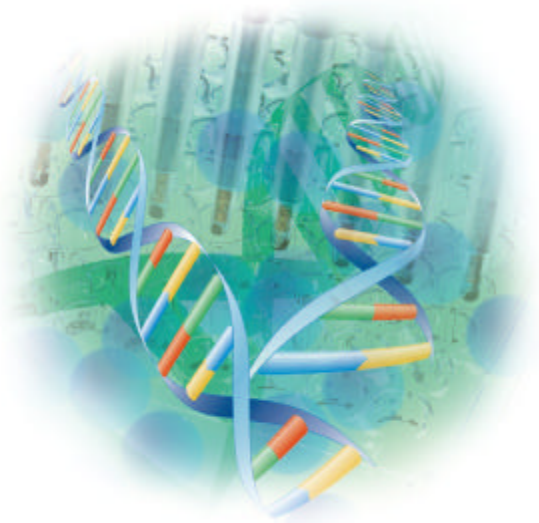


## **QuickPick™ SML gDNA**

- 51002 • genomic DNA purification kit, 8 preps
- 51012 • genomic DNA purification kit, 24 preps
- 51022 • genomic DNA purification kit, 96 preps



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**1 INTRODUCTION**

These are the instructions for use for the QuickPick™ SML gDNA purification kits. Please read the entire instructions carefully before starting the work. Also refer to the MagRo™ 8-M robotic workstation operating manual or PickPen® 1-M or PickPen® 8-M instructions for use.

The QuickPick™ SML gDNA purification kits are intended for universal genomic DNA purification from various sample materials. DNA can be purified for example from human or animal whole blood, buffy coat, leukocytes, dried blood spots, tissue, cultured cells, bacteria, hair, buccal swabs, urine, saliva, milk and seminal fluid. The reagents are suitable for use with whole blood which has been treated with commonly used anticoagulants like EDTA, heparin or citrate. The technique provides a fast and simple means of DNA purification and does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The size of DNA purified using the QuickPick™ SML gDNA purification kit is typically at least 30 kbp. DNA fragments of this length denature completely during thermal cycling and can be used for downstream applications such as PCR amplifications, restriction enzyme digestions and sequencing.

The reagent volumes can be scaled up or down to be used for different sample amounts either with the PickPen® manual tools or the MagRo™ 8-M robotic workstation.

**1.1 Principle of the method**

DNA in the sample is released using Proteinase K solution and Lysis Buffer. Released DNA is bound to Magnetic Particles in the presence of Binding Buffer. Magnetic Particles with the bound DNA are washed with Wash Buffers 1 and 2 and then the DNA is eluted from the Magnetic Particles with the Elution Buffer.

**2 SPECIFICATIONS**

Table 1: Specifications for QuickPick™ SML gDNA purification kits.

Sample	Amount	Yield <sup>(1)</sup>	Amount	Yield <sup>(1)</sup>	Amount	Yield <sup>(1)</sup>
Whole Blood	50 µl	Up to 1.5 µg	100 µl	Up to 3 µg	200 µl	Up to 6 µg
Buffy Coat	50 µl	Up to 3 µg	100 µl	Up to 6 µg	200 µl	n.d. <sup>(4)</sup>
Leukocytes <sup>(2)</sup>	5 x 10 <sup>5</sup> cells	Up to 4 µg	1 x 10 <sup>6</sup> cells	Up to 8 µg	2 x 10 <sup>6</sup> cells	n.d. <sup>(4)</sup>
Cultured cells	1 x 10 <sup>6</sup> cells	Up to 2.5 µg	2 x 10 <sup>6</sup> cells	Up to 5 µg	4 x 10 <sup>6</sup> cells	Up to 10 µg
Tissue (Liver)	2.5 mg	Up to 8 µg	5 mg	Up to 16 µg	10 mg	Up to 32 µg
Hair	2 - 4 strands	Up to 0.5 µg	6 - 8 strands	Up to 1 µg	12 - 16 strands	n.d. <sup>(4)</sup>
Typical purity <sup>(3)</sup>		= 1.7				
Size of purified DNA		= 30 kbp				

<sup>1</sup>The purifications are carried out by using MagRo™ 8M. Yields of DNA depend on the species, growth conditions used etc.

<sup>2</sup>Leukocytes are suspended in 50 - 200 µl of PBS depending on sample amount.

<sup>3</sup>Ratio of absorbance at 260/280 nm is corrected with absorbance at 320 nm.

<sup>4</sup>Not determined.

### 3 KIT CONTENTS

#### 3.1 Reagents for the SML kits

Reagent:	51002 <sup>(1)</sup>	51012	51022
gDNA Magnetic Particles <sup>(1)</sup>	64 µl	230 µl	2 x 430 µl
gDNA Proteinase K solution	80 µl	370 µl	1.2 ml
gDNA Lysis Buffer	800 µl	3.8 ml	11.6 ml
gDNA Binding Buffer <sup>(1)</sup>	2 ml	7.2 ml	2 x 13.5 ml
gDNA Wash Buffer 1 <sup>(1,2)</sup>	8 ml	30 ml	2 x 58 ml
gDNA Wash Buffer 2 <sup>(1)</sup>	4 ml	15 ml	2 x 28 ml
gDNA Elution Buffer	1 ml	7 ml	2 x 11 ml

<sup>(1)</sup>Contains 0.02 % NaN<sub>3</sub>.

<sup>(2)</sup>gDNA Wash Buffer 1 is delivered without ethanol. The volume indicated here is the total volume after addition of ethanol.

The reagents for QuickPick™ SML gDNA purification kits can also be bought separately:

Reagent:	Volume	Product No:
QuickPick™ XL gDNA Magnetic Particles <sup>(1)</sup>	3.2 ml	51100
QuickPick™ XL gDNA Proteinase K solution	5.0 ml	51200
QuickPick™ XL gDNA Lysis Buffer	41 ml	51400
QuickPick™ XL gDNA Binding Buffer <sup>(1)</sup>	100 ml	51300
QuickPick™ XL gDNA Wash Buffer 1 <sup>(1,2)</sup>	220 ml	51510
QuickPick™ XL gDNA Wash Buffer 2 <sup>(1)</sup>	210 ml	51520
QuickPick™ XL gDNA Elution Buffer	85 ml	51600

<sup>(1)</sup>Reagent contains 0.02 % NaN<sub>3</sub>.

