

# NucleoFast 96 PCR Purified DNA for Single Nucleotide Polymorphism Analysis Using the NanoChip<sup>®</sup> Technology (Nanogen)

The analysis of single nucleotide polymorphisms (SNPs) is becoming more important in clinical research and clinical diagnostics, e.g. in respect to screening for certain traits, like susceptibility to diseases.

The NanoChip<sup>®</sup> Molecular Biology Workstation (Nanogen, San Diego, USA), consisting of the NanoChip<sup>®</sup> Loader, the NanoChip<sup>®</sup> Cartridges, and the NanoChip<sup>®</sup> Reader, provides a convenient tool for SNP analysis. As samples are drawn to the test sites on the NanoChip<sup>®</sup> Electronic Microarray by electricity and are attached to the permeation layer by a biotin-streptavidin binding, removal of salts and other contaminants from the DNA is very crucial for optimized concentration and binding of the sample on the chip.

## Efficient PCR clean-up

MACHEREY-NAGEL's NucleoFast 96 PCR system allows rapid manual and automated purification of PCR products in a high-throughput environment. During the NucleoFast 96 PCR procedure (Fig. 1) the PCR sample is applied to the ultrafiltration membrane. Under vacuum or centrifugation contaminants (primers, dNTPs, salts, detergents) are filtered to waste while the PCR product can be recovered from the membrane after the addition of water or buffer and a short incubation. PCR products are ready-to-use for common downstream applications, e.g. sequencing and microarray analysis. NucleoFast 96 PCR is easily adapted to most common liquid handling instruments.

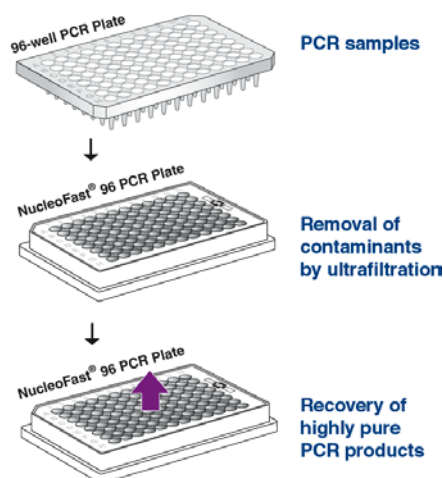


Fig. 1: NucleoFast 96 PCR Procedure

PCR products purified with NucleoFast 96 PCR have been used successfully for SNP analysis utilizing the NanoChip<sup>®</sup> technology.

A system was developed for the analysis of three different SNPs in three different amplicons (PCR 7, PCR 8, PCR 9). Three primer pairs (one pair for each amplicon) were used in a standard PCR. For two of the amplicons addition of DMSO (3% or 10%, respectively) to the PCR reaction was necessary to achieve the desired amplification yield. One primer of each pair was 5'-biotinylated for later binding to the test site on the NanoChip<sup>®</sup>. The PCR products were purified using NucleoFast 96 PCR with no adverse effect on the recovery by DMSO or the biotin label (Fig. 2).

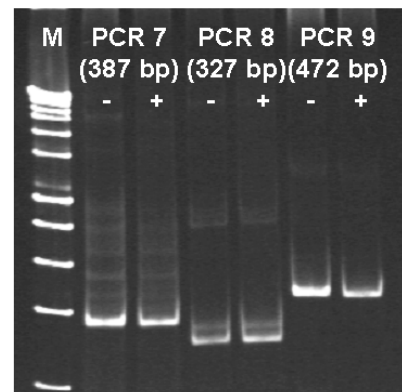


Fig. 2: Three amplicons were produced in standard PCR reactions containing different concentrations of DMSO for optimal yield (PCR 7: 0% DMSO; PCR 8: 3% DMSO; PCR 9: 10% DMSO). Products were purified manually using NucleoFast 96 PCR according to the standard protocol including the optional washing step. 15  $\mu$ l of each purified sample (+) were compared to the unpurified sample (-). Recovery of PCR products is not effected by DMSO or the biotin labels attached to one primer of each primer pair.

## SNP Analysis

For evaluation of the system the three amplicons were produced from all possible genotypes (*wt/wt*; *wt/mut*; *mut/mut*) and attached to separate test sites on the NanoChip<sup>®</sup>. Binding occurs via the primer-attached biotin to the streptavidin on the permeation layer of the chip.

Three fluorescently labeled reporter pairs (Rep 7, Rep 8, Rep 9) were constructed, one pair specific for each amplicon. The respective *wt*-reporter was labeled with Cy3 (green), the *mut*-reporter with Cy5 (red).

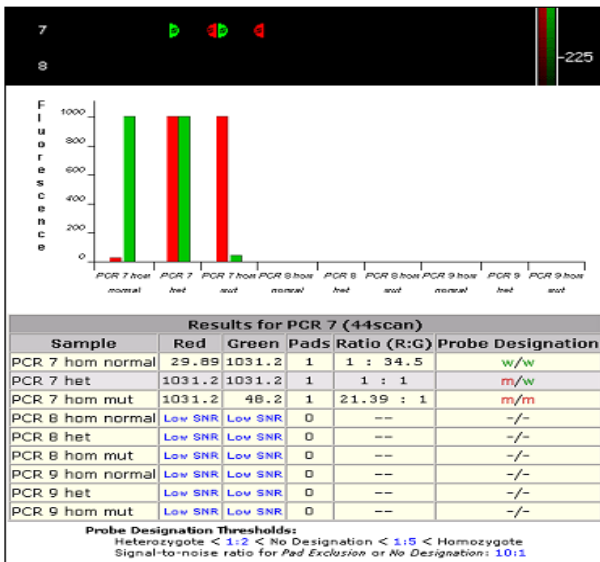
The reporter pairs were added to the samples on the NanoChip<sup>®</sup> in the manner that each reporter pair was added to all three amplicons.

