TECAN.

Application Guide

MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit

Genesis Freedom
Genesis RSP
Genesis RWS

Document Name	Application Guide MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit
Version	V1.0
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Table of Contents

Revision History

Index:	Date:	Author:	Changes:
1.0	20.03.03	FeKr	First release

1 Application Description

1.1 Introduction

1.1.1 Gemini Script for the MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit

The “MN-NucleoSpin Robot-96 Extract_G8” Gemini script runs on the Genesis Freedom, RSP or RWS. It allows fully automated purification of PCR products in a multi-well format with the MACHEREY-NAGEL NucleoSpin Robot-96 Extract Kit in approximately 60 minutes. The script can be obtained from your local Tecan application specialist.

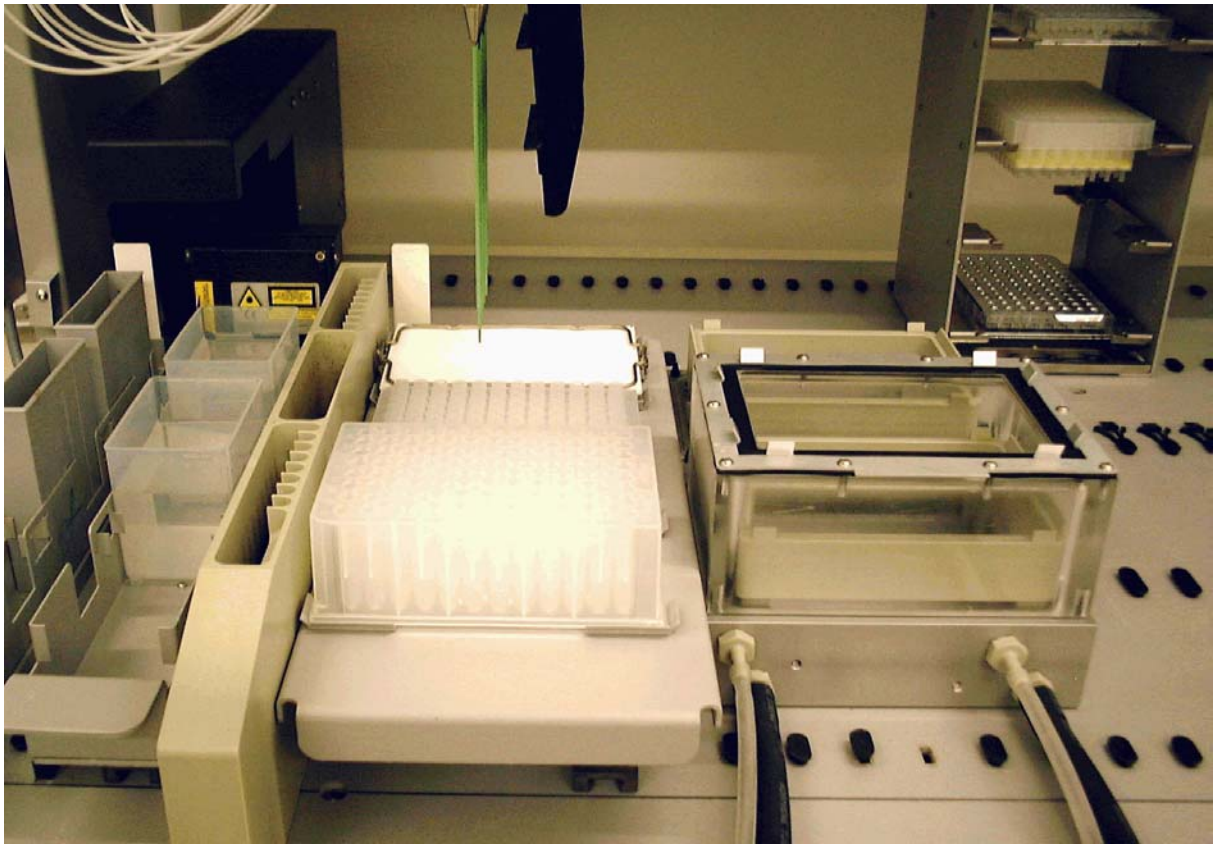

Fig 1-1 Automation of the MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit on Genesis Freedom, RSP or RWS.

