# The road from micro to macro in preparative isotachophoresis.

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Many clinical and diagnostic applications require high quality nucleic acids for downstream analytical methods such as quantitative PCR, microarrays, and/or next-generation sequencing. A complex matrix, such as blood and other bodily fluids, samples from the scene of crime, fossil samples, etc., very often compromise isolation and purification of nucleic acids.
The most common nucleic acids isolation techniques are based on extraction with inherent limitations, with regard to quantitative results. In recent years, there is an increasing interest in sorbent free alternatives. Here, we report on two instrumental systems for processing of large sample volumes by discontinuous electrophoresis with theoretically unlimited concentration factor.
In the first system, we used capillary instrument with large bore (0.8 mm ID) fluoropolymer capillary, conductivity detector and fraction collection valve. Sample volumes up 150 ?l could be injected, focused and collected. In order to achieve a high recovery and enrichment, factors including electric current, sample amount and matrix were investigated experimentally as well as by computer simulation.
The second, laboratory constructed, system was designed in a flat arrangement where sample zones migrated towards a fraction collection well. This allowed focusing of 15 ml sample volumes in a 110 mm device in less than 1 hour. Position of the migrating zone was monitored by laser-induced fluorescence. While a discontinuous electrolyte system was used, the selected geometry did not lead to a typical isotachophoretic migration when operated at constant current, constant voltage or constant power modes. These experimental findings were confirmed by theoretical descriptions derived for each operation mode. Samples dissolved in the terminating electrolyte or in saline solutions were processed in both systems. The DNA content in the collected fractions were further analyzed by fluorescence spectrometry and chip capillary electrophoresis.
In conclusion, we have developed two simple, preparative methods for DNA concentration and purification. The first method, based on a capillary ITP, can process sample volumes up to 150 µl. The second method, based on the flat channel design, allows processing of 15 ml sample volumes. This is by far the largest described focusing capacity. In the discontinuous electrolyte system the

sample enrichment factor is limited only by the system geometry and even higher loading capacities are possible if needed.