COSMO BIO CO., LTD.
Inspiration for Life Science

IR-MAR-DNA01 **For research use only**

IR/MAR Gene Amplification Reagent

Protein expression system in mammalian cells has the great advantage to produce mammalian protein representing proper three-dimensional conformation as well as modifications after translation. The structure found in mature protein is essential in the process of proteomics study and protein engineering. However, levels of protein expression in mammalian cells are relatively low in comparison with the other expression systems using bacteria or insect cells, so that the lower expression levels make difficulties in further application. In order to obtain a high-producer mammalian cell line, multiple copies of an expression vector might be introduced into a host cell.

IR/MAR gene amplification is a technology based on a novel mechanism of gene amplification discovered in cancer cell studies. A DNA fragment containing IR (Initiation Region) and MAR (Matrix Attachment Region) effectively amplifies the copy number of a co-transfected expression vector, leading to high protein production rate. Stable cell lines with high protein expression can be easily obtained by standard transfection methods and drug selection.

Material	7.5kbp DNA fragment containing IR and MAR
Quantity	10 µg DNA/vial, 20 µL TE (sterilized)
Storage	- 20 degree C Handle with care to avoid DNA degradation by nuclease contamination

Structure

BSR : Blasticidin S deaminase from *Escherichia coli*
IR : Mammalian replication initiation region
MAR : Matrix Attachment Region

【Protocol】

1. Expression vector preparation

Linearize expression vector encoding a protein by a proper restriction enzyme; subsequently, purify the vector in standard procedure.

2. Transfection

On one day before transfection, plate cells in a well of 6 wells plate. According to the protocol attached to a standard transfection reagent, cells cultured up to 70-80% confluency are co-transfected with the “IR/MAR Gene Amplification Reagent” (1.0-2.0 g) and a linearized expression vector (1.0-2.0 g). For the best result, the detail of transfection protocol should be optimized by users. A transfection without the “IR/MAR Gene Amplification Reagent” can work as a negative control to estimate the effect of IR/MAR gene amplification.

3. Drug selection

On the next day of transfection, transfer the cells to 10cm dish; subsequently, start double drug selection using the selection marker of expression vector and blasticidin S. Effective dosages of drugs should be determined in advance. After double drug selection for a few days, dosage of blasticidin S increases up to 5-20 folds from initial dosage so as to stimulate gene amplification. Stable cell lines with high protein expression can be obtained by drug selection for one month.

4. Cloning of high producer

Limiting dilution method can be used to obtain high producer. As a procedure to select clones, the copy number of an expression vector as well as mRNA expression levels is the possible indicator, which is determined by realtime-PCR analysis.

【References】

N.Shimizu *et al. Cancer Res* **61**, 6987–6990, 2001
N.Shimizu *et al. Cancer Res* **63**, 5281–5290, 2003
N.Shimizu *et al. Exp Cell Res* **302**(2), 233-2433, 2005
N.Shimizu *et al. Nucleic Acids Res* **33**(19), 6296-6307, 2005
T.Hashidume *et al. J Cell Biochem* **101**, 552-565, 2007

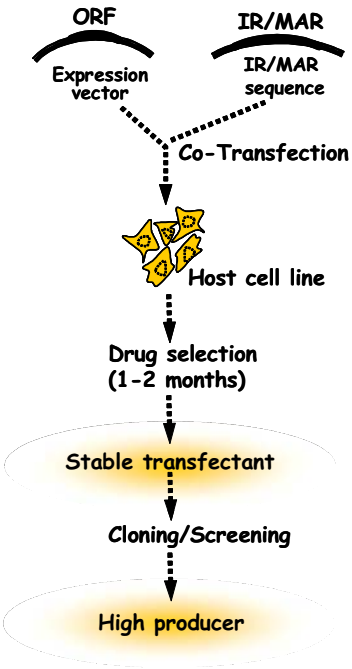
【License statements】

【An example of experimental procedure】

1. Establishment of stable transfectant

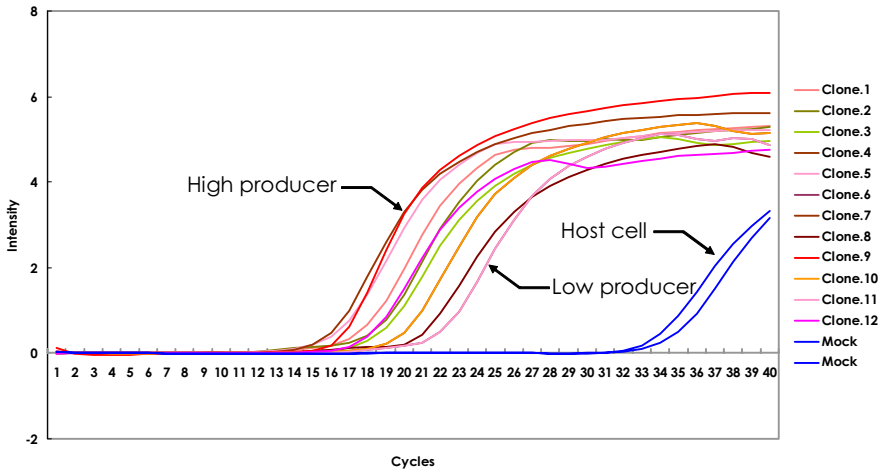
Sub-confluent HEK293 cells were transfected with the “IR/MAR Gene Amplification Reagent” (1.0-2.0 g) and a linearized expression vector (1.0-2.0 g).

On the next day of the transfection, the transfected cells were transferred to 10cm dish and cultured in double drug selection medium (DMEM+10% FCS, 0.5 mg/mL Neomycin, 10 g/mL Blasticidin S). The cells were transferred every 4-7 days. Blasticidin S was added at 100 g/ml (final concentration) in selection medium from the second passage.



2. Screening of high producer by RealTime-PCR

Twelve independent clones derived from CHO cells were isolated as stable transfectants. The relative ratio of mRNA expression of each clone is determined by RealTime-PCR. Mouse -Actin gene is used as internal standard. In the calculation based on Ct, the expression level of the highest producer line was 235-fold higher than that of lowest producer.



Amplification curve of integrated gene
The relative ratio of mRNA expression is determined by RealTime-PCR

Manufactured by TransGenic Inc.

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