Cat. #3653-3658

For Research Use

TaKaRa

pDON-AI-2 Neo DNA	**(Cat. #3653)**
pDON-AI-2 DNA	**(Cat. #3654)**
pMEI-5 Neo DNA	**(Cat. #3655)**
pMEI-5 DNA	**(Cat. #3656)**
pDON-5 Neo DNA	**(Cat. #3657)**
pDON-5 DNA	**(Cat. #3658)**

Product Manual

v202008Da

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I. Description

The pDON-AI, pMEI-5 and pDON-5 series are retroviral vectors. They contain only LTR and packaging signal (Ψ sequence) of MoMLV genome, but not *gag*, *pol* and *env* coding sequence.

[pDON-AI-2 Series]

The plasmids are for performing high-titer preparation of retrovirus.
The U3 region of 5′ LTR has been substituted with a more powerful promoter derived from cytomegalovirus, giving it a high transcription efficiency and allowing it to be used to generate high titer-recombinant retroviruses and accordingly efficient gene transductions. Moreover, it carries a human actin-derived intron and splice acceptor upstream of the cloning site to increase the efficiency of target gene expression after gene transduction. pDON-AI-2 Neo DNA (Cat. #3653) has a neomycin resistance gene as a drug selection marker.

[pMEI-5 Series]

The plasmids are for preparing high-expression retrovirus.
It carries a human EF1 ***α*** -derived intron with high splicing activity upstream of the cloning site designed to increase the efficiency of target gene expression after gene transduction. These characteristics give the vector a very high transcription efficiency. pMEI-5 Neo DNA (Cat. #3655) has a neomycin resistance gene as a drug selection marker.

[pDON-5 Series]

The plasmids are for performing high-titer preparation of high-expression retrovirus.
It carries a human EF1 ***α*** -derived intron with high splicing activity upstream of the cloning site and the U3 region of 5′ LTR substituted with a more powerful promoter derived from cytomegalovirus. pDON-5 Neo DNA (Cat. #3657) has a neomycin resistance gene as a drug selection marker.
This product is superior to the pMEI-5 series in enabling the production of high titer-recombinant retroviruses.

II. Product Information

pDON-AI-2 Neo DNA	(Cat. #3653)	20 μg
pDON-AI-2 DNA	(Cat. #3654)	20 μg
pMEI-5 Neo DNA	(Cat. #3655)	20 μg
pMEI-5 DNA	(Cat. #3656)	20 μg
pDON-5 Neo DNA	(Cat. #3657)	20 μg
pDON-5 DNA	(Cat. #3658)	20 μg

Concentration: 1 μg/μl
Form: 10 mM Tris-HCl (pH8.0), 1 mM EDTA

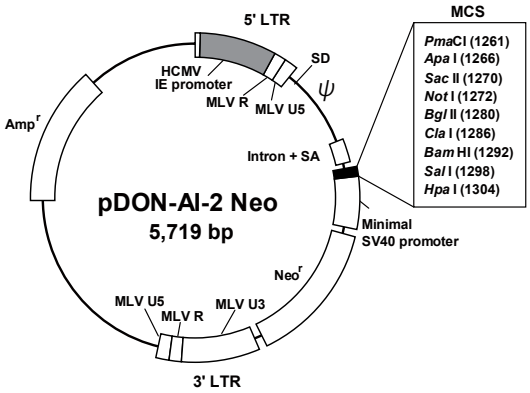
III. Storage -20℃

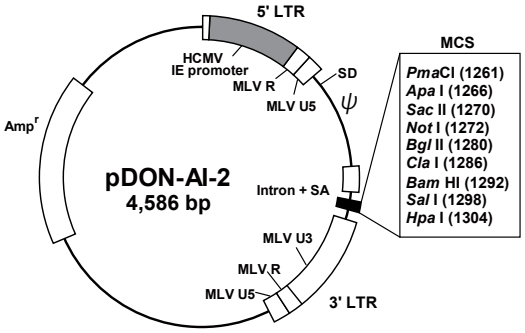
IV. Vector Map and Cloning Sites

The vectors have 9 common multicloning sites (with pMEI-5 DNA and pDON-5 DNA each having one additional site), which makes it easy to insert the target gene and perform comparisons among the vectors.