<sup>(2)</sup>gDNA Wash Buffer 1 is delivered without ethanol. The instructions for ethanol addition are also shown in the XL Reagent insert. The volume shown here is the total volume after addition of ethanol.

See Chapter 3.2 "Preparation of reagents" for the instructions of ethanol addition into Wash Buffer 1.

#### 3.2 Preparation of reagents

Wash Buffer 1 is supplied as a concentrate. Before use, add appropriate volume of ethanol (96 - 100 %) into the Wash Buffer 1 bottle(s) as follows:

##### Wash Buffer 1 (QuickPick™ SML gDNA 8 preps):

Add 7 ml ethanol (96 – 100 %) into the Wash Buffer 1 bottle. Mix thoroughly. The volume of ethanol to be added is also shown on the bottle label.

##### Wash Buffer 1 (QuickPick™ SML gDNA 24 preps):

Add 24 ml ethanol (96 – 100 %) into the Wash Buffer 1 bottle. Mix thoroughly. The volume of ethanol to be added is also shown on the bottle label.

##### Wash Buffer 1 (QuickPick™ SML gDNA 96 preps):

Add 43 ml ethanol (96 – 100 %) into each of the Wash Buffer 1 bottles. Mix thoroughly. The volume of ethanol to be added is also shown on the bottle label.

##### Wash Buffer 1 (QuickPick™ XL gDNA):

Add 170 ml ethanol (96 - 100 %) into the Wash Buffer 1 bottle. Mix thoroughly. The volume of ethanol to be added is also shown on the bottle label.

The gDNA Magnetic Particles should be thoroughly mixed before pipetting. Vortexing of the Magnetic Particles is not recommended.

#### 3.3 Scaling of sample amounts

For both manual and automated protocols the sample amount can be scaled yielding to different number of preparations (Table 2) and reagent consumption. The reagent volumes are linearly dependent on the used sample amount. The reagent volumes for the manual purifications are shown in Tables 3 and 4 (see Chapter 6 "Protocols for manual PickPen® 1-M and 8-M tools"). In MagRo™ 8-M protocols the reagent volumes are adjusted to be suitable for automation.

Table 2: The effect of sample amount to number of preparations for the purifications with QuickPick™ gDNA SML kits.

Sample	Amount of sample per preparation			
	25 µl	50 µl	100 µl	200 µl
Whole Blood	25 µl	50 µl	100 µl	200 µl
Buffy Coat	25 µl	50 µl	100 µl	200 µl
Leukocytes <sup>(1)</sup>	2.5 x 10 <sup>5</sup>	5 x 10 <sup>5</sup>	1 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>
Cultured cells	5 x 10 <sup>5</sup>	1 x 10 <sup>6</sup>	2 x 10 <sup>6</sup>	4 x 10 <sup>6</sup>
Tissue	1.25 mg	2.5 mg	5 mg	10 mg
Hair	1 - 2 pcs	2 - 4 pcs	4 - 8 pcs	8 - 16 pcs
<b>Number of preps:</b>				
51002 <sup>(2)</sup>	32	16	8	4
51012	96	48	24	12
51022	384	192	96	48

<sup>(1)</sup>Leukocytes are suspended in 25 - 200 µl PBS depending on amount of sample.

<sup>(2)</sup>For manual use only.

### 4 SAMPLE PREPARATION

DNA yield is dependent on the sample type and number of cells in the sample. Elution into smaller volume increases the final concentration of DNA in the eluate, but slightly reduces the yield of DNA.

#### 4.1 Sample preparation from whole blood, buffy coat fraction and leukocytes

##### Human whole blood

Genomic DNA can be purified from human whole blood which has been treated with commonly used anticoagulants; EDTA, heparin or citrate. Both fresh and frozen EDTA and heparin blood can be used but only fresh citrate blood is recommended. Frozen samples should be thawed quickly in +37°C water bath with mild agitation before starting the purification. Typically, fresh blood samples results in better yields.

For automated protocols whole blood samples are lysed manually as follows:

1. Equilibrate the samples to room temperature.
2. Add appropriate amount of Proteinase K solution into tubes (1.5 - 2.0 ml).
3. Add appropriate amount of Lysis Buffer into the same tubes.
4. Invert the tubes several times and mix by pulse-vortexing for 15 s.
5. Spin shortly the mixed solutions and transfer the suspensions into the Sample plate (96-well plate) starting from the wells of column 1.

Genomic DNA can also be successfully purified from blood of other animal species but the suitability for particular sample types should be separately established. (See also "Additional protocols" at [www.bio-nobile.com](http://www.bio-nobile.com)).

#### Buffy coat fraction

Centrifuge 2 ml fresh whole blood for 10 minutes at 2500 x g at room temperature. After centrifugation there are three layers; the yellow transparent upper layer is plasma, the dark red bottom layer is red blood cells and in the middle of these two is a pale red layer which is the buffy coat fraction. Remove the upper layer and carefully pipette the buffy coat layer (approximately 200 µl) into a tube. Use an appropriate volume in the purification protocol.

#### Leukocytes

All reagents used in the preparation of leukocytes should be at room temperature. Pipette 1 ml of fresh whole blood (EDTA is recommended as an anticoagulant) into a 15 ml centrifuge tube. Add 10 ml of 0.83 % NH<sub>4</sub>Cl, pH 7.4 (adjust pH to 7.4 using 2 M Na<sub>2</sub>CO<sub>3</sub>). Mix the tube by inverting it gently several times. Incubate the solution for 10 minutes or until the solution becomes translucent at room temperature. During the incubation, red cells are lysed. Collect the leukocytes by centrifuging for 10 minutes at 300 x g. Remove the supernatant and wash the leukocytes with 1 ml of PBS. Centrifuge for 10 minutes at 300 x g. Resuspend the leukocytes in PBS. (If the sample is difficult to solubilize, addition of 0.1% gelatin to the PBS is suggested). Count the leukocytes with a hemocytometer (Bürker chamber) and use an appropriate amount in the purification protocol.

## 4.2 Sample preparation from cultured cells

Harvest the needed amount of cultured cells (see specifications in Table 1) and pellet them by centrifuging for 5 minutes at 300 x g. Remove the supernatant carefully. Wash the cells once with PBS and centrifuge for 5 minutes at 300 x g. Take PBS away and add appropriate amount of Lysis Buffer. Proceed with the purification protocol.

## 4.3 Sample preparation from hair

Place the roots of the hair strands into bottom of tube. Add appropriate volume of Proteinase K solution and Lysis Buffer into the tube. Mix the suspension well and gently. After lysis spin the tubes shortly and take away the hair strands by using sterile forceps. Proceed with the purification protocol.

