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# Application Manual

# MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit
# Genesis Freedom
# Genesis RSP, RWS

**Document Status Sheet**

| **Title:** | Application Manual for MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit automated on the Genesis Freedom/RSP/RWS | | |
|---|---|---|---|
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| **Version** | **Revision** | **Issue** | **Major changes** |
| 1 | 0 | | first released version |

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# 1 Product Description

**Purpose of This Chapter**

This chapter introduces the reader to the automation of the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit, its intended use and requirements.

## 1.1 Introduction

### 1.1.1 Gemini Script for MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit

The MN-NucleoSpin® Robot-96 Plasmid_G8 script runs on the Genesis Freedom, RSP or RWS equipped with a Te-VacS. It allows fully automated plasmid purification of batches of 96 samples with the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit in approximately 60 minutes. The script can be obtained from your local Tecan application specialist.

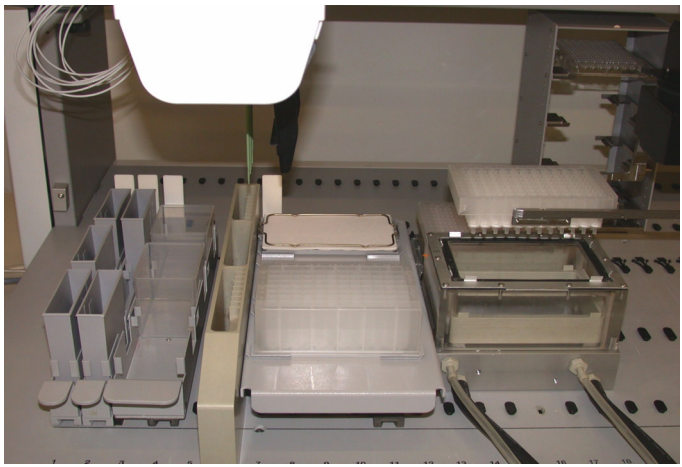

***Fig. 1-1*** *Genesis Instrument Processing the MN-NucleoSpin® Robot-96 Plasmid_G8 Script*

### 1.1.2 Product Use

The MN-NucleoSpin® Robot-96 Plasmid_G8 Gemini script is intended for automated processing of the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit on a Tecan Genesis Freedom, RSP or RWS equipped with a Te-VacS. The script is intended for research purposes only.

## 1.2 Materials

Refer to the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit manual for a complete list of materials delivered with the kit.

## 1.3 Requirements

| | |
|---|---|
| **Software** | Gemini V3.5 or later |
| **Instrument** | ◆ Genesis Freedom 100, 150 or 200 with LiHa (8 tips) and RoMa *or*<br>◆ Genesis RSP 100/8 or 150/8 or 200/8 *or*<br>◆ Genesis RWS 100/8 or 150/8 or 200/8 |
| **Instrument Configuration** | ◆ 2.5 ml syringes<br>◆ fixed tips (standard tip or 384well adjustable standard tip)<br>◆ wide bore tips can be used optionally |
| **Modules** | ◆ Te-VacS-B with vacuum block C and adapter frame 6 (2x) |
| **Carriers** | ◆ two trough racks for three 100 ml troughs each<br>◆ one trough rack for three 200 ml troughs each<br>◆ microplate carrier<br>◆ SPE hotel with four shelves |

**Ordering Information**

Refer to chapter 8 “Re-ordering”, 🗎 23.

# 2 Principle

**Purpose of This Chapter**

This chapter explains the basic principle of the automation of the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit.

## 2.1 Principle of Application

Refer to the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit manual.

## 2.2 Principle of Automation

The Genesis instrument equipped with a Te-VacS allows fully automated isolation of plasmid DNA from bacterial cultures. All pipetting is performed by the liquid handling arm (LiHa). The robotic manipulator arm (RoMa) assembles the vacuum unit and removes the cartridges after the extraction procedure has finished. 96 samples can be processed simultaneously. Due to different equipment usage, the automated process may not be completely congruent with the manual process.

# 3 Installation

**Purpose of This Chapter**

This chapter describes how the script for processing the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit is installed and gives instructions on initial operation and how the performance qualification can be achieved.

