Gaining experience in genomics to study heavy metal tolerance in bacteria

Colyn Crane-Robinson
(University of Portsmouth, UK)
Nelly Sapojnikova
(Tibilisi State University, Georgia)

From 11 June to 17 July 2022, Dr Nelly Sapojnikova visited the University of Portsmouth from her institution Tibilisi State University, Georgia. The principal aim of the visit was for Dr Sapojnikova to obtain experience in the manipulation of genomic data using bioinformatics freeware and accessible websites. This theme was stimulated by the fact that she works on strains of bacteria that thrive on very high levels (typically toxic) of heavy metals such as copper and zinc. Genome sequencing of the indigenous bacterial species reveals the genes responsible for heavy metal tolerance and detoxification, and thereby improving understanding of the potential of such bacteria for bioremediation. Defining the mutations that facilitate such bacterial lifestyles requires appropriate bioinformatic, i.e., genomic, comparisons. For this reason, Dr Sapojnikova worked extensively with the Integrative Genomics Viewer (IGV) that is readily downloaded from the web onto a desktop computer. To become familiar with open servers that offer wide-ranging facilities, she also made some use of the website of the University of California San Diego, in particular the mouse browser.

One can learn the manipulation of genomic data only by handling real results, so we turned to data from a project of our own at the University of Portsmouth to which Dr Sapojnikova earlier made contributions in both ChIP-Seq and ATAC-Seq experiments. The project aims to investigate the re-programming of fully differentiated cells into pluripotential stem cells. For this, we use the (commercially available) mouse that harbours a cassette expressing the four Yamanaka factors (Oct4, Sox2, KLF4, Myc) in response to treatment of cells with doxycycline. Mouse embryonic fibroblasts (MEFs) from these mice remain synchronous for 2 days following Dox treatment, so at this point in the re-programming one can monitor genes essential for pluripotency (silent in the MEFs) and also genes characteristic of differentiated MEFs that must be supressed for pluripotency, i.e., one can study both the commissioning and decommissioning of genes and their enhancers. For this system, we have ChIP-Seq data for two histone modifications (H3K4me2 and acH3K27), one variant histone (H2A.Zac), plus

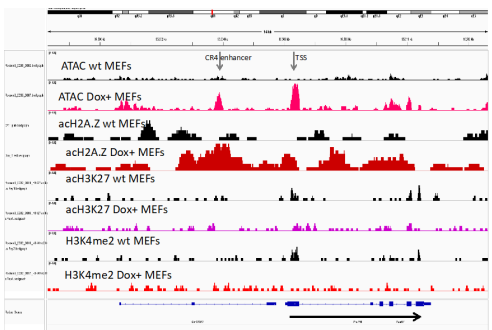


Figure 1. OCT4/POU5F1 is critically involved in the self-renewal of undifferentiated embryonic stem cells. As such, it is frequently used as a marker for undifferentiated cells. There is no transcriptional activity in wtMEFs.

the results of ATAC-Seq experiments that monitor chromatin accessibility. So there are data from four different experiments – for each of which we compare untreated wtMEFs and Dox-treated cells (Dox+).

We decided to concentrate on genes that become activated on Dox treatment with the aim of defining the primary targets of the four episomally expressed factors, as this represents the key initiating step towards pluripotency. This report is not the place to elaborate the large number of interesting observations made in Dr Sapojnikova's analyses but two stand out. The endogenous gene for Oct4 is totally silent (repressed) in wtMEFs but on Dox treatment – although not yet transcribed – there are two new signs of activity: ATAC accessibility of the promoter and distal enhancer and the appearance of acH2A.Z on the gene and on its enhancers (see Figure 1). Oct4 is critical for pluripotency, so these observations are not unexpected, but observations of the FGF4 gene (also silent in wtMEFs) are novel.

Figure 2 shows that Dox treatment leads to new chromatin accessibility of FGF4 and the appearance of acH2A.Z and H3K4me2, covering all the gene not just the promoter and enhancers. This is new as FGF4 has not previously been deemed critical for the induction of pluripotency.

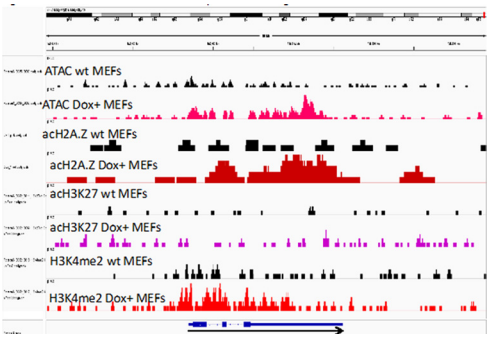


Figure 2. Mouse FGF4 gene. In wtMEFs, there is no transcription and no pol-II on the gene, but there are significant amounts of H3K4me1, so not a poised bivalent gene in MEFs. Expression of OSKM results, after 2 days, in an increase in gene accessibility (ATAC) and further H3K4 methylation and acH2A.Z, but not H3K27ac.

Figure 3. Dr Sapojnikova and Professor Crane-Robinson in the tissue culture laboratory at the University of Portsmouth.

These observations were made by downloading our own bedgraph files into the IGV platform, though the status of these (and other genes) in different cell types were checked by Dr Sapojnikova consulting the UCSC mouse browser.

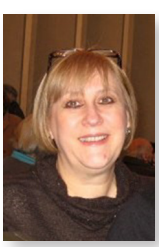
Dissemination within Georgia of the knowledge obtained during the visit to the University of Portsmouth will include talks at the regular monthly meeting of the Department of Physics of Biological Systems, Andronikashvili Institute of Physics, I. Javakhishvili Tibilisi State University, and also at the regular monthly sessions of the Biochemical Society of Georgia, both in Tibilisi.

Dr Sapojnikova also spent 3 days helping research students (M.Res.) who were working to establish protocols for CUT&RUN experiments for the same MEF re-programming system. Her experience with ATAC-Seq proved very helpful to these Portsmouth postgraduates. Figure 3 shows Dr Sapojnikova and Professor Colyn Crane-Robinson in the tissue culture laboratory at the University of Portsmouth.

It can certainly be stated that, from the point of view of UoP, and most certainly by Dr Sapojnikova herself (and subsequently Tibilisi State University), the visit was a considerable success – for which much gratitude from all participants goes to the Biochemical Society.

Colyn Crane-Robinson and Nelly Sapojnikova received a Travel Award for International Skills and Knowledge Exchange to support this visit. If you are planning a similar research-led trip, visit our website to see how we can help! https://www.biochemistry.org/grants-and-awards/grants-and-bursaries/travel-award-for-international-skills-and-knowledge-exchange/

Colyn Crane-Robinson has spent a scientific lifetime devoted to histones and other chromosomal protein domains such as HMG boxes. He was present at the birth of histone modification mapping by ChIPs. Recent interests centre on the thermodynamics of protein–DNA interactions. Email: cranerobinsonc@hotmail.com



From 2010 to present, Nelly Sapojnikova has been principal investigator at I. Javakhishvili Tibilisi State University, Andronikashvili Institute of Physics. Research interests include genetic identification of micro-organisms for ecological and medical diagnostics using biochips; their genetic instability caused by toxic agents; and structure and function of transcriptionally active and inactive chromatin. She has participated in Biochemical Society Visiting Fellowships (2014, 2022) to University of Portsmouth, UK.

