

10. APPENDIX

I: Abbreviations

Abbreviation	Full expression
5-azaC	5-azacytidine
Ac	acetylation
APS	ammonium persulphate
bp	base pair
BSA	bovine serum albumin, fraction V
Cass	transcription cassette
ChIP	chromatin immunoprecipitation
CMV	human cytomegalovirus
CpG	cytosine and guanine dinucleotide
CS	cleavage site
ddH ₂ O	double distilled water
DMEM	Dulbeco's Modified Eagle's Medium
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic acid
DNMT	DNA methyltransferase
EF (EF-1a)	human elongation factor-1 alpha
EGFP	enhanced green fluorescent protein
FACS	fluorescence-activated cell scanning
FACSorting	fluorescence-activated cell sorting
h	hour
HDAC	histone deacetylase
hrGFP	humanized recombinant GFP
Hyg ^{R/S}	hygromycin resistant/sensitive
IRES	internal ribosomal entry site
LB	Luria Bertani
LCR	locus control region
M	Molar
MACS	magnetic assistant cell sorting
Me	methylation

MEM	Minimum Essential Medium Eagle
Min	minute
mRNA	messenger RNA
ms.	multiple copy
no.	number
O/N	over night
PBS	phosphate buffered saline
PCNA	proliferating cellular nuclear antigen
PEV	position effect variegation
PI	propidium iodide
RIGS	repeat-induced gene silencing
RMCE	recombinase-mediated cassette exchange
RNA	ribonucleic acid
rRNA	ribosome RNA
RT	room temperature
SAP	shrimp alkaline phosphatase
SDS	sodium dodecyl sulphate
SSC	standard saline citrate
SV40	simian virus 40
TEMED	N,N,N',N'- tetramethylethylene diamine
tRNA	transport RNA
TSA	trichostatin
UAS	upstream activating sequence
UV	ultraviolet
X-gal	5-bromo-4-chloro-3-indolyl- β -D-galactoside
Zeo ^{R/S}	Zeocin resistant/sensitive

II. List of Figures

Fig. 1. Diagrammatic representation of the nucleosome and potential amino acid residues of modification on histone H3 and H4.....	7
Fig. 2. Rheostat versus on/off models for transcriptional activation by enhancers.....	18
Fig. 3. The location of the FRT site in the host cell line genome.	37
Fig. 4. Scheme of Flp-In system.	38
Fig. 5. The feature of the FRT site. CS, cleavage site.....	39
Fig. 6. The flow chart of the “Sorting-Subcloning” approach.....	40
Fig. 7. The presence of the FRT site in the host cell line (Flp-In 293 cells) genome.....	42
Fig. 8. The copy number of the transgene at FRT site examined by Southern blotting.....	44
Fig. 9. Stability of the transgene expression under antibiotic selection.	47
Fig. 10. Unstable expression of the transgene under nonselective conditions.....	49
Fig. 11. Gradual loss of transcription at the FRT site.....	50
Fig. 12. The irreversible expression state of the transgene after FACSorting.....	51
Fig. 13. The variance of transgenic expression between clones.	54
Fig. 14. Transgenic expression of the clone with multiple copies of the transgene.....	55

