

实验研究

## 犬贾第虫病毒转染载体介导的绿色荧光蛋白在犬贾第虫体内的表达

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摘要

目的 构建犬贾第虫病毒 (*Giardia canis virus*, GCV) 转染载体。方法 根据GCV基因组 (DQ238861) 的转录起始位点、复制起始位点及包装位点的序列特征和表达外源基因的顺式作用元件, 将绿色荧光蛋白 (GFP) 基因替换GCV基因部分编码区, 构建GCV基因与 GFP基因的嵌合体, 并置T7启动子之下。用T7 RNA聚合酶体外转录后经电穿孔转染犬贾第虫滋养体, 并用荧光显微镜检测GFP表达情况, 间接ELISA测定转染后GFP的表达量。结果 构建了犬贾第虫病毒重组质粒

pGCV634/GFP/GCV2174, 经Sac I 和Not I 双酶切得到约2.0和3.5 kb两条带, 与预计值相符。由其介导的绿色荧光蛋白在犬贾第虫体内得到了高效表达, 其表达量在转染后第1天达高峰( $A_{490}=1.8$ ); 以后随着时间的延长而逐渐下降, 14 d后绿色荧光信号基本消失。结论 成功构建了犬贾第虫病毒转染载体, 为贾第虫细胞基因表达调控的研究提供了方法。

关键词 [犬贾第虫病毒](#) [转染载体](#) [绿色荧光蛋白](#)

分类号

## *Giardia canis* Virus Transfection Vector-Mediated Expression of Green Fluorescent Protein in the Parasite

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**Abstract**  
Objective To construct *Giardia canis virus* (GCV) transfection vector. Methods According to transcriptional start site, replication origin and packaging site of GCV genome (DQ238861), a system was developed for the expression of a foreign gene in this organism by flanking the green fluorescent protein (GFP) gene with the fragments of GCV positive-strand RNA. The transcript of the construct was synthesized *in vitro* with T7 RNA polymerase and used to transfect GCV-infected trophozoites by electroporation. Results The recombinant plasmid pGCV634/GFP/GCV2174 was constructed. The expression of green fluorescent protein mediated by GCV transfection vector in *Giardia canis* peaked at 1 d after electroporation ( $A_{490}=1.8$ ), and slowly decreased until 14 d post-transfection. Conclusion The engineered GCV vector can be successfully used to introduce and efficiently express a heterologous gene in the eukaryotic microorganism.

**Key words** [Giardia canis virus](#) [Transfer vector](#) [Green fluorescent protein](#)

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