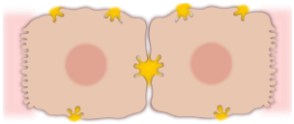
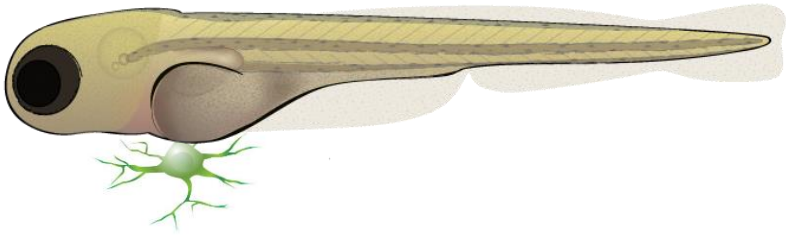
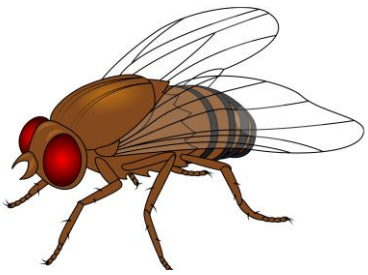
International summer school on model systems and IPSCs





Malta 18th – 22nd September 2017



Train MALTA Horizon 2020

TrainMALTA Partners:

University of Malta
University of Cambridge
Katholieke Universiteit Leuven

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 692041

Day 1 - Monday, 18 September 2017
Centre for Molecular Medicine and Biobanking (CMMB), University Campus, Msida

9.30 - 10.00	**Welcome and introduction Room 402**
10.00 - 10.30	**Coffee break**: Room 401
10.30 - 12.30	**Lab practical**: Seed Mammalian Cells Lab 307
12.30 - 13.30	**Lunch:** Room 401
13.30 - 15.00	**CRISPR theory** Lecture room 402
15.00 - 15.30	**Coffee break**: Room 401
15.30 - 17.30	**Lab practical**: Transfect Mammalian Cells Lab 307

Day 2 - Tuesday, 19 September 2017
Lab 307,
Centre for Molecular Medicine and Biobanking (CMMB),
University of Malta, Msida

9.30 – 10.00	**Lab practical**: Media Change: Room 307
10.00 - 10.30	**Coffee break**: Room 401

Day 2 - Tuesday, 19 September 2017
Conference Hall 402

10.30 - 11.30	**Madhavsai GAJJAR**: Recent advances in the field of induced pluripotent stem cell (iPSC)
11.30 - 12.30	**Joke Terryn**: Genome engineering in stem cell research
12.30 - 13.50	**Lunch**: Room 401
14.00 - 15.00	**Daniel Ortmann**: Control of human embryonic stem cell fate by directed differentiation and inducible gene modulation
15.00 - 16.00	**Rute Tomaz**: Generation of Hepatocytes from Pluripotent Stem Cells
16.00 - 16.30	**Coffee break**: Room 401
16.30 - 17.30	**Amanda Dalby**: Forward Programming of iPSCs to generate Megakaryocytes and Erythrocytes in vitro

Day 3 - Wednesday, 20 September 2017
Labs: 344/337a
Centre for Molecular Medicine and Biobanking (CMMB), University of Malta, Msida

9.00 – 9.30	**Meet room 402**
9.30 – 11.00	**Lab practical**: Visualize and Harvest Cells: Room 114 and Extract gDNA: Room 344
	Or **Practical:** Design your own IsgRNA Guide: Room 402 *bring your own computer for this session
11.00 - 12.30	**Lab practical**: Quantify and PCR Amplify: Room 344
	Or **Lab practical**: Visualize and Harvest Cells: Room 114 and Extract gDNA: Room 344
12.30 - 13.50	**Lunch:** Room 401
14.00 - 15.30	**Lab practical**: Gel Electrophoresis: Room 337a and Recover DNA: Room 344
	Or **Lab practical**: Quantify and PCR Amplify: Room 344
15.00 - 15.30	**Coffee break**: Room 401
15.30 - 17.30	**Practical:** Design your own sgRNA Guide: Room 402 *bring your own computer for this session
	Or **Lab practical**: Gel Electrophoresis: Room 337a and Recover DNA: Room 344

Day 4 - Thursday, 21 September 2017
Conference Hall 402
Centre for Molecular Medicine and Biobanking, University of Malta, Msida