## 3.1 Installing the Script

### 3.1.1 Check in Advance

Make sure that the required hardware and software have been properly installed.

### 3.1.2 Script Installation

We recommend to copy the Gemini script onto your local harddisk into a separate data folder. In addition to the Gemini script file, you will need the carriers, racks and liquid classes used by the script. These are contained by the files Carrier.cfg, LClasses.dat, Gemini.cfg and Gemini.opt. Copy these files into the same data folder as the Gemini script.

## 3.2 Putting into Action

Before running any Gemini script, perform the following checks:

- Z-Adjust LiHa
  Precise LiHa movements are very important for proper functioning of a Gemini script. Check the precision of the liquid handling arm in the Genesis Instrument Software. If necessary adjust reference positions, scalefactor, absolute z-positioning and z-offset (refer to Genesis Instrument Software Manual or call your local Tecan representative). Check the rack positions with the LiHa in Gemini.
- RoMa vectors
  Due to individual RoMa properties it may be necessary to adjust predefined RoMa vectors. If you find the predefined RoMa vectors to deviate from the situation on your instrument, calibrate your RoMa or adjust the RoMa vectors.

Perform test runs with water before running the script(s) with real samples.

***Note:*** *The RoMa vectors in the MN-NucleoSpin® Robot-96 Plasmid_G8 script are defined for a Genesis RSP 150 instrument. When using a Genesis RSP/RWS 100 or 200 or Genesis Freedom instrument, the RoMa vectors must be transcribed to the respective instrument type. When working with a different syringe size than 2.5 ml syringes, the Gemini script has to be adapted.*

# 4 Preparation

**Purpose of This Chapter**

This chapter describes the prerequisites for running a script.

## 4.1 Sample Preparation

1. Grow cells as described in the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit manual.
2. Pellet bacteria by centrifuging at 1000 x g for 10 minutes.
3. Remove supernatants.
4. Place Square-well Block (culture plate) on the worktable.

## 4.2 Working Solutions

Refer to the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit manual for complete instructions on preparation of working solutions.

Refer to “Liquids in Containers”, 11 for the amount of liquid to be provided on the worktable.

## 4.3 Worktable

***Note:*** *If you change the worktable layout you will have to make changes in the script such that it fits to the new worktable layout.*

Before starting the MN-NucleoSpin® Robot-96 Plasmid_G8 script make sure that the worktable is arranged properly.

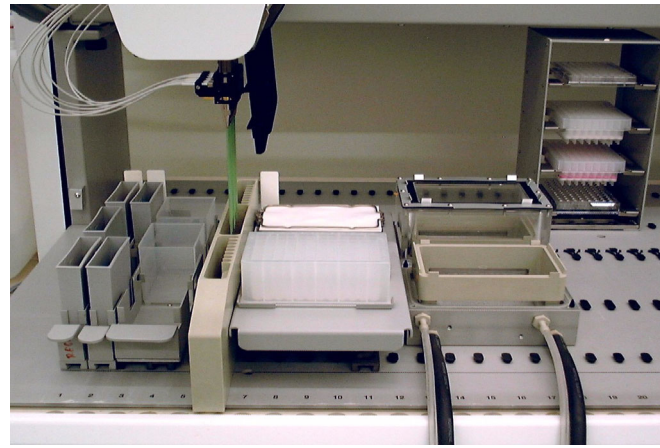

***Fig. 4-1*** *Worktable Layout*

**Carriers on the Worktable**

Carriers are placed on the worktable as shown in Fig. 4-1, 🗎 8.

| | |
|---|---|
| Grid 1 | carrier for three 100 ml troughs |
| Grid 2 | carrier for three 100 ml troughs |
| Grid 3 | carrier for three 200 ml troughs |
| Grid 6 | wash station |
| Grid 7 | microplate carrier |
| Grid 13 | Te-VacS |
| Grid 20 | SPE hotel with 4 shelves in the rear of the worktable (fourth grid row) |

## Racks and Containers on Carriers

Racks and containers are placed on the carriers as shown in Fig. 4-1, 🗎 8.

