5. OUTLOOK

The Flp-In system can repeatedly deliver transgene vectors into the identical genomic locus in every cell. Therefore, it appears ideally suitable to dissect the epigenetic events related to transgene silencing, and the roles that prokaryotic sequences have on transgene expression.

Bisulfite sequencing can be applied to thoroughly examine the DNA methylation at the transgene and its proximal regions. Thus, the hypothesis that DNA methylation starts on the flanking prokaryotic region and gradually extends to the transgene region can be easily tested. If DNA methylation examination is performed at different time points relating to the transgene expression state, one could find out whether DNA methylation causes or is caused by transgene silencing.

In order to test if the heterochromatinization process is initiated by the prokaryotic sequences at the transgene region, ChIP (chromatin immunoprecipitation) assay can be applied to detect the various histone modifications and binding of the HP (heterochromatin-binding protein) on the prokaryotic sequences and the transgene DNA sequences, respectively. This investigation should be performed at different time points in relation to the transgene expression state. Thus, one can reveal in which order the chromatin modifications go with transgene suppression. By comparing the occurrence of DNA methylation and histone modification, one can gain more insight to the sequence of these two epigenetic events.

Undoubtedly, it is important to know how the “Sorting-Subcloning” approach brings about homogeneous and steady transgene expression. It will be interesting to find out how many copies of the transgene are inserted in those stable clones obtained by bypassing antibiotic selection, or if the stable and homogeneous expression is actually in inverse proportion to the transgene copy number. This can be easily done by Southern hybridization. If it is the case, strategies should be explored for low copy number integration of the transgene. Viruses and transposons can be introduced into this approach and meet the

request of single copy integration. Additionally, it is necessary to test the "Sorting-Subcloning" approach devised in this study in other cell lines for its general applicable competence. Moreover, in order to gain insight into the integration site of the transgene, it is critical to detect in which region the transgene is inserted by *in situ* hybridization, and what specific chromatin characteristics and DNA methylation states the integration region possesses. Results obtained will allow us to isolate integration sites that are epigenetic inert. Ultimately, one can upgrade the recombinase based gene delivery systems with the "Sorting-Subcloning" approach so that the transgene of interest introduced would be immune to epigenetic modulations.

It is curious to know if these clones isolated either with EGFP or cell surface marker as a sorting marker can maintain the transgene expression over a long period of time. Now I am using ß-galactosidase and luciferase genes with EGFP as a FACSorting marker to test the reliability of the approach devised in this thesis. In parallel, the MACS approach using a cell surface marker gene is also at the developmental stage. If these trials prove practical and successful, the "Sorting-Subcloning" approach will provide valuable means to obtain cell lines with steady and homogeneous transgene expression and greatly benefit the transgenic researches in cell lines.