## 4.4 Sample preparation from tissue

Complete disruption of cell walls, plasma membranes, and organelle membranes is essential to release all the nucleic acids contained in the tissue. Insufficient disruption of starting material will lead to low DNA yield. Cell wall properties vary widely between species and proper homogenization method should be applied to achieve complete disruption. The homogenization can be performed for example by mechanical grinding or with liquid nitrogen. Other homogenization methods can also be used.

Keep tissue sample on ice during the sample preparation. Homogenize the tissue with tissue grinder (Pellet Pestle or equivalent device) or with liquid nitrogen using mortar and pestle.

#### Homogenization using tissue grinder

Tissue grinder disrupts samples efficiently and helps in rapid preparation of the sample homogenate. Weight the tissue sample into 2 ml tube. Add appropriate volume of Lysis Buffer (Table 3 and 4) and homogenize the tissue manually or with Pellet Pestle or equivalent device. A homogeneous suspension should be obtained within 5 - 10 minutes. Keep the tissue homogenate on ice, add appropriate amount of Proteinase K solution and proceed according to the purification protocol.

#### Homogenization with liquid nitrogen using mortar and pestle

One of the most common homogenization methods involves freezing samples in liquid nitrogen and grinding with a mortar and pestle.

Typical protocol for disrupting tissue sample using mortar and pestle:

1. Freeze the tissue sample in liquid nitrogen immediately after harvesting. Do not let the tissue sample to thaw at any time during disruption.
2. Pre-cool equipments by pouring liquid nitrogen into mortar and placing the pestles grinding end in the liquid nitrogen.
3. Place frozen tissue sample in mortar and grind until fine whitish powder results.
4. Add liquid nitrogen as necessary but be careful not to spill the tissue sample out of the mortar.
5. Using a pre-cooled spatula transfer the powdered tissue sample into pre-cooled tubes. Use several tubes for large samples to avoid thawing.
6. Ensure all the liquid nitrogen has evaporated before closing the tube.
7. If the tissue samples are not processed immediately the tube should be kept on dry ice or liquid nitrogen or stored at -80°C, to prevent the tissue sample from thawing after evaporation.
8. If the tissue samples are processed immediately after homogenization, add appropriate volume of Proteinase K solution and Lysis Buffer (Table 3 and 4) before the sample thaws. Proceed with the purification protocol.

## 4.5 Other sample materials

Genomic DNA can also be purified from other sample materials, for example dried blood spots, mouse tail, feather, saliva, urine, buccal swabs, bacteria, milk, and seminal fluid using QuickPick™ SML gDNA purification kit. Protocols for different sample preparations are available upon request. (See also "Additional protocols" at [www.bio-nobile.com](http://www.bio-nobile.com)).

## 5 PICKPEN® TIPS

The tips packed in bulk quantities in plastic bags are not sterile. Sterilize the tips in PickPen® Tip box by autoclaving.

## 6 PROTOCOLS FOR MANUAL PICKPEN® 1-M AND 8-M TOOLS

### 6.1 PickPen® 1-M protocol

#### Notes

1. All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved.
2. gDNA Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the Magnetic Particles is not recommended.
3. Repeat pipettors should not be used when dispensing Magnetic Particles.
4. If RNA-free genomic DNA preparation is required add RNase solution into the samples before starting the lysis step.
5. Lysis time can be increased if using difficult samples.
6. Water can also be used for elution.

- Ensure that ethanol (96 - 100 %) has been added into the Wash Buffer 1 (see section 3.2 "Preparation of reagents").
- If the magnetic particle lump does not disperse into Elution Buffer completely increase the elution time to achieve complete dispersion.
- For purifications with 200 µl of whole blood (or respective amount of other sample materials) use 2 ml tubes during the lysis step.

### Reagent volumes

Table 3: Reagent volumes for PickPen® 1-M purifications.

Reagent	Reagent volume per preparation			
	25 µl	50 µl	100 µl	200 µl
Sample amount <sup>(1)</sup>	25 µl	50 µl	100 µl	200 µl
Lysis Buffer	25 µl	50 µl	100 µl	200 µl
Proteinase K solution	2.5 µl	5 µl	10 µl	20 µl
Binding Buffer	62.5 µl	125 µl	250 µl	500 µl
Magnetic Particles	2 µl	4 µl	8 µl	16 µl
Wash Buffer 1	2 x 125 µl	2 x 250 µl	2 x 500 µl	2 x 750 µl
Wash Buffer 2	125 µl	250 µl	500 µl	750 µl
Elution Buffer	25 µl	50 µl	100 µl	200 µl

<sup>1</sup>Whole blood used as an example, for the amounts of other sample materials, see Table 2.

### Materials required

- Sterile 1.5– 2.0 ml tubes.
- Pipettes and sterile micropipettor tips.
- PickPen® 1-M tool and PickPen® tips in box or in 8-Packs.

### Protocol

- Number tubes from 1 to 5.
- Pipette appropriate volumes of Proteinase K solution, sample and Lysis Buffer into tube 1 according to the Table 3. Mix well by inverting the tube several times and pulse-vortexing for 15 seconds. Lyse the sample at 56°C for 10 - 30 minutes.

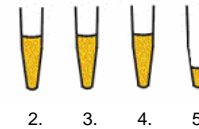


Incubate for 10 - 30 minutes at 56°C

- During the lysis step, pipette QuickPick™ SML gDNA purification kit reagents (according to the Table 3) into tubes 2 - 5 as follows:

Tube 2: gDNA Wash Buffer 1  
 Tube 3: gDNA Wash Buffer 1  
 Tube 4: gDNA Wash Buffer 2

Tube 5: gDNA Elution Buffer



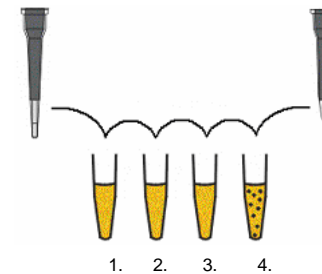
- Remove tube 1 from 56°C. Pipette correct volumes of gDNA Binding Buffer and gDNA Magnetic Particles into tube 1 according to the Table 3.
- Mix tube 1 gently and incubate at room temperature for 2 - 10 minutes. Mix the suspension continuously during this step (use a tube rotator or mix manually). Normally, the Magnetic Particles form a lump when DNA binds to them.



1.

