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Construction and efficiency evaluation of replicating nonviral minicircle for gene therapy

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Background and Aims: Current vectors used in gene therapy and vaccination can be divided into viral vectors with higher cell transduction efficiency, and nonviral vectors with less toxicity without DNA insert size limitations. Bacterial backbone sequences are dispensable for gene transfer application which reduces the efficiency of gene expression.Thus, producing of episomal minicircle vectors, devoid bacterial backbone was developed. New generation of these vehicles carrying Scaffold/matrix attachment region (S/MAR) elements causes long-term expression of transgene in the absence of selection. Presence of S/MAR elements in minicircle DNA can exploit the cellular replication machinery for episomal replication. Construction of replicating non-viral minicircle can be achieved by site specific recombination in parental plasmid between two copies of ΦC31 recognition sites in bacterial cells. This study was designed for constructing an efficient vehicle containing S/MAR elements creating minicircle DNAs in purpose of long term expression of target genes in different cell lines.
Methods: At the first step, a DNA fragment containing EGFP CDS, S/MAR elements and SV40 promoter was amplified using pGL268 pEpi-FGM18F plasmid as a template, and inserted into pTZ57R/T. Next this fragment was treated with restriction enzymes and inserted into the parental plasmid originated from pBAD/gIIIA plasmid, between two ΦC31 recognition sites. Eventually after an induction of parental plasmid to produce integrase, minicircle DNA formation was resulted and checked in CHO cell line by measuring EGFP expression.
Results: Data indicated that both parental plasmid and minicircle DNA carrying EGFP-S/MAR were constructed successfully. Moreover, generated minicircle retains functionally to express EGFP in severalpassages and generations for at least 2 months.
Conclusions: Here we reported construction of an efficient minicircles which contain S/MAR elements applicable for long-term transfection of different cell lines in episomal state and efficient modification of dividing cells.

Keywords: Minicircle DNA; S/MAR elements; Nonviral vector; ΦC31