| | |
|---|---|
| Grid 1 | three 100 ml troughs |
| Grid 2 | two 100 ml troughs |
| Grid 3 | two 200 ml troughs |
| Grid 7 | paper holder with 5 pieces of paper on rear position of microplate carrier |
| Grid 13 | adapter frame type 6 and vacuum block type C on rear position of Te-VacS<br>adapter frame type 6 on front position of Te-VacS |
| Grid 20 | Elution plate U-bottom, NucleoSpin® Plasmid Binding Plate (transparent), NucleoSpin® Plasmid Filter Plate (purple) and MN Wash Plate in the SPE hotel as shown in Fig. 4-2, 🗎 10 |

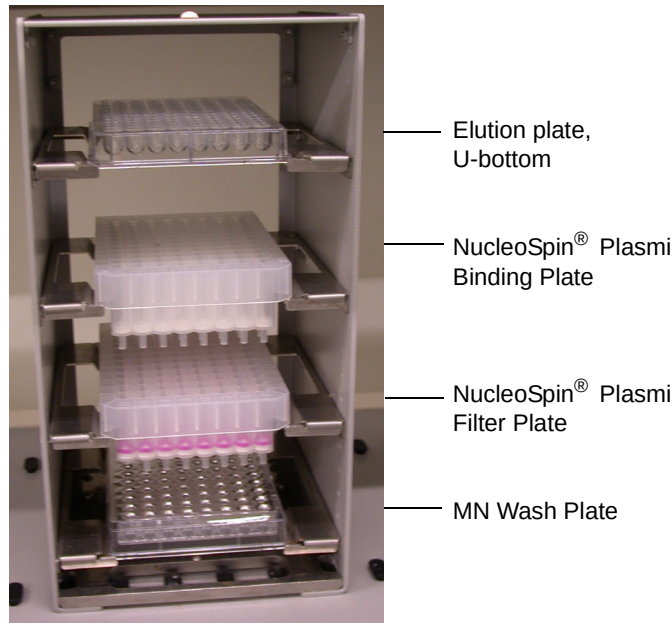


***Fig. 4-2*** *Placement of Plates in the Hotel*

**Liquids in Containers**

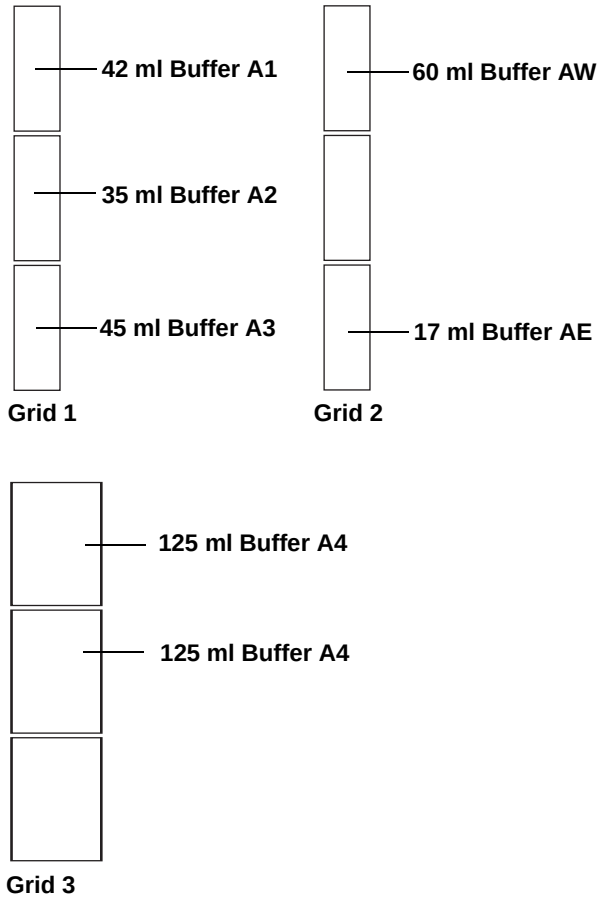


***Fig. 4-3*** *Placement of Liquids in Containers*

# 5 Running the Script

**Purpose of This Chapter**

This chapter gives instructions on how to run the Gemini script properly.

