

peqGOLD Tissue DNA Mini Kits (Safety Line)

– Instruction Manual –

Contents

Introduction	3
Principle.....	3
Kit components	4
Storage.....	4
Binding Capacity.....	4
Before Starting	5
peqGOLD Tissue DNA Mini Kit Protocols	6
A. Eucaryotic Cells and Tissue	6
B. Paraffin-embedded Tissue.....	9
C. Dried Blood	10
D. Mouse Tail Snips.....	11
Concentrating the DNA.....	13
Quantification and Storage of the DNA.....	13
Ordering Information.....	14
Troubleshooting Guide.....	14

Introduction

The peqGOLD Tissue DNA Mini Kits provide a rapid and easy method for the isolation of up to 30 µg of genomic DNA from eucaryotic cells, fresh, frozen or paraffin-embedded tissue, dried blood or mouse tail snips. The kit allows simultaneous processing of single or multiple samples from up to 1 x 10⁷ cells, 30 mg of tissue material, 200 µl of dried blood spots or 1 cm of mouse tail snip.

There is no need for extractions with organic solvents like phenol or chloroform or time consuming steps such as precipitation with isopropyl or ethanol. Although the kits are principally also suited for the isolation of genomic DNA from blood, Buffy Coat, serum or plasma, we recommend the use of peqGOLD Blood DNA Kits for these purposes. The peqGOLD Blood DNA Kits are optimised for an efficient hemolysis and removal of hemoglobin and prove therefore better yields and higher quality of the isolated DNA.

Genomic DNA purified using the peqGOLD Tissue DNA Mini Kits is ready for a range of applications such as PCR, Southern Blotting, and restriction digestion.

peqGOLD Tissue DNA Mini Kits are available as S- or C-line columns (Safety-Line, Best.-Nr. 12-3396-xx or Classic Line, Best.-Nr. 12-3496-xx). S-line columns have a slender form and a tube-like outlet at the bottom, ensuring that all positions in centrifuges and vacuum manifolds can be occupied. Lids close the columns tightly and avoid cross-contamination. C-line columns have a bigger diameter and a flat bottom, allowing higher sample volumes to be processed. These columns possess no lid.

Principle

Die peqGOLD Tissue DNA Mini Kits use the reversible binding properties of HiBind[®] matrix, a silica-based material, combined with the speed of mini-column spin technology. A specifically formulated buffer system allows up to 30 µg of genomic DNA up to a size of 60 kbp to bind to the matrix.

Samples are first homogenised, lysed under denaturing conditions and then applied to the HiBind[®] spin columns where the DNA is effectively bound to the silica membrane. Cellular debris, proteins and other contaminants are washed away by specific buffers. The high quality DNA is finally eluted in sterile deionized water or low salt buffer.

Kit components

peqGOLD Tissue DNA Mini Kits	5 Preparations	50 Preparations	200 Preparations
Best.-Nr. Safety-Line	12-3396-00	12-3396-01	12-3396-02
Kit components			
HiBind®-DNA columns	5	50	200
2 ml Collection Tubes	15	150	600
Buffer TL	5 ml	20 ml	50 ml
Buffer BL	5 ml	20 ml	60 ml
DNA Wash Buffer	12 ml	40 ml	3 x 40 ml
Elution Buffer	2 ml	30 ml	100 ml
OB™ Protease	3 mg	30 mg	120 mg
10 mM TE buffer	1.5 ml	1.5 ml	4 x 1.5 ml

Storage

Dissolved OB™ Protease should be stored at $-20\text{ }^{\circ}\text{C}$, all other components of the peqGOLD Tissue DNA Mini Kits should be stored at room temperature. The components of the kits remain stable at an ambient temperature of $22 - 25\text{ }^{\circ}\text{C}$ for at least 12 months after the date of purchase. Precipitates can form in Buffer BL and should be dissolved by heating of the bottle at $37\text{ }^{\circ}\text{C}$.

Binding capacity

Each HiBind® column can bind approximately $30\text{ }\mu\text{g}$ of DNA. Using greater than 30 mg of tissue is not recommended.

Before starting

Please read the protocol carefully before the first use of the peqGOLD Tissue DNA Mini Kit and keep all required materials available before starting the preparation.

- ! Buffer BL contains a chaotropic salt. Use gloves and protective eyewear when handling this solution.
- ! Under low ambient temperatures, precipitates can form in Buffer BL. These precipitates are normal, but should be dissolved by heating the bottle at 37 °C before use.
- ! DNA Wash Buffer is delivered as a concentrate and must be diluted with absolute ethanol before use:

Kit 12-3396-00	Mix 12 ml Wash Buffer with 18 ml 100 % EtOH.
Kit 12-3496-00	
Kit 12-3396-01	Mix 40 ml Wash Buffer with 60 ml 100 % EtOH.
Kit 12-3496-01	
Kit 12-3396-02	Mix 3 x 40 ml Wash Buffer with 3 x 60 ml 100 % EtOH.
Kit 12-3496-02	

Diluted DNA Wash Buffer should be stored at room temperature. If stored at lower temperatures, it should be heated to room temperature before use.

