ABSTRACT

GENE MANIPULATION AND EXPRESSION USING GENOMIC ELEMENTS

Expression of mammalian target genes is achieved by employing chromosomal target DNA, either native primary cells or YACs in a yeast host, where the YACs include a fragment of a mammalian chromosome, the fragment comprising the target gene. Employing homologous recombination, an amplifiable gene is integrated into the mammalian fragment at a site to allow for amplification. In the same step, or one or more steps, as desired, the mammalian gene and/or the transcriptional system may be modified by in vivo mutagenesis. The resulting construct from homologous recombination may then be transformed into a mammalian expression host and integrated into the host genome, either randomly or by homologous recombination, The amplifiable gene may then be amplified by an appropriate agent providing for multiple copies of the target gene and the expression host grown to provide for high yields of the desired wild-type or modified protein.