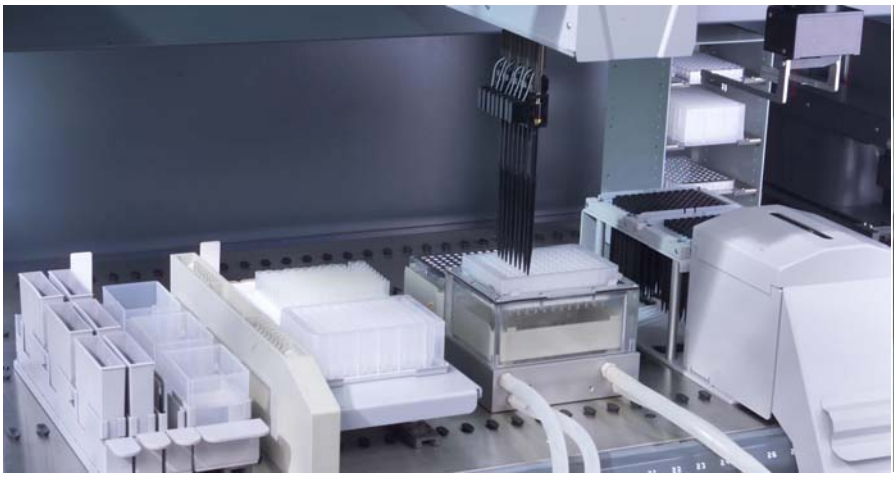
Automated PCR Clean-up

The MACHEREY-NAGEL NucleoFast 96 PCR Kit on a Tecan Freedom Nucleic Acid Sample Preparation Workstation



Introduction

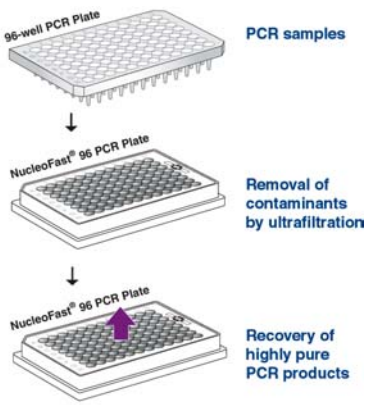
Polymerase Chain Reaction (PCR) clean-up is a prerequisite for several downstream applications, e.g. sequencing or microarray spotting. Ultrafiltration technology is a cost-effective and efficient method to remove undesired components like salts, detergents, primers, or additives from PCR reactions. In contrast to classical precipitation or size-exclusion chromatography methods, no centrifugation steps are required. Due to the fact that neither salt nor ethanol is required for clean-up, the risk of salt or ethanol carry-over is excluded compared to glass-milk or silica filter based purification methods.

The NucleoFast 96 PCR kit from MACHEREY-NAGEL is optimized for use on liquid handling workstations, e.g. the Tecan Freedom Nucleic Acid Sample Preparation Workstation equipped with a Te-VacS vacuum module. The separation plate seals to the vacuum block without additional push down steps. Furthermore, the plate design allows sealing of barcode labels for plate identification. The high stability of the membrane tolerates touching the membrane during the recovery step with disposable tips or even fixed tips. The NucleoFast 96 PCR ultrafiltration membrane is permanently hydrophilized. Therefore, no detergents are washed out of the membrane during the clean-up procedure.

Purification Process

PCR samples are loaded onto the NucleoFast 96 PCR plate. Vacuum is applied for 10-15 minutes. An optional wash step with sterile water can be performed for further removal of contaminants. Finally, recovery buffer is dispensed onto the membrane. After some mixing steps or a short incubation (optionally with a shaker) purified samples are recovered from the membrane.

Fig. 1: Purification principle of NucleoFast 96 PCR Kit



Automation

The NucleoFast 96 PCR kit was fully automated on a Tecan Freedom Nucleic Acid Sample Preparation Workstation equipped with a Te-VacS vacuum option. The Robotic Manipulator Arm (RoMa) was used for plate transfers to the vacuum block. Pipetting of samples was performed with disposable tips whereas buffer dispense was carried out with fixed tips. Tecan Gemini™ software was used for programming of all process steps.

