

## 紫花苜蓿光敏色素B基因片段克隆及RNA干扰表达载体的构建

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## Cloning of *Medicago sativa* Phyochrome B cDNA and Establishment of Its RNA Interference Expression Vector

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摘要

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**摘要** 根据紫花苜蓿2级标准品种Vernal *PhyB*基因的序列(GenBank登录号为GQ379903.1), 设计2对含有酶切位点的特异性引物F1/R1和F2/R2, 以紫花苜蓿总RNA为模板, 通过RT-PCR法扩增得到*PhyB*基因正向、反向目的片段, 将片段连接到pGEM-T Easy载体上得到重组载体pGEMB-1和pGEMB-2, 再以中间载体pHANNIBAL和植物双元表达载体pART27为基础, 通过多次酶切和连接, 成功地构建了紫花苜蓿*PhyB*的RNAi表达载体。为进一步研究光敏色素B与苜蓿秋眠性之间的关系奠定了基础。

**关键词:** 紫花苜蓿 秋眠性 光受体 光敏色素 RNA干扰

**Abstract:** The short day is one of the main factors affecting alfalfa fall dormancy, which is called as the photoperiodic effect. Study on the relationship between the main photoreceptors-*PhyB* gene and alfalfa fall dormancy may reveal the regulation mechanism of alfalfa fall dormancy radically, and provide a scientific reference for the application of alfalfa varieties differing in fall dormancy in forage production. The objective of this study was to establish an RNAi expression vector harboring *PhyB* gene of *Medicago sativa*. Two pairs of specific primers containing different enzyme sites were designed on the basis of *PhyB* gene sequence of alfalfa variety "Vernal" (GenBank accession number: GQ379903.1). With the template of total RNA, positive sense strand and antisense strand were amplified by RT-PCR and cloned into pGEM-T Easy vector to obtain recombinant vectors pGEMB-1 and pGEMB-2, then based on the intermediate vector pHANNIBAL and the plant binary expression vector pART27, we constructed the RNAi expression vector pART27-RNAi containing a hairpin structure by many times of enzyme digestion and connection. The results provide a foundation for further studying the relationship between alfalfa dormancy and *PhyB*.

**Keywords:** *Medicago sativa* Fall dormancy Photoreceptor Phytochrome RNA interference

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