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Automated fluorescence-activated collecting duct isolation for genomics and proteomics

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The purpose of this study was to develop an automated method for isolating collecting ducts (CD) from mouse kidneys using the Complex Object Parametric Analyzer and Sorter (COPAS, Union Biometrica). CD from the B1-EGFP transgenic mice, which express enhanced green fluorescent protein in intercalated cells throughout the entire CD, were enzymatically digested, filtered and sorted based on fluorescence intensity using the COPAS method. 500 CD fragments with an average length of 220 ± 25 µm were routinely isolated from a single mouse within 10 minutes (~3000 total per mouse), which yielded an average of 3.5 ± 1.0 µg total RNA and 40 ± 3.2 µg protein. Confocal fluorescence/brightfield microscopy showed that COPAS accurately isolated nephron fragments and distinguished between GFP-positive and negative nephrons ≥ 95% and ≥ 97% of the time, respectively. Quantitative real-time RT-PCR showed that AQP2 and H+-ATPase B1-subunit mRNA were enriched ≥ 40 fold in CD verse non-CD, while AQP1 mRNA was enriched ≥ 35 fold in non-CD verses CD; western-blot analysis showed similar findings. CD primary cultures from COPAS isolated CD fragments established confluent monolayers and expressed AQP2 in response to dDAVP/IBMX. COPAS represents a novel automated method for the isolation of fluorescently labeled nephron fragments for RNA and protein analysis and establishing nephron-specific primary cultures.