ASSEMBLING THE TURBOID-CONTAINING PLASMID CONSTRUCT FOR INVESTIGATING THE *IN VIVO* PROTEIN-PROTEIN INTERACTIONS

S.A. Makhsatova, A.B. Kurmanbay, I.A. Akhmetollayev, A.T.Kulyyassov

National Center for Biotechnology, Republic of Kazakhstan, 000010, Astana, Qorgalzhyn highway 13/5
e-mail: kulyyasov@biocenter.kz

In vivo interactions between biomolecules (Proteins, RNA, and DNA) are the basis of cellular functionality including cell cycle, signaling pathways, cellular metabolism, and other biological processes. The traditional methods for detecting protein-protein interactions, such as affinity purification and two-hybrid analysis have limitations for the in-depth study of the cellular proteome. Besides, proteomics of the organelle protein components is still challenging to study, due to the spatial and temporal dynamics of proteins. To address these problems, proximity labeling technology was introduced. This technology not only surpasses all the limitations of traditional methods but also has unique advantages in the qualitative and quantitative analysis of the proteome of living cells.

Proximity labeling is one of the most commonly used methods for detecting the functional features and protein composition of target proteins and neighboring ones, through biotin labeling (biotinylation). Biotin is employed due to its high affinity to streptavidin and avidin, which is an efficient procedure to purify and isolate fractions containing biotinylated proteins for further analysis. During the *in vivo* biotinylation study, we used the mutant biotin ligase TurboID which is the modified enzyme of BirA. BirA is an enzyme found in *E. coli* bacteria, capable of catalyzing the attachment of biotin to specific lysine residues on a single cellular protein. The development of TurboID involved introducing specific mutations into the BirA sequence to improve its performance. These mutations result in the inability of the TurboID biotin ligase to maintain biotinyl-5'-adenylate in its active form, causing its release from the active center into the surrounding environment. This released substance contains a reactive mixed anhydride bond, enabling it to readily modify lysine residues of nearby proteins within 10nm in the cell. So, the advantage of TurboID-X (X-any protein of interest (POI)) is that it has a high efficiency for *in vivo* proximity labeling. We use the PTF (pluripotency transcription factors) SOX2, OCT4, and NANOG for X as model systems.

In our study, we used genetic engineering methods to obtain recombinant plasmid DNA containing the nucleotide sequence of TurboID and fused protein of interest X. DNA plasmid constructs have next key structural elements: Kozak sequence at the beginning of the fragment, His-Tag, and diglycine sequence at the end, BglII and XhoI restriction sites respectively to replace the wild-type biotin ligase BirA by TurboID. Two rounds of PCR amplification were performed, the first one using terminal primers that amplify only the TurboID ORF. The resulting amplicon of TurboID was applied as a matrix in the second PCR round by using long primers that contain the structural elements mentioned above. Expression of recombinant proteins from the resulting plasmid constructs will be demonstrated in HEK293T (Human embryonic kidney) cells using transient transfection with calcium phosphate method or Lipofectamine 2000.

In conclusion, further research will consist of affinity purification, detection of labeled proteins by Western blotting, and their identification by the LC-MS/MS. We hope that our research work will help us to better understand the mechanisms of the early stages of the reprogramming process.