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M2 internship proposal

Research Team : Mutagenesis in Single cells and Evolution (MuSE team), Micalis institute, INRAE
https://www.muselab.fr/

Supervisors : Lydia Robert & Marina Elez

This project could be pursued with a PhD funded by Impulscience (fondation Bettencourt Schueller ; https://www.fondationbs.org/notre-communaute/laureats-et-projets/lydia-robert)

Project title : Impact of antibiotics on mutations in bacteria

Our fight against infectious diseases is underpinned by an evolutionary arms race with pathogens, in which mutations fuel the evolution of virulence and antibiotic resistance. Similar issues arise in cancer therapy, where cancer cells accumulate mutations and escape chemotherapy. It has been proposed that mutation rates are environment-dependent. For instance, **mutagenesis in bacteria was proposed to increase in presence of sublethal concentrations of antibiotics**, which are often found in our environment due to our extensive use of antibiotics in agriculture and medicine. Such a phenomenon is both fundamental to our understanding of evolution, and of high clinical importance.

However, previous studies on the environmental control over mutagenesis lead to many controversies, due to the limitations of classical experimental approaches. We recently developed a new experimental approach to overcome these limitations, combining genetics and molecular biology with microfluidics and microscopy, and allowing **detecting mutations and assessing their effects directly in single bacterial cells** (figure 1). In this approach, bacteria grow in a microfluidic chip called the “mother machine” (figure 1A), and they express a fluorescent marker which tags mutations (mutations are visualized as fluorescent spots; figure 1B). The growth of the bacteria and the occurrence of mutations is followed on a long time-scale (several days) by time-lapse microscopy.

In this project we will use this approach to **visualize and quantify mutations in the bacterium *Escherichia coli* in presence of sublethal doses of antibiotics**.

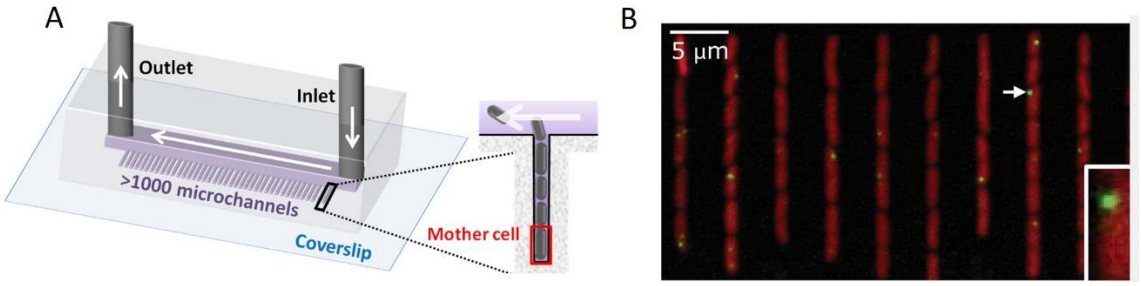

Figure 1: Mutation Visualization experiments. A) The "mother machine" microfluidic chip used to grow bacteria in precisely controlled conditions on a long time-scale. B) Overlay of red (to see the bacteria growing in the microchannels) and yellow fluorescence images (YFP-MutL tags the mutations). The inset shows a magnified image of a cell with a YFP-MutL spot (arrow), i.e. a mutation.

Related Publications :

Robert L, et al. (2018) Mutation dynamics and fitness effects followed in single cells *Science* 359(6381):1283-1286

Robert L, Ollion J, Elez M, (2019) Real-time visualization of mutations and their fitness effects in single bacteria. *Nat. Protoc.* 14(11):3126-3143

Ollion J, Elez M, Robert L, (2019) High-throughput detection and tracking of cells and intracellular spots in mother machine experiments. *Nat. Protoc.* 14(11):3144-3161.

Elez M. (2021) Mismatch Repair: From preserving genome stability to enabling mutation studies in real-time single cells. Cells 10(6):1535

Ludvikova L, et al. (2023) Near-infrared co-illumination of fluorescent proteins reduces photobleaching and phototoxicity Nat.Biotechnol doi: 10.1038/s41587-023-01893-7