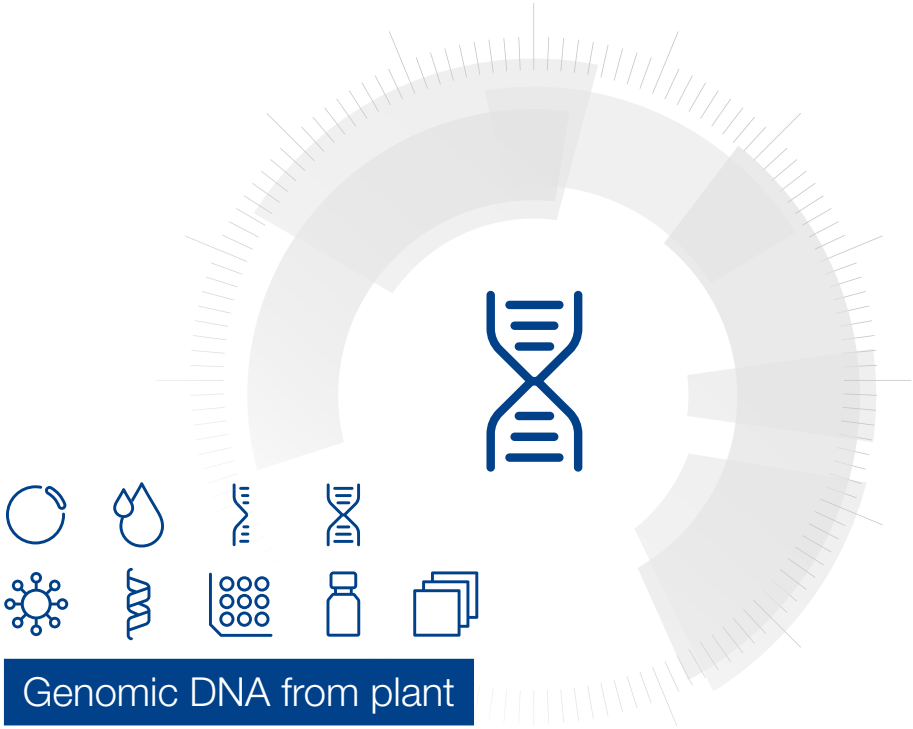
Bioanalysis

MACHEREY-NAGEL

User manual



Genomic DNA from plant

- NucleoSpin® Plant II
- NucleoSpin® Plant II Midi
- NucleoSpin® Plant II Maxi

April 2023 / Rev. 14

Genomic DNA from plant

Protocol at a glance (Rev. 14)

NucleoSpin® Plant II		Mini	Midi	Maxi
1 Homogenize samples		100 mg	100 mg	100 mg
2 Cell lysis		400 µL **PL1** 10 µL RNase A 65 °C, 10 min	1.7 mL **PL1** 25 µL RNase A 65 °C, 15 min	6 mL **PL1** 100 µL RNase A 65 °C , 20 min
		ALTERNATIVELY	***ALTERNATIVELY***	***ALTERNATIVELY***
		300 µL **PL2** 10 µL RNase A 65 °C, 10 min 75 µL **PL3** on ice, 5 min	1.5 mL **PL2** 25 µL RNase A 65 °C, 15 min 200 µL **PL3** on ice, 5 min	5.3 mL **PL2** 100 µL RNase A 65 °C, 20 min 700 µL **PL3** on ice, 5 min
3 Filtration / Clarification of lysate		≥ 11,000 x *g*, 2 min	4,500 x *g*, 10 min	4,500 x *g*, 10 min
4 Adjust DNA binding conditions		450 µL PC	2.3 mL PC	10 mL PC
5 Bind DNA		≥ 11,000 x *g*, 1 min	4,500 x *g*, 2 min	4,500 x *g*, 2 min
6 Wash and dry silica membrane		**1st** 400 µL PW1 ≥ 11,000 x *g*, 1 min **2nd** 700 µL PW2 ≥ 11,000 x *g*, 1 min **3rd** 200 µL PW2 ≥ 11,000 x *g*, 2 min	**1st** 1 mL PW1 4,500 x *g*, 2 min **2nd** 3 mL PW2 4,500 x *g*, 2 min **3rd** 1 mL PW2 4,500 x *g*, 10 min	**1st** 4 mL PW1 4,500 x *g*, 2 min **2nd** 10 mL PW2 4,500 x *g*, 2 min **3rd** 2 mL PW2 4,500 x *g*, 10 min
7 Elute DNA		50 µL PE 65 °C, 5 min ≥ 11,000 x *g*, 1 min Repeat elution step	200 µL PE 65 °C, 5 min 4,500 x *g*, 2 min Repeat elution step	1000 µL PE 65 °C, 5 min 4,500 x *g*, 2 min Repeat elution step

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1 Components

1.1 Kit contents

	NucleoSpin® Plant II		
REF	**10 preps 740770.10**	**50 preps 740770.50**	**250 preps 740770.250**
Lysis Buffer PL1	5 mL	25 mL	125 mL
Lysis Buffer PL2	4 mL	20 mL	100 mL
Precipitation Buffer PL3	1 mL	10 mL	25 mL
Binding Buffer PC	6 mL	30 mL	125 mL
Wash Buffer PW1	6 mL	30 mL	125 mL
Wash Buffer PW2 (Concentrate)*	6 mL	25 mL	50 mL
Elution Buffer PE**	13 mL	13 mL	30 mL
RNase A (lyophilized)*	1.5 mg	6 mg	2 x 15 mg
NucleoSpin® Plant Filters (violet rings)	10	50	250
NucleoSpin® Plant II Columns (green rings)	10	50	250
Collection Tubes (2 mL)	20	100	500
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* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer PE: 5 mM Tris/HCl, pH 8.5

Kit contents continued

	NucleoSpin® Plant II Midi	NucleoSpin® Plant II Maxi
REF	**20 preps 740771.20**	**10 preps 740772.10**
Lysis Buffer PL1	75 mL	75 mL
Lysis Buffer PL2	60 mL	60 mL
Precipitation Buffer PL3	10 mL	10 mL
Binding Buffer PC	60 mL	125 mL
Wash Buffer PW1	30 mL	75 mL
Wash Buffer PW2 (Concentrate)*	25 mL	50 mL
Elution Buffer PE**	13 mL	30 mL
RNase A (lyophilized)*	6 mg	10 mg
NucleoSpin® Plant Plant Filters Midi/Maxi (plus Collection Tubes)	20	10
NucleoSpin® Plant II Midi / Maxi Columns (plus Collection Tubes)	20	10
Collection Tubes (15 mL / 50 mL)	20	10
User manual	1	1

* For preparation of working solutions and storage conditions see section 3.