1.1.2 Intended Use

The “MN-NucleoSpin Robot-96 Extract_G8” script is intended for automated processing of the MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit on a Tecan Genesis Freedom, RSP or RWS. The script is intended for research purposes only.

1.2 Materials

Refer to the MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit user manual (Rev. 01/August 2002 or later) for a complete list of materials delivered with the kit.

1.3 Requirements

Software/Instrument

- Gemini V3.5 or later
- Genesis Freedom 100, 150 or 200 with LiHa (8 tips) and RoMa *or*
- Genesis RSP 100/8 or 150/8 or 200/8 *or*
- Genesis RWS 100/8 or 150/8 or 200/8

Instrument Configuration

- 2.5ml syringes (or larger)
- fixed tips (standard tip or 384well adjustable standard tip)

Module

- Te-VacS-B with vacuum block C
- Adapter frame 6 (2x)

Carriers

- one trough rack for three 100ml troughs
- one trough rack for three 200ml troughs
- one microplate carrier

Other

- PCR Plate for cooling (positioning of unskirted PCR plate types)

Order Information

- Refer to chapter 8 “Reordering Information”

2 Principle

2.1 Principle of Application

The NucleoSpin® Robot-96 Extract purification kit is designed for the post-PCR clean up in 96well microplate format on a Genesis RSP robotic platform using vacuum filtration technology. The principle is based on reversible disruption of the hydrate shell surrounding nucleic acids in the presence of chaotropic salts. PCR products are adsorbed to the silica membrane while residual primers, primer dimers, nucleotides, Taq-polymerase do not bind and are removed in subsequent wash steps. After removal of ethanol by drying in vacuum, purified DNA is eluted in water or low salt elution buffer. Purified PCR products are ready to use for down stream applications like sequencing, restriction analysis or spotting on arrays

2.2 Principle of Automation

The Genesis Instrument equipped with a Te-VacS allows fully automated purification of PCR products. All pipetting is performed by the Genesis Liquid Handling Arm (LiHa). The Robotic

Manipulator Arm (RoMa) moves microplates between Hotel and Te-VacS. Due to proprietary equipment usage, the automated process may not be completely congruent with the manual process described in the MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit user manual.

3 Installation

3.1 Installing the Script

- Make sure the required software (Gemini V3.5 or later) is properly installed.
- Unzip the “NucleoSpin Robot-96 Extract Kit (V1.0)” data folder and create a backup copy.

Note: In addition to the Gemini script file, also carriers, racks and liquid classes used by the script are needed (Carrier.cfg, Lclasses.dat, Gemini.cfg, Gemini.opt) for proper functioning.

3.2 Check and Adjust Hardware

- Check instrument configuration required for “MN-NucleoSpin Robot-96 Extract Kit _G8” script (see chapter 1.3)
- Check precision of liquid handling arm in Genesis Instrument Software. Adjust reference positions, scale factor, absolute z-positioning and z-offset, if necessary
- Adjust predefined RoMa vectors
- Preferably perform test runs with water before running the script with real samples

Note: The RoMa vectors are defined for the Genesis RSP 150. When using a different Genesis Instrument, the RoMa vectors must be adapted to the respective instrument type. Script adaptation also may be required when working with a different syringe size.

4 Preparation

4.1 Sample Preparation

- Place the PCR plate with PCR samples on Genesis Microplate Carrier (un-sealed)

4.2 Working Solutions

Refer to MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit user manual for complete instructions on preparing working solutions.
Refer to “Liquids in Containers” for amount of liquid to be provided on the worktable.

4.3 Worktable

Note: If changing the worktable layout, it may be necessary to adapt the script to the new worktable