<Vector Map>

● **pDON-AI-2 series**





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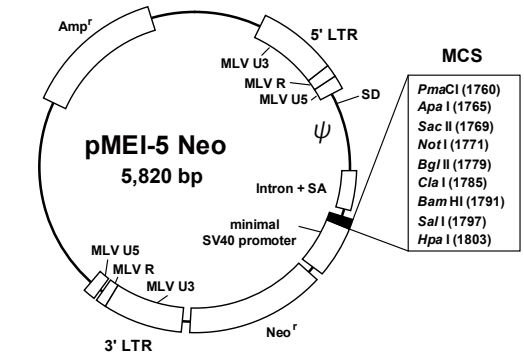

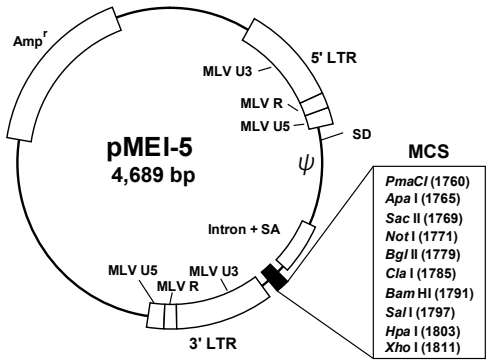

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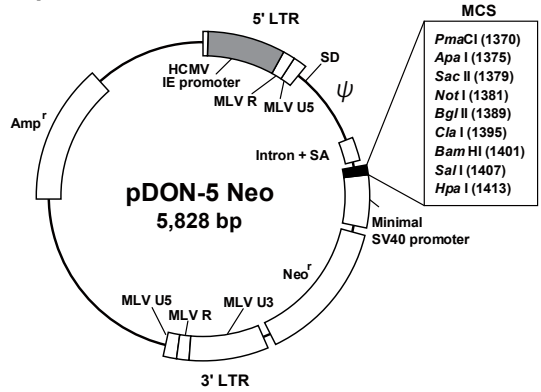
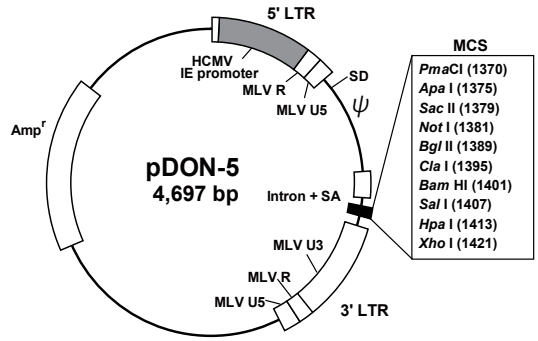
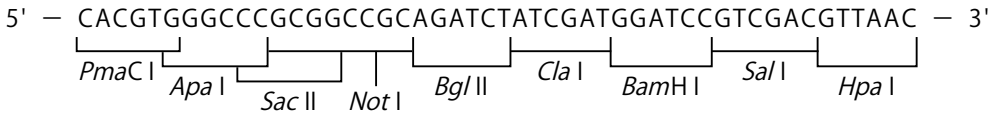


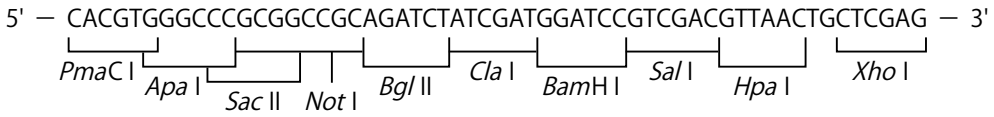


<Multi cloning site>

pDON-AI-2 Neo, pDON-AI-2, pMEI-5 Neo, pDON-5 Neo



pMEI-5, pDON-5



V. Advantages of Retroviral Vectors

Retroviral vectors offer the following advantages in transformation of a host cell with a target gene for expression.

