



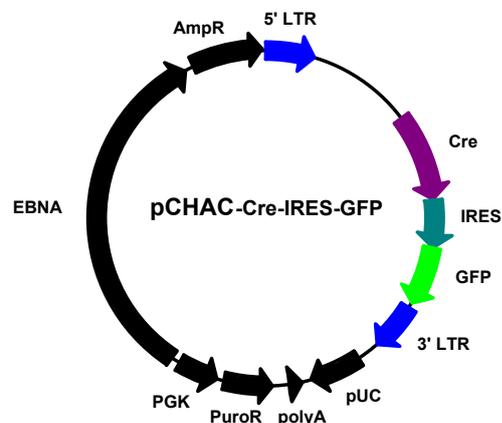
Expression Ready Cre-IRES-GFP Retrovirus

The bacterial phage P1 Cre/lox recombination system mediates DNA excision, integration, inversion and translocation in a sequence specific manner involving only two genetic components: the 38-kDa Cre (cyclization recombination) recombinase and two 34 bp loxP (locus of X-over of P1) sites. The most efficient Cre/lox reaction is DNA excision from a "floxed" locus (flanked by loxP) and this reaction has been frequently applied for mouse genome modification.

Viral vectors are widely used vehicles to bring genetic material inside eukaryotic cells. Compared to transfection methods such as cationic lipids, electroporation, or microinjection, induction with viral particles is generally more efficient if an appropriate viral system with correct tropism is selected.

Allele Biotech provides custom retrovirus or lentivirus packaging services using proprietary technologies that are unique and highly efficient, yielding 10^8 to 10^9 TU/ml, without any concentrating steps (US patents 6,207,455 and 6,531,123). These technologies and optimal operation procedures enable Allele Biotech to package viruses at < 1% of the market price in certain categories on per million particle basis. Right now, based on this strong platform, Allele Biotech offers pre-packaged retroviral particles ready to infect target cells for Cre/loxP system.

The Expression Ready Cre-IRES-GFP retroviral particles encode Cre for Cre/LoxP experiments. Retrovirus also expresses GFP as a marker. Viral Vector map shown below:



Protocols

Storage: -80°C. Avoid repeated freeze thaw cycles

Steps:

1. Plate target cells to 40-60% confluence in a 24-well plate before infection.
2. Place virus stock (virus titer: 1×10^8 IU/ml) in a 37°C water bath and before totally thaw transfer onto ice immediately.
3. Make a serial dilution of 3-4 different multiplicity of infection (MOI = infectious units/ cell number) to test the target cells. Example 1×10^4 TE671 cells in each well of a 24-well plate:

	10MOI	20MOI	30MOI	60MOI
Culture Medium (ul):	198	197	195	189
Polybrene (2mg/ml):	1	1	1	1
Virus (ul):	1	2	4	10
Total volume (ul):	200	200	200	200

4. Tilt the plate gently every 30 min. After 3hrs, add 1 ml fresh medium.
5. After 48-72 hrs, evaluate GFP expression by using fluorescence microscope or flow cytometry.

Related Products

◆ pCHAC Retroviral Vectors

- mWasabiGFP Control
- LacZ Control

◆ Gryphon Retroviral Packaging Cell Lines

- Amphi Cell
- Eco Cell

◆ Gryphon Packaging Cell Selection Medium

Gryphon Hexamethrine Bromide for viral infection enhancement

Safety Issues: Retroviral vectors should be handled using NIH BSL-2 safety guidelines. For more information, please see Biosafety in Microbiological and Biomedical Laboratories (4th edition) which is available on the Web sites of the National Institutes of Health at <http://bmbi.od.nih.gov>.

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