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Generation of repetitive sequence-depleted microdissected chromosome arm painting probe. *H. He[1], W. Huang[1], XY. Guan[2]*. 1) American Lab Technologies, Inc, Rockville, MD; 2) Department of Clinical Oncology, University of Hong Kong, Hong Kong, China.

The application of fluorescence in situ hybridization (FISH) with whole chromosome painting probes, chromosome arm painting probes, and chromosome band-specific painting probes has greatly facilitated to detect chromosome rearrangements in both hereditary diseases and cancers. To meet the increasing demand for the high quality FISH painting probes, a subtraction strategy has been applied in our company to remove repetitive sequences from microdissected DNA probes before hybridization. Chromosome arm 5p has been chosen to test our new method. Briefly, 10 copies of 5p were microdissected and amplified using a degenerate oligo primer (UN1) by PCR. UN1 primer was then replaced by a unique sequence primer (R1) by PCR. These PCR products were then hybridized with biotin-labeled human repetitive sequences derived from a 120 kb BAC containing various human repetitive sequences. After hybridization, avidin was added into the reaction and phenol/chloroform subtraction was performed to remove proteins in the reaction solution including all avidin-bound biotin-labeled repetitive sequences and their specifically hybridized repetitive sequences in the microdissected DNA. The remaining unique DNA fragments were recovered by PCR with R1 primer. The intensity and specificity of the repeat-depleted 5p arm painting probe have been characterized by FISH without adding block DNA Cot-1. The intensity and hybridization specificity of the fluorescence signal was similar between a regular 5p arm painting probe with Cot-1 block and our repeat-deplete 5p painting probe. This repeat-depleted painting probe which no longer require adding block DNA will be used for FISH and will provide cheaper and quicker resource for the increasing demand of the high quality FISH painting probes.