** Composition of Elution Buffer PE: 5 mM Tris/HCl, pH 8.5

1.2 Reagents, consumables, and equipment to be supplied by user

Reagents

- 96 – 100 % ethanol

Consumables

- 1.5 mL microcentrifuge tubes (**NucleoSpin® Plant II**) or 15 / 50 mL tubes (**NucleoSpin® Plant II Midi / Maxi**) for elution
- Disposable pipette tips

Equipment

- Manual pipettors
- Centrifuge for microcentrifuge tubes (**NucleoSpin® Plant II**) or an appropriate centrifuge with swing-out rotors capable of reaching 4,500 x *g* for 15 mL / 50 mL tubes (**NucleoSpin® Plant II Midi / Maxi**)
- Thermal heating-block or water bath for incubation and preheating of Elution Buffer PE (to 65 °C)
- Equipment for sample disruption and homogenization (see section 2.4)
- Personal protection equipment (lab coat, gloves, goggles)

1.3 About this user manual

It is strongly recommended reading the detailed protocol sections of this user manual if the **NucleoSpin® Plant II / Midi / Maxi** kit is used for the first time. Experienced users, however, my refer to the Protocol at a glance instead. The Protocol at a glance is designed to be used only as a supplemental tool for quick referencing while performing the purification procedure.

All technical literature is available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous revisions.

2 Product description

2.1 The basic principle

The plant samples are homogenized by mechanical treatment. Then the DNA can be extracted with Lysis Buffers PL1 or PL2 containing chaotropic salts, denaturing agents, and detergents. Crude lysates should be cleared by centrifugation and/or filtration using the **NucleoSpin® Plant Filters** provided with the kits in order to remove polysaccharides, contaminations, and residual cellular debris. The clear flowthrough is mixed with Binding Buffer PC to create conditions for optimal binding of DNA to the silica membrane. After loading this mixture onto the spin column, contaminants are washed away using Wash Buffers PW1 and PW2. The genomic DNA can finally be eluted with low salt Elution Buffer PE (5 mM Tris/HCl, pH 8.5) or nuclease-free water and is ready to use for subsequent reactions.

2.2 Kit specifications

- **NucleoSpin® Plant II** kits are designed for the isolation of genomic DNA from plant tissue using two optimized lysis buffer systems based on the established CTAB and SDS methods.
- **NucleoSpin® Plant Filters** are included for conveniently clearing the crude lysates.
- RNase A is included to remove RNA and to allow photometric quantification of pure genomic DNA.
- The optimized Binding Buffer PC and the chaotropic Wash Buffer PW1 completely remove proteins, RNA, metabolites, and other PCR inhibitors.
- The eluted DNA is ready to use for subsequent reactions like PCR, restriction analysis, Southern Blot etc.
- For research use only

Table 1: Kit specifications at a glance

Parameter	NucleoSpin® Plant II	NucleoSpin® Plant II Midi	NucleoSpin® Plant II Maxi
Technology	Silica membrane technology	Silica membrane technology	Silica membrane technology
Format	Mini spin columns	Midi spin columns	Maxi spin columns
Sample material	Up to 100 mg wet weight Up to 20 mg dry weight	Up to 400 mg wet weight Up to 80 mg dry weight	Up to 1500 mg wet weight Up to 300 mg dry weight
Lysate clarification	NucleoSpin® Plant Filters	NucleoSpin® Plant Filters Midi	NucleoSpin® Plant Filters Maxi
Fragment size	50 bp – approx. 50 kbp	50 bp – approx. 50 kbp	50 bp – approx. 50 kbp
Typical yield	1 – 30 µg	10 – 100 µg	50 – 300 µg
A_{260}/A_{280}	1.8 – 1.9	1.8 – 1.9	1.8 – 1.9
Elution volume	2 x 50 µL	200 – 400 µL	1000 – 2000 µL
Preparation time	30 min/6 preps	90 min/6 preps	90 min/6 preps
Binding capacity	50 µg	200 µg	> 500 µg

2.3 Storage of plant samples

Plant samples can be stored in ethanol, lyophilized, or frozen. Fresh material can be kept at 4 °C for one day but should be frozen at -20 °C for longer storage.

2.4 Homogenization of plant samples

As plant tissue is very robust, the lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills using steel or glass beads. However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields.

After homogenization and treatment of the sample with lysis buffer, the crude lysate can be cleared easily either with **NucleoSpin® Plant Filters** or by centrifugation.

Methods to homogenize samples

- Grinding with mortar and pestle in the presence of liquid nitrogen: Freeze plant material in liquid nitrogen and do not let the sample thaw at any time during homogenization. Precool mortar and pestle using liquid nitrogen. Grind frozen sample thoroughly to a fine powder and refill mortar occasionally with liquid nitrogen to keep the sample

frozen. Use a precooled spatula to transfer the sample in precooled tubes. Make sure no liquid nitrogen is transferred or all nitrogen has evaporated before closing the tube.

