6. SUMMARY

Sustained and homogeneous transgene expression is a prerequisite for many biological studies that rely on the manipulation of genomes by introduction of additional genetic information. However, transgenes stably integrated into a cell's genome are frequently subjected to progressive decrease of their expression over time, and eventually become silenced. This phenomenon has long been noticed and created great attention in both the cell biology and transgenic animal research fields. This is also a serious limitation to gene therapy research. Increasing evidence indicates that heterogeneous or silenced transgene expression is largely caused by epigenetic modulations, in particular, DNA methylation and chromatin modification, on the transgene itself or at the integration site. Epigenetic control is the underlying mechanisms on several regulatory pathways, such as position effect variegation, repeat-induced gene silencing and host defense mechanism. In addition, promoter choices and the nature of the transgene are among other factors contributing to the epigenetic control of transgene expression.

The work presented in this thesis focused initially on using a single cell assay (Fluorescence-Activated Cell Scanning, FACS analysis) to determine the effects of single copy integration on the stability of EGFP transgene expression in the Flp recombinase-based transgenic system. By repeatedly targeting the predetermined FRT site in Flp-In 293 cells, sustained and uniform EGFP expression driven either by the viral CMV promoter or the housekeeping EF-1a gene promoter was monitored under antibiotic selective conditions. After removal of the antibiotic from the culture, however, progressive transgene suppression took place. The use of EF-1a housekeeping gene promoter did not show any advantage over the viral CMV promoter to sustain the transgene expression. Moreover, the suppression of the transgene expression was even not ameliorated when substituting the EGFP gene by a novel allele free of CpG dinucleotides ($EGFP^{CpG-}$). I thereby hypothesized that prokaryotic sequences integrated along with the transgene into the host genome triggered epigenetic modulations at the local chromatin domain, resulting in the transgene region become engrossed by the spreading of heterochromatin structure and gradually being silenced.

Additionally, two different regulation modes were observed during the course of transgene silencing: the CMV promoter is associated with the rheostat mode; the EF-1 a promoter has an on/off mode.

The newly introduced “Sorting-Subcloning” approach is designed to obtain EGFP transgene positive clones by bypassing the traditional antibiotic selection. This approach directly selects integration events that escape epigenetic modulations and permit unforced transgene expression. Systematic studies presented in this thesis proved this novel approach is a successful gateway to homogeneous, prolonged, and even enhanced transgene expression. Furthermore, it provides strong evidence that antibiotic selection protocol is a decisive factor contributing to transgene silencing. These results will greatly facilitate transgene studies in cultured cell lines. Future investigation is necessary to decipher how epigenetic modulations are controlled on the integrated transgene sequences. The outcome will facilitate analyzing transcription programs of the transgene without the dominant effect of the epigenetic environment.