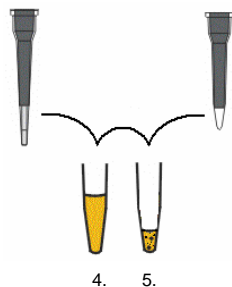
Incubate for 2 - 10 minutes at room temperature with continuous mixing

- Pick up the PickPen® tip with the PickPen® 1-M. Extend the magnet 2 - 3 times to check that the tip is firmly in place. Collect the Magnetic Particles from tube 1 with PickPen® 1-M and release them into tube 2 (Wash Buffer 1). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the PickPen® tip. Note that the magnet has to be withdrawn at this point. Repeat the washing steps in tubes 3 and 4 (Wash Buffer 1 and Wash Buffer 2).



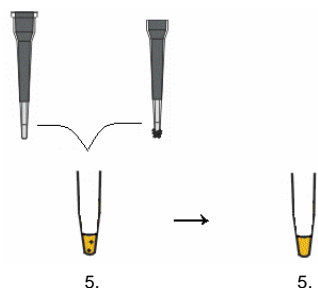
Washing steps

- Collect the Magnetic Particles from tube 4 with PickPen® 1-M and release them into tube 5 (Elution Buffer). Mix tube 5 continuously, and incubate at room temperature for 2 - 10 minutes (use a tube rotator or mix manually). During elution the lump of Magnetic Particles should disperse completely.



Incubate for 2 - 10 minutes at room temperature with continuous mixing

8. Collect the Magnetic Particles from tube 5 and discard them and the tip. The eluate in tube 5 containing the purified genomic DNA is ready to be used in downstream applications. If the purified DNA is not used on the same day, store at -20°C until use.



Collect the Magnetic Particles and discard them

## 6.2 PickPen® 8-M protocol

### Notes

- All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved.
- gDNA Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the Magnetic Particles is not recommended.
- Repeat or 8-channel pipettors should not be used when dispensing Magnetic Particles.
- If RNA-free genomic DNA preparation is required add RNase solution into the samples before starting the lysis step.
- When using 96-well plates, the use of an orbital shaker is recommended. Adjust the speed to the highest possible level without causing liquid spill but still keeps Magnetic Particles in suspension.
- Lysis time can be increased with difficult samples.
- Water can also be used for elution.
- Ensure that ethanol (96 - 100 %) has been added into Wash Buffer 1 (see Chapter 3.2 "Preparation of reagents").

- If the magnetic particle lump does not disperse into Elution Buffer completely increase the elution time to achieve complete dispersion.
- For 200 µl whole blood samples (and corresponding amounts of other sample materials) the protocol is changed as follows:
  - Binding Buffer and Magnetic Particles are dispensed into wells of columns 1 and 2.
  - After the lysis step the suspensions from each tube are divided in equal portions into the respective wells of columns 1 and 2.
  - There are three Wash Buffer 1 steps. Wash Buffer 1 is dispensed in the wells of columns 3, 4, and 5.
  - Wash Buffer 2 is dispensed in the wells of column 6.
  - Elution Buffer is dispensed in the wells of column 7.

### Reagent volumes

Table 4: Reagent volumes for PickPen® 8-M purifications.

Reagent	Reagent volume per preparation				
	Sample amount <sup>(1)</sup>	25 µl	50 µl	100 µl	200 µl
Lysis Buffer		25 µl	50 µl	100 µl	200 µl
Proteinase K solution		2.5 µl	5 µl	10 µl	20 µl
Binding Buffer		62.5 µl	125 µl	250 µl	2 x 250 µl
Magnetic Particles		2 µl	4 µl	8 µl	2 x 8 µl
Wash Buffer 1		2 x 125 µl	2 x 250 µl	2 x 500 µl	3 x 750 µl
Wash Buffer 2		125 µl	250 µl	500 µl	750 µl
Elution Buffer		30 µl	50 µl	100 µl	200 µl

<sup>1</sup>Whole blood used as an example, for the amounts of other sample materials, see Table 2.

### Materials required

- Sterile U-bottom 96-well plates (for example, Nunc 500 µl 96-well microplate or 1 ml 96 deep-well plate, both available also from BN Products & Services Oy).
- Pipettes and sterile micropipettor tips.
- PickPen® 8-M tool and PickPen® tips in box or in 8-Packs.
- Orbital shaker for 96-well plates.

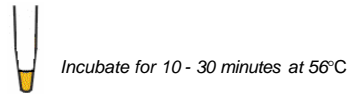
Table 5: Recommended plates for different sample amounts.

Sample amount	Recommended plate	BN Products & Services Product No.
Up to 50 µl	Nunc 96-well microplate, 500 µl	M1-267334, 10 plates
	Sterile	M-267334, 120 / case
100 µl and 200 µl	Nunc 96 deep-well plate, 1 ml	M1-260251, 10 plates
	Sterile	M-260251, 50 / case

## Protocol

The following instructions are for 8 samples. Samples are lysed in tubes and transferred into 96-well plates (U-bottom) where the rest of the protocol is carried out. The lysis step can also be performed in a thermal shaker using suitable adapter for 96-well plate.

1. Prepare the samples by pipetting appropriate volumes of Proteinase K solution, sample and Lysis Buffer into tubes 1-8 according to the Table 4. Mix the tubes 1-8 well by inverting the tube several times and pulse-vortexing for 15 seconds. Lyse samples at 56°C for 10 - 30 minutes.



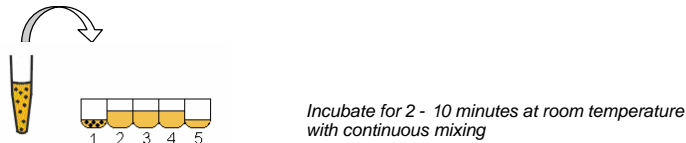
2. During the lysis step, pipette QuickPick™ SML gDNA purification kit reagents (according to the Table 4) into 96-well plate columns 1 - 5 as follows:

- Column 1: gDNA Magnetic Particles and gDNA Binding Buffer
- Column 2: gDNA Wash Buffer 1
- Column 3: gDNA Wash Buffer 1
- Column 4: gDNA Wash Buffer 2
- Column 5: gDNA Elution Buffer



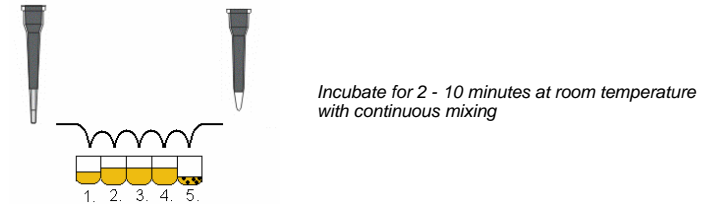
3. Remove the tubes from 56°C and spin shortly. Gently transfer the whole suspension from each tube into the respective wells of column 1 (Binding Buffer, Magnetic Particles) by mixing the lysed sample and Magnetic Particles carefully by pipetting up and down few times. Mix the 96-well plate on the orbital shaker for 2 - 10 minutes at room temperature. Make sure that the Magnetic Particles are in suspension during this step. Normally, the Magnetic Particles form a lump when DNA binds to them.

