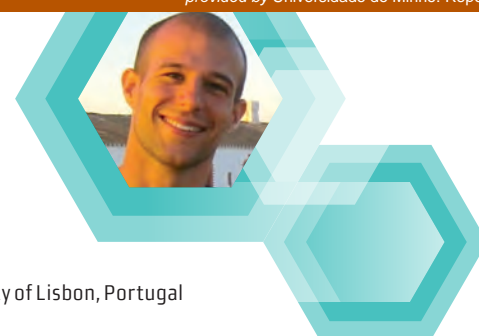


# Investigating regulatory T cells by single cell RNA-sequencing

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**Background:** Master's degree in Genetics and Molecular Biology, Faculty of Sciences of the University of Lisbon, Portugal  
**Starting year:** 2012  
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## OBJECTIVES

Single-cell transcriptomics is an emergent and powerful approach to understand the complexity inherent in many cell populations. Its relevance has been growing as increasing evidence supports the existence of marked heterogeneity within cell populations previously considered as homogeneous. This is particularly important for many immune cell types, where single cell RNA-sequencing has the potential to provide profound new insights into subpopulation structure.

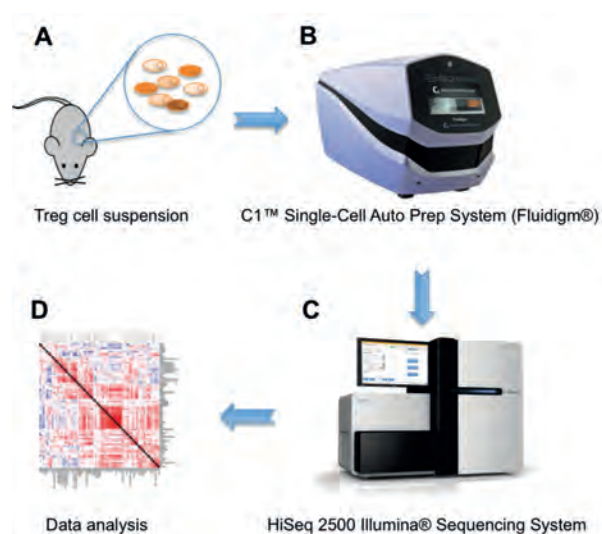
This project focuses on a particular immune lineage - Regulatory T (Treg) cells - which play a central role in peripheral tolerance to self- and non-self-antigens. By single-cell mRNA sequencing of Treg cells, we intend to address their remarkable gene expression plasticity, determine Treg identity, define subsets, and investigate their functions, surface markers and key features. Results from this work will provide a more comprehensive overview of how immune homeostasis is regulated.

## WORK PLAN

The initial phase of this project consists of characterizing Treg identity at the single-cell level. As a first step, we are focusing on Treg isolation protocols from diverse tissues in collaboration with research groups with previous expertise on the field. The resulting single cell suspensions are then run on the C1™ microfluidics systems (Fluidigm®) for single cell capture, reverse transcription and cDNA amplification. Next, we barcode and prepare the Illumina libraries for sequencing. Using this methodology, we plan to get single-cell transcriptomics data from immunologically relevant tissues. Extensive bioinformatics

analysis on these data will provide new insights into Treg biology.

In a second stage, the most interesting features of Treg biology will be extracted and prioritised for further study. A combination of adoptive transfers of newly discovered Treg subsets and manipulations targeting newly identified Treg genes will be used as validation assays.



**FIGURE 1**

**Project Workflow.** (A) Regulatory T (Treg) cell suspensions are obtained from mouse tissues. (B) Single cells are then captured in separate compartments on C1™ chips and lysed. The C1™ system is responsible for the reverse-transcription of the mRNA released from each cell, as well as the amplification of the resultant cDNA. After appropriate Library Prep, (C) the cDNA is sequenced on the Illumina® platforms. (D) The single-cell transcriptomics data obtained presents itself as a brand new open window to Treg biology. After thorough analysis, interesting findings will be tested in vivo and/or in vitro.