Research report visit Gregor Mendel Institute in Vienna 7-4-2013 till 19-4-2013, (note: the trip was originally intended on week earlier).

ConGenOmics - Short Visit Grant – 4973

C.A.M. Wagemaker, Radboud Universiteit Nijmegen

Visit lab: Gregor Mendel Institute, Dr. Ortrun Mittelsten Scheid, Vienna, AT

At the Experimental Plant Ecology group of the Faculty of Science of the Radboud University Nijmegen(The Netherlands) we do research on the interaction of inbreeding, environment and epigenetic factors. Until this date epigenetic analysis was performed only by absolute and/or relative quantification of methylated Cytosine’s in the *Scabiosa columbaria* genome, our model plant. In The Netherlands this species is on the red list of endangered species; a small number of mostly small populations still excists and are suitable for our experiments on inbreeding in ecological context as well as common garden and greenhouse experiments.

First our aim was to go to Vienna to learn Bisulphite sequencing, a technique suitable for more elaborate investigation of the *Scabiosa columbaria* methylome. But the successful development of a related technique, we call epi-GBS (a combination of BS sequencing en Genotyping by sequencing), gave us the opportunity to learn another technique at the GMI in Vienna named CHIP.

CHIP, or Chromatin Histone Immuno Precipitation, combined with QPCR or HT Sequencing is a powerful tool to find (epigenetic) histone modifications that can regulate gene expression. Histones are protein complexes in the nuclei of the cell and is nature’s way to pack the enormous amount of genetic code in a small volume (the nuclei). The protein modifications on the different subunits of the histones determine the density of this packaging and thereby the accessibility of the DNA by enzymes transcribing the DNA to mRNA for further processing into active proteins. The cell is capable of changing the modifications on the histones, making it possible i.e. to regulate processes of different life stages or environmental conditions. Possibly it even is a way of nature to be able to react quickly to “new conditions”, in a way genetics can never do…

The group of Ortrun Mittelsten Scheid at the GMI is very experienced in this technique and gave me the opportunity to play around with this technique and getting familiar with the in’s and out’s. It has been a wonderful experience that our group can benefit from in the near future. I was lucky that, during my stay, four very good talks were organized on this topic as well.

We were able to determine two basic parameters for the first two and very important steps of the CHIP protocol; crosslinking of the DNA to the histone complexes and sonification of the cross linked DNA. Also a basic experiment was started, just to go thru the whole protocol. DNA-Histon complexes were Immuno precipitated with Antibodies agains H3 histon subunit, H3K4Me3 modified histon subunit (for precipitation of euchromatin) and H3K9Me2 modified histon subunit (for precipitation of heterochromatin). Analysis of the end product has yet to be done in Nijmegen by QPCR.

Also we performed the very first analysis of the Scabiosa columbaria nuclei by FACS, a technique that can sort the nuclei by weight and (if you want) sort them for further analysis (this we did not). This technique can give you an idea of the endoreplication of the genome in different tissues. I.e. in *Arabidopsis thaliana* which genome is 2n can in later stages of life undergo a endoreplication to reach 4x, 8x and even 16x endoreplication level and, thereby, further fine-tuning cell metabolism and cell size. In Scabiosa columbaria we found only a low level of endoreplication, most of the cells contained 2n nuclei and only a relative small portion had a 4 x endoreplication levels.

I was also able to attend to 5 very interesting lectures of international speakers and I gave a talk myself the last day of my trip.

The experience in Vienna has been a wonderful opportunity to get experience with these techniques and will help us a lot with the future research plans of our group.