SOP for Detection by PCR of a Recombinant Gene in Cells Infected with the MFG Retrovirus Vector (ψ-CRIP series)

1. Scope

1.1 This procedure describes a method for the detection by PCR of a target recombinant gene in cells infected with the MFG vector.

1.2 For preparation of MFG vector-infected cells, see _____.

1.3 For preparation of genomic DNA from MFG vector-infected cells, see Virus Bank SOP-MFG-001.

2. Principles

2.1 The MFG retrovirus vector is derived from murine moloney leukemia virus (MMLV) and is packaged in ψ-CRIP cells. The vector is constructed by insertion of foreign DNA between the *Nco*I or *Xba*I restriction site and the *BamH*I restriction site in the env region of the genome of the wild-type virus. The inserted DNA can be specifically amplified and detected by PCR using primers that correspond to these restriction sites.

3. Reagents

3.1 Sterile water; 10x Ex Taq Buffer, dNTP mixture, and TaKaRa Ex Taq DNA polymerase (Takara Shuzo), mineral oil (Sigma, St. Louis, MO); and Molecular Weight Marker 1-kb Ladder (#15615-016; GIBCO-BRL).

4. Preparation of primers for PCR

4.1 Primers for the amplification of inserted DNA: primer #12, 5'-CTTCTCTAGGCGCCCATATG-3'; and primer #13, 5'-GCCTGGACCACTGATATCCT-3'. Dissolve each primer in sterile water to a final concentration of 10 μM.

5. Procedure (wear gloves and perform all manipulations on ice)

5.1 Prepare the reaction mixture for PCR, which contains the following components:

Sterile water	34.75 μL

10x Buffer	5.0 µL
dNTP mixture	4.0 µL
Primer #12	2.0 µL
Primer #13	2.0 µL
Genomic DNA from cells (0.1 µg)	2.0 µL
TaKaRa Ex Taq DNA polymerase	0.25 µL
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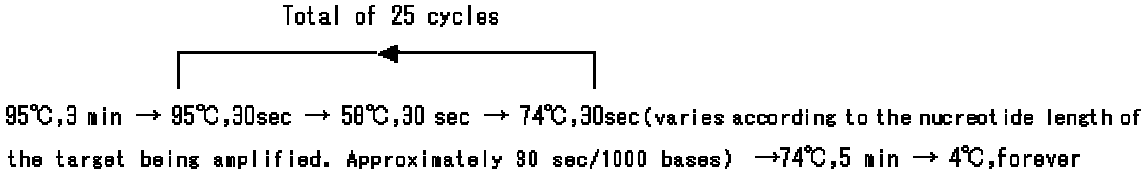
	50.0 µL

Overlay with this mixture with 25 µL of mineral oil and centrifuge in a microfuge for 2 sec at top speed.

5.2 Program for the thermal cycler for PCR



5.3 Fractionate the reaction mixture after PCR on a 2% agarose gel and detect the band of the target DNA after reaction with ethidium bromide. The size of the product should equal the length of the insert plus 382 nucleotides.

5.4 Example of detection of products of PCR.