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采用Gateway™系统构建人Rb94基因重组腺病毒载体

李进，王芹，宋力，刘强，王月英，唐卫生，张宁，王蕾

300192天津，中国医学科学院放射医学研究所, 天津市分子核医学重点实验室

Construction of Recombinant Adenovirus Vector for hRb94 Gene Using Gateway™ Clone Technology

LI Jin, WANG Qin, SONG Li，LIU Qiang，WANG Yue ying, TANG Wei sheng, ZHANG Ning, WANG Lei

Tianjin Key Laboratory of Molecular Nuclear Medicine, Institute of Radiation Medicine,Chinese Academy of Medical Sciences and Peking Union Medical College,Tianjin 300192, China

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摘要 目的 采用Gateway™技术构建人Rb94基因重组腺病毒载体(Ad-hRb94)。方法 从人类胚胎中提取总RNA,经反转录得到目的cDNA，PCR扩增Rb94目的基因片段。设计含有attB侧翼序列的引物，用于重组片段的PCR扩增，在BP重组酶的作用下，将含attB位点的PCR产物与受体载体pDONR™221发生重组反应，产生入门克隆。在LR重组酶作用下，将入门克隆与带attR1、attR2位点的目的载体 Ad／CMV／V5-DEST体外重组形成表达克隆 Ad-hRb94。经PCR和测序鉴定，将 Ad-hRb94线性化后转入 293A细胞进行病毒的包装、扩增及病毒滴度测定。结果 经PCR和测序证实目的基因Rb94片段按正确方向重组入目的载体中，带Rb94基因的目的载体在 293A细胞中包装成功，获得高滴度的病毒颗粒，滴度为9.41×1010pfu／ml。结论本实验利用Gateway™技术成功构建了Ad-hRb94，为进一步进行肿瘤基因治疗研究奠定了实验基础。

关键词： 人视网膜母细胞瘤94基因 Gateway™技术 重组腺病毒

Abstract： Abstract: Objective To establish a recombinant adenovirus vector with hRb94 by λ phage-site specific recombination systems. Methods Total RNA was extracted from human embryo and reversed transcript to get object cDNA, the hRb94 gene fragment was amplified by PCR.The attBflanked PCR primers were designed and used to amplify hRb94 gene by PCR. An entry clone was performed by a BP recombination reaction with attB-PCR products and donor vector pDONR™221. Then the entry clone and the target vector Ad／CMV／V5-DEST with attR1 and attR2 sites was recombined together in vivo to create the expression clone (Ad-hRb94)by an efficient LR recombination reaction. After the expression clone was confirmed by PCR and sequencing.Ad-hRb94 was digested with Pac I and transferred into 293A cells to be packaged into adenovirus stock. Ad-hRb94 was amplified by infection of 293A cells and the titer was measured. ResultsThe target gene of hRb94 was transferred into Ad／CMV／V5-DEST vector correctly with the right ORF (open reading frame) by LR recombination reaction and it was confirmed by PCR and sequencing. The expression clone Ad-hRb94 was packaged into maturated adenovirus successfully. The titer of Ad-hRB94 was 9. 41×1010pfu／ml. Conclusion Ad-hRB94 was constructed with Gateway™ clone technology，which lays an experimental foundation for the further research on the genetic therapy of hRb94.

Key words： Human retinoblastoma 94 gene GatewayTM clone technology Recombinant adenovirus vector

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[1] 谢富华;王润秀;李昌武;覃燕梅;梁念慈 . *survivin* shRNA重组腺病毒的构建及其对人肺癌细胞A549的作用[J]. 肿瘤防治研究, 2009, 36(4): 277-281.

[2] 吴建辉;张勇;黎玮;马洪顺 . 腺病毒介导的HSV-TK/GCV自杀基因治疗前列腺癌的体外实验[J]. 肿瘤防治研究, 2009, 36(11): 920-924.

[3] 齐进春;张勇;黎玮;蔡文清;王亚轩;刘凯隆. hTERT启动子联合HSV-tk/GCV对人前列癌裸鼠移植瘤的治疗作用[J]. 肿瘤防治研究, 2009, 36(10): 815-817.

[4] 张盛周;张宏霞;潘飞燕;李朝军;. 腺病毒介导的siRNA下调c-Met表达抑制肝癌细胞生长[J]. 肿瘤防治研究, 2008, 35(05): 309-312, .

[5] 匡志鹏;谢裕安;梁安民;罗小玲;吴继宁. 一种新的重组腺病毒载体**pAdBM5-GFP-mAFP**的构建[J]. 肿瘤防治研究, 2007, 34(4): 274-276,.

[6] 施明;王福生;刘明旭;金磊;雷周云;邱兆华;高兰兴;吴祖泽;. 重组腺病毒介导的人野生型p53、GM-CSF和B7-1基因在肝癌细胞中的表达 [J]. 肿瘤防治研究, 2002, 29(2): 121-122.