Before starting the script, make sure that the worktable is arranged properly

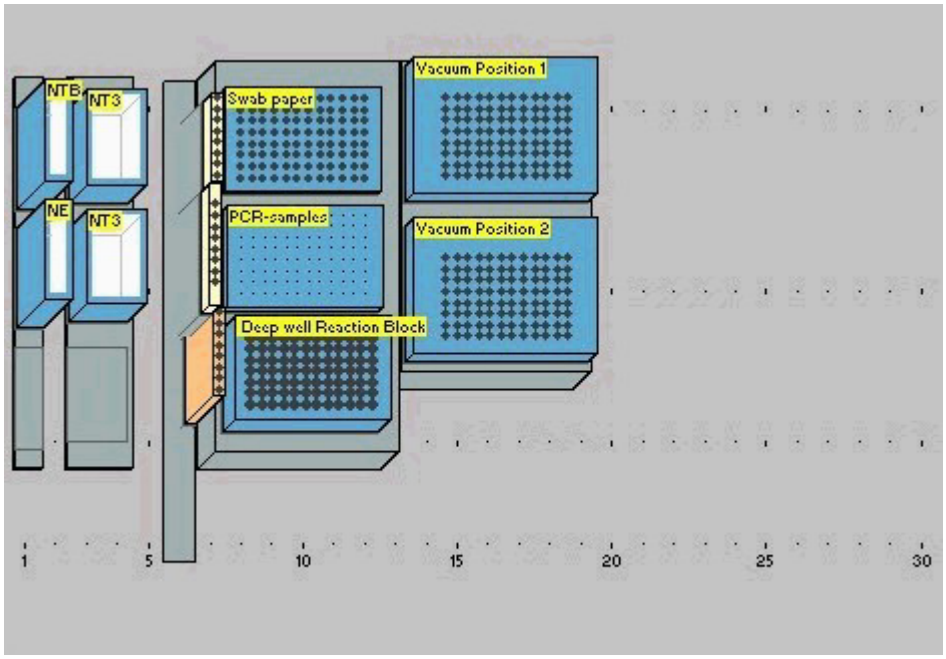

Fig. 4-1 Gemini Worktable Layout for "MN-NucleoSpin Robot-96 Extract_G8" script on Genesis Instrument (Hotel not visible)

Carriers on the worktable

Carriers are placed on the worktable as shown in Fig. 4-1:

Grid 1	Carrier for three 100ml troughs
Grid 3	Carrier for three 200ml troughs
Grid 6	Wash Station
Grid 7	Microplate Carrier (RoMa Version)
Grid 13	Te-VacS Vacuum Block
Grid 20	Hotel (MN-Type_2)

Racks and Containers on Carriers

Racks and containers with buffers are placed on the carriers as shown in Fig. 4-1 and 4-2. Plates are prepared in hotel as shown in Fig. 4-3

Grid 1	two 100ml troughs	(rear position) (middle position)
Grid 2	two 100ml troughs	(rear position) (middle position)
Grid 7	Paper holder with 5 pieces of paper PCR sample plate Round-well Block (96wells)	(rear position) (middle position) (front position)

Grid 13	adapter frame type 6 and vacuum block C	(rear position)
	adapter frame type 6	(front position)
Grid 20	Elution plate, U-Bottom	(position 1)
	NucleoSpin® Extract Binding Plate (yellow)	(position 2)
	MN Wash Plate	(position 4)

Liquids in Troughs

Fig. 4-2 Placement of Liquids in Containers

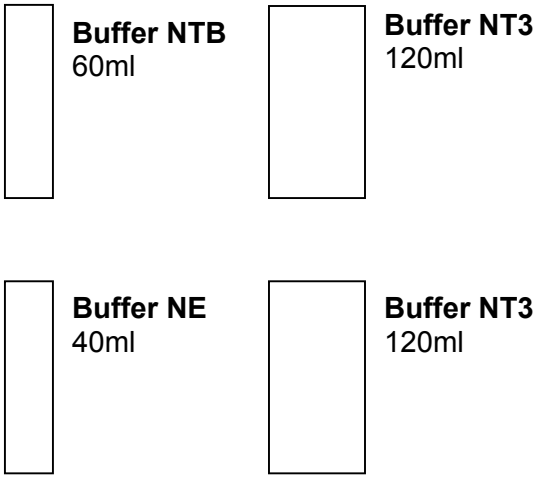

Fig. 4-3 Plates in Hotel

5 Running the Script

5.1 MN-NucleoSpin Robot-96 Extract_G8 Script

Before Running the Script

- Start Gemini.exe
- Set data path to folder “NucleoSpin Robot-96 Extract (V1.0)”
- Open script
- Prepare Genesis Instrument worktable with appropriate carriers, racks, troughs, plates and reagents
- Flush liquid system until it is free of air bubbles
- Start the script

After Running the Script

- Purified PCR samples ready for use in further applications
- Flush liquid system (> 30ml)
- Remove buffers from worktable
- Clean Genesis worktable and carriers

5.2 Customizing the Gemini Script

To facilitate modifying the Tecan Gemini script, the following table gives an overview of the structure of the script. Please refer to the Gemini Software Manual for more information of script writing in Gemini.