Pipette

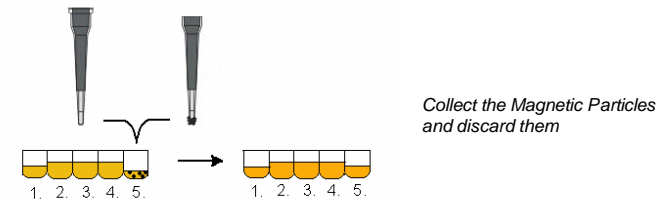


4. Pick up the PickPen® tips using PickPen® 8-M. Extend the magnets 2 - 3 times to check that the tips are firmly in place. Collect the Magnetic Particles from column 1 with PickPen® 8-M and release them into column 5 (Elution Buffer). Mix the suspensions gently for 10 - 20 seconds using the PickPen® tips. Note that the magnets have to be withdrawn at this point. Repeat the washing steps in columns 3 and 4 (Wash Buffer 1 and Wash Buffer 2).

5. Collect the Magnetic Particles from column 4 with PickPen® 8-M and release them into column 5 (Elution Buffer). Mix the 96-well plate on the orbital shaker for 2 - 10 minutes at room temperature. Make sure that the Magnetic Particles are in a suspension during this step. During elution the lump of Magnetic Particles should disperse completely.



6. Collect the Magnetic Particles from column 5 and discard them and the tips. The eluates in column 5 contain the purified genomic DNA and are ready to be used in downstream applications. If the purified DNA is not used on the same day, store at -20°C until use.



## 7 PROTOCOLS FOR MAGRO™ 8-M ROBOTIC WORKSTATION

### 7.1 Notes for working with MagRo™ 8-M

1. All solutions should be clear when used. If precipitates have formed warm the solutions gently until the precipitates have dissolved.
2. Mix the Magnetic particles bottle thoroughly (do not vortex) and empty it into the Binding Buffer bottle. Store this MP / Binding Buffer mix at room temperature with other reagent bottles. If using XL Reagents refer to the QuickPick™ XL gDNA Reagents instructions for use.
3. Before starting any process make sure that:
  - a. You have enough of disposable tips (DiTi) for the AutoPickPen™ (APP) and pumps.
  - b. You have enough reagents in the reagent bottles.
  - c. Waste box is empty.
  - d. The APP and the gripper are correctly positioned in their place.
  - e. There are no tips attached on APP or the pumps.
  - f. Correct Heat Adapter is properly placed on Thermo-ShakeX.
  - g. Wash Buffer 1 contains correct amount of ethanol (96 - 100 %).
4. See also MagRo™ 8-M manual for proper use of the instrument. There are more instructions on how to use the software and how to execute processes.
5. If RNA-free genomic DNA preparation is required add RNase solution into the samples before starting the lysis step.
6. Water can also be used for elution.
7. Use sterile U-bottom 96-well plates (500 µl and 1 ml) for purifications (see Table 5).

## 7.2 Executing MagRo™ 8-M protocols (macros)

1. Switch on the instrument and start the Lirix software.
2. Each macro has an individual name. Look for correct macro name in the Table 6. To find a macro from MagRo™ 8-M open Lirix Shortcuts and click Start Macro icon.



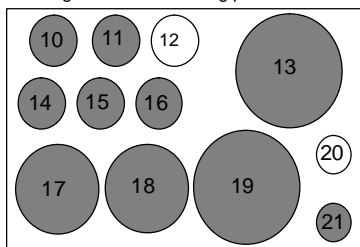
Table 6: The names of the macros for MagRo™ 8-M used with QuickPick™ SML gDNA purification kits.

Preps	Macro names		
	Up to 50 µl <sup>(1)</sup>	100 µl <sup>(1)</sup>	200 µl <sup>(1)</sup>
1 – 24	gDNA_50µl_24 (Chapter 7.3)	gDNA_100µl_24 (Chapter 7.6)	gDNA_200µl_24 (Chapter 7.9)
25 – 48	gDNA_50µl_48 (Chapter 7.4)	gDNA_100µl_48 (Chapter 7.7)	gDNA_200µl_48 (Chapter 7.10)
49 – 96	gDNA_50µl_96 (Chapter 7.5)	gDNA_100µl_96 (Chapter 7.8)	Not available

<sup>1</sup>Whole blood used as an example, for the amounts of other sample materials, see Table 2.

3. Click the correct macro name in “Macro Names” –list to open the process layout in the window.
4. Place trays, tip boxes, Sample plate and other 96-well plates and racks as described in Chapters 7.3 to 7.10.
5. Mix all reagent bottles, open and place them in the Reagent rack in following positions:

Positions 10 and 11: MP/Binding Buffer mix  
 Position 21: Proteinase K solution  
 Position 14: Lysis buffer  
 Position 13 and 19: Wash Buffer 1  
 Positions 17 and 18: Wash Buffer 2  
 Positions 15 and 16: Elution Buffer



**NOTE:** If preparing up to 48 samples, only one bottle of each reagent is needed for the run (place in positions 10, 13, 15 and 17).

6. Prepare the samples according to Chapter 4 “Sample preparation”. Use appropriate volumes of Lysis Buffer and Proteinase K solution (see Table 3) with the samples.
7. Start the macro by double-clicking the name.
8. A dialog window opens, in which you may enter the run specific information. You can also choose to start with refilled DiTi boxes by ticking the box. Press Next to continue.
9. Macro asks you to verify that all the items placed on the deck correspond to the displayed layout. Press Start Run to continue.
10. A dialog window asking for number of samples and other variables appears. Enter the number of samples and press OK. MagRo™ 8-M starts the macro.