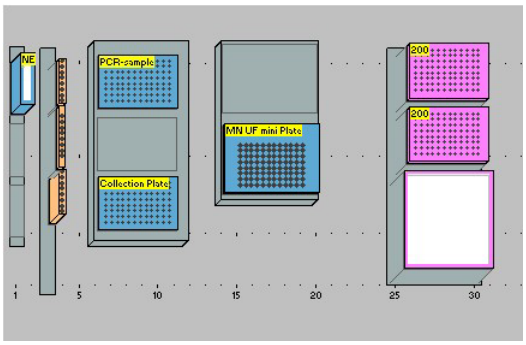


Fig. 2: Worktable layout
Typical worktable layout of NucleoFast 96 PCR kit on the Tecan Freedom Sample Preparation Workstation (Software screenshot).

Material and Methods

A set of eight different PCR fragments with sizes from 164 bp to 1484 bp was created in standard PCR reaction (2 ng template DNA, 0.4 µM forward primer, 0.4 µM reverse primer, 1.5 mM $MgCl_2$, 0.2 mM dNTP mixture, 2.0 U TAQ Polymerase (Invitrogen)). Purification was carried out on a Genesis RSP 150/8. 25 µl from each PCR reaction were transferred to the NucleoFast 96 PCR plate and a vacuum of –400 mbar was applied for 10 min. Samples were recovered in 50 µl recovery buffer RB. Purified samples were incubated for 1 min. in recovery buffer prior to the transfer to the elution plate. Recovery was facilitated by 5 mixing cycles (pre-aspirate mix). Purified DNA was analyzed on 1 % agarose gel (TAE, ethidium bromide staining).

Fig. 4: Reproducible yields of pure products
PCR fragments of different length have been purified. 25 µl of each reaction have been purified according to the standard procedure and were analyzed on a 1% agarose gel. Highly reproducible yields were obtained.

Results

Automation of the NucleoFast 96 PCR kit on the Tecan Freedom Sample Preparation Workstation allows fast and reliable clean-up for PCR fragments. Consistent DNA recovery of 50-95% for fragments of 150 to 1500 bp could be achieved. Efficient removal of contaminants was demonstrated by the excellent sequencing results of the purified DNA fragments.

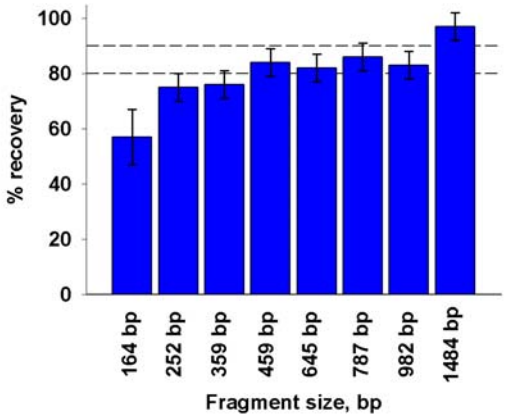


Fig. 3: Recovery rate of PCR products
Sets of PCR fragments (164 to 1484 bp) were purified using NucleoFast 96 PCR according to kit protocol. Each bar represents the average recovery of at least 24 purifications. Typically, recovery rates for PCR products >250 bp are at least 70-90%. Recovery rates for fragments <250 bp are 60-80%.