## 5.1 MN-NucleoSpin® Robot-96 Plasmid_G8

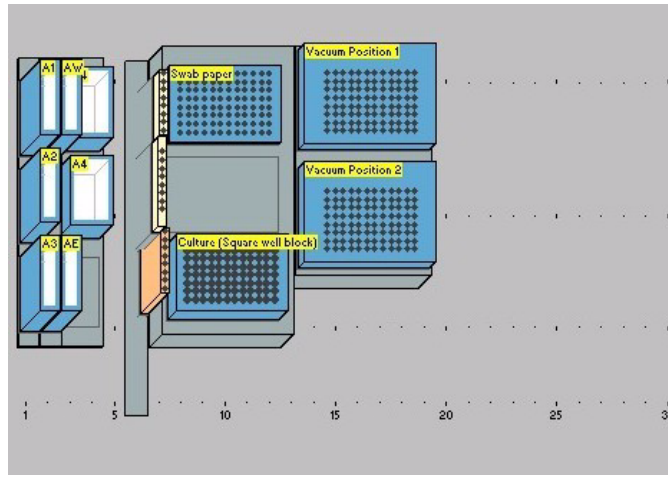


***Fig. 5-1*** *Gemini Worktable Layout (hotel for plates not visible)*

**Before Running the Script**

1. Prepare the Genesis instrument worktable.
2. Open the script.
3. Flush the liquid system until it is free of airbubbles.
4. Start the script.

**After Running the Script**

1. Eluted plasmid DNA is ready for use in further applications.
2. Flush the liquid system (> 30 ml).
3. Flush the tubing system of the Te-VacS with water to avoid salt precipitation (> 100 ml).
4. Remove buffers from the worktable (for reuse, store each buffer in a separate bottle).
5. If necessary, clean the Genesis worktable and carriers.

## 5.2 Customizing the Gemini Script

To facilitate modifying of 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 on script writing in Gemini.

***Tab. 5-1*** *Structure of the Gemini Script*

| Comment in Script | Explanation |
|---|---|
| Preparation of Te-VacS Vacuum Block | Transfer of Plasmid Binding Plate from hotel into vacuum block and Plasmid Filter Plate onto vacuum block on front position. MN Wash plate is placed into vacuum block on rear position. |
| Resuspension of Pellet with Buffer A1 | 250 µl of resuspension buffer is pipetted into the Square-well Block (culture plate). Centrifuged bacterial cells are resuspended by pipetting up and down. |
| Lysis of Bacteria with Buffer A2 | 250 µl of lysis buffer is added to the resuspended cells. |

***Tab. 5-1*** *Structure of the Gemini Script*

| Comment in Script | Explanation |
|---|---|
| Neutralization of Lysate with Buffer A3 | The cell lysate is neutralized by addition of 350 µl of neutralization solution. |
| Transfer of Lysate | The neutralized lysate is transferred onto filter membranes (Plasmid Filter Plate). |
| Filtration of Lysate | Vacuum is applied to filter crude lysate through filter. Filtered lysate is caught on membranes of Plasmid Binding Plate. |
| Rearrangement of Te-VacS Vacuum Block | Plasmid Filter Plate is brought back to the hotel. Plasmid Binding Plate is prepared for subsequent process steps on the vacuum block rear position. |
| DNA Adsorption | Vacuum is applied to drain liquid from Plasmid Filter Plate. Plasmid DNA is bound to the membrane. |
| Wash DNA with Buffer AW | 500 µl of Buffer AW is pipetted onto Plasmid Binding Plate.Vacuum is applied. |
| 1st wash of plasmid DNA with Buffer A4 | 1000 µl of Buffer A4 is pipetted onto Plasmid Binding Plate. Vacuum is applied. |
| 2nd wash of plasmid DNA with Buffer A4 | 1000 µl of Buffer A4 is pipetted onto Plasmid Binding Plate. Vacuum is applied. |
| Dry Cartridge on Absorbent Paper | RoMa step; drying wet nozzles of Plasmid Binding Plate on absorbent paper. |

***Tab. 5-1*** *Structure of the Gemini Script*

| Comment in Script | Explanation |
|---|---|
| Vacuum Drying Step | Additional airflow through Plasmid Binding Plate is generated by vacuum. Residual ethanol is removed. |
| Elution Step | Prepare vacuum block for elution. 75-150 µl buffer AE is pipetted onto silica plate and vacuum is applied. |
| Rearrangement of TeVacS Vacuum Block for new run | Plasmid Binding Plate and Elution plate are brought back to the hotel. The vacuum block is prepared for a new run. |
| Process Completed | The automated plasmid purification procedure is completed. The Elution plate can be removed and sealed and stored or used directly for subsequent analysis experiments. |

## 5.3 Extended Customization

**WithTeShake**

- Reduced resuspension time.
- Throughput will increase.
- Worktable must be adapted (enough space on the 100 sized instrument).

