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Construction of an Insertion Vector for Gene Targeting of Chicken Lens-specific Gene

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Abstarc :

Production of gene-targeted chickens is considered to be valuable for studies in both biological science and industry, but it is yet to be achieved. In this study, an insertion vector for gene targeting of chicken lens-specific gene, delta1-crystallin (d1cry), was constructed as a useful tool to evaluate homologous recombination (HR) in chickens. A promoter-less d1cry-homologous DNA and DsRed with an artificial linker sequence (linkerDsRed) were amplified by PCR from genomic DNA and pCMV-DsRed-Express, respectively. The amplified fragments were then cloned and sequenced. The homologous DNA was 7,402bp in size and contained no amber and frameshift mutations. The linkerDsRed fragment had accurate sequence without artificial errors. Floxed marker genes composed of enhanced green fluorescent protein (EGFP) gene, internal ribosome entry site (IRES) and puromycin resistance gene (Pac) regulated by CAG promoter (*P*CAGEIP) was constructed using standard genetic engineering methods. Finally, these DNA materials were ligated to pCC1BAC vector. The accuracy of construction was confirmed by sequencing of ligated portions, and a 20.7-kb insertion d1cry-targeting vector was accomplished. By gene targeting in the pluripotent stem cells using this vector and transplantation of the cells into recipient embryos, chicken transformation would be observed rapidly and easily by the mutant gene-derived red fluorescence in the lens at an early stage of embryogenesis.

Key Word :
chicken, delta1-crystallin, gene targeting, insertion vector

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