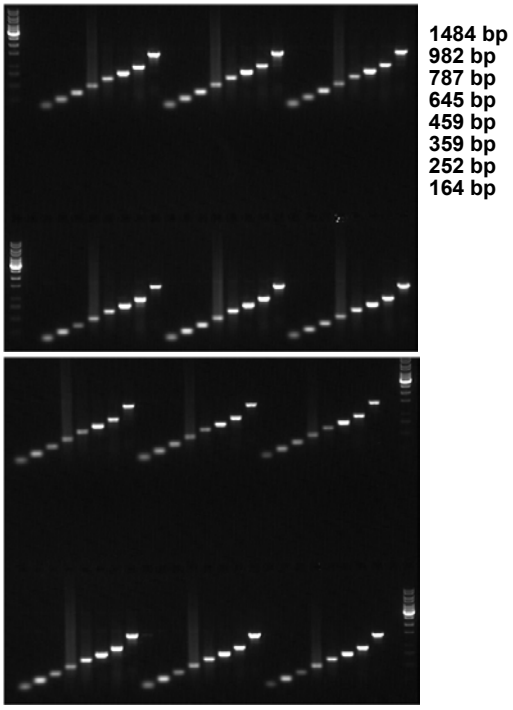
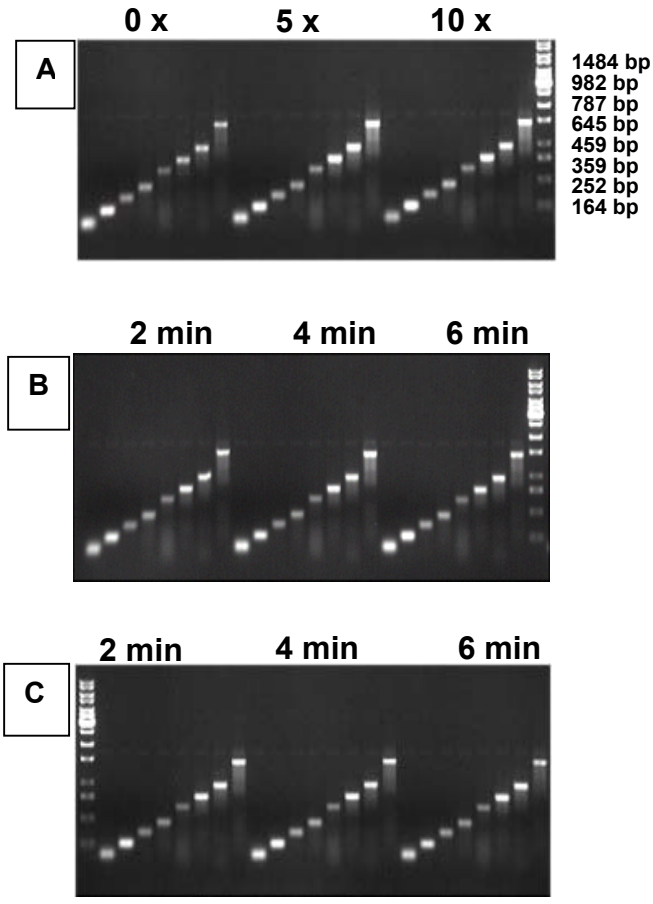


Fig. 5: Process optimization
Methods for recovery of purified PCR fragments. PCR fragments (sizes as indicated) were purified according to the standard protocol. Samples were recovered after the indicated number of mixing steps (A), after the indicated minutes of incubation (B), or after the indicated minutes of incubation with shaking (C). Fragments <500 bp can be eluted directly after addition of recovery buffer. For fragments >500 bp recovery rates can be increased by mixing, incubation or shaking. 5 mixing steps or 2 min incubation with shaking is sufficient for recovery. Mixing more than 5 times or prolonged incubation do not result in higher recovery rates.