Table 5-2 Structure of the Gemini Script

Comment in Script	Explanation
Dilute PCR reaction to 100 µl with buffer NE	Buffer NE is used to dilute to final volume (100µl).
Dispense binding buffer NTB	Buffer NTB is pre-dispensed into the reaction block (Round-well Block)
Transfer PCR-Mix, mix and transfer to Silica plate	Samples are mixed with Buffer NTB and applied onto NucleoSpin® Extract Binding Plate (yellow).
DNA adsorption step	Vacuum is applied to drain liquid from NucleoSpin® Extract Binding Plate. DNA binds to silica membrane
Wash of silica membrane with buffer NT3	Contaminants removal
Air-drying	Residual ethanol is evaporated from membranes by extensive vacuum driven airflow
Dry cartridge outlets on absorbent paper	Removes droplets from cartridge
Elution	Purified PCR fragments are eluted from NucleoSpin® Extract Binding Plate with Buffer NE

5.3 Extended Customization

With Te-Shake

- Faster mixing without tip washing; throughput will increase
- Gemini script and worktable must be adapted

Multiple Batches

- Gemini script and worktable must be adapted. Additional Hotels required
- Recommended to use a Te-Shake with shaker plate for two microplates

Disposable Tips

- Gemini script and worktable must be adapted

6 Troubleshooting

Table 6-1 Troubleshooting

Problem	Possible Cause and Suggestions
Insufficient vacuum	*Plate not positioned correctly* Check RoMa-Vector *Block sealing dirty or broken* Check and clean or replace sealing (upper; lower) *Te-VacS failure* Check Te-VacS functionality
RoMa drops plates	*RoMa vectors are not correctly configured* Adjust RoMa vectors (especially grip distance and gripper force) *Plates stick to carrier or Te-VacS block* Clean carrier or Vacuum block
Cross contamination observed in downstream process	*Tips not clean enough* Increase wash volume and/or implement decontamination steps (e.g. bleach) *Drop of residual liquid from cartridge outlets in neighboring plate wells* Increase vacuum pressure and duration for drying step *Spraying/aerosol formation during elution step* Check cartridge outlet position in relation to liquid level in the Elution plate.
Weak yield or purity	Refer to the MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit user manual

7 Results

7.1 Primer Removal Efficiency

PCR products purified with the MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit on TECAN Genesis were analyzed by agarose gel electrophoresis. Figure 7-1 shows PCR products of various lengths before and after purification. This figure also illustrates the size dependency of the recovery rate.

