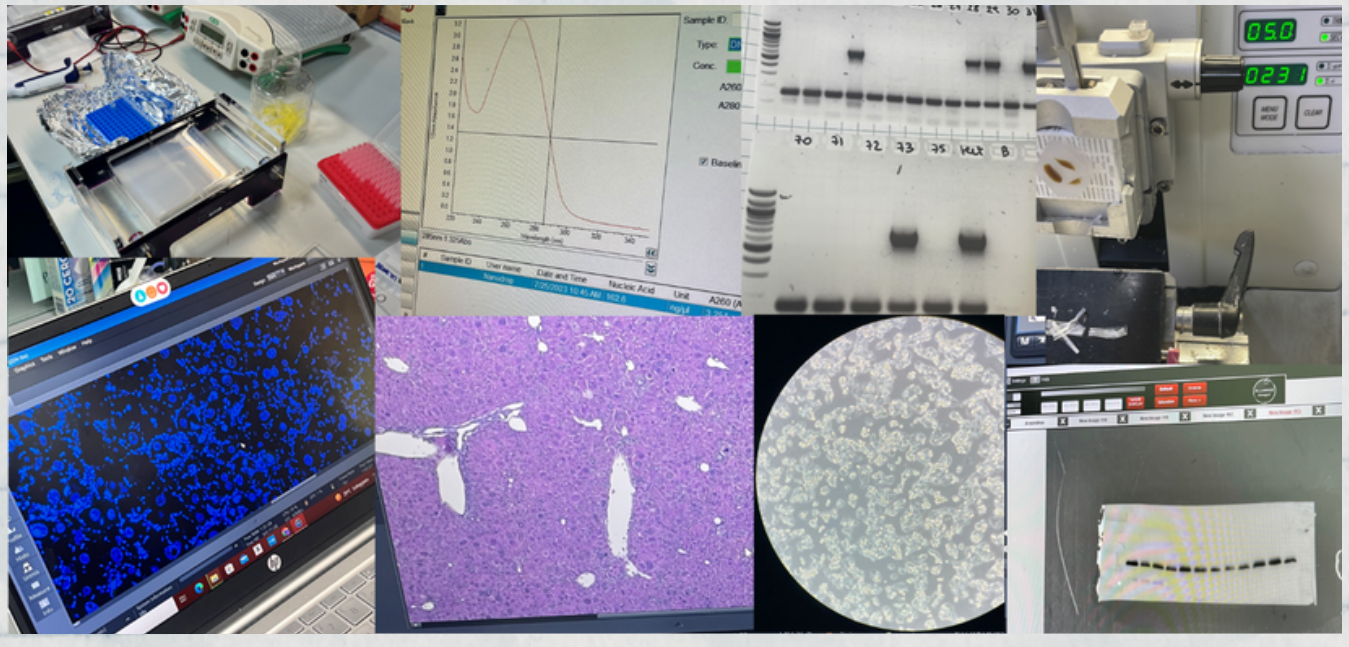
Adelaide Pia Sicignano
Liceo Statale R. Caccioppoli,
Scafati (SA)

From 19th to 28th July we had the chance to interface directly with many innovative biotechnological procedures, such as PCR, Western Blot, electrophoresis and many others. I was paired with Pasquale Piccolo's research group. Piccolo is a P.I. and he mainly focuses on gene therapy and he studies cases of fibrosis in which gene therapy isn't efficient. he group I was paired with focuses on Wilson disease, which is an autosomical recessive disorder of copper metabolism, due to a genetic mutation of the ATP7B gene.

This copper accumulation causes chronic hepatitis, fibrosis, cirrhosis, liver failure and it can also lead to psychiatric and neurological deficits including Parkinsonism. My group particularly focused on autophagy, because recent in vitro studies identified autophagy as a novel pro-survival process that helps ATP7B-deficient hepatocytes to handle copper build up through sequestration of damaged and, hence, potentially toxic cell components. I was paired with Maria Battipaglia, a PhD student whose research focused on the modulation of TRPML1/TFEB pathway for the treatment of Wilson disease. TFEB and TRPML1 play an important role in the regulation of autophagy and lysosomal exocytosis. In particular, TRPML1 is a lysosomal Ca channel that activates calcineurin, which dephosphorylates TFEB, thus promoting its nuclear translocation and transcriptional activity. TRPML1 also modulates autophagy through a non-transcriptional and TFEB-independent mechanism. In particular, it rapidly induces autophagosome and lysosome biogenesis and their fusion. Interestingly, TRPML1 is a druggable target that has been investigated in various diseases. So TFEB/TRPML1 axis may be a new targets for the therapy of WD. At first, through gel electrophoresis, mice genotype was analyzed, so that scientists could focus on ATP7B knock-out mice, so that they could be targeted through different types of molecular therapy and their liver cells can later be compared with healthy mice which are in heterozygosity for this gene mutation.

A sample of DNA used to analyze the mice genotype is usually token from the mice fingers. Each mice, right after their birth, gets one of their finger and toe removed so that they can be identified. Six weeks old Atp7b knock-out mice were previously injected with 3 different AAV serotype 8 vectors expressing TFEB, TRPML1 or GFP, under the control of a hepatocyte-specific thyroxin binding globulin promoter, TBG. Every 4 weeks they were put in metabolic cages to collect their urines, feces and blood. Mice were sacrificed at 12 weeks post-injection. So we analysed the overexpression effects on mice. Liver damage and cholestastis were monitored through serum transaminases and alkaline phosphatase activities, respectively. We observed a significative reduction of these markers in treated mice. We performed different types of staining on liver sections that we cut at the microtome. Liver fibrosis was analized by Sirius red staining to assess collagen deposition in the extracellular matrix.

After the staining, the sections were analyzed through a microscope. In the non-treated mice, hepatocytes are more rich in collagen than treated mice. To evaluate necroinflammation, we performed the hematoxylin and eosin staining and we observed a significant improvement of liver inflammation in mice overexpressing TFEB and, to a larger extent, TRPML1. Then, I estracted DNA from mice phalanges, using two different protocols, and I perform a PCR to amplify the genetic material. After I did an agarose gel and I used electrophoresis to determinate the mice genotype. Moreover, I estracted proteins from mice liver, I quantized them through Bradford method. Western Blot was used to determinate the levels of TFEB in both nucleic and cytosolic fractions. Naturally, I've at first, been explained every aspect of those techniques in a way that was quite underst for me and my education level. Then, after many theoretical explanations, I've started to perform some of those procedures by myself, supervised by my tutor Maria Battipaglia.



Every sequencing, western blotting, electrophoresis and cell culture analyzed at the microscope is a little yet so important step in finding a cure for a disease that can cause many other related symptoms, even depression. I personally found it extremely fascinating, and it made me realize how important research is. We should never underestimate researchers, because everyone of them gives their important contribute in the developing of modern medicine, especially in the “invisible” part of medicine, that deals with disease that seems impossible to cure. I’ve found it so fascinating, inspiring and captivating and thanks to this experience I now have a much more clear idea on my future career path. I will always thank ABE for giving me this opportunity.

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