11. After finishing the macro a Message box appears notifying that the run is finished. Click OK.
12. The eluates containing the purified genomic DNA (deck tray 4 at position 3) are ready to be processed in downstream applications.
13. If the purified DNA is not used on the same day, store at -20°C until use.

### 7.3 MagRo™ 8-M purification from 50 µl of sample, 1 - 24 preps

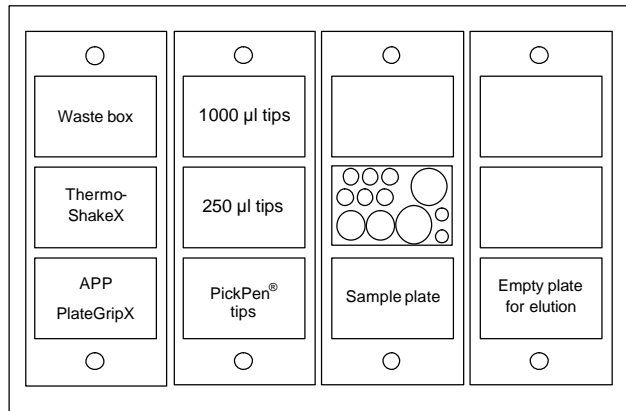
#### Specifications

Sample material amount: 1 – 50 µl  
 Preps in one run: 1 – 24  
 Macro name: gDNA\_50µl\_24

#### Materials required

1. Samples in a 96-well microplate
2. One sterile 96-well microplate
3. Reagent rack
4. Sterile Rainin 250 µl and 1000 µl tips in DiTi boxes
5. Sterile PickPen® tips in 96 tip box
6. Heat Adapter for 96-well microplates

#### Deck layout



### 7.4 MagRo™ 8-M purification from 50 µl of sample, 25 - 48 preps

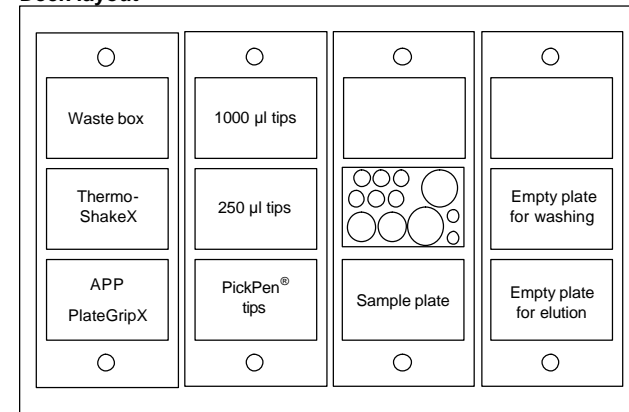
#### Specifications

Sample material amount: 1 – 50 µl  
 Preps in one run: 25 – 48  
 Macro name: gDNA\_50µl\_48

#### Materials required

1. Samples in a 96-well microplate
2. Two sterile 96-well microplates
3. Reagent rack
4. Sterile Rainin 250 µl and 1000 µl tips in DiTi boxes
5. Sterile PickPen® tips in 96 tip box
6. Heat Adapter for 96-well microplates

#### Deck layout



### 7.5 MagRo™ 8-M purification from 50 µl of sample, 49 – 96 preps

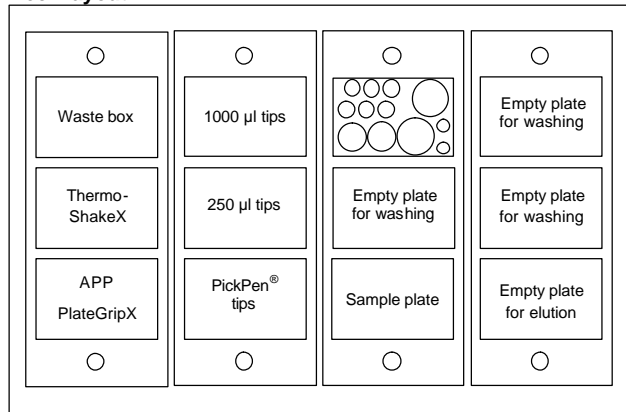
#### Specifications

Sample material amount: 1 – 50 µl  
 Preps in one run: 49 – 96  
 Macro name: gDNA\_50µl\_96

#### Materials required

1. Samples in a 96-well microplate
2. Four sterile 96-well microplates
3. Reagent rack
4. Sterile Rainin 250 µl and 1000 µl tips in DiTi boxes
5. Sterile PickPen® tips in 96 tip box
6. Heat Adapter for 96-well microplates

#### Deck layout



### 7.6 MagRo™ 8-M purification from 100 µl of sample, 1 - 24 preps

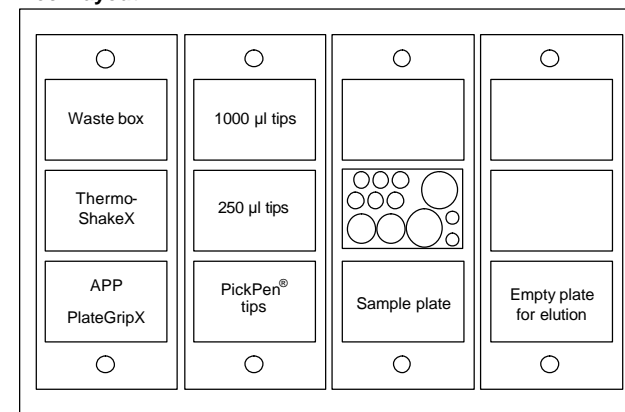
#### Specifications

Sample material amount: 51 - 100 µl  
 Preps in one run: 1 – 24  
 Macro name: gDNA\_100µl\_24

#### Materials required

1. Samples in a 96 deep-well plate
2. One sterile 96-well microplate
3. Reagent rack
4. Sterile Rainin 250 µl and 1000 µl tips in DiTi boxes
5. Sterile PickPen® tips in 96 tip box
6. Heat Adapter for 96 deep-well plates

#### Deck layout



### 7.7 MagRo™ 8-M purification from 100 µl of sample, 25 - 48 preps

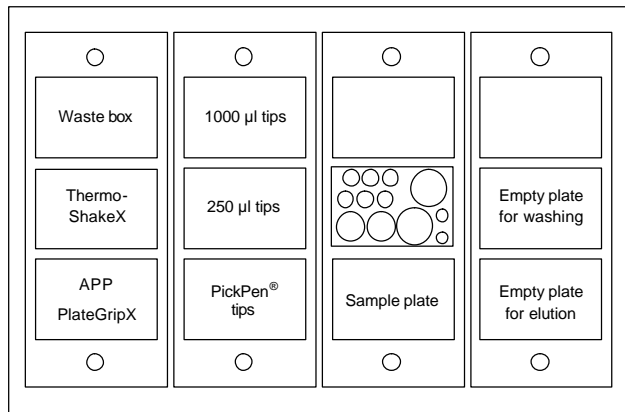
#### Specifications

Sample material amount: 51 - 100 µl  
 Preps in one run: 25 – 48  
 Macro name: gDNA\_100µL\_48

