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基础医学

流式细胞术测定蠕形螨基因组大小

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摘要:

目的 采用流式细胞术(FCM)测定蠕形螨基因组大小, 为构建蠕形螨基因文库和筛选功能基因奠定基础。方法 自然沉降法清洗收集蠕形螨, 组织匀浆器和超声波破碎螨体, 在柠檬酸介质中分离蠕形螨的细胞核。以家鸡血细胞核DNA含量为内标, 用FCM测定经碘化丙啶(PI)染色的家鸡血细胞核和蠕形螨细胞核混合样品发出的PI荧光强度, 通过比较蠕形螨与家鸡血细胞DNA含量峰值的倍数关系, 计算出蠕形螨的基因组大小。结果 采用FCM测得蠕形螨DNA含量约为1.05pg/2C, 以1pg DNA相当于978Mb计算得出蠕形螨基因组大小约为512Mb。结论 流式细胞术可快速、精确测定蠕形螨基因组大小, 为蠕形螨基因组研究奠定了基础工作。

关键词: 蠕形螨;基因组大小;基因组学;碘化丙啶染色;流式细胞术

Determination of Demodex genome size by flow cytometry

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Abstract:

Objective To determinate the genome size of demodex by flow cytometry(FCM), which could lay the foundation for the construction of gene bank and the screening of functional genes of demodex. Methods Clean mites washed with cleanser essence and selected under microscope were collected. After smashing the mites by combination of tissue grinder and ultrasonic wave, mites' nuclei were extracted in citric acid solution. Chicken erythrocytes were served as an internal reference. The mixed sample of demodex and chicken erythrocytes was dyed by propidium iodide (PI). The peak values of the sample fluorescence intensities were measured by FCM. Comparing the multiple relationships between the two peak values, mites' genome size was calculated. Results The DNA content of demodex was about 1.05pg/2C determinated by FCM. 1pg converts to 978Mb, so the mite' s genome size was the equal of 512Mb. Conclusion Flow cytometry could rapidly and accurately determinate the genome size of Demodex, which was the foundation work for the research of mite' s genomics.

Keywords: Demodex; Genome size; Genomics; PI dyeing; Flow cytometry

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