The present inventors have attempted to develop a new gene expression system using promoters that allow genes to be expressed with higher efficiency. Specifically, they have compared and examined promoter activity resulting from combinations of promoters and enhancers for various genes. As a result, the present inventors have discovered that a gene can be expressed with high efficiency by causing such gene to be flanked by two promoters or a promoter and an enhancer with the use of a gene expression cassette. The gene expression cassette comprises a DNA construct containing a gene to be expressed and a poly A addition sequence that are located downstream of a 1st promoter, as well as an enhancer or a 2nd promoter ligated downstream of the DNA construct. The present inventors have further discovered that a gene can be expressed with even higher efficiency by causing the above expression cassette to further contain an element such as a poly A addition sequence, RU5', UAS, or SV40-ori.

Through the development of "a system for increasing gene expression and the vector comprising the system" of the present invention, protein expression can be strongly increased in almost all cells and genes.

The present invention is as follows.

In one aspect, there is provided a gene expression cassette, which comprises:

(i) a DNA construct in which a 1st promoter, a gene to be expressed, and a polyA addition sequence are linked in such order; and

(ii) an enhancer or an enhancer with UAS ligated to the upstream portion thereof, in such order,

wherein the enhancer or the enhancer with UAS ligated to the upstream portion thereof is ligated immediately downstream of the polyA addition sequence, and

can enhance the protein expression from the gene compared with a case in which an enhancer is inserted upstream of the 1st promoter.

In another aspect, there is provided a vector, containing the expression cassette for a foreign gene as defined herein.

In yet another aspect, there is provided a host cell, containing the vector as defined herein.

In yet another aspect, there is provided a preparation for disease detection or treatment, comprising the vector as defined herein.

In yet another aspect, there is provided an in vitro method for expressing a gene to be expressed using the expression cassette as defined herein or the vector as defined herein.

In yet another aspect, there is provided an in vitro method for producing a protein encoded by a gene to be expressed, comprising introducing the expression cassette as defined herein or the vector as defined herein into a cell, and then culturing the cell.

In yet another aspect, there is provided an in vitro method for enhancing protein expression from a gene compared with a case in which an enhancer is inserted upstream of a 1st promoter by: ligating an enhancer or an enhancer with UAS ligated to the upstream portion thereof to a site immediately downstream of a polyA addition sequence of a DNA construct in which a 1st promoter, a gene to be expressed, and the polyA addition sequence are linked in such order, so as to insert the enhancer to a site immediately downstream of the DNA construct comprising the gene to be expressed, in an expression cassette for gene expression comprising the 1st promoter, the gene to be expressed, the polyA addition sequence, and the enhancer.

Also disclosed are:

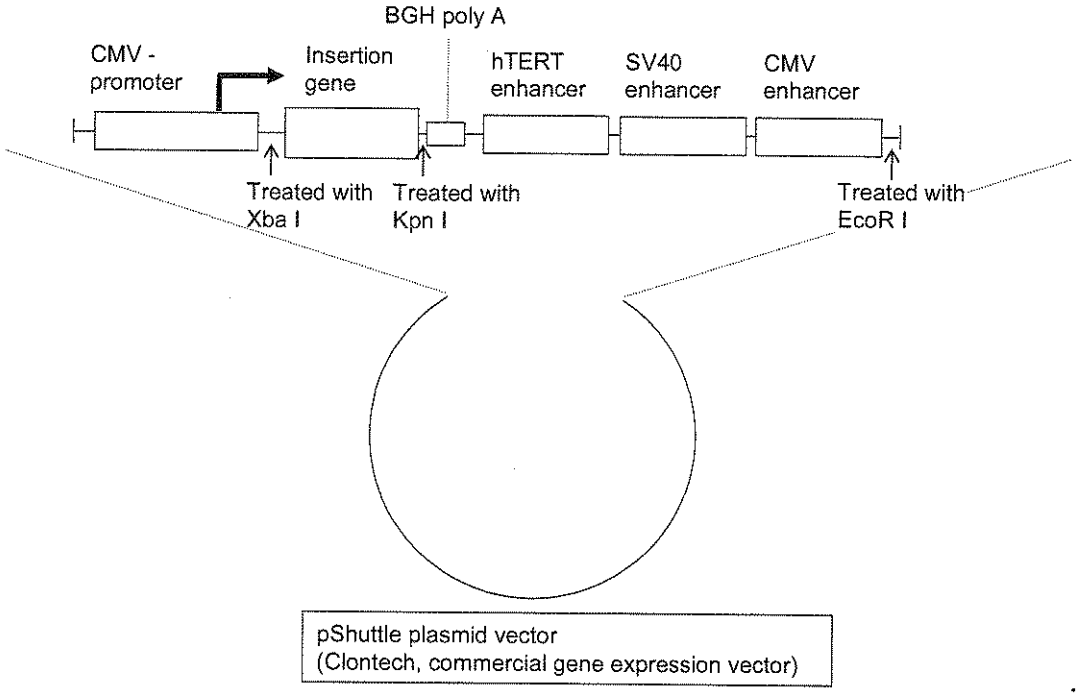
[1] A gene expression cassette, comprising

a DNA construct containing a gene to be expressed and a poly A addition sequence that are located downstream of a 1st promoter, and further comprising an enhancer or a 2nd promoter downstream of the DNA construct.

[2] The expression cassette according to [1], which does not have another mechanism for gene expression downstream of the ligated enhancer or the 2nd promoter, but has a structure in which a gene to be expressed is flanked by one 1st promoter and one enhancer, or one 1st promoter and one 2nd promoter.

[3] The expression cassette according to [1], wherein the promoter is selected

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11. A vector, containing the expression cassette for a foreign gene according to any one of claims 1 to 10.

12. The vector according to claim 11, which is an adenovirus vector or an adeno-associated virus vector.

13. A host cell, containing the vector according to claim 11 or 12.

14. A preparation for disease detection or treatment, comprising the vector according to claim 11 or 12.

15. An in vitro method for expressing a gene to be expressed using the expression cassette according to any one of claims 1 to 10 or the vector according to claim 11 or 12.

16. An in vitro method for producing a protein encoded by a gene to be expressed, comprising introducing the expression cassette according to any one of claims 1 to 10 or the vector according to claim 11 or 12 into a cell, and then culturing the cell.

17. An in vitro method for enhancing protein expression from a gene compared with a case in which an enhancer is inserted upstream of a 1st promoter by: ligating an enhancer or an enhancer with UAS ligated to the upstream portion thereof to a site immediately downstream of a polyA addition sequence of a DNA construct in which a 1st promoter, a gene to be expressed, and the polyA addition sequence are linked in such order, so as to insert the enhancer to a site immediately downstream of the DNA construct comprising the gene to be expressed, in an expression cassette for gene expression comprising the 1st promoter, the gene to be expressed, the polyA addition sequence, and the enhancer.

18. The method according to claim 17, wherein the enhancer ligated downstream of the polyA addition sequence is at least one enhancer selected from the group consisting of a CMV enhancer, an SV40 enhancer, and an hTERT enhancer.

19. The method according to claim 17 or 18, wherein the enhancers ligated downstream of the polyA addition sequence are a TERT enhancer, an SV40 enhancer, and a CMV enhancer that are linked in such order.