**Syringes size**

- The use of smaller syringes enables pipetting of low volumes in downstream processes.
- Pipetting script must be adapted.

| | |
|---|---|
| **Multiple Batches** | ◆ Worktable must be adapted.<br>◆ Te-Shake with shakerplate for two microplates is recommended. |
| **Disposable Tips** | ◆ Worktable must be adapted.<br>◆ Not enough space on the 100 sized instrument (because of DiTi carriers). |

# 6 Troubleshooting

**Purpose of This Chapter**

This chapter helps to resume operation after a minor problem has occurred with the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit. It lists possible occurrences, their probable cause and suggests how to remedy the problem.

***Tab. 6-1*** *Troubleshooting*

| Problem | Possible Cause and Suggestions |
|---|---|
| Incomplete lysis of bacterial cells | *Cell pellet not properly resuspended*<br>It is essential that the cell pellet is completely resuspended prior to lysis. No cell clumps should be visible before addition of Buffer A2. If necessary, increase the number of mixing cycles.<br>*Too many bacterial cells used*<br>We recommend usage of LB as growth medium. When using extremely rich media like TB, cultures reach very high cell densities. |
| DNA degraded | *Cultures overgrown*<br>We recommend usage of LB as growth medium. When using extremely rich media like TB, cultures reach very high cell densities. |
| Not all samples were sucked through completely during filtration | *Inhomogeneous samples*<br>Make sure to use the same amount of cells in each well. Use the same bacterial host in each well.<br>*Not enough vacuum*<br>Increase vacuum time or number of vacuum cycles. |

***Tab. 6-1*** *Troubleshooting*

| Problem | Possible Cause and Suggestions |
|---|---|
| RoMa drops plates | *Plates stick to carrier / vacuum block*<br>Clean carrier / vacuum block and adapter frames. Treat the sealing of the vacuum block with silicone grease.<br>*RoMa vectors are not correctly teached*<br>Adjust RoMa vectors (especially grip distance and gripper force). |
| Clogged tips | *Tip opening too narrow*<br>Use wide bore tips. Be aware that wide bore tips cannot be used for pipetting downstream applications with small volumes (PCR mix pipetting, cycle sequencing setup, etc.) |
| Other | refer to MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit manual. |

# 7 Results

**Purpose of This Chapter**

This chapter shows some typical results obtained with the automation of MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit and possible downstream applications. Typical downstream use of purified plasmid DNA is radioactive and fluorescent DNA sequencing, library screening, cloning and PCR.

## 7.1 DNA Quality and Yield Reproducibility

Plasmid DNA extraction automated on a Tecan Genesis instrument is analyzed via agarose gel electrophoresis and UV measurement. Fig. 7-1, 20 shows the high quality of the DNA and the reproducibility of DNA yields. A high percentage of supercoiled DNA (95%) is obtained. Depending on the bacterial strain and growing conditions, >5 µg of plasmid DNA can be obtained from 1 ml *E.coli* culture. The concentration of the eluted DNA is between 100 and 300 ng/µl. The purity (A260/A280) of the isolated DNA is about 1.8. Purified samples are completely free of RNA.

