I was in Munich at the Ludwig-Maximilians University in Munich from 27th of February to 27th of March. During this training period I got introduced to production, pseudotyping and handling of lentiviral vectors. Namely, I produced different kind of lentiviral vectors with particular envelope or expression plasmids. However, all the vectors carry the construct for eGFP. First, I produce two different kind of LCMV pseudotyped lentiviral vectors (Lymphocytic choriomeningitis virus) that have a strong tropism in vivo for neural stem cells in the astroglia lineage independent of their proliferative state. They were produced using two different expression plasmids, one with the classical CMV promoter and a second with the CAG promoter. The latter is a CMV early enhancer/chicken β actin promoter, known to possess a strongest activity. Vectors containing the CAG promoter offer a valuable tool for the long term expression of transgenes, conversely CMV promoter activity slightly decreases with time. The third lentiviral vector produced was the VSVG (vesicular stomatitis virus G) pseudotyped lentiviral vectors, the classical lentiviral type pseudotyped able to infect a broad phenotypic spectrum of non-cycling and cycling cells. Moreover, I got introduced to the basic cloning procedure and to the propagation of viral plasmids that due to their big size requires specific procedures. Growing viral plasmids is a critical issue. Unlike ordinary plasmids, viral plasmids are notoriously unstable in E. coli - due to the viral long terminal repeats, LTR's. I also performed the purification of plasmid DNA for viral transfection. Viral plasmids are often too large to elute properly, from ordinary Maxi columns. That's why we used a very old method called CsCl density gradient banding. It selects specifically for the intact, supercoiled conformation of the plasmid. All damaged forms (nicked, linearized, fragmented) will get sorted out.

The full protocol of viral purification involves two rounds of ultracentrifugation. If the virus will be needed only for standard cell lines, and long-term storage of virus is not desired, one round is usually enough. For transduction of primary cells and in-vivo expression, two rounds are recommended to prevent toxicity, and immune responses to bovine serum proteins.

As a first step, the HEK 293T packaging cells were expanded and harvested at about 80% confluence. The packaging reaction is one of the critical step of the viral production. Using Lipofectamin-based transfection, we introduced the packaging plasmid, the expression plasmid and envelope in the packaging cells in order to obtain viral particles. After two days, the supernatant
was collected, spun, filtered and ultracentrifuged. The viral pellets were resuspended, aliquoted and frozen.

The transduction capability of the generated viral lots was tested in vivo after injection of the viral particles in the adult subependymal zone of the lateral ventricles and analyzed 4 days later. As expected, LCMV pseudotyped vectors transduced astrocytes in vivo.

Now in Turin, we are ready to start with the viral production and to employ it in our research. I had not only the great opportunity to get introduced into the practical aspect of the production techniques, but I also learnt how to manage a viral lab. The knowledge acquired during this month will greatly improve our research quality and my personal career.

Chiara Rolando