

Enhancement of the Cre-loxP System

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Summary:

Vanderbilt researchers have enhanced the Cre-loxP system by reconstituting Cre activity from Cre protein fragments. This reconstitution recovers substantial Cre activity, 8 fold more than other reconstructive systems. In addition this invention allows for restricting Cre activity selectively in cells that express dual protein markers, allowing greater specificity of Cre expression.

Potential Market Size:

The Cre-loxP system is an essential research tool used by almost all biologists. Thus almost all researchers currently using this system can benefit from this technology due to the greater cell type specificity and improved activity it offers.

Current Competitive Product(s):

One of the major challenges that researchers who utilize the Cre-loxP system face is the difficulty in properly regulating Cre expression. Currently the Cre-loxP system utilizes one promoter to drive Cre expression in specific cell types however all cells in which that promoter is active will drive Cre expression. Toward this end it may be difficult to find promoters that are strictly limited to a particular cell type. Inappropriate or nonspecific Cre activity can hinder data interpretation in cell lineage studies. Additionally in cell types where little is known, such as stem cell populations, Cre activity maybe driven incorrectly.

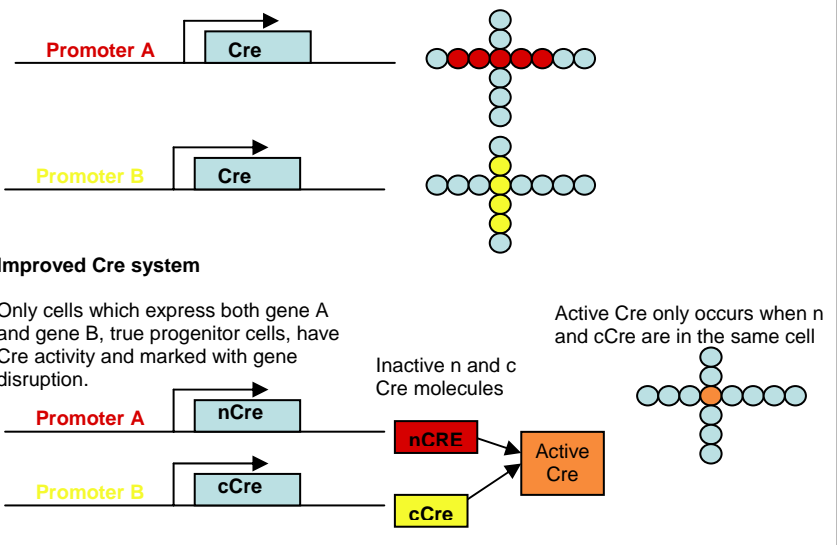
Description of Technology/Products:

The Cre-lox P system has been widely used since its conception in 1984 and is a key research tool allowing the analysis of essential genes in specific organs by gene inactivation or ectopic expression. When combined with visible marker-gene activation, this system allows for cell marking and cell lineage analysis in cultured cells and living animals.

Cre is a recombinase enzyme which can recognize specific DNA sequences termed loxP sites. Upon encountering two separate loxP sites, Cre can excises and exchange the DNA strands containing the loxp sequences and result in excision, integration, or inversion of the DNA sequences next to the loxP sites, depending upon whether the loxP sites were localized on a same DNA molecule, and the orientation of the target sites. For most applications, loxp sites are localized in a same DNA molecule and excision occurs. The excision reaction is effectively irreversible, and normal gene expression is considerably compromised or eliminated if a cells contains a gene floxed by loxP sites and the cell express the Cre recombinase. Specific gene promoters can drive Cre expression in desired cells or tissues. This type of control is not ideal since many promoters drive gene expression in several cell types, which can lead to inappropriate Cre activation in cells which are not of interest (Figure 1). This is particularly problematic for stem/progenitor cells that can only be defined by expression of several markers. Thus, the normal one-promoter-based Cre-loxP system can not be utilized to selctively manipulate genes in these cell types do to the nonspecific Cre expression that may occur. This newly developed technology overcomes these barriers and allows for a more cell type specificCre-loxP operation.

Figure 1.

Disrupt gene activity in the progenitor stem cell only. Gene A and gene B are expressed in progenitor stem cells but are also expressed in other cells. Thus how does one know which cells are true progenitor cells under the current Cre system?



Essentially Vanderbilt researchers have cleaved the Cre open-reading frame into two cDNA fragments. Each of these fragments encodes an inactive portion of the Cre molecule. Each cleaved Cre fragment was then fused with cDNAs that encode a pair of peptides that could form antiparallel leucine zippers, respectively. Each fusion was then driven by a different cell-type specific promoter. Only when both promoters are present within the same cell will the fully active Cre protein assemble (Figure 1). This group has shown that activity of Cre increased by over 8 fold when the assembly is facilitated by the leucine zippers. The improved features of this system allowed researchers to utilize the power of the Cre-loxP system to manipulate genes in cell types that only co-express dual protein markers. Such a cell type specificity are not possible to achieve using the traditionally Cre expression approach.

Value Proposition:

This technology provides significant advantage over the conventional Cre/LoxP system in that it vastly improves the cell-selectivity for Cre expression, so that DNA recombination is restricted to cells that co-express dual promoters, instead of the current system in which only one promoter is used. Further, this system improves Cre activity by 8 fold, over similar Cre systems in which Cre is reconstituted. Taken together these improvements make this technology ideal for cell lineage studies especially in progenitor stem cell studies.

Intellectual Property Status:

One United States Patent is pending with composition and method claims. International rights are still available with this priority date.