- VA steel beads: Put 4 – 5 beads (diameter: 7 mm) and plant material into a 15 mL plastic tube (Falcon), chill the tube in liquid nitrogen and vortex for about 30 seconds (e.g., with a Multi Pulse Vortexer, Schütt Labortechnik GmbH, www.schuett-labortechnik.de). Repeat the chilling and vortexing procedure until the entire plant material is ground to a fine powder. Chill the tube once more and remove the beads by rolling them out gently or using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube since this leads to sticking and loss of plant material attached to the beads.
- Rotor-stator homogenizers are only useful to disrupt soft plants in the presence of lysis buffer. Keep homogenizer submerged at all times to reduce foaming.

2.5 Lysis of plant samples

Increasing the amount of starting material

The standard protocols of **NucleoSpin® Plant II / Midi / Maxi** kits allow processing of 10 – 1500 mg of plant material. This usually yields 1 – 300 µg of high quality DNA. However, the amount of DNA that can be expected per mg of sample depends on the size and ploidy of the genome. For example 100 mg fresh wheat with a hexaploid genome (1.7×10^{10} bp) contains 30 µg DNA, whereas the same amount of Arabidopsis with a smaller diploid genome (1.9×10^{8} bp) only yields 3 µg DNA.

Thus, it might be advantageous to process even more than the recommended sample mass (up to 5-fold) to obtain a reasonable DNA yield. However, to ensure a complete lysis, all lysis buffer volumes of protocol step 2 have to be increased proportionally and multiple loading steps are necessary. Additional lysis buffers (PL1, PL2, PL3, RNase) have to be purchased separately (see ordering information).

Choosing the optimal lysis buffer system

Plants are very heterogeneous and contain varying amounts of polyphenols, acidic components, or polysaccharides which can lead to suboptimal DNA extraction or performance in downstream applications. Therefore, we offer two different lysis buffers for optimal processing, high yields, and an excellent DNA quality with most common plant species.

The standard protocol uses Lysis Buffer PL1, which is based on the established CTAB procedure. Additionally, the SDS based Lysis Buffer PL2 is provided which requires subsequent protein precipitation by potassium acetate (Precipitation Buffer PL3). For some plant species Lysis Buffers PL1 and PL2 can be used with similar results. However, for most plant material the lysis efficiency is different due to the negative charge of SDS and the positive charge of CTAB.

Table 2 gives an overview about customer data on different plant species and the corresponding buffer system that has been tested successfully using NucleoSpin® Plant II. **Important! For a large variety of plant species, both lysis buffers allow good results.**

Use Table 2 only for a rough orientation and guideline which buffer system has already been tested. In order to find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with both lysis buffers.

Table 2: Plant species tested with NucleoSpin® Plant II

		Lysis buffer successfully tested	
Plant species	**Plant tissue / organ**	**PL1**	**PL2**
Abies alba (fir)	Needle	✓	✓
Acer griseum	Leaf	✓	✓
Amorphophallus titanum	Leaf	✓	Not tested
Apium graveolens (celery)	Corm	✓	✓
Arabidopsis thaliana	Leaf	✓	✓
Boreava orientalis	Leaf, herbarium sample	✓	✓
Carex annectens	Leaf	✓	✓
Carex waponahkikensis	Leaf, silica-gel dried	✓	✓
Cleisostoma racemiferum	Inflorescence rachis, silica-gel dried	✓	Not tested
Doritis pulcherrima	Leaf, silica-gel dried	✓	Not tested
Eichornia azurea	Leaf	✓	Not tested
Encephalartos natalensis	Leaf	✓	Not tested
Galium aparine	Leaf	✓	✓
Hordeum sp. (barley)	Leaf	✓	✓
Isatis kotschyana	Leaf, herbarium sample	✓	✓
Laurus azorica (laurel)	Leaf	✓	Not tested
Lupinus sp. (lupin)	Leaf	✓	✓
Lycopersicon esculentum (tomato)	Stem	✓	✓
Myagrum perfoliatum	Leaf, herbarium sample	✓	✓
Oryza sativa (rice)	Leaf	✓	✓
Persea feru./caerulea	Leaf	✓	Not tested
Pteridium sp.	Leaf	✓	Not tested
Pterocarya fraxiniofolia	Leaf	✓	Not tested

Table 2: Plant species tested with NucleoSpin® Plant II			
Quercus cerris	Leaf	✓	✓
Quercus frainetto	Leaf	✓	✓
Rosa sp. (rose)	Leaf	✓	✓
Rubus fruticosus (blackberry)	Leaf	✓	✓
Sameraria nummularia	Leaf, herbarium sample	✓	✓
Secale sp. (rye)	Leaf	✓	✓
Stereochilus sp.	Leaf, silica-gel dried	✓	Not tested
Tauscheria lasiocarpum	Leaf, herbarium sample	✓	✓
Trachycarpus takil	Leaf	✓	Not tested
Trichoglottis sp.	Leaf, silica-gel dried	✓	Not tested
Triticum aestivum (wheat)	Leaf	✓	✓
Vigna radiata (mung bean)	Root	✓	✓
Zea mays (maize)	Leaf	✓	✓
Zea mays (maize)	Grain, dried, ground coarsely	✓	✓
Fungal mycel (not specified)		✓	Not tested
Green algae (not specified)		✓	Not tested

2.6 Elution procedures

The following graphs show DNA yields (solid line) and the resulting DNA concentrations (dotted line) in dependence on elution buffer volume. Elution is done with either one elution step (A) or two subsequent elution steps with the indicated elution buffer volume each (B).