9.30 – 11.30	**Ruben Cauchi and Maia Lanfranco**: Learning to *fly*: exploring gene function in Drosophila
11.30 - 12.30	**Annelii Ny**: (Zebra)Fishing for new and safe drugs to cure diseases
12.30 - 13.50	**Lunch**: Room 401
14.00 - 15.00	**Ana Cvejic**: Single-cell RNA-Sequencing uncovers transcriptional states and fate decisions in haematopoiesis
15.00 - 15.30	**Coffee break**: Room 401
15.30 - 17.30	**Kathleen Lambaerts**: Housing and care of Zebrafish

Day 5 - Friday, 22 September 2017
Labs 344/337a
Centre for Molecular Medicine and Biobanking, University of Malta, Msida

9.30 – 11.00	**Lab practical**: Denaturation and Hybridization: Room 334
10.00 - 10.30	**Coffee break**: Room 401
11.00 - 12.30	**Lab practical**: Gel Electrophoresis: Room 337a
12.30 - 13.50	**Lunch:** Room 401
14.00 - 15.30	**Lab practical**: Gel Electrophoresis continued: Room 337a
15.30 - 15.45	**Coffee break**: Room 401
15.45 - 17.30	**Concluding remarks**: Q&A session: Room 402

Abstracts

Recent advances in the field of induced pluripotent stem cell (iPSC).

Madhavsai GAJJAR

Stem cells have the remarkable ability to self–renew and simultaneously differentiate towards various matured cell lineages in the human body. Because of this immense potential, they are a great tool to study not only human development but also model human diseases, drug discovery and regenerative medicine. With recent advance in the field of stem cell research, it has been shown that with help of reprogramming factors, small molecules and proteins one can convert the somatic cells into iPSC, which resembles *in vivo* stem cells. However, there remains some major hurdles in obtaining fully matured and functional differentiated cells, resembling their *in vivo* counterpart. Recent advances in various fields, for example, iPSC technology, chemical/bio engineering, transcriptomics and computational modelling has helped in improving the current knowledge for generating greater maturation of differentiated tissue cells.

Genome engineering in stem cell research

Joke Terryn MD[1,2,3], Arefe Nami[1], Wietse Decraene[1],Susanna Raitano PhD[1], Laura Ordovas[1] PhD, Philip Van Damme MD PhD[2,3], Catherine M. Verfaillie MD PhD[1]

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[2] University Hospitals Leuven, Department of Neurology, Belgium
[3] KU Leuven, VIB Center for Brain & Disease Research, Belgium

Presenting author: Joke Terryn MD

The advent of iPSC technology offers a new approach to characterize and decipher disease mechanisms. Because stem cells can be genetically modified, patient-derived lines can be corrected or altered to contain a reporter or overexpression system.

We have generated multiple patient derived iPSC lines, carrying the progranulin gene mutation (IVS1 +5 G>C), and seamlessly inserted this point mutation into a control iPSC line and embryonic stem cell line. Genome engineering was performed using TALE nucleases and the Piggybac transposon system.

In a second gene targeting project, a cassette suitable for FLPe recombinase-mediated cassette exchange (RMCE) was inserted in the *AAVS1* locus of patient iPSC lines, using Zinc-finger nucleases. The cassette in this system is exchangeable and allows for the highly efficient generation of stable reporter and overexpression cell lines.

In this lecture, a detailed overview of the genome engineering process will be given, to illustrate the steps leading to successful gene targeting.

Control of human embryonic stem cell fate by directed differentiation and inducible gene modulation

Daniel Ortmann

Human pluripotent stem cells (hPSCs) represent an invaluable source of various differentiated cell types that can be used for basic research and ultimately clinical applications.

I will be talking about a few classic approaches of cellular differentiation following developmental pathways and how to potentially improve such systems using reporter cell lines. After this, I will present an alternative way of producing cells through forward programming using inducible expression of transcription factors. As proof of principle, we demonstrated rapid, robust and deterministic reprogramming of neurons (iNGN2) and skeletal myocytes (iMYOD1). We also developed a forward programming strategy to efficiently and expeditiously generate human oligodendrocytes. Finally, I will highlight some further applications of this inducible system to knock-down or knock-out genes of interest in a controlled way.

Generation of Hepatocytes from Pluripotent Stem Cells

Rute Tomaz

The liver performs over 500 different vital functions, some of which include fighting off infections, producing bile and detoxifying blood from alcohol and drugs. Liver disease is one of the main causes of death in the western world and the shortage of organ donors makes it urgent to develop novel therapies. However, the lack of appropriate *in vitro* models for disease modelling and drug screening represents a major drawback. Human induced pluripotent stem cells (hiPSCs) have emerged has a promising tool for regenerative medicine, given their ability to differentiate into all the tissues of the body. Their unlimited growth capacity allows large scale production of cell types relevant for cell-based therapies, drug screening and disease modelling. Here, we will review the progress made on the development of protocols for differentiation of hiPSCs into hepatocytes – the main liver cell type. Furthermore, we will address the limitations of existing methods and the steps being taken in our lab to tackle such limitations.