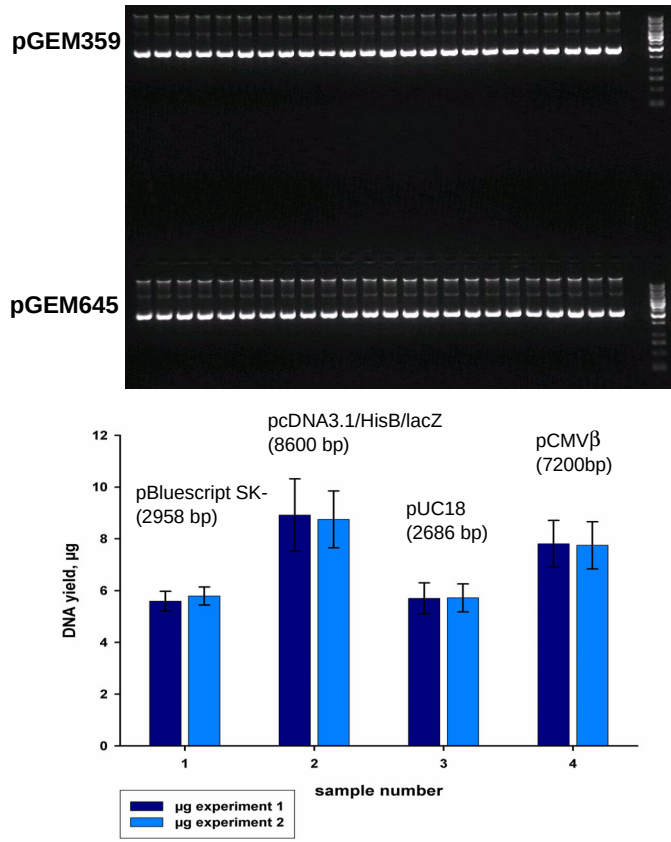


***Fig. 7-1*** *Top: Agarose Gel Analysis of purified plasmid DNA. (10/125µl of eluted plasmid DNA were analysed on 1% TAE agarose gel; 24 of 48 samples each). Upper row: pGEM359 (pGEM vector with 359 bp insert). Lower row: pGEM645 (pGEM vector with 645 bp insert). Homogenous results were obtained with both plasmids. Bottom: Four different plasmid DNAs were purified from 1.2 ml LB grown E. coli cultures. DNA yield was determined by UV measurement. Data are shown from two experiments with 24 samples per experiment of each plasmid.*

## 7.2 Restriction Enzyme Analysis

Plasmid DNA isolated with the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit can directly be analyzed with restriction enzymes. Fig. 7-2, 21 shows the restriction pattern of pBluescript SK (Stratagene) isolated with the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit.

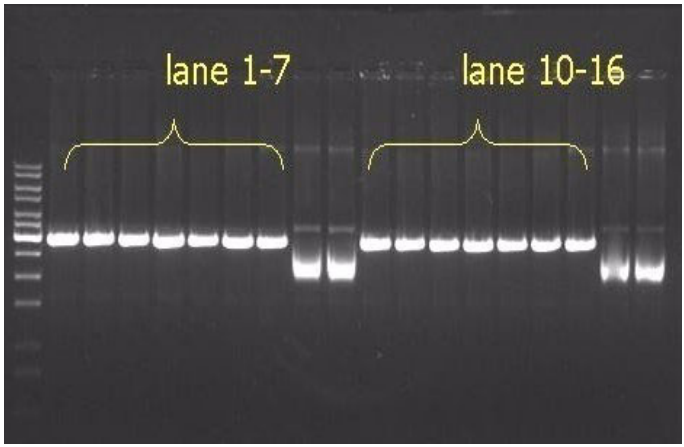


***Fig. 7-2*** *Restriction Enzyme Analysis of pBluescript SK: Plasmid DNA was digested with 2 U restriction enzyme (EcoRI) for 2 hours at 37°C and was analyzed on a 1% TAE agarose gel (lane 1-7, 10-16) in comparison to undigested plasmid DNA stored at 37°C (lane 8,17) or stored at 4°C (lane 9, 18) in incubation buffer each.*

## 7.3 Sequencing Plasmid DNA

Plasmid DNA isolated with the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit can directly be used for sequencing analysis. Automated fluorescent sequencing was performed on ABI 3700 using BigDye-terminator chemistry. Typical reading length was consistently higher than 550-800 bp (seeFig. 7-3, 22 ).