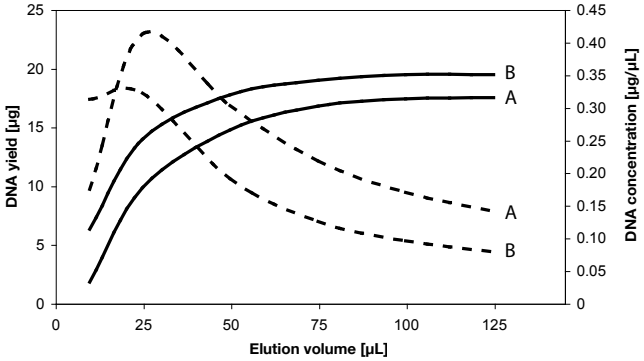

Figure 1 NucleoSpin® Plant II elution profile
Genomic DNA from 100 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 10 – 125 µL Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

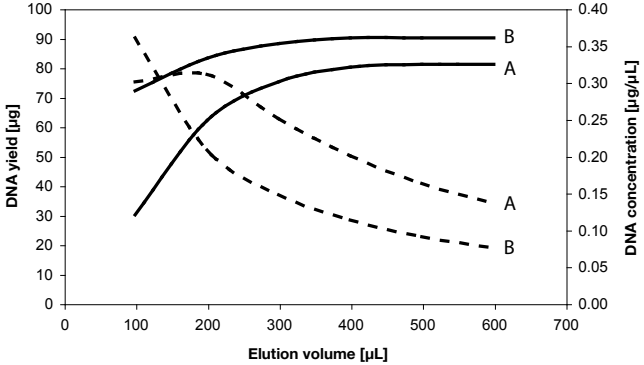

Figure 2 NucleoSpin® Plant II Midi elution profile
Genomic DNA from 400 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 100 – 600 µL Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

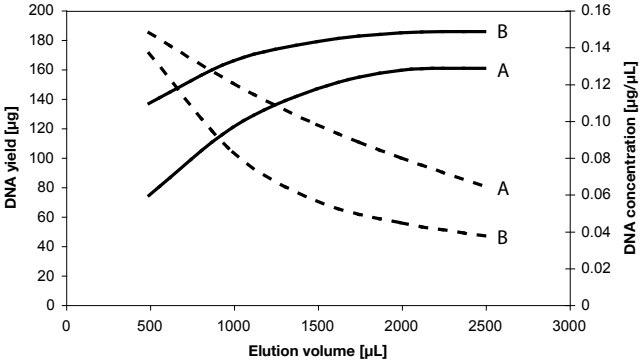

Figure 3 NucleoSpin® Plant II Maxi elution profile
Genomic DNA from 1000 mg fresh wheat leaves was purified and eluted once (A) or twice (B) with 500 – 2500 µL Buffer PE. Resulting yield and concentration is shown as solid and dotted lines, respectively.

A two-fold elution generally yields more DNA than just one elution with the same total buffer volume. This is most important for small buffer volumes. However, large volumes or eluting two times results in a lower DNA concentration.

The standard elution procedure is already optimized to yield 80 – 90 % by eluting two-fold at elevated temperatures. However, if even higher yields, a higher concentration, or maximum speed is required, the elution procedure can be adapted:

Table 3: Elution parameters

Procedure (% of exp. yield)	NucleoSpin® Plant II	NucleoSpin® Plant II Midi	NucleoSpin® Plant II Maxi
Standard elution (85 – 90 %)	50 µL + 50 µL 65 °C, 5 min	200 µL + 200 µL 65 °C, 5 min	1000 µL + 1000 µL 65 °C, 5 min
Maximum yield (95 – 100 %)	100 µL + 100 µL 65 °C, 5 min	400 µL + 400 µL 65 °C, 5 min	2000 µL + 2000 µL 65 °C, 5 min
High concentration (75 %)	25 µL + 25 µL 65 °C, 5 min	100 µL + 100 µL 65 °C, 5 min	500 µL + 500 µL 65 °C, 5 min
Fast elution (60 – 70 %)	100 µL RT / 65 °C, 1 – 5 min	400 µL RT / 65 °C, 1 – 5 min	2000 µL RT / 65 °C, 1 – 5 min

3 Storage conditions and preparation of working solutions

Attention:

Buffers PL1, PL2, PC, and PW1 contain guanidine hydrochloride and/or detergents like CTAB or SDS! Wear gloves and goggles!

CAUTION: Buffers PC and PW1 contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

All kit components can be stored at 15 – 25 °C and are stable until: see package label.

Before starting any **NucleoSpin® Plant II** protocol prepare the following:

- **Lysis Buffer PL1 / PL2:** Check for precipitated detergent especially after storage at temperatures below 20 °C. If necessary incubate the bottle for several minutes at 30 – 40 °C and mix well until the precipitate is re-dissolved completely.
- **Wash Buffer PW2:** Add the given volume of ethanol (96 – 100 %) as indicated on the bottle or in the table below to **Buffer PW2 Concentrate** before first use. Mark the label of the bottle to indicate that the ethanol is added. Buffer PW2 at is stable at 15 – 25 °C for at least one year.
- **RNase A:** Add the given volume of water as indicated on the vial and in the table below to lyophilized **RNase A**. Store the **RNase A** solution at 4 °C for up to 3 months. For longer storage (up to 1 year), the RNase A solution should be divided into small aliquots and stored at **-20 °C**.

	NucleoSpin® Plant II		
REF	**10 preps 740770.10**	**50 preps 740770.50**	**250 preps 740770.250**
Wash Buffer PW2 (Concentrate)	6 mL add 24 mL ethanol	25 mL add 100 mL ethanol	50 mL add 200 mL ethanol
RNase A	1.5 mg dissolve in 150 µL H_2O	6 mg dissolve in 600 µL H_2O	2 x 15 mg dissolve in 1500 µL H_2O each

	NucleoSpin® Plant II Midi	**NucleoSpin® Plant II Maxi**
REF	**20 preps 740771.20**	**10 preps 740772.10**
Wash Buffer PW2 (Concentrate)	25 mL add 100 mL ethanol	50 mL add 200 mL ethanol
RNase A	6 mg dissolve in 600 µL H_2O	10 mg dissolve in 1100 µL H_2O

4 Safety instructions

When working with the **NucleoSpin® Plant II** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

Caution: Guanidine hydrochloride in buffer PC and buffer PW1 can form highly reactive compounds when combined with bleach! Thus, do not add bleach or acidic solutions directly to the sample preparation waste

The waste generated with the **NucleoSpin® Plant II** has not been tested for residual infectious material. A contamination of the liquid waste with residual infectious material is highly unlikely due to strong denaturing lysis buffer but it cannot be excluded completely. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

4.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

5 NucleoSpin® Plant II protocols

5.1 Genomic DNA from plant

Before starting the preparation:

- Check that Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 65 °C.