(1) Retroviral vectors are integrated into chromosomes, enabling stable long-term gene expression. Transgenes introduced into a cell by a vector lacking the chromosome-integration ability are degraded intracellularly or diluted along with cell division. Expression of such transgenes, therefore, is transient and not long lasting. Retroviral vectors are integrated into the host chromosome, ensuring their stability and propagation even after cell division.

(2) Construction of other viral vectors require complicated procedures. Retroviral vectors, on the other hand, can be produced with relative ease.

(3) Retroviral vectors enable transduction of a large number of cell types logarithmic growth phase. Transfection of hematopoietic stem cells is challenging using physical or chemical methods. The use of a fibronectin fragment (RetroNectin® (Recombinant Human Fibronectin Fragment) (Cat. #T100A/B)) however, allows high-efficiency gene transduction of hematopoietic stem cells with retroviral vectors.

VI. Construction of Recombinant Retroviral Vectors Using pDON-AI-2, pMEI-5, and pDON-5 Series

Figure 1 shows the procedure for producing recombinant retroviruses by transiently transfecting G3T-hi cells using the Retrovirus Packaging Kit Eco/Ampho (Cat. #6160/6161). Recombinant retroviruses can also be produced by transfecting packaging cells.

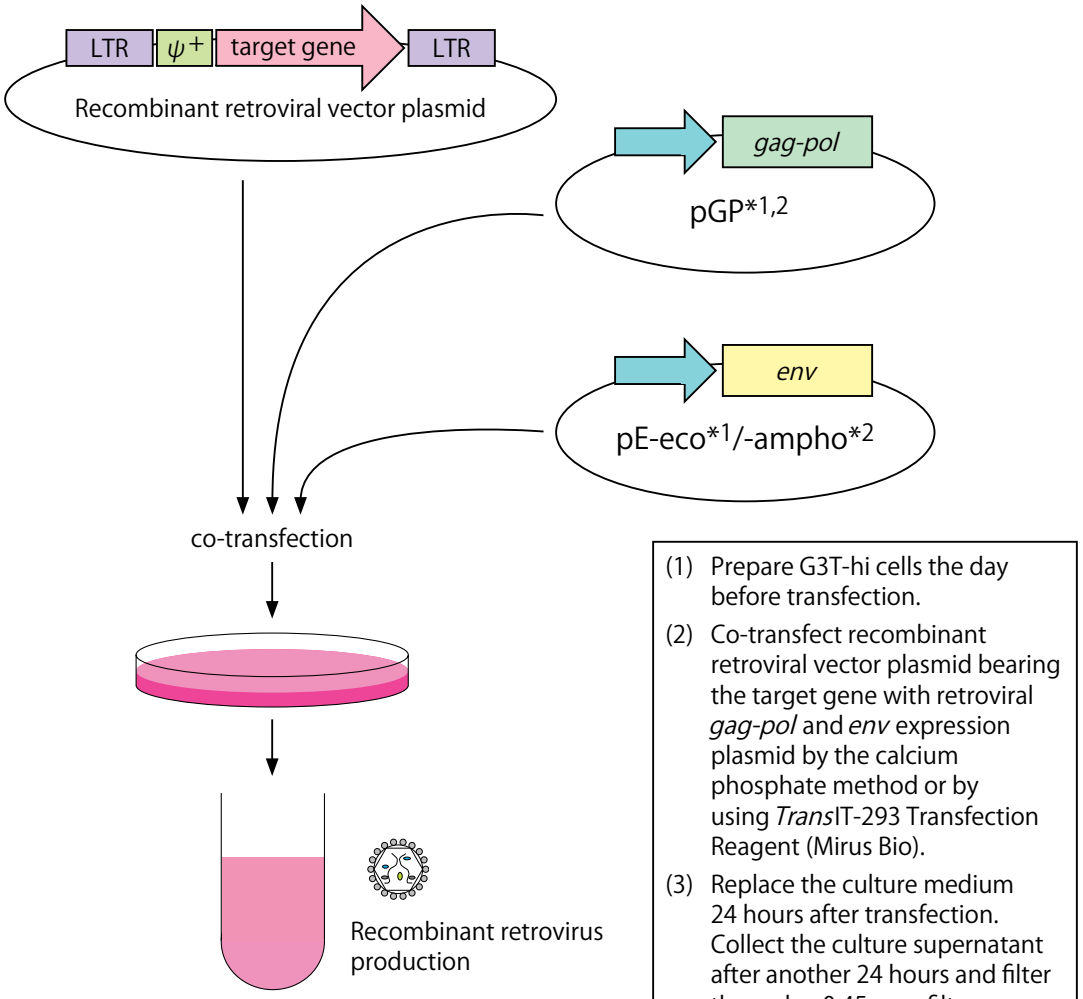


Figure 1. Procedure for Producing Recombinant Retroviruses Using the Retrovirus Packaging Kit

*1 It is included in Retrovirus Packaging Kit Eco (Cat. #6160).
