# A modified RMCE-compatible Rosa26 locus for the expression of transgenes from exogenous promoters

**JournalArticle (Originalarbeit in einer wissenschaftlichen Zeitschrift)**

**ID** 1195136
**Author(s)** Tchorz, J. S.; Suply, T.; Ksiazek, I.; Giachino, C.; Cloetta, D.; Danzer, C. P.; Doll, T.; Isken, A.; Lemaistre, M.; Taylor, V.; Bettler, B.; Kinzel, B.; Mueller, M.
**Author(s) at UniBasel** Bettler, Bernhard ; Taylor, Verdon ;
**Year** 2012
**Title** A modified RMCE-compatible Rosa26 locus for the expression of transgenes from exogenous promoters
**Journal** PLoS ONE
**Volume** 7
**Number** 1
**Keywords** Animals; Attachment Sites, Microbiological/genetics; Embryonic Stem Cells/metabolism; Genes, Reporter/genetics; Genetic Loci/*genetics; Green Fluorescent Proteins/metabolism; Integrases/*metabolism; Luciferases/metabolism; Mice; Mice, Inbred BALB C; Mice, Transgenic; Mutagenesis, Insertional/*methods; Organ Specificity/genetics; Promoter Regions, Genetic/*genetics; Proteins/*genetics; Transgenes/*genetics
Generation of gain-of-function transgenic mice by targeting the Rosa26 locus has been established as an alternative to classical transgenic mice produced by pronuclear microinjection. However, targeting transgenes to the endogenous Rosa26 promoter results in moderate ubiquitous expression and is not suitable for high expression levels. Therefore, we now generated a modified Rosa26 (modRosa26) locus that combines efficient targeted transgenesis using recombinase-mediated cassette exchange (RMCE) by Flipase (Flp-RMCE) or Cre recombinase (Cre-RMCE) with transgene expression from exogenous promoters. We silenced the endogenous Rosa26 promoter and characterized several ubiquitous (pCAG, EF1? and CMV) and tissue-specific (VeCad, ?SMA) promoters in the modRosa26 locus in vivo. We demonstrate that the ubiquitous pCAG promoter in the modRosa26 locus now offers high transgene expression. While tissue-specific promoters were all active in their cognate tissues they additionally led to rare ectopic expression. To achieve high expression levels in a tissue-specific manner, we therefore combined Flp-RMCE for rapid ES cell targeting, the pCAG promoter for high transgene levels and Cre/LoxP conditional transgene activation using well-characterized Cre lines. Using this approach we generated a Cre/LoxP-inducible reporter mouse line with high EGFP expression levels that enables cell tracing in live cells. A second reporter line expressing luciferase permits efficient monitoring of Cre activity in live animals. Thus, targeting the modRosa26 locus by RMCE minimizes the effort required to target ES cells and generates a tool for the use exogenous promoters in combination with single-copy transgenes for predictable expression in mice.
**Publisher** Public Library of Science
**ISSN/ISBN** 1932-6203
**edoc-URL** http://edoc.unibas.ch/dok/A6005322
**Full Text on edoc** No;
**Digital Object Identifier DOI** 10.1371/journal.pone.0030011
**PubMed ID** http://www.ncbi.nlm.nih.gov/pubmed/22253858
**ISI-Number** WOS:000301361500030
**Document type (ISI)** Article