Note: The NucleoSpin® Plant II kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

1 **Homogenize sample**

Homogenize samples

Homogenize up to 100 mg wet weight or up to 20 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.4).

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2 a **Cell lysis using Buffer PL1**

+ 400 µL PL1

+ 10 µL RNase A

65 °C, 10 min

Transfer the resulting powder to a new tube and add **400 µL Buffer PL1**. Vortex the mixture thoroughly.

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of RNase A (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.*

Add **10 µL RNase A** solution and mix sample thoroughly.

Incubate the suspension for 10 min at 65 °C.

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2 b **Cell lysis using Buffer PL2**

+ 300 µL PL2

Transfer the resulting powder to a new tube and add **300 µL Buffer PL2**. Vortex the mixture thoroughly.

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of RNase A, Buffer PL3 (step 2b), **and Buffer PC** (step 4) have to be increased proportionally.*

+ 10 µL RNase A

Add **10 µL RNase A** solution and mix sample thoroughly.

65 °C, 10 min

Incubate the suspension for **10 min at 65 °C**.

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

+ 75 µL PL3 on ice 5 min

Add **75 µL Buffer PL3**, mix thoroughly and incubate for 5 minutes on ice to precipitate SDS completely.

Proceed with step 3.

3 **Filtration / Clarification of crude lysate**

Place a **NucleoSpin® Filter** (violet ring) into a new Collection Tube (2 mL) and load the lysate onto the column. Centrifuge for **2 min** at **11,000 x *g***, collect the clear flowthrough and discard the NucleoSpin® Filter.

11,000 x *g*, 2 min

If not all liquid has passed the filter, repeat the centrifugation step.

If a pellet is visible in the flowthrough, transfer the clear supernatant to a new 1.5 mL microcentrifuge tube (not provided).

Alternatively, centrifuge the crude lysate for 5 min at 11,000 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter to remove solid particles completely.

4 **Adjust DNA binding conditions**

+ 450 µL PC

Add **450 µL Buffer PC** and mix thoroughly by pipetting up and down (5 times) or by vortexing.

5 Bind DNA

Place a **NucleoSpin® Plant II Column** (green ring) into a new Collection Tube (2 mL) and load a maximum of 700 µL of the sample.

Load lysate

Centrifuge for **1 min** at **11,000 x *g*** and discard the flowthrough.

11,000 x *g*, 1 min

The maximum loading capacity of the NucleoSpin® Plant II Column is 700 µL. For higher sample volumes repeat the loading step.

6 Wash and dry silica membrane

1st wash

Add **400 µL Buffer PW1** to the NucleoSpin® Plant II Column. Centrifuge for **1 min** at **11,000 x *g*** and discard flowthrough.

+ 400 µL PW1

11,000 x *g*, 1 min

Note: Although washing with Buffer PW1 increases purity it can in some cases slightly reduce the final yield.

2nd wash

Add **700 µL Buffer PW2** to the NucleoSpin® Plant II Column. Centrifuge for **1 min** at **11,000 x *g*** and discard flowthrough.

+ 700 µL PW2

11,000 x *g*, 1 min

3rd wash

Add another **200 µL Buffer PW2** to the NucleoSpin® Plant II Column. Centrifuge for **2 min** at **11,000 x *g*** in order to remove wash buffer and dry the silica membrane completely.

+ 200 µL PW2

11,000 x *g*, 2 min

7 Elute DNA

Place the NucleoSpin® Plant II Column into a new 1.5 mL microcentrifuge tube (not provided).

+ 50 µL PE 65 °C, 5 min

Pipette **50 µL Buffer PE (65 °C)** onto the membrane. Incubate the NucleoSpin® Plant II Column for 5 min at 65 °C. Centrifuge for **1 min** at **11,000 x *g*** to elute the DNA.

11,000 x *g*, 1 min

Repeat this step with another **50 µL Buffer PE (65 °C)** and elute into the same tube.

+ 50 µL PE 65 °C, 5 min

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.

Elution Buffer PE does not contain EDTA. If DNA degradation is observed after storage of purified DNA, adjust EDTA in Buffer PE to 1 mM before elution.

11,000 x *g*, 1 min

5.2 Genomic DNA from fungi

Attention: *Additional reagents and equipment necessary!*

- Ethanol (96 – 100 %)
- Chloroform
- Micro pistill
- MN Bead Tubes Type B or sea sand

1 Homogenize sample

Wash **50 – 200 mg mycelium** (fresh weight) or material from a fruiting body of macro fungi in **ethanol**. Mycelium can be obtained from a liquid culture or scraped off (with or without agar) from the surface of a solid medium.

Cover sample completely with **ethanol** and mix carefully. Short washing in ethanol is sufficient in most cases, although incubation overnight sometimes increases DNA yield. (Long-term storage in ethanol is also possible).

Remove ethanol by pipetting and squeezing the mycelium.

2 Cell lysis

Place the sample into MN Bead Tubes Type B or into a 1.5 mL microcentrifuge tube (not provided) with 150 mg **sea sand** and add **200 µL Buffer PL1**. Homogenize sample using a micro pistil and vortex regularly. Add additional **100 µL Buffer PL1** and continue to homogenize the sample.