Forward Programming of iPSCs to generate Megakaryocytes and Erythrocytes in vitro

Amanda Dalby

Forward programming, a method developed in the Ghevaert lab, relies on the over-expression of three key hematopoietic transcription factors (FLI1, GATA1 and TAL1) in human induced pluripotent stem cells (iPSCs). This method generates bi-potent progenitor cells, allowing differentiation towards both the megakaryocyte and erythrocyte lineages in vitro. Megakaryocytes are the precursor cells to platelets, and we aim to use forward programming to produce platelets for future use in transfusion medicine. My talk will discuss forward programming, gene editing, the development of an inducible iPSC system and future directions of this technology.

Learning to Fly: Exploring Gene Function in Drosophila

Ruben Cauchi and Maia Lanfranco

The fruit fly (*Drosophila melanogaster*) has been an attractive and effective genetic model system since Thomas Hunt Morgan and his proteges made seminal discoveries with them more than a century ago. There are many technical advantages of using *Drosophila* over vertebrate models but the most appealing is that fruit flies can be genetically modified in numerous ways. Work with *Drosophila* is advancing several fields including neuroscience mostly because the fly model system allows us to probe gene function in swift and effective ways. In the first part of our talk, we will give a brief history of the fly model system, highlight the principles of 'flypushing' and introduce the infamous Muller's Morphs. In the second part, we will discuss strategies through which we explore gene function in *Drosophila* mainly forward or reverse genetic approaches. We will also underscore the use of genetic screens to identify genetic pathways, hence allowing us to gain further insights into gene function.

(Zebra)Fishing for new and safe drugs to cure diseases

Annelii Ny

Zebrafish (Danio rerio) combines the high through-put capacity of in vitro screening approaches with the high physiological and genetic homology to humans. Hence, this species has become increasingly useful in translational biomedical research. This lecture will focus on how zebrafish can be used to model human disease for drugs discovery and safety assessment of drug candidates. The first part of the lecture will introduce zebrafish as an experimental animal model, including a brief historical description, development, and advantages/limitations of this species. The second part of the lecture will give concrete examples of the ongoing zebrafish-based drug discovery and safety assessment projects at the Laboratory for Molecular Biodiscovery.

Single-cell RNA-Sequencing uncovers transcriptional states and fate decisions in haematopoiesis

Ana Cvejic

The success of marker-based approaches for dissecting haematopoiesis in mouse and human is reliant on the presence of well-defined cell-surface markers specific for diverse progenitor populations. An inherent problem with this approach is that the presence of specific cell surface markers does not directly reflect the transcriptional state of a cell. Here we used a marker-free approach to computationally reconstruct the blood lineage tree in zebrafish and order cells along their differentiation trajectory, based on their global transcriptional differences. Within the population of transcriptionally similar stem and progenitor cells our analysis revealed considerable cell-to-cell differences in their probability to transition to another, committed state. Once fate decision was executed, the suppression of transcription of ribosomal genes and up-regulation of lineage specific factors co-ordinately controlled lineage differentiation. Evolutionary analysis further demonstrated that this haematopoietic program was highly conserved between zebrafish and higher vertebrates.

Housing and Care of Zebrafish

Kathleen Lambaerts

During the past few decades, zebrafish (*Danio rerio*) has become a powerful vertebrate model system for studying development, modelling disease and screening for novel therapeutics. Given this increasing importance of these animals in biomedical research and the fact that their housing and care but also their health status can significantly affect research outcomes, husbandry techniques must be considered carefully in order to optimize fish health and reproductive success. Although zebrafish are relatively easy to maintain, optimal husbandry requirements are still far from fully understood and protocols concerning their housing and care vary considerably from facility to facility. During the talk, several aspects of maintaining a productive zebrafish housing facility will be discussed. At first, the different components of a housing system, the most important water parameters and some environmental factors (e.g. enrichment, stocking density etc.) will be highlighted. Additionally, tips and tricks on how to feed, import/export, breed and raise zebrafish will be given. Next, health monitoring and some health problems will be discussed. To end, some guidelines concerning anaesthesia and euthanasia will be provided.