Only the adequate amplicons are detected (Fig. 3 - 5), where the homozygous *wt/wt* is seen in form of a green signal, the homozygous *mut/mut* in form of a red signal, and the heterozygote *wt/mut* is seen as a red-green signal.

### Conclusion

NucleoFast 96 PCR provides an efficient and cost-sensitive tool for the high-throughput purification of PCR products (manually or automated) to be used with the NanoChip® technology. Salts, which might interfere with the electro-kinetic movement of the samples to the charged test sites on the NanoChip® are effectively removed from PCR reactions during clean-up. Furthermore, additives like detergents, Betain, or DMSO, are reduced or completely removed, so no adverse effect on the downstream application is seen. The recovery rate of PCR products is not effected by the addition of DMSO to the PCR reaction.

The purified amplicons show strong signals on the Nanogen Workstation, with a signal-to-noise ratio greater than 10:1 for all experiments.



**Fig. 3:** SNP analysis for amplicon 7: After immobilization of all amplicons in all possible genotypes the reporter set Rep 7 (sensitive for amplicon 7) was introduced to the NanoChip® Electronic Microarray. A distinct green signal is detected for the homozygous *wt/wt* of amplicon 7. A distinct red signal is detected for the homozygous *mut/mut*. The heterozygous *mut/wt* gives the expected mixed red-green signal. No signals are visible from the other two amplicons (amplicon 8 and 9) as Rep 7 is only specific for amplicon 7. The heterozygous sample is used as the standard for the fluorescence levels of the homozygous samples. In all experiments a signal-to-noise ratio (SNR) of greater than 10:1 was achieved.

### Ordering Information:

Product	Preps	Cat. No.
NucleoFast 96 PCR Clean-up Kit	4 x 96	743 500.4
NucleoFast 96 PCR Clean-up Kit	24 x 96	743 500.24
NucleoFast 96 PCR Plates (10)	10 x 96	743 100.10
NucleoFast 96 PCR Plates (50)	50 x 96	743 100.50

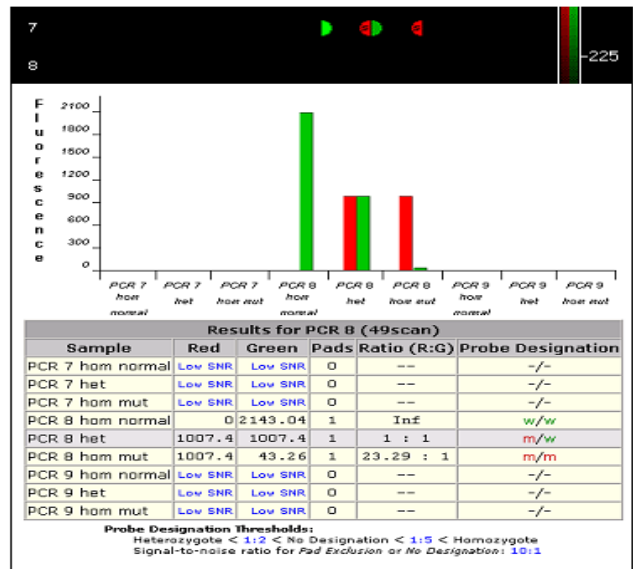
For more information regarding the use of MN products, please contact your local representative or visit MN directly under [www.mn-net.com](http://www.mn-net.com).

Trademarks: NanoChip® is a registered trademark of Nanogen.

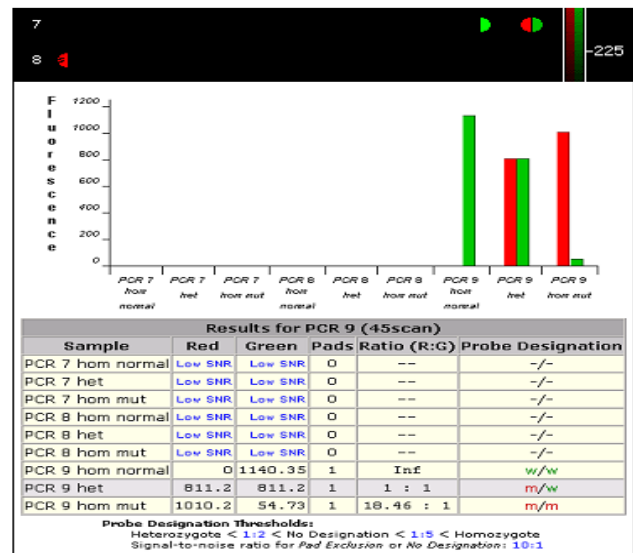
# MACHEREY-NAGEL



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**Fig. 4:** SNP analysis for amplicon 8: Rep 8 was added to the NanoChip® Electronic Microarray. Signals are detected for all three genotypes of amplicon 8, no signals are detected for amplicons 7 and 9.



**Fig. 5:** SNP analysis for amplicon 9: Rep 9 was added to the NanoChip® Electronic Microarray. Signals are detected for all three genotypes of amplicon 9, no signals are detected for amplicons 7 and 8.

All data shown was kindly provided by Angel Barroso, PhD, Cultek S. L., Spain.