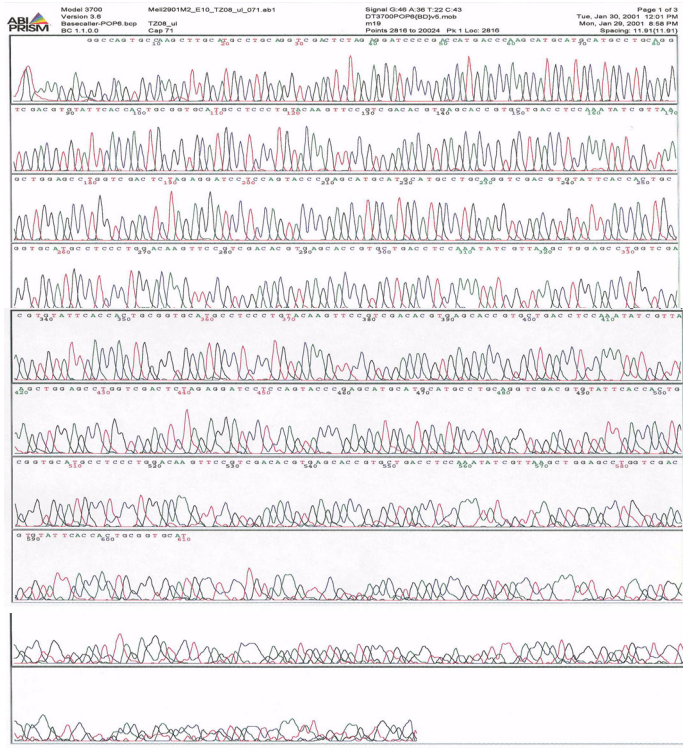


***Fig. 7-3*** *Sequence Obtained with the ABI 3700 Sequencer*

# 8 Re-ordering

**Purpose of This Chapter**

This chapter lists materials that are used in connection with the MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit including their ordering information.

**Hardware**

**Order from Tecan:**

| | |
|---|---|
| SPE plate adapter frame 6, (2x) | 760666 |
| SPE vacuum block, type C | 760626 |
| Carrier for microplates | 612604 |
| SPE hotel with 4 shelves | 612687 |
| Carrier for three reagent troughs 100 ml | 613020 |
| Carrier for three reagent troughs 200 ml | 760645 |
| Reagent troughs 100 ml (100 pcs) | 613021 |
| Reagent troughs 200 ml (32 pcs) | 760646 |
| Paper holder | 760653 |
| Filter paper | 760656 |
| Standard tips (set of 8) | 612501 |
| 384well standard tip, adjustable (set of 8) | 612532 |

If you want to order a complete Te-VacS or Te-Shake module, refer to their respective order configuration sheets.

**Kit**

**Order from MACHEREY-NAGEL:**

| | |
|---|---|
| NucleoSpin® Robot-96 Plasmid Kit, Pack of 2 x 96 preps | 740 708.2 |

| | | |
|---|---|---|
| | NucleoSpin® Robot-96 Plasmid Kit, Pack of 4x 96 preps | 740 708.4 |
| | NucleoSpin® Robot-96 Plasmid Kit, Pack of 24x 96 preps | 740 708.24 |
| | NucleoSpin® Robot-96 Plasmid Core Kit, Pack of 24 x 96 preps | 740 616.24 |
| **Optional Materials** | **Order from Tecan:** | |
| | Covers for reagent troughs 100 ml, set of 6 | 612548 |
| | Wide bore tips (set of 8) | 760644 |

# 9 Literature

**Purpose of This Chapter**

This chapter contains a list of literature referenced in this manual.

**Tecan Manuals**

- Genesis Freedom Operating Manual 391588
- Genesis RSP Operating Manual 390783
- Genesis RWS Operating Manual 391197
- Te-VacS Operating Manual 391236
- Gemini Software Manual 391201

**MACHEREY -NAGEL Manual**

- NucleoSpin ® Robot Plasmid Kit

**Technical Literature**

Refer to MACHEREY-NAGEL NucleoSpin® Robot-96 Plasmid Kit manual.

# 10 Customer Support

**Purpose of This Chapter**

This chapter informs you how to contact us in case help is needed. It lists addresses and telephone numbers of the manufacturer's representatives.

**How to get Help**

Tecan and its representatives maintain a fully trained staff of technical specialists around the world. For any technical question, contact your system integrator or nearest Tecan representative.