*2 It is included in Retrovirus Packaging Kit Ampho (Cat. #6161).

VII. Experimental Examples

Producing recombinant retroviruses using pDON-AI-2 Neo DNA, pDON-AI-2 DNA, pMEI-5 Neo DNA, pMEI-5 DNA, pDON-5 Neo DNA, and pDON-5 DNA

1. Method

The ZsGreen gene was inserted into the *Bam* H I/*Hpa* I site of pDON-AI-2 Neo DNA, pDON-AI-2 DNA, pMEI-5 Neo DNA, pMEI-5 DNA, pDON-5 Neo DNA, and pDON-5 DNA, yielding pDON-AI-2 Neo-ZsGreen, pDON-AI-2-ZsGreen, pMEI-5 Neo-ZsGreen, pMEI-5-ZsGreen, pDON-5 Neo-ZsGreen, and pDON-5-ZsGreen vectors, respectively. G3T-hi cells were transiently transfected using the Retrovirus Packaging Kit Ampho (Cat. #6161) and then recombinant retroviruses were produced.

2. Results

<2-1. Comparison of virus titers (Table 1, Figure 2)>

(1) HT1080 cells were infected with serially diluted retroviral vectors in the presence of polybrene and the transfer efficiencies of the ZsGreen gene were measured using a flow cytometer to calculate virus titers.

(2) HT1080 cells were infected with serially diluted retroviral vectors, and the colony numbers formed after drug selection with G418 were determined to calculate virus titers. (Only for those having the Neo gene)

Table 1. Comparison of Virus Titers

	(1) ZsGreen/HT1080	**(2) G418/HT1080**
	ivp/ml	**cfu/ml**
DON-AI-2-Neo	4.08×10^6	4.43×10^6
DON-AI-2	7.35×10^6	–
MEI-5 Neo	3.96×10^5	5.88×10^5
MEI-5	1.31×10^6	–
DON-5 Neo	2.65×10^6	3.83×10^6
DON-5	3.26×10^6	–

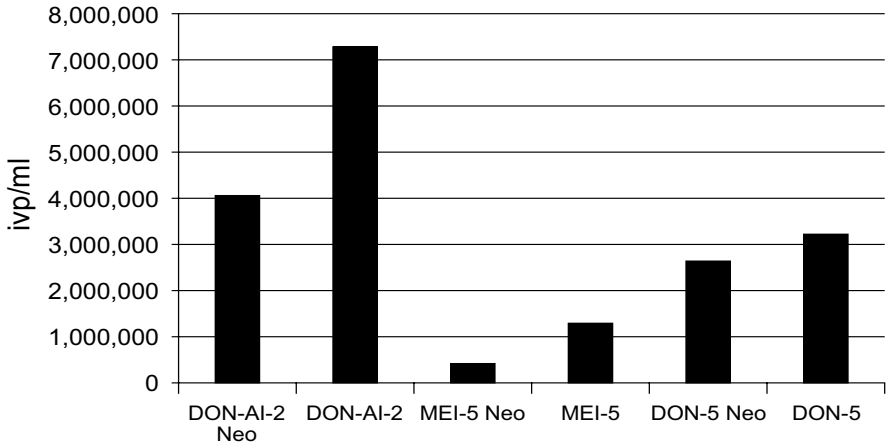


Figure 2. Comparison of Virus Titers (ZsGreen/HT1080)