Note: If the sample can not be handled easily because e.g. the sample material is soaking up too much buffer, additional Buffer PL1 can be added. Note that the volume of Buffer PC (step 4) has to be increased proportionally.

Optional: If the sample is rich in RNA or protein, we recommend adding ***10 µL RNase A and/or Proteinase K*** *(5 – 10 mg/mL stock solution, see ordering information), respectively, to the* ***PL1 lysis solution*** *in order to minimize contaminants.*

Incubate for **10 min** at **65 °C**.

For some fungi it might be advantageous to increase the incubation time to 30 – 60 min.

Add **100 µL chloroform**. Vortex for 10 s and separate phases by centrifugation for **15 min** at **20,000 x *g***. Pipette the top aqueous layer into a new 1.5 mL microcentrifuge tube (not provided).

Proceed with section 5.1, step 3.

5.3 Genomic DNA from soil, compost, dung, and animal excrements

Attention: *Additional equipment necessary!*

- Bead mill (e.g., Pulverisette 0, Fritsch – Idar-Oberstein) or mortar and pestle
- Sea sand
- Extraction buffer: 2 M NaCl, 20 mM EDTA, 100 mM Tris/HCl, 2 % (w / v) CTAB, 2 % (w / v) Polyvinylpyrrolidon (MW 40,000), pH 8.0

1 Homogenize sample

Weigh **5 g soil** or **2 g dung** into a petri dish. Add extraction buffer until the sample is completely soaked. Heat the sample in a **microwave oven** (400 W) for a few seconds until the extraction buffer is foaming.

Extraction buffer may be added to keep the sample in a slushy state.

2 Cell lysis

Transfer sample into a bead mill or mortar. Add **0.5 mL sea sand** and disrupt the sample.

3 Filtration / Clarification of lysate

Transfer the homogenized sample into a centrifuge tube (e.g., Sorvall SS34) and centrifuge for **10 min** at **5,000 x *g***. Pipette **300 µL** of the clear supernatant into a new 1.5 mL microcentrifuge tube (not provided).

Proceed with section 5.1, step 3.

6 NucleoSpin® Plant II Midi protocol

Before starting the preparation:

- Check that Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 65 °C.
- A centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,500 x *g* is required.

Note: The NucleoSpin® Plant II Midi kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

1 Homogenize sample

Homogenize samples

Homogenize up to 400 mg wet weight or up to 80 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.4).

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2 Cell lysis using Buffer PL1

+ 1.7 mL PL1

Transfer the resulting powder to a new tube and add 1.7 mL Buffer PL1. Vortex the mixture thoroughly.

*Note: If the sample can not be resuspended easily because e.g. the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of **RNase A** (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.*

+ 25 µL RNase A

Add **25 µL RNase A** solution and mix sample thoroughly.

65 °C, 15 min

Incubate the suspension for **15 min** at **65 °C**.

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2 b **Cell lysis using Buffer PL2**

+ 1.5 mL PL2

Transfer the resulting powder to a new tube and add **1.5 mL Buffer PL2**. Vortex the mixture thoroughly.

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of **RNase A, Buffer PL3** (step 2b), and **Buffer PC** (step 4) have to be increased proportionally.*

+ 25 µL RNase A

Add **25 µL RNase A** solution and mix sample thoroughly.

65 °C, 15 min

Incubate the suspension for **15 min** at **65 °C**.

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

+ 200 µL PL3 on ice, 5 min

Add **200 µL Buffer PL3**, mix thoroughly and incubate for **5 min on ice** to precipitate SDS completely.

Proceed with step 3.

3 **Filtration / Clarification of crude lysate**

Transfer the lysate to a **NucleoSpin® Filter Midi**. Centrifuge for **10 min** at **4,500 x *g***, collect the clear flowthrough and discard the NucleoSpin® Filter Midi.

If not all liquid has passed the filter, repeat the centrifugation step.

4,500 x *g*, 10 min

If a pellet is visible in the flowthrough, transfer the clear supernatant to a new 15 mL microcentrifuge tube (not provided).

Alternatively, centrifuge the crude lysate for 5 min at 4,500 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter Midi to remove solid particles completely.

4 **Adjust DNA binding conditions**

+ 2.3 mL PC

Vortex 30 s

Add **2.3 mL Buffer PC** to the cleared lysate and mix immediately by vortexing for **30 s**.

5 Bind DNA

Load sample

Load sample on a **NucleoSpin® Plant II Midi Column**.

Centrifuge for **2 min** at **4,500 x *g*** and discard the flowthrough.

The maximum loading capacity of the NucleoSpin® Plant II Midi Column is 5 mL. For higher sample volumes repeat the loading step.

4,500 x *g*, 2 min

6 Wash and dry silica membrane

+ 1 mL PW1

4,500 x *g*, 2 min

1st wash

Add **1 mL Buffer PW1** to the NucleoSpin® Plant II Midi Column. Centrifuge for **2 min** at **4,500 x *g*** and discard flowthrough.

Note: Although washing with Buffer PW1 increases purity it can in some cases slightly reduce the final yield.

+ 3 mL PW2

4,500 x *g*, 2 min

2nd wash

Add **3 mL Buffer PW2** to the NucleoSpin® Plant II Midi Column. Centrifuge for **2 min** at **4,500 x *g*** and discard flowthrough.

+ 1 mL PW2

4,500 x *g*, 10 min

3rd wash

Add another **1 mL Buffer PW2** to the NucleoSpin® Plant II Midi Column. Centrifuge for **10 min** at **4,500 x *g*** in order to remove wash buffer and dry the silica membrane completely.

7 Elute DNA

+ 200 µL PE 65 °C, 5 min

4,500 x *g*, 2 min

Place the NucleoSpin® Plant II Midi Column into a new Collection Tube (15 mL)

Pipette **200 µL Buffer PE (65 °C)** onto the membrane. Incubate the NucleoSpin® Plant II Midi Column for **5 min** at **65 °C**. Centrifuge for **2 min** at **4,500 x *g*** to elute the DNA.

