Martina Arends

Comparing two vector-based CRISPR/Cas9-systems for Targeting Myelinating Proteins in Zebrafish

In the last few years, the CRISPR/Cas9 system was engineered to a powerful tool in life science. It is widely used for genome editing, but it has a wide range of applications in genome manipulation. The simplest way of using the CRISPR/Cas9 system for genome editing is the introduction of single guided RNAs and the Cas9 protein into an organism. Different groups optimized the CRISPR/Cas9 system for application in zebrafish. Two different vector-based, tissue-specific CRISPR/Cas9 systems were engineered for zebrafish application: the CRISPR/Cas9 Gateway system [Ablain et al., 2015] and the CRISPR/ Cas9 Golden Gate system [Yin et al., 2015b]. A future aim is to establish one of these systems to target myelinating proteins like Caspr and Caspr2 in the zebrafish. These proteins maintain the paranodal and juxtaparanodal structure of myelin and form a diffusion barrier to keep Nav and Kv channels at place which is a precondition for saltatory conduction. In this study, these two different systems were compared for their applicability on some planned experiments by investigating the construction efficiency and comparing the properties of the vectors. It seems that the CRISPR/Cas9 Golden Gate system fits the purposes of short generation time and cost efficiency better than the CRISPR/ Cas9 Gateway system. In addition, its possibility of targeting multiple genes simultaneously is especially useful for our applications as parts of the zebrafish genome are duplicated and caspr2 is affected by this. As a consequence the pGGDestTol2LC vectors of the CRISPR/Cas9 system will be modified to target caspr and caspr2 in zebrafish.