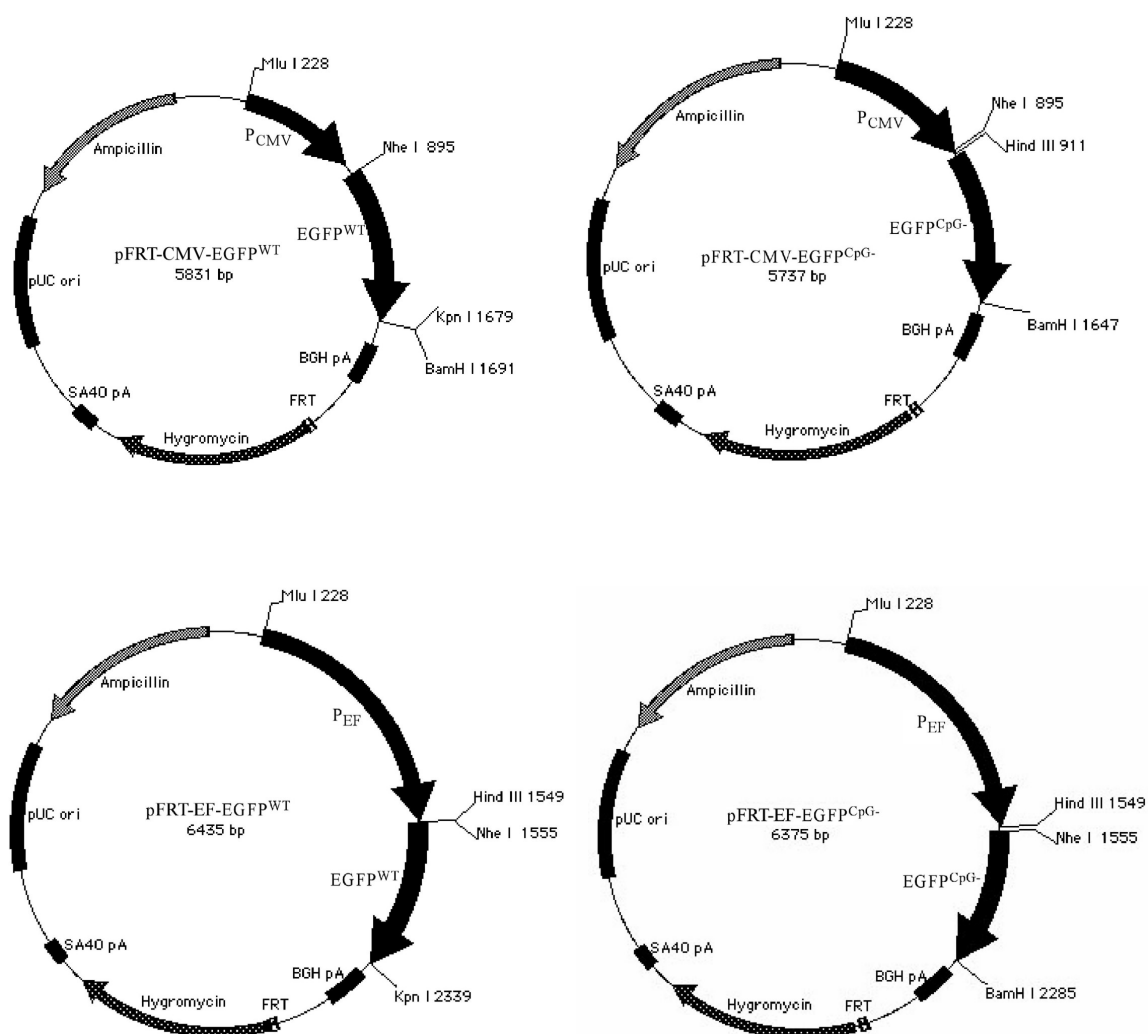
Fig. 15. Transgene cassettes used to generate stable clones with the “Sorting-Subcloning” approach.....	57
Fig. 16. Comparison of clonal expression patterns obtained by the “Sorting-Subcloning” approach.....	59
Fig. 17. Stable expression of clones derived from transfection with the “cass” by the “Sorting-Subcloning” approach.....	61
Fig. 18. Expression pattern comparison of clones isolated by “Sorting-Subcloning” and G418 selection.....	63
Fig. 19. Expression stability analysis of clones isolated by either the “Sorting-Subcloning” approach or G418 selection.	65
Fig. 20. Illustration for the inability of multiple copy transgenes to dissect gene regulation modes.	75

III. List of Tables

Table 1. Additional enzymes.....	27
Table 2. Kits.....	28
Table 3. Vectors	28
Table 4. Antibiotics	29
Table 5. Statistics of the clones derived from each construct with different features.....	45
Table 6. Summary of clones with different expression pattern obtained by the “Sorting-Subcloning” approach.....	58