#### Materials required

1. Samples in a 96 deep-well plate
2. One sterile 96 deep-well plate
3. One sterile 96-well microplate
4. Reagent rack
5. Sterile Rainin 250 µl and 1000 µl tips in DiTi boxes
6. Sterile PickPen® tips in 96 tip box
7. Heat Adapter for 96 deep-well plates

#### Deck layout



### 7.8 MagRo™ 8-M purification from 100 µl of sample, 49 - 96 preps

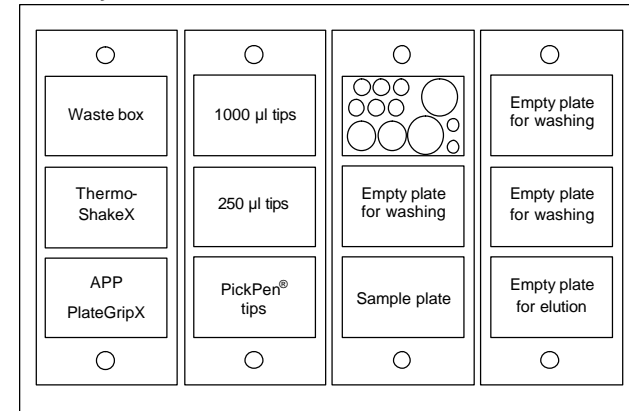
#### Specifications

Sample material amount: 51 - 100 µl  
 Preps in one run: 49 – 96  
 Macro name: gDNA\_100µL\_96

#### Materials required

1. Samples in a 96 deep-well plate
2. Three sterile 96 deep-well plates
3. One sterile 96-well microplate
4. Reagent rack
5. Sterile Rainin 250 µl and 1000 µl tips in DiTi boxes
6. Sterile PickPen® tips in 96 tip box
7. Heat Adapter for 96 deep-well plates

#### Deck layout



## 7.9 MagRo™ 8-M purification from 200 µl of sample, 1 - 24 preps

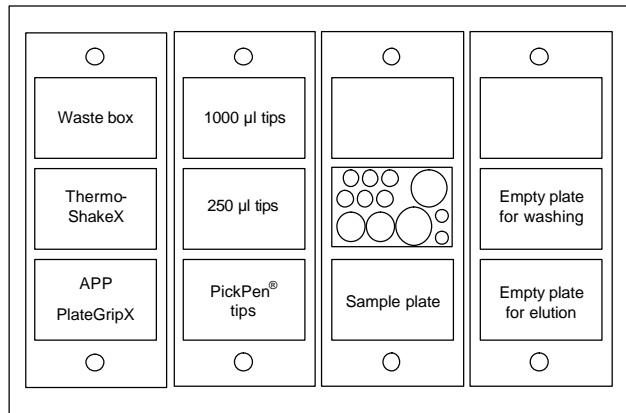
### Specifications

Sample material amount: 101 - 200 µl  
 Preps in one run: 1 – 24  
 Macro name: gDNA\_200µl\_24

### Materials required

1. Samples in a 96 deep-well plate
2. One sterile 96 deep-well plate
3. One sterile 96-well microplate
4. Reagent rack
5. Sterile Rainin 250 µl and 1000 µl tips in DiTi boxes
6. Sterile PickPen® tips in 96 tip box
7. Heat Adapter for 96 deep-well plates

### Deck layout



## 7.10 MagRo™ 8-M purification from 200 µl of sample, 25 - 48 preps

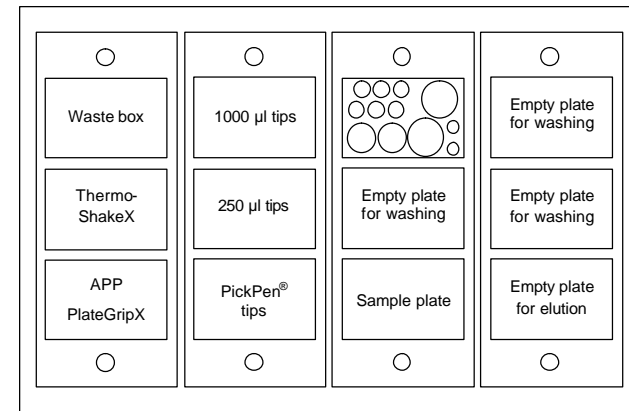
### Specifications

Sample material amount: 101 - 200 µl  
 Preps in one run: 25 – 48  
 Macro name: gDNA\_200µl\_48

### Materials required

1. Samples in a 96 deep-well plate
2. Three sterile 96 deep-well plates
3. One sterile 96-well microplate
4. Reagent rack
5. Sterile Rainin 250 µl and 1000 µl tips in DiTi boxes
6. Sterile PickPen® tips in 96 tip box
7. Heat Adapter for 96 deep-well plates