<2-2. Comparison of gene expression intensity (Figure 3)>

HT1080 cells were infected with retroviruses vector at various dilution rates by the polybrene method. Three days after transduction, gene transfer efficiency and ZsGreen expression intensity were measured using a flow cytometer. Expression intensities were compared, and it was assumed that the expression intensity at the dilution rate showing less than 20% transfection efficiency is that of a single-virus infected cell. The values shown are relative to the expression intensity of DON-AI-2 virus vector (value set to 1).

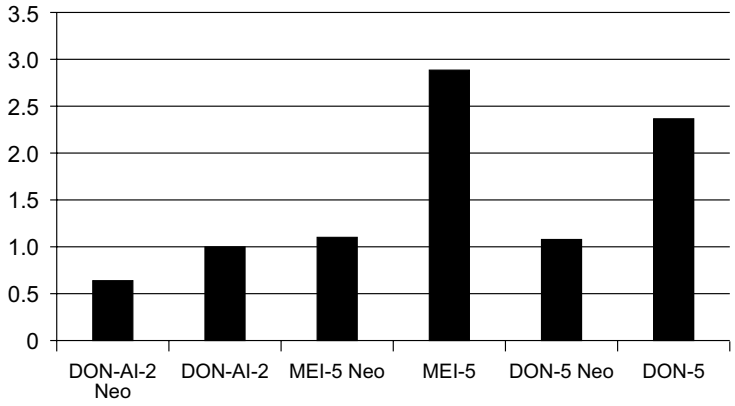


Figure 3. ZsGreen Expression Intensity
(relative value to the mean fluorescence intensity of 1 copy/cell)

VIII. References

1) Kim, S.H., Yu, S.S., Park, J.S., Robbins, P.D., and An, C.S. *J Virol*. (1998) **72**: 994-1004.
2) Yu, S.S., Kim, J.M., and Kim, S. *Gene Ther*. (2000) **7**: 797-804.
3) Lee, J.T., Yu, S.S., Han, E., Kim, S., and Kim, S. *Gene Ther*. (2004) **11**: 94-99.

IX. Related Products

RetroNectin® (Recombinant Human Fibronectin Fragment) (Cat. #T100A/B)
RetroNectin® Dish (RetroNectin Pre-coated Dish, 35 mmφ) (Cat. #T110A)
Retrovirus Packaging Kit Eco/Ampho (Cat. #6160/6161)
Retro-X™ Universal Packaging System (Cat. #631530)
Retrovirus Titer Set (for Real Time PCR) (Cat. #6166)
Provirus Copy Number Detection Primer Set, Human (for Real Time PCR) (Cat. #6167)
Retro-X™ qRT-PCR Titration Kit (Cat. #631453)
Retro-X™ Integration Site Analysis Kit (Cat. #631467)

X. Precautions for Use

(1) Users must contact Takara Bio when they plan to use these products for purposes other than research use.
Any inquiries about the licenses relating to these products should be made to Takara Bio Inc. directly or through Takara Bio's subsidiaries.
(Please refer to Takara Bio's website. https://www.takarabio.com)

(2) Virus supernatants produced using this retrovirus vector system may contain potentially hazardous viruses, depending on the inserted fragment.

(3) Use caution when producing or handling recombinant retroviruses. Please follow the guidelines for experiments using recombinant DNA established by relevant authorities and the safety committee of your organization.

(4) Please be advised that Takara Bio is not liable for any accident or damage stemming from the use of this product.