BioFeedback

The Biofeedback section accommodates letters to the editor, addenda, errata and other comments on previously published articles.

Comments on an Article by M.P. Burton et al.

The recent short technical report (1998, *BioTechniques* 24:86-92) by M.P. Burton et al. entitled, "Comparison of Histological Stains for Use in PCR Analysis of Microdissected, Paraffin-Embedded Tissue", evaluated the effect of six different histological stains on the productivity of PCR amplification of DNA isolated from paraffin-embedded tissue samples. The authors state that their "most significant finding was that a commonly used histological stain, hematoxylin, failed to produce DNA templates that could be consistently amplified by PCR" and "it is prudent to avoid hematoxylin stains when preparing tissues as starting material for PCR". The fact that hematoxylin may be one of, if not the most common stains used in pathology today makes this a most important issue to address.

We would like to add to their findings by addressing this issue and commenting on our own comparison of histologically stained tissue as a source of template DNA for PCR. In a recent report (1), we showed that DNA from hematoxylin and eosin-stained clinical tissue could be reproducibly amplified following a destaining procedure that eliminates the deparaffinizing process that Burton et al. utilize and involves a similar amount of time (1 day).

Briefly, slides were soaked in xylene overnight, and the coverslips were removed. Sections still on the slide were soaked for 1 h each in absolute ethanol twice and in water twice (alternatively, the tissue can be removed in a sterile manner from the slides, after the xylene treatment, and processed in microcentrifuge tubes). The tissues were then destained by placing the slides in 1% HCl/95% ethanol for 1 h and then rinsed in distilled, deionized water twice. Samples of tissue were removed from the slides, vacuum-centrifuged and subjected to Chelex[®] extraction (1).

Reproducible "multiplex" amplification of 6 loci was successful on these destained samples as opposed to samples that underwent just a deparaffinizing procedure. The same kind of results were obtained for samples stained with Masson's Trichrome and Periodic Acid

Schiff with Diastase. By eliminating the deparaffinizing step, and replacing it with a destaining procedure, hematoxylin-stained tissues do not need to be avoided and are useful as a source of PCR template DNA.

Our study also showed that tissue samples stained by Kinyoun's method for acid fast bacteria, Grimoris' method for iron, Gridely's silver method for reticulum and Grocott's methenamine silver could also serve as reproducible sources for PCR template DNA after undergoing paraffin removal (1).

REFERENCE

1. Medintz, I., L. Chiriboga, H. Hotaling and L. Kobilinsky. 1997. DNA identity testing in stained tissue samples. *Anal. Lett.* 30:909-916.

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Response to Comments by I. Medintz, L. Chiriboga and L. Kobilinsky

We are gratified to hear that the adverse effects of hematoxylin can be reversed by destaining. Your finding is especially relevant to investigators whose only source of tissue is one that has already been stained with hematoxylin and eosin.

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