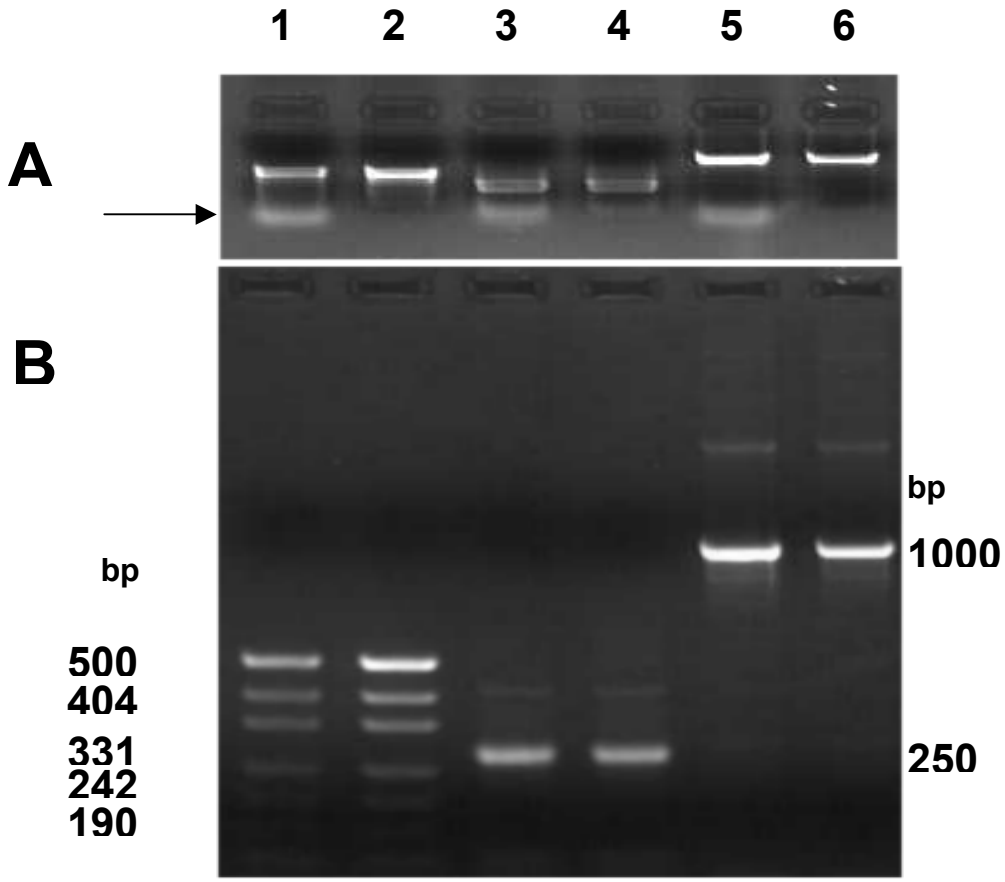

Fig. 7-1 Primer removal: A set of PCR products (sizes as indicated, 20 µl) were purified using the NucleoSpin® Robot-96 Extract kit on Tecan Genesis RSP150 instrument. After elution purified samples and unpurified controls were dried and separated on 1 % agarose gel (ethidium bromide stain). Lane 1: PCR mix unpurified, lane 2: PCR mix purified (2 purified samples were combined before drying to demonstrate primer removal). lane 3: 250 bp PCR fragment unpurified, lane 4: 250 bp PCR fragment purified, lane 5: 1000 bp PCR fragment unpurified, lane 6: 1000 bp PCR fragment purified. Separation was stopped after 5 min (A) to check primer removal. Primers are completely removed after purification (see arrow). Electrophoresis was continued for 60 min (B) for complete separation of PCR products.

7.2 PCR Fragment Recovery

Excellent homogeneity in PCR product recovery was achieved when using the MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit on TECAN Genesis. Results for a set of different fragment length is visualized in Figure 7-2.

