

利用种子序列检测山羊皮肤中microRNA靶基因分子方法的建立

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摘要 文章旨在建立一种种子序列介导的可控遗传操作—microRNA靶基因指纹图谱(MicroRNA targets finger print, MTFP), 用于在基因表达检测中筛选与特定microRNA相关的靶基因。在设定上游种子序列的互补序列和下游锚定序列的基础上添加特殊接头, 通过反转录和特殊二步PCR将microRNA的靶基因扩增; 扩增后的microRNA靶基因在聚丙烯酰胺凝胶电泳中检测其片段大小和表达丰度, 用于筛选在不同生理状态或试验条件下特异表达的基因; 特定的靶基因序列通过DNA回收和测序方法得到。以miR-203为例, 在不同生理状态的山羊皮肤样品中获得了5条大小分别为718 bp(JN709494)、349 bp(JN709495)、243 bp(JN709496)、156 bp(JN709497)和97 bp(JN709498)的靶基因序列。MTFP经济适用、可操作性强, 可用于探索microRNA调节的靶基因, 或用来评估靶基因的表达谱特征。

关键词: microRNA 表达丰度 靶基因指纹图谱 山羊 皮肤

Abstract: The purpose of the present study was to establish a new microRNA seed mediated controllable genetic operation, namely MicroRNA Targets Finger Print (MTFP), for screening microRNA targets and detecting target gene expression profiles. Based on combining the complementary sequence of seed sequence, upstream and downstream anchor sequence including special adaptor, microRNA targets were amplified by means of the reverse transcription and special two step PCR. The polyacrylamide gel electrophoresis was used to analyze the sizes of amplified microRNA targets and their abundance of expression, which were used to screen specifically expressed genes in different physiological or experimental conditions. Specific target genes were obtained through isolation of DNA fragments and sequencing methods. As an example, by screening the targets of miR-203 in goat skin, five genes were amplified and sequenced with the sizes of 718 bp (JN709494), 349 bp (JN709495), 243 bp (JN709496), 159 bp (JN709497), and 97 bp (JN709498) from goat skin collections. The novel universal MTFP method could be applied for finding microRNA regulation targets or assessing target gene expression profile.

Keywords: microRNA, expression abundance, microRNA targets finger print (MTFP), goat, skin

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