Noriaki Shimizu, J Bioanal Biomed 2014, 6:2
http://dx.doi.org/10.4172/1948-593X.S1.015

OMICS Group Conferences
Accelerating Scientific Discovery

3rd International Conference and Exhibition on
Biowaivers, Biologics & Biosimilars

October 27-29, 2014 Hyderabad International Convention Centre, Hyderabad, India

The IR/MAR gene amplification technology and biopharmaceuticals production

Noriaki Shimizu
Hiroshima University, Japan

Gene amplification has been frequently used for the production of biopharmaceuticals from cultured animal cells. The most widespread method is the amplification of Dhfr gene-bearing plasmid in hamster CHO cells by the addition of methotrexate (Mtx) to the culture. However, this method requires long time, much labor. Furthermore, the high producer cells developed by this method frequently become unstable during cultivation. Whereas, we found that the plasmid with a mammalian replication initiation region (IR) and a matrix attachment region (MAR) is "spontaneously" amplified to high copy number in animal cells. This is a completely novel gene amplification technology and it requires both IR and MAR sequence in a special plasmid construct. Because the IR/MAR promote the replication initiation, the IR/MAR plasmid is initially amplified in the transfected cells at the extrachromosomal context to be a giant circular molecule in which the plasmid sequences are arranged as a direct repeat. Such giant circles are then integrated into multiple chromosomal sites in a cell. Thus, the plasmid multimer have a chance to integrate to chromosome site that support both high expression and further amplification. Such plasmid repeats in the chromosomal arm may be further elongated to a large homogeneously staining region (HSR), spontaneously or upon stimulation by Mtx, depending on the cell type used. The combined method of the IR/MAR and the conventional Dhfr/Mtx resulted in the faster plasmid amplification under the lower concentration of Mtx that alleviate Mtx-induced instability of amplification. The high producer clones from CHO DG44 cells produced recombinant antibody in a suspension culture at a production rate of 45 pg/cell/day, a highly competitive value. Taken together, our technology provides a rapid and easy way to produce CHO cell clone that stably produce higher amount of biopharmaceuticals.

Biography

Noriaki Shimizu graduated and obtained his PhD from Kyoto University, Japan. He has worked at Yamanouchi (now Astellas) Pharmaceuticals Co. for more than 5 years (1983 to 1988), where he engaged in the development of protein pharmaceuticals. He then moved to Hiroshima University in 1988, and has been working at there until now as a full Professor. He also worked at Geoff Wahl lab in the Salk Institute, CA, as a visiting Scientist during 1994 to 1995. He has been enthusiastic on the study of gene amplification for about 22 years. He studied the intracellular behavior and elimination of the autonomously replicating extrachromosomal elements, which mediate gene amplification. During such study, he serendipitously found "the IR/MAR gene amplification" method and first published on 2001 in Cancer Research. He used the method to uncover the mechanism of gene amplification, and he applied the method to the production of recombinant protein pharmaceuticals.

shimizu@hiroshima-u.ac.jp