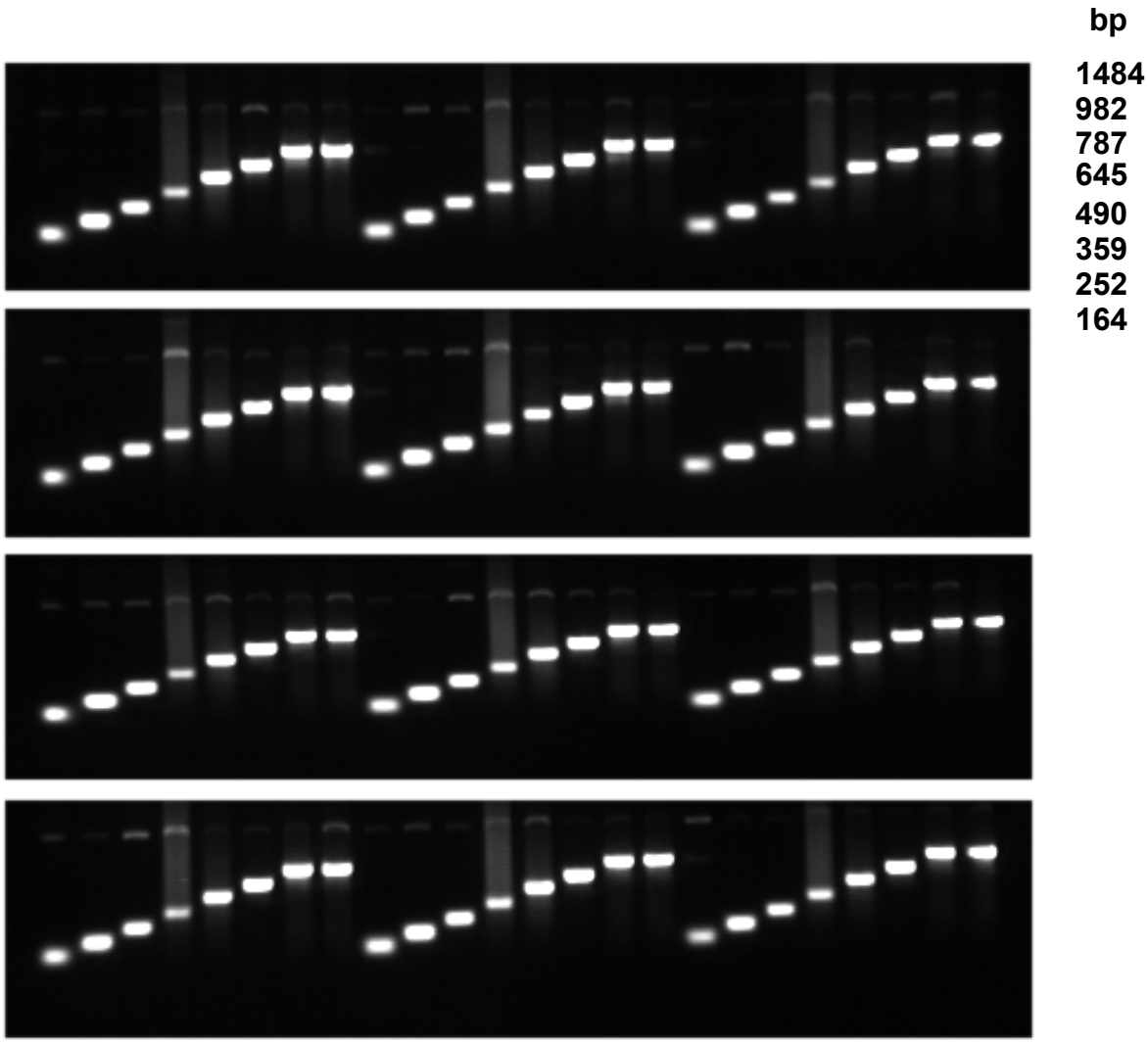

Fig 7-2: Homogeneity of purification: 96 PCR samples (25 µl each, sizes as indicated) were purified using the NucleoSpin® Robot-96 Extract kit on Tecan Genesis. Samples were eluted with 125 µl elution buffer NE dried and separated on 1 % agarose gel (ethidium bromide stain).

7.3 Sequencing PCR Products

PCR samples purified with the “MN-NucleoSpin Robot-96 Extract Kit _G8” script can be used for automated fluorescent DNA sequencing, micro arrays and restriction digestion. Sequencing a 1500 bp PCR on ABI PRISM® 377 DNA sequencer results in more than 600bp confirmed sequence data (see Fig. 7-3).