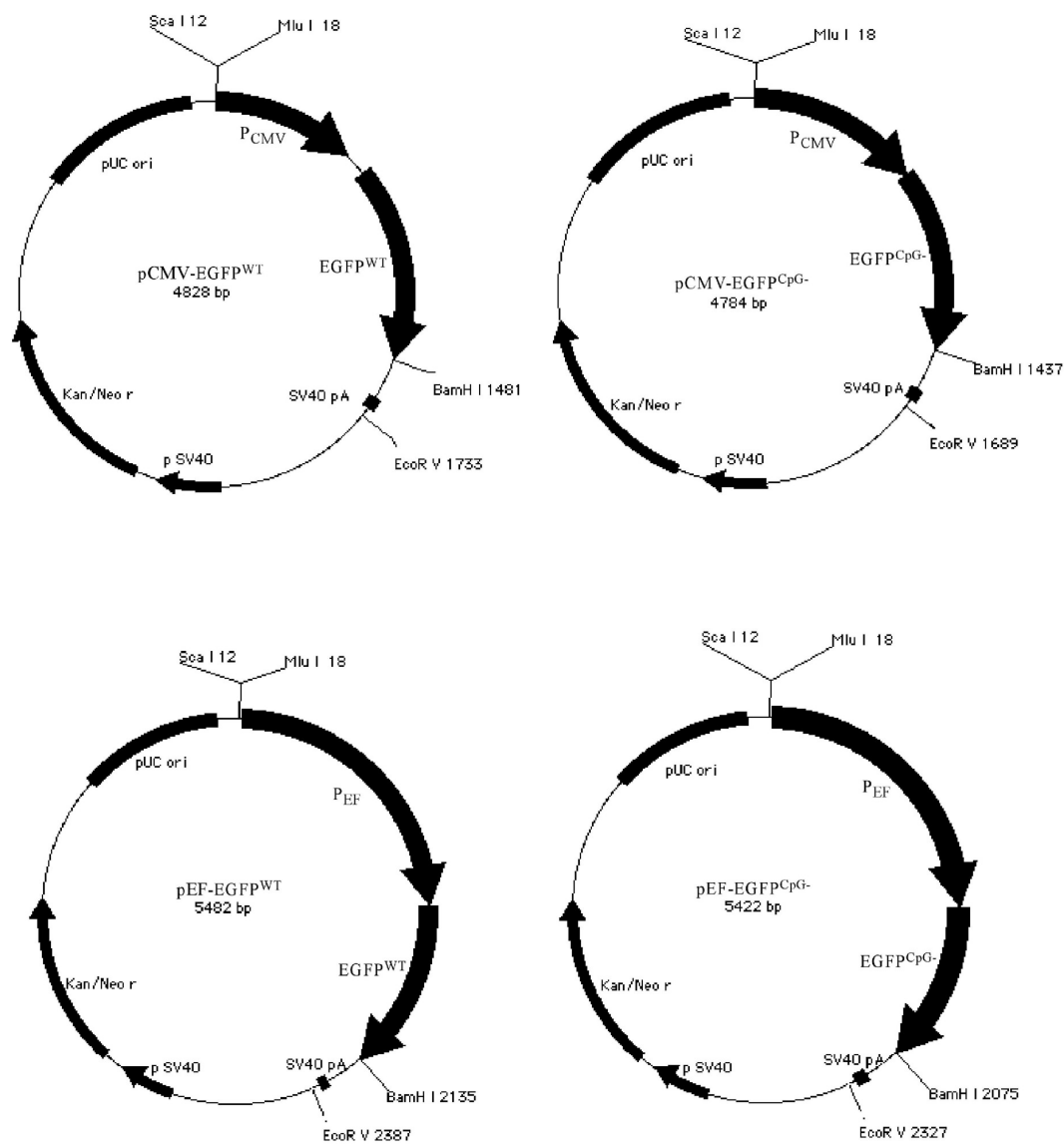
IV. Maps of Plasmid Constructs

Expression plasmids used in the Flp-In system:



Note: The above four plasmids were constructed basing on pcDNA5/FRT (Invitrogene Co., the Netherlands).

Expression vectors used in the experiments by means of “Sorting-Subcloning”:



Note: the above four plasmids were constructed based on pEGFP-C1 (BD Biosciences, Clontech).