

## 人工microRNAs对拟南芥*At1g13770*和*At2g23470*基因的特异沉默

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**摘要** DUF647 (Domain of unknown function 647) 蛋白家族是在真核生物中广泛存在的、高度保守的蛋白家族。拟南芥中该基因家族共有6个成员, 迄今为止拟南芥DUF647家族中4个成员的功能尚不清楚。文章以拟南芥内源*MIR319a*前体为骨架, 构建了敲减DUF647家族中2个基因*At1g13770*和*At2g23470*表达的人工microRNAs(Artificial microRNAs, amiRNAs)。利用WMD(Web microRNA designer)平台设计分别靶向*At1g13770*和*At2g23470*基因的amiRNAs序列, 通过重叠PCR置换拟南芥*MIR319a*前体序列。构建融合amiRNAs前体的植物表达载体pCHF3-amiRNAs, 在农杆菌介导下转化拟南芥。RT-PCR分析表明, amiRNAs能够显著抑制*At1g13770*和*At2g23470*基因的表达, 获得了抑制效果明显的转基因株系。*At2g23470*-amiRNA转基因植株*At2g23470*转录水平的下调导致育性严重下降。文章为进一步研究这两个基因的功能奠定了良好的基础。

**关键词:** 拟南芥 DUF647基因家族 人工microRNA 基因沉默

**Abstract:** DUF647 (domain of unknown function 647) protein family is found in diverse eukaryotic organisms and highly conserved in eukaryotes. It has 6 members in *Arabidopsis* genome. So far, the function of 4 members of *Arabidopsis* DUF647 family is unknown. In this report, using an endogenous *Arabidopsis MIR319a* precursor as the backbone, we constructed two artificial microRNAs (amiRNAs) to knock down the expression of two DUF647 family genes *At1g13770* and *At2g23470*. Using the WMD (Web microRNA Designer) platform, we designed two amiRNAs targeting *At1g13770* and *At2g23470* genes, respectively. Both amiRNAs sequences were engineered into the *MIR319a* precursor using overlapping PCR and the amiRNAs backbones were transferred into the binary vector pCHF3. The resulting plasmids that harbor amiRNAs stem loop fragments were transformed into *Arabidopsis* by *Agrobacterium*-mediated floral dipping. Upon constitutive expression of these two amiRNAs, the target genes were efficiently down-regulated in transgenic line. The decreased level of *At2g23470* transcript in *At2g23470*-amiRNA transgenic plants resulted in severe sterility. This work will facilitate the functional analysis of *At1g13770* and *At2g23470* genes in *Arabidopsis* growth and development.

**Keywords:** *Arabidopsis*, DUF647 gene family, artificial microRNAs, gene silencing

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