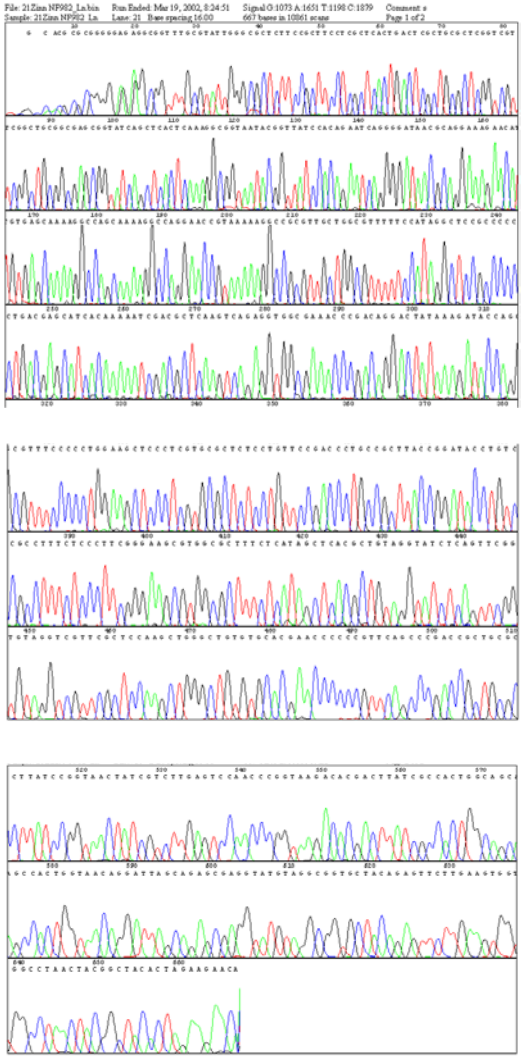


Fig. 6: Automated fluorescent sequencing.
200 ng of a 982-bp fragment purified with NucleoFast 96 PCR have been cycle-sequenced using BigDye terminator chemistry and analyzed on an ABI PRISM 3100 DNA analyzer. No double signals are observed at the beginning of the chromatogram; primers have been completely removed during the purification process.

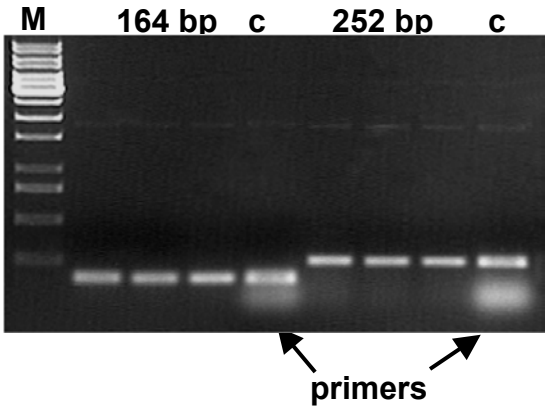


Fig. 7: Primer removal
Two PCR fragments (164 bp and 252 bp) were amplified with an excess of primers. PCR products were purified using NucleoFast 96 PCR and recovered in 100 µl sterile water. Samples were analyzed on a 1 % TAE agarose gel. M: marker lane, c: unpurified control samples. Small fragments are recovered with high efficiency whereas primers are removed completely.

Conclusions

Automation of the NucleoFast 96 PCR kit on a Tecan Freedom Sample Preparation Workstation allows fast and reliable clean-up of DNA fragments in a true "walk-away mode". The automation friendly design of the filter plate allows easy adaptation to the Tecan TeVacS system. The permanently hydrophilized membrane reduces the risk of detergent carryover into the purified product. Excellent DNA recovery and efficient removal of primers and other contaminants could be demonstrated for a broad range of fragment sizes.
Automated processing of multiple batches is enabled by simple integration of additional plate storage modules.

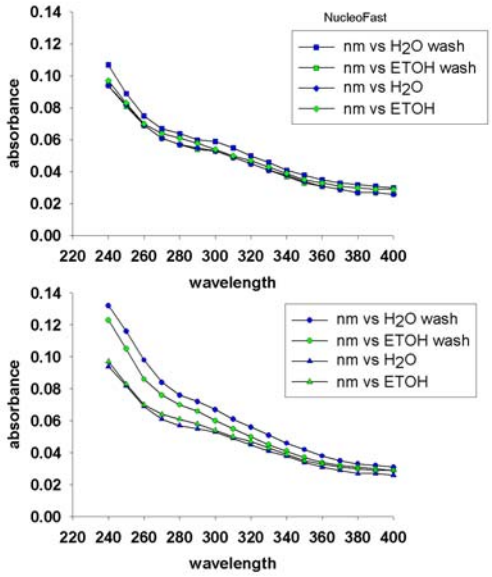


Fig. 8: Detergent-free hydrophilized membrane
The NucleoFast 96 PCR ultrafiltration membrane is permanently hydrophilized. In contrast to other competitive membranes no detergents are used for hydrophilization. Upper panel: NucleoFast 96 PCR membrane washed with water or ethanol respectively. Lower panel: detergents were used for hydrophilization of the membrane. In contrast to the detergent hydrophilized membrane (lower panel) no detergents are detected using UV/Vis spectroscopy (absorbance at <300 nm indicates presence of detergents). Reagent carry-over can interfere with downstream applications, e.g. microarray spotting.

Data and text material were kindly provided by
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