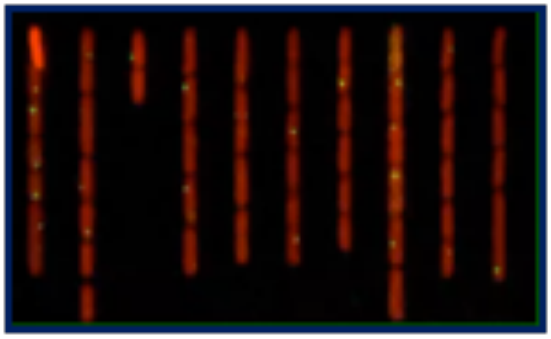
Postdoctoral position: Origin of spontaneous mutations in phages

A 2-year postdoctoral position is available in the team Mutagenesis in single cells and evolution of the MICALIS Institute (INRAE, AgroParisTech, University Paris-Saclay) in Jouy-en-Josas, in Paris area (20 km in the south-east of Paris). The position is funded by a grant from the French National Research Agency (ANR-20-CE12-0008-02).

TEAM RESEARCH: Our research focuses on mutation generating processes and their impact on evolution and adaption in bacteria and phages. We use new quantitative approaches, such as the one we have recently developed, which allows to visualise the accumulation of mutations in single bacterial cells in real time over hundreds of generations [1, 2, 3]. Our projects are interdisciplinary and combine experimental techniques from molecular biology, genetics, high-throughput sequencing, video microscopy, microfluidics and microfabrication, with data analysis and modelling.



Visualization of mutations (yellow spots) at the single cell level in *E. coli* (red) growing in the "mother machine" microfluidic chip

PROJECT: In this project, we aim to investigate the origin of spontaneous mutations (SM) in phage genomes. In growing cells, SMs occur mainly through replication errors (RE) and endogenously induced oxidative damage to nucleotides. In most organisms, the vast majority (>90%) of REs are repaired by the evolutionarily conserved mismatch repair (MMR) pathway. However, MMR does not function efficiently on phage DNA, even for phages that use their host machinery for DNA replication. The reason for this phenomenon is not clear. The consequence is a much higher mutation rate of phages compared to their hosts [4].

The aim of the project is to address these questions using *Escherichia coli* and its phage lambda as models. Our work on the visualisation of REs in living cells suggests that MMR efficiently detects REs on lambda DNA, suggesting inefficiency in the downstream steps. The latter depends critically on the methylation status of d(GATC) sequences as insufficient or too rapid d(GATC)s methylation interferes with the proper functioning of MMR. Importance of incomplete methylation of d(GATC)s in phage DNA has been suggested to explain MMR inefficiency in phages but this have not been established [5]. To test this, we propose in a first step to investigate the impact of alteration of the methylation status of d(GATC)s on lambda mutagenesis, using visualization of mutations but also high-throughput Duplex sequencing. These results have potential implications for antibiotic resistance and phage therapy efficiency.

PROFILE AND APPLICATION: Applicants should have obtained a PhD in biology. We welcome applications from candidates with background in biophysics, microbiology, DNA repair,

mutagenesis, or evolution. Previous experience with fluorescent microscopy and microfluidics, bacteria and phages will be valued. Applicants should send a CV, a description of their research interests and the contact details of at least two referees to Marianne De Paepe (marianne.depaepe@inrae.fr) and Marina Elez (marina.elez@inrae.fr).

STARTING DATE: The position is available from December 2021. Salary will be in accordance with INRAE regulations.

RELATED PUBLICATIONS:

1. Robert L, Ollion J, Robert J, Song X, Matic I, Elez M. Mutation dynamics and fitness effects followed in single cells. Science. 2018.
2. Robert L, Ollion J, Elez M. Real-time visualization of mutations and their fitness effects in single bacteria. Nature Protocols. 2019.
3. Ollion J, Elez M, Robert L. High-throughput detection and tracking of cells and intracellular spots in mother machine experiments. Nature Protocols. 2019.
4. M. Pereira-Gomez, R. Sanjuan, Effect of mismatch repair on the mutation rate of bacteriophage varphiX174. Virus Evol 1, vev010 (2015).
5. R. Sanjuan, M. R. Nebot, N. Chirico, L. M. Mansky, R. Belshaw, Viral mutation rates. J Virol 84, 9733-9748 (2010).