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3种碱性染液对显微捕获联合低体积扩增技术的影响

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【摘 要】目的：比较3种碱性染液对显微捕获联合低体积扩增（Low volume polymerase chain reaction，LVPCR）技术的影响，为细胞分离检验中细胞核标记物的选择提供依据。方法：显微捕获联合LVPCR中，用苏木素、龙胆紫、亚甲基蓝3种染液对口腔上皮细胞进行标识。参照龙胆紫染色法，比较0.5、1、2、5、8 μl苏木素染液及2、5、8、10、15 μl亚甲基蓝染液的染色效果，优化染色条件；并比较优化后的细胞核标识效果。显微操作仪捕获3种染液染色后的细胞进行DNA检验，比较检测结果；并捕获染色10、30、60 min口腔上皮细胞，比较染色时间对DNA检测结果的影响。结果：100 μl口腔上皮细胞悬液加入2 μl苏木素、0.5 μl龙胆紫或5 μl亚甲基蓝染液，细胞核均着色明显，与胞浆对比清晰。5个细胞的分离检验结果示有效分型率分别为90%、100%、66.7%，成功分型率为20%、100%、11.1%；龙胆紫、苏木素染色时间（≤60 min）对DNA检测结果无明显影响，而亚甲基蓝染色时间延长导致等位基因丢失等现象加重，影响检测结果。结论：3种染液均可清晰标识细胞核；而龙胆紫染液对LVPCR技术抑制最弱，为三者中较优的细胞核标记物。

【关键词】法医物证学；碱性染料；低体积扩增；显微捕获单细胞分离检验技术

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Comparative study of three basic dyes in micromanipulation and LVPCR

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【Abstract】*Objective*: To compare the effect of three basic dyes in micromanipulation and LVPCR in order to set the ground for the choice of nucleus stain. *Methods*: Oral epithelium cell was stained using Haematoxylin solution, Gentian violet solution and Methylthionine chloride solution with the technique of micromanipulation and LVPCR System. Based on Gentian violet stain-method, the effect of 0.5, 1, 2, 5 μl and 8 μl Haematoxylin solution and 2, 5, 8, 10 μl and 15 μl Methylthionine chloride solution were compared to optimize staining condition. The staining effects of the three solutions under optimized condition were compared to define the best one. Cells stained by those solutions were captured by micromanipulators and DNA test results were compared; oral epithelium cells stained for 10, 30 mins and 60 mins were captured to check the effect of staining time on final results. *Results*: Best staining effect was achieved by adding 2 μl Haematoxylin, 0.5 μl Gentian violet or 5 μl Methylthionine chloride solution to 100 μl oral epithelium cells suspension, the nuclei being clearly distinguishable from cytoplasm. Useful rates of STR genotyping in 5 cell micromanipulation were 90%, 100% and 66.7% respectively, with success rates of 20%, 100% and 11.1% respectively. No negative influence was detected upon the extension of staining time using Haematoxylin and Gentian violet, while the extension of time in using Methylthionine chloride resulted in ADO, exerting negative effect on final result. *Conclusion*: Nuclei can be clearly stained using all the three solutions, and least negative effect on LVPCR can be detected using Gentian violet solution, making it the optimized choice.

【Key words】forensic genetic; basic dyes; LVPCR; testing technique of micromanipulation method of single cell

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