Repeat this step with another **200 µL Buffer PE (65 °C)** and elute into the same tube.

+ 200 µL PE 65 °C, 5 min

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.

4,500 x *g*, 2 min

Elution Buffer PE does not contain EDTA. If DNA degradation is observed after storage of purified DNA, adjust EDTA in Buffer PE to 1 mM before elution.

7 NucleoSpin® Plant II Maxi protocol

Before starting the preparation:

- Check that Wash Buffer PW2 and RNase A were prepared according to section 3.
- Preheat Elution Buffer PE to 65 °C.
- A centrifuge with a swing-out rotor and appropriate buckets capable of reaching 4,500 x *g* is required.

Note: The NucleoSpin® Plant II Maxi kits include two different lysis buffers for optimal results with most common plant species. Please refer to section 2.5 for choosing the optimal lysis buffer system for your individual plant sample and for information on how to process even more sample material than recommended in the following protocol.

1 Homogenize sample

Homogenize samples

Homogenize up to 1500 mg wet weight or up to 300 mg dry weight (lyophilized) plant material (for homogenization methods see section 2.4).

Proceed with cell lysis using **Buffer PL1** (step 2a) or alternatively **Buffer PL2** (step 2b).

2 Cell lysis using Buffer PL1

+ 6 mL PL1

Transfer the resulting powder to a new tube and add **6 mL Buffer PL1**. Vortex the mixture thoroughly.

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL1** can be added. Note that the volumes of **RNase A** (step 2a) and **Buffer PC** (step 4) have to be increased proportionally.*

+ 100 µL RNase A

Add **100 µL RNase A** solution and mix sample thoroughly.

65 °C, 20 min

Incubate the suspension for **20 min** at **65 °C**.

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Proceed with step 3.

2 b **Cell lysis using Buffer PL2**

Transfer the resulting powder to a new tube and add **5.3 mL Buffer PL2**. Vortex the mixture thoroughly.

+ 5.3 mL PL2

*Note: If the sample can not be resuspended easily because for example the plant powder is soaking up too much buffer, additional **Buffer PL2** can be added. Note that the volumes of **RNase A, Buffer PL3** (step 2b), and **Buffer PC** (step 4) have to be increased proportionally.*

Add **100 µL RNase A** solution and mix sample thoroughly.

+ 100 µL RNase A

Incubate the suspension for **20 min** at **65 °C**.

65 °C, 20 min

Note: For some plant material it might be advantageous to increase the incubation time to 30 – 60 min.

Add **700 µL Buffer PL3**, mix thoroughly and incubate for **5 min on ice** to precipitate SDS completely.

+ 700 µL PL3 on ice, 5 min

Proceed with step 3.

3 **Filtration / Clarification of crude lysate**

Transfer the lysate to a **NucleoSpin® Filter Maxi**. Centrifuge for **10 min** at **4,500 x *g***, collect the clear flowthrough and discard the NucleoSpin® Filter Maxi.

If not all liquid has passed the filter, repeat the centrifugation step.

4,500 x *g*, 10 min

If a pellet is visible in the flowthrough, transfer the clear supernatant to a new 50 mL microcentrifuge tube (not provided).

Alternatively, centrifuge the crude lysate for 5 min at 4,500 x g and transfer the supernatant to a new tube or pass the precleared supernatant through the NucleoSpin® Filter Maxi to remove solid particles completely.

4 **Adjust DNA binding conditions**

Add **10 mL Buffer PC** to the cleared lysate and mix immediately by vortexing for **30 s**.

+ 10 mL PC

Vortex 30 s

5 Bind DNA

Load sample on a **NucleoSpin® Plant II Maxi Column**

Centrifuge for **2 min** at **4,500 x *g*** and discard the flowthrough.

The maximum loading capacity of the NucleoSpin® Plant II Maxi Column is 15 mL. For higher sample volumes repeat the loading step.

Load sample

4,500 x *g*, 2 min

6 Wash and dry silica membrane

1st wash

Add **4 mL Buffer PW1** to the NucleoSpin® Plant II Maxi Column. Centrifuge for 2 min at 4,500 x *g* and discard flowthrough.

Note: Although washing with Buffer PW1 increases purity it can in some cases slightly reduce the final yield.

+ 4 mL PW1

4,500 x *g*, 2 min

2nd wash

Add 10 mL Buffer PW2 to the NucleoSpin® Plant II Maxi Column. Centrifuge for **2 min** at **4,500 x *g*** and discard flowthrough.

+ 10 mL PW2

4,500 x *g*, 2 min

3rd wash

Add another **2 mL Buffer PW2** to the NucleoSpin® Plant II Maxi Column. Centrifuge for **10 min** at **4,500 x *g*** in order to remove wash buffer and dry the silica membrane completely.

+ 2 mL PW2

4,500 x *g*, 10 min

7 Elute DNA

Place the NucleoSpin® Plant II Maxi Column into a new Collection Tube (50 mL)

Pipette **1000 µL Buffer PE (65 °C)** onto the membrane. Incubate the NucleoSpin® Plant II Maxi Column for **5 min** at **65 °C**. Centrifuge for **2 min** at **4,500 x *g*** to elute the DNA.

+ 1000 µL PE 65 °C, 5 min

4,500 x *g*, 2 min

Repeat this step with another **1000 µL Buffer PE (65 °C)** and elute into the same tube.

+ 1000 µL PE 65 °C, 5 min

Note: To achieve maximum yield or higher concentrations refer to section 2.6 for alternative elution procedures.

4,500 x *g*, 2 min

Elution Buffer PE does not contain EDTA. If DNA degradation is observed after storage of purified DNA, adjust EDTA in Buffer PE to 1 mM before elution.