**Addresses**

Contact your local distributor or one of the addresses below.
Also see our homepage on the web: **www.tecan.com**

| Country/ Region | Address | Telephone/Telefax/E-mail |
|---|---|---|
| Switzerland | Tecan Sales Switzerland AG<br>Seestrasse 103<br>CH-8708 Männedorf<br>Switzerland | Phone +41/1 922 82 82<br>Fax +41/1 922 84 84<br>E-mail helpdesk-ch@tecan.com |
| Asia | Tecan Asia (Pte) Ltd.<br>80, Marine Parade<br>#13-04<br>Singapore 449 269 | Phone +65 644 41 886<br>Fax +65 644 41 836<br>E-mail tecan@tecan.com.sg |
| Austria | Tecan Austria GmbH<br>Untersbergstrasse 1a<br>5082 Grödig / Salzburg | Phone +43 62 46 89 33 444<br>Fax +43 62 46 89 33 39<br>E-mail helpdesk-at@tecan.com |
| Belgium | Tecan Benelux B.V.B.A.<br>Vaartdijk 55<br>B-2800 Mechelen | Phone +32 15 42 13 19<br>Fax +32 15 42 16 12<br>E-mail tecan-be@tecan.com |

| Country/ Region | Address | Telephone/Telefax/E-mail |
|---|---|---|
| France | Tecan France S.A.<br>Parc d'Activités de Pissaloup<br>Bâtiment Hermes II<br>27, Rue Edouard Branly<br>F-78190 Trappes | Phone +33 1 30 68 81 50<br>Fax +33 1 30 68 98 13<br>E-mail helpdesk-fr@tecan.com |
| Germany | Tecan Deutschland GmbH<br>Theodor-Storm-Strasse 17<br>D-74564 Crailsheim | Phone +49 7951 94 170<br>Fax +49 7951 50 38<br>E-mail helpdesk-de@tecan.com |
| Great Britain | Tecan UK<br>Theale Court<br>11-13 High Street<br>Theale, UK-Reading<br>Berkshire RG7 5AH | Phone +44 118 930 0300<br>Fax +44 118 930 5671<br>E-mail helpdesk-uk@tecan.com |
| Italy | Tecan Italia, S.r.l.<br>Via Fratelli Cerci<br>Palazzo Bernini<br>Centro Direzionale Milano 2<br>I-20090 Sergate (Mi) | Phone +39 02 215 21 28<br>Fax +39 02 215 97 441<br>E-mail helpdesk-it@tecan.com |
| Japan | Tecan (Japan) Co. Ltd.<br>Meiji Seimei Fuchu Building<br>10F<br>1-40, Miyamachi<br>Fuchu City<br>Tokyo | Phone +81 66 30 58 511<br>Fax +81 42 33 40 401<br>E-mail helpdesk-jp@tecan.com |
| Netherlands | Tecan Benelux B.V.B.A.<br>Industrieweg 30<br>NL-4283 Giessen | Phone +31 018 34 48 174<br>Fax +31 018 34 48 067<br>E-mail tecan-nl@tecan.com |
| Scandinavia | Tecan Nordic AB<br>Box 208<br>SE-43123 Mölndal<br>Sweden | Phone +46 31 75 44 000<br>Fax +46 31 75 44 010<br>E-mail tecan.nordic@tecan.com |

| Country/ Region | Address | Telephone/Telefax/E-mail |
|---|---|---|
| Spain | Tecan Spain<br>Sabino de Arana<br>32<br>E-08028 Barcelona | Phone +34 93 490 01 74<br>Fax +34 94 411 24 07<br>E-mail tecanbcn@terra.es |
| USA | Tecan U.S.<br>P.O. Box 13953<br>Research Triangle Park<br>NC 27709 | Phone +1 919 361 5200<br>Fax +1 919 361 5201<br>E-mail helpdesk-us@tecan.com |
| USA | Tecan Systems Inc.<br>(formerly Cavro)<br>2450 Zanker Road<br>San Jose<br>CA 95131 | Phone +1 408 953 31 00<br>Fax +1 408 953 31 07<br>E-mail tecansystemsinfo@tecan.com |