

Automated Cycle Sequencing of DNA Samples Using an AVISO „TheOnyx Liquid Performer“ Robotic Platform

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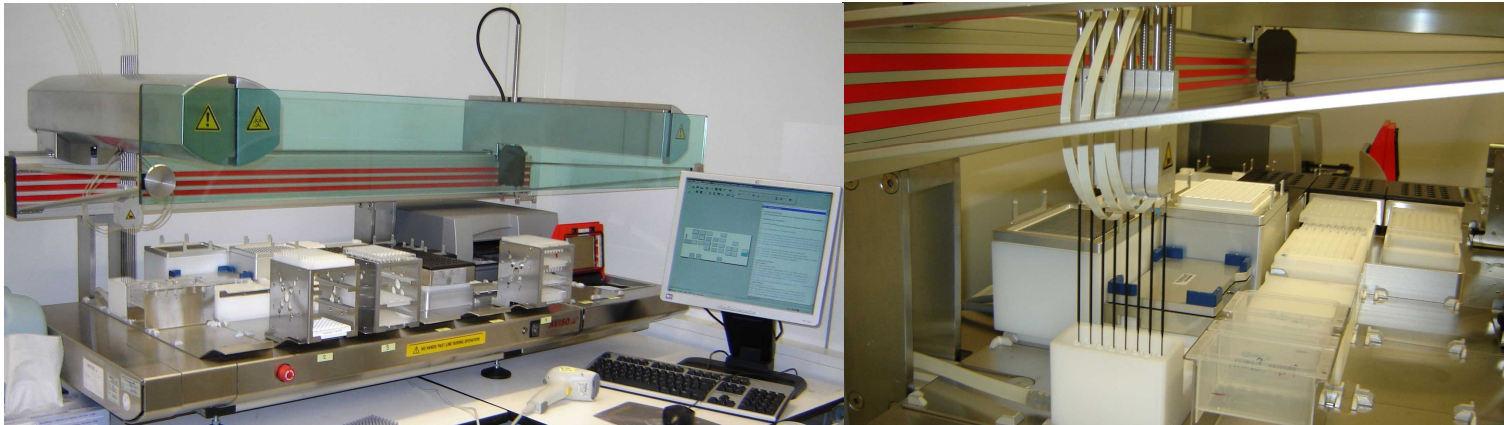


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Introduction

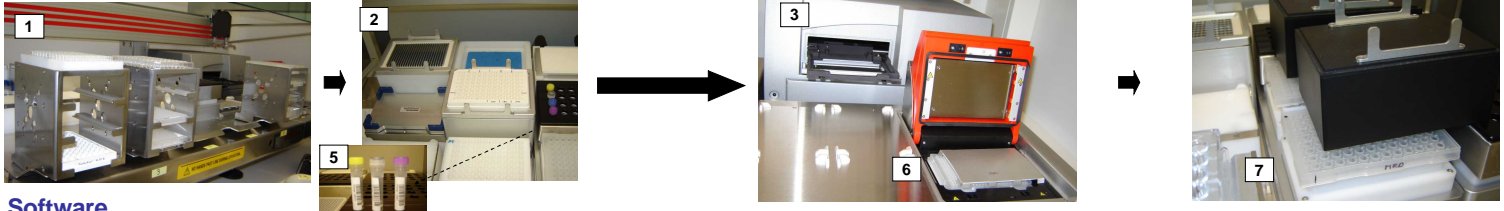
Increasing numbers of sequence reactions to be set up are a growing problem in molecular diagnostics. Since automated capillary electrophoresis has helped to reduce hands-on time and sequence analysis software assists in finding causative mutations in hereditary diseases, manual setup of sequencing reactions is the limiting factor of the whole process. We have successfully adapted a „TheOnyx 440H Liquid Performer“ robotic platform with an 8-needle liquid handling arm to the demands of automated DNA sequencing in a sequencing core setting, supporting a wide range of laboratories at the University Hospital of Heidelberg.



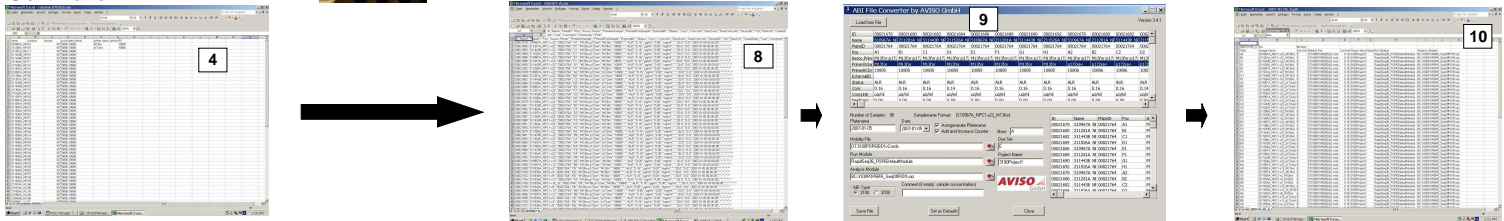
Schedule of Automated DNA Sequencing

PCR products are provided in 0.2ml reaction tubes (strip/cap system) in a tube rack (1), and vacuum-purified by filtration plates (2). Subsequently, purified PCR products are spectrophotometrically measured providing a definite template input (3). Sequencing reactions are automatically pipetted in a total volume of 10µl into a 384well sample plate on the basis of an input EXCEL file (4) providing sample position and associated primers defined by primer barcodes (5) and transferred into a 384well Thermal Cycler (6). After thermal cycling sequencing sequence reactions are vacuum-purified by ultrafiltration and eluted into ABI 3100-compatible sample plates which are kept under light-protection hoods at cooled rack positions (7). The robot output data files (8) have to be converted into an ABI importable format (9). Thereafter, the sequence reactions/ output file(s) (10) can be loaded on an ABI 3100 sequencer.

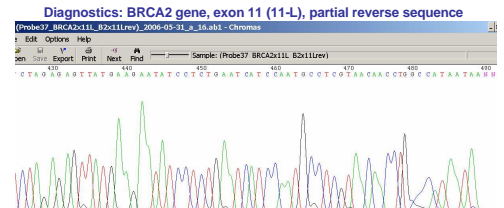
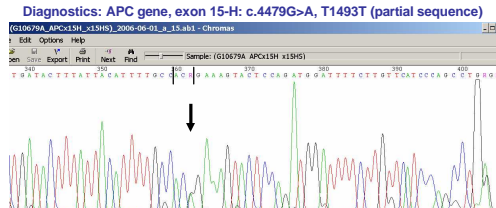
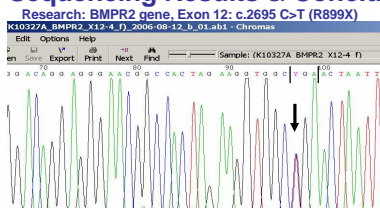
Hardware



Software



Sequencing Results & Conclusions



Various sequencing protocols for routine diagnostics (TSC1/2, BRCA1/2, APC, ret/MEN2, DMD, various genes of metabolic enzymes) as well as research projects have been adapted to automated sequencing on a TheOnyx 440H Liquid Performer. Sequences show very low background, on average, with removal of almost all dye blobs and read lengths up to 500bp. Up to 192 sequences can be generated using bidirectional sequencing primers in approximately 6 hours and electrophoresed on an ABI 3100 in additional 12 hours, thus enabling full sequencing of average genes in several days. Further optimization will be achieved by the use of universal primer tags and by upgrading the ABI 3100 sequencer to the ABI 3130XL technology using POP-7 polymer under ultrarapid run conditions, allowing run times of 30 minutes.