### Deck layout



## 8 TROUBLESHOOTING GUIDE

### 8.1 Reagents

Low DNA yield	
Poor sample preparation	Thaw frozen blood samples quickly in water bath / heating block (+37°C) with occasional shaking Blood is clotted. Start from the beginning by using fresh blood.
Too small sample amount	Frozen blood samples were not mixed properly after thawing
	Repeat the purification with fresh sample Resuspend leukocytes/cultured cells properly in PBS
	Make sure that hair roots are in solution during lysis
	Try another homogenization method for tissue samples Use larger sample amount or smaller reagent volumes (see Chapter 6 "Protocols for manual PickPen® 1-M and 8-M tools")
Too large sample amount	Use smaller sample amount or diluted sample. Too high sample amount interfere with the purification. For larger sample amounts use more reagents (see Chapter 6 "Protocols for manual PickPen® 1-M and 8-M tools").
Insufficient lysis	Add correct volume or increase the volume of Proteinase K solution and Lysis Buffer Use increased lysis time and/or improve mixing
	Make sure to mix sample, Proteinase K solution and Lysis Buffer thoroughly by inverting tube several times and pulse vortexing for 15 seconds before starting the lysis incubation. Also during the lysis the solution may be pulse-vortexed occasionally.
	Ensure that the heating step is done at 56°C
	Use the appropriate Heat Adapter for the sample 96-well plate
Insufficient binding	Make sure the Magnetic Particles are in suspension during incubations Suspend the Magnetic Particles gently by pipetting up and down before binding step Increase the binding time
	Make sure the volume of the Binding Buffer correlates with the sample amount used
	Make sure the Magnetic Particles are in suspension during incubations
No shaking during incubations	Make sure the Magnetic Particles are in suspension during incubations
Insufficient washes	Ensure that ethanol (96 - 100 %) is added to Wash Buffer 1 concentrate
	Increase the washing times in each Wash Buffer
	Use eluate as the sample and repeat the purification
Inappropriate Elution Buffer	DNA will only be eluted in the presence of low salt (e.g. 10 mM Tris-Cl, pH 8.5) or water. Check the pH and salt concentration of the Elution Buffer.
	Increase the lysis time and make sure to mix the sample, Proteinase K solution and Lysis Buffer thoroughly by inverting tube several times and pulse vortexing for 15 seconds before starting the lysis incubation
	Ensure that the lysis step is done at +56 °C
	Wash the Magnetic Particles with bound DNA properly in Wash Buffers before elution
	Ensure that correct volume of ethanol has been added into Wash Buffer 1

	Increase the volumes of Lysis and Binding Buffers in the same ratio to each others During wash and elution steps avoid transferring liquid droplets into the next tube/well
Insufficient elution	Increase elution time
	Ensure that Magnetic Particles are in suspension during elution Continue elution until Magnetic Particles are uniformly dispersed
	Use heating during elution (max +65°C)
Magnetic particles	Optimize the Magnetic Particle amount
	Use only gDNA Magnetic Particles
	Do not freeze Magnetic Particles
	Make sure that Magnetic Particles are uniformly suspended before dispensing
Purified DNA too concentrated / too diluted	
Too small elution volume	Use more Elution Buffer to achieve optimal concentration
	Dilute final eluate by adding sufficient amount of Elution Buffer
Too large elution volume	Use less Elution Buffer to achieve optimal concentration

### 8.2 Manual Tools

Magnetic Particles are not collected from the suspension	
Magnet inside	Push the magnet out
Sample too viscous	Make sure to use correct sample amounts and that the homogenization and the lysis steps are adequately performed
	Decrease the amount of sample material Add 1 – 5 µl of 1 M DTT into sample before lysis step
	Dilute the sample and use Lysis Buffer, Proteinase K solution and Binding Buffer in correct ratio
Visible Magnetic Particles in all vessels/wells	Repeat the collections
Visible Magnetic Particles in Elution Buffer	Centrifuge the sample for 1 minute with maximum speed Increase the collection time

Magnetic Particles are not released from the tip	
Magnet out	Pull the magnet inside
No tip	Use PickPen® tip
Sample amount too high	Make sure to use correct sample amounts and that the homogenization and the lysis steps are adequately performed
	Decrease the amount of sample material
	Dilute the sample and use Lysis Buffer, Proteinase K solution and Binding Buffer in correct ratio Increase the suspension time and rub the PickPen® tip with Magnetic Particles against the vessel wall
Too small elution volume	Use larger volume

### 8.3 MagRo™ 8-M robotic workstation

MagRo™ 8-M does not work	
Instrument connections	Check that instrument is connected to the main power source and that all the cables are connected Check that the power is turned on both in the back and in the front of the instrument
Home-made process or macro doesn't start	Check the process in Edit process or Edit macro Check that all the parameters are correctly written

Magro™ 8-M protocol problems	
No liquid aspirated	The tip might be blocked or broken. Do not reuse tips The pump piston may have some liquid inside, clean the pump
"No liquid" signal	Some reagents might have air bubbles that interfere with the aspiration Check that reagent bottles contain liquids. Fill the bottles and continue the process.
The tips were not dropped in to waste box	Empty the waste box after each run Check that the waste slide is positioned at the right edge
Gripper malfunction	Check that the grippers are correctly placed on the holder
APP malfunction	Check that the APP is correctly placed on the holder with no tips attached
Pump malfunction	Make sure that there are enough tips for the APP Check that there are no tips attached to the pumps before the run Make sure that there are enough tips for both pumps
Shaker	Check that the cables are connected Ensure the Heat Adapter is firmly placed on the shaker Use only new plates on the Heat Adapters (autoclaving may alter the plate dimensions) Check the shaking speed and maximum liquid volumes for each plate to prevent liquid spill Check the temperature for each reagent and volume
Labware	Check the plate dimensions
No tips taken	Place the plates in a correct orientation (A1 is in the upper left corner) Ensure that the tip boxes are tightly in place Ensure that the tip boxes are filled Use only Rainin tips

### 8.4 Downstream applications

Downstream applications	
High absorbance at 320 nm	Eluates may contain Magnetic Particles. Centrifuge the sample for 1 min with maximum speed. Wash the Magnetic Particles with bound DNA properly in Wash Buffers
No PCR product	Repeat purification using fresh sample Residual ethanol carried from Wash Buffer 1 Sequencing enzymes, polymerases and other Mg <sup>2+</sup> - dependent enzymes: EDTA inhibits the enzymes, use water as elution buffer Optimize the DNA amount for the application Wash the Magnetic Particles containing the bound DNA properly in Wash Buffers
RNA contamination	Add RNase A to sample before lysis step Optimize the amount of RNase A used

## 9 STORAGE AND STABILITY

The QuickPick™ SML gDNA purification kits should be stored at room temperature. Magnetic Particles should not be frozen.

## 10 WARNINGS AND LIMITATIONS

The QuickPick™ SML gDNA purification kits are intended for research use only, and are not intended for use in human diagnostic or therapeutic procedures. Standard methods for preventing contamination with DNases during preparation of DNA must be taken. Precautions should also be taken to avoid contamination of opened vessels. Do not pipet by mouth.

gDNA reagent, Wash Buffer 2 and Binding Buffer contain 0.02 % sodium azide (NaN<sub>3</sub>) as a preservative. When in contact with acid or heavy metal ions, it forms a highly toxic gas. Preservatives such as NaN<sub>3</sub> are toxic if ingested. Do not pipet by mouth. Direct skin contact must be avoided. Appropriate precautions should be taken when handling these solutions.

## 11 DISCLAIMERS AND WARRANTIES

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