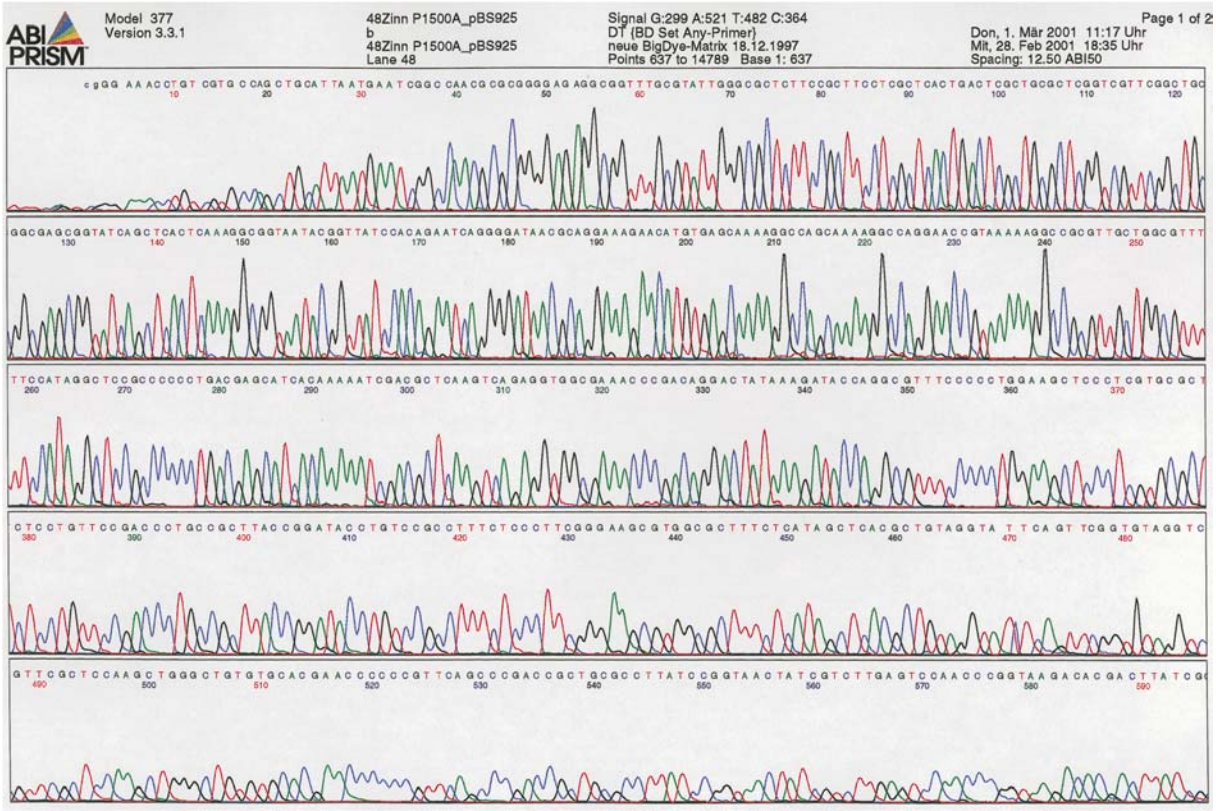

Fig. 7-3 Sequencing of purified PCR products: A 1500 bp PCR product was purified using the NucleoSpin® Robot-96 Extract Kit on Tecan Genesis RSP 150 Instrument. Purified DNA was sequenced using the ABI PRISM BigDye Terminator cycle sequencing ready reaction kit according to the manufacturer’s instructions. No background (resulting from not removed primers) was detected.

8 Reordering

Hardware Order from **Tecan**

• SPE plate adapter frame 6, (2x)	760666
• SPE vacuum block, type C	760626
• Carrier for microplates	612604
• Carrier for three reagent troughs 100ml	613020
• Carrier for three reagent troughs 200ml	760645
• Reagent troughs 100ml (100 pcs)	613021
• Reagent troughs 200ml (32 pcs)	760646
• Standard tips (set of 8)	612501
• 384well standard tip, adjustable	612532
• Paper holder	760653
• PCR-Plate Adaptor for Cooling	623037
• Filter Paper	760656
• Te-VacS B	(*)
• *Te-Shake (optional)*	(*)

Note: Optional hardware for extended customization can be ordered from your local Tecan representative.
(*) If you want to order a complete Te-VacS module, please refer to the respective order configuration sheet.

Kit Order from MACHEREY-NAGEL

• NucleoSpin® Robot-96 Extract Kit	2x96	740707.2
• NucleoSpin® Robot-96 Extract Kit	4x96	740707.4
• NucleoSpin® Robot-96 Extract Kit	24x96	740707.24

Available separately from MACHEREY-NAGEL

• Square-well Block (pack of 20 pcs.)	740670
• MN Wash Plate (pack of 20 pcs.)	740674
• Self-adhering PE foil (pack of 50 pcs.)	740676

9 Literature

Tecan Manuals

• Genesis Freedom Operating Manual	391588
• Genesis RSP Operating Manual	390783
• Genesis RWS Operating Manual	391197
• Gemini Software Manual	391201
• *Te-Shake Operating Manual (optional)*	*391496*

MACHEREY-NAGEL Technical Note

- MACHEREY-NAGEL NucleoSpin® Robot-96 Extract Kit user manual (Rev. 01/August 2002 or later)

10 Customer Support

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