8 Appendix

8.1 Troubleshooting

Problem	Possible cause and suggestions
DNA yield is low	*Homogenization of plant material was not sufficient* • For most species we recommend grinding with steel beads or mortar and pestle (see section 2.4). For disruption of the cell wall it is important to homogenize the plant material thoroughly until the sample is ground to a fine powder. • Instead of freezing in liquid nitrogen the sample can also be lyophilized and easily ground at 15 – 25 °C. *Suboptimal lysis buffer was used* • Lysis efficiencies of Buffer PL1 (CTAB) and Buffer PL2 (SDS) are different and depend on the plant species. Try both buffers in a side-by-side purification to find the best detergent system to lyse your plant material. *Suboptimal lysis buffer volume was used* • Cell lysis might be insufficient and too much DNA might get lost during lysate clarification if e.g. dry material soaks up too much lysis buffer. Use more lysis buffer and increase the volume of Binding Buffer PC proportionally. *Suboptimal binding buffer volume was used* • Increase Binding Buffer PC proportionally if more lysis buffer was used. *Extraction of DNA from plant material during lysis was insufficient* • Increase incubation time in lysis buffer (up to overnight). *Suboptimal Elution* • The DNA can either be eluted in higher volumes or by repeating the elution step up to three times. Incubate NucleoSpin® Plant II Column with elution buffer at 65 °C for at least 5 minutes. • Also check the pH of the elution buffer, which should be in the range of pH 8.0 – 8.5. To ensure correct pH, use supplied Elution Buffer PE (5 mM Tris/HCl, pH 8.5).

Problem	Possible cause and suggestions
NucleoSpin® Filter or NucleoSpin® Plant II Column is clogged	*Sample was too viscous due to too much sample material or material carry-over.* • Centrifuge large amounts of sample material before loading it onto the NucleoSpin® Filter or Filter Midi / Maxi. • Make sure the cleared lysate is absolutely free of resuspended matter before loading it onto the NucleoSpin® Plant II or Plant II Midi / Maxi Column. • Increase centrifugation speed and time. • Use more Lysis Buffer PL1 or PL2.
DNA is degraded	*Sample is contaminated with DNase* • Adjust Elution Buffer PE to 1 mM EDTA. *Centrifugation speed was too high* • Centrifuge at a maximum speed of 11,000 x *g*. Higher velocities may lead to shearing of the DNA.
DNA quality is low	*Elution buffer contains EDTA* • EDTA may disturb subsequent reactions. Use water or the supplied Elution Buffer PE (5 mM Tris/HCl, pH 8.5) for elution. *Salt or ethanol carry-over* • Make sure the last two wash steps were done with Wash Buffer PW2 and the membrane was dried according to the protocol.

8.2 Ordering information

Product	REF	Pack of
NucleoSpin® Plant II	740770.10 / .50 / .250	10 / 50 / 250 preps
NucleoSpin® Plant II Midi	740771.20	20 preps
NucleoSpin® Plant II Maxi	740772.10	10 preps
Buffer PL1	740918	125 mL
Buffer Set PL2 / PL3 (100 mL Buffer PL2 + 25 mL Buffer PL3)	740919	1 set
Buffer PC	740937	125 mL
Buffer PW1	740938	125 mL

Product	REF	Pack of
Buffer PW2 Concentrate (for 250 mL Buffer PW2)	740939	50 mL
RNase A	740505.50 740505	50 mg 100 mg
Proteinase K	740506	100 mg
NucleoSpin® Plant Filters (for filtration of cell homogenates)	740606	50
Collection Tubes (2 mL)	740600	1000
NucleoSpin® Bead Tubes Type A	740786.50	50
NucleoSpin® Bead Tubes Type B	740812.50	50
NucleoSpin® Bead Tubes Type C	740813.50	50
NucleoSpin® Bead Tubes Type D	740814.50	50
NucleoSpin® Bead Tubes Type E	740815.50	50
NucleoSpin® Bead Tubes Type F	740816.50	50
NucleoSpin® Bead Tubes Type G	740817.50	50
MN Bead Tube Holder	740469	1

8.3 Product use restriction / warranty

All MACHEREY-NAGEL products are designed for their intended use only. They are not intended to be used for any other purpose. The description of the intended use of the products can be found in the original MACHEREY-NAGEL product leaflets. Before using our products, please observe the instructions for use and the safety instructions from the respective Material Safety Data Sheet of the product.

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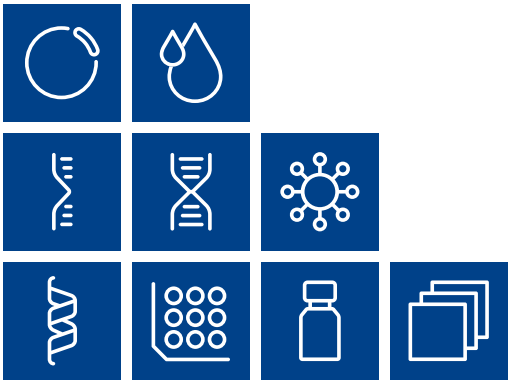
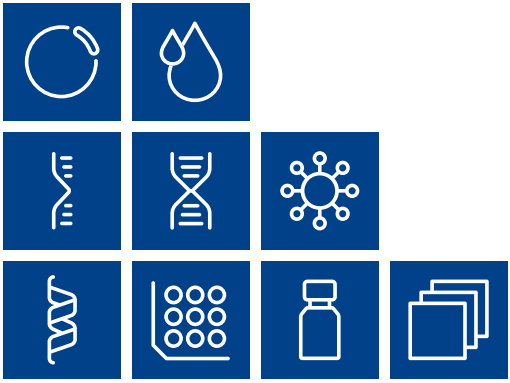


Plasmid DNA
Clean up
RNA
DNA
Viral RNA and DNA
Protein
High throughput
Accessories
Auxiliary tools



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