- ! OB™ Protease is delivered as a powder and must be dissolved before use by thoroughly vortexing in TE buffer:

Kit 12-3396-00	3 mg OB™ Protease in 150 µl TE buffer.
Kit 12-3496-00	
Kit 12-3396-01	30 mg OB™ Protease in 1.5 ml TE buffer.
Kit 12-3496-01	
Kit 12-3396-02	120 mg OB™ Protease in 6 ml TE buffer.
Kit 12-3496-02	

Dissolved OB™ Protease should be aliquoted à 25 µl, 50 µl or 100 µl, stored at -20 °C and be freshly thawed before use.

- ! All centrifugation steps have to be performed at 22 – 25 °C.

peqGOLD Tissue DNA Mini Kit Protocols

A. Eucaryotic Cells and Tissue

Required materials to be supplied by the user:

- ! RNase A (25 mg/ml in 10 mM Tris-HCl, pH 8.0)
- ! 100 % ethanol
- ! Sterile pipette tips and centrifuge tubes

1. Homogenisation and Lysis

a. Tissue

Cut up to 30 mg of tissue (ca. 2 to 3 mm³) in small pieces, place the sample in a 1.5 ml tube and add 200 µl TL Buffer.

Principally, a mechanical homogenization of the starting material is not required when isolating genomic DNA with the peqGOLD Tissue DNA Mini Kit isolation protocols. Nevertheless, mechanical grinding under liquid nitrogen helps for a better lysis with shorter incubation times. Freeze the samples in a mortar and grind them with a pestil. After evaporation of the liquid nitrogen, take up the sample in 200 µl TL Buffer and continue the preparation as described in the following steps. Please note: liquid nitrogen can cause severe injuries. Always wear gloves and protective eyewear when using it.

Add 25 µl of OBTM Protease, mix thoroughly by vortexing and incubate at 55 °C in a shaking water bath. The incubation time depends on the type and amount of tissue to be prepared and is usually less than 3 hours. If no shaking water bath is available, mix the solutions every 20 to 30 minutes by vortexing. The incubation can also last over night.

Use a maximum of 30 mg of tissue per HiBind[®] DNA column to be prepared, otherwise the column will be overloaded. If you need to isolate higher amounts of DNA, divide the lysate on more than one column. Adapt the volumina of buffers and solutions on the bigger sample volume. For example add 400 µl TL Buffer and 50 µl OBTM Protease to 60 mg of tissue material. Also adapt the volumes of BL Buffer and absolute ethanol in the following steps.

b. Monolayer cells

Cell culture cells growing in monolayers are directly lysed in the culture flask or dish. Discard the culture medium and add 800 µl TL Buffer per T35-flask or 10-cm-dish. For smaller vessels add only 400 µl TL Buffer. Spread the buffer evenly over the whole surface to ensure complete lysing of the cells. Mix the lysate by pipetting up and down several times. Fill the lysate in a 5 ml centrifuge tube. Add 25 µl OBTM Protease, mix thoroughly by vortexing and incubate at 55 °C for 1 hour in a shaking water bath as described in section a.

Use a maximum of 1 x 10⁷ cells per HiBind[®] DNA column for the preparation of genomic DNA, otherwise the column will be overloaded. If a higher number of cells needs to be processed, divide the lysate on more than one column. Adapt the volume of buffers and solutions on the bigger sample volume.

c. Suspension cells

Pellet down the cells by centrifugation (5 minutes at 1.500 rpm or 400 x g). Discard the supernatant and resuspend the cells in 400 µl TL Buffer per 1×10^7 cells. Lyse the cells by pipetting up and down several times and fill the lysate in a 1.5 ml centrifuge tube. Add 25 µl OB™ Protease, mix thoroughly by vortexing and incubate at 55 °C for 1 hour in a shaking water bath as described in section a.

Use a maximum of 1×10^7 cells per HiBind® DNA column for the preparation of genomic DNA, otherwise the column will be overloaded. If a higher number of cells needs to be processed, divide the lysate on more than one column. Adapt the volume of buffers and solutions on the bigger sample volume.

2. RNA Digest (optional!)

Together with genomic DNA, RNA is isolated from the mouse tails. Depending on the downstream application, it can disturb the application more (standard enzymatic reactions) or less (PCR), and should therefore be digested with 20 µl RNase A (25 mg/ml) per 30 mg of tissue or 1×10^7 cells for 2 minutes at room temperature if required.

3. Loading and Binding

Add 220 µl BL Buffer per 200 µl TL Buffer and mix thoroughly by vortexing. Incubate for 10 minutes at 70 °C.

After addition of the BL Buffer and isopropyl a precipitate can form, but which has no influences on the DNA isolation.

Add 220 µl absolute ethanol per 200 µl TL Buffer and mix thoroughly by vortexing. Place a HiBind® DNA column in a 2 ml collection tube and load up to 650 µl of the preparation (inclusive all precipitates) on the column. Centrifuge the column with the collection tube for 1 Minute at 8.000 x g. Repeat this step until the entire preparation is loaded. Discard the flow-through and the collection tube.

4. Washing I

Place the column in a new 2 ml collection tube and add 600 µl of the diluted DNA Wash Buffer (Buffer concentrate plus 1.5 volume absolute ethanol) on the column. Centrifuge the column with the collection tube for 1 Minute at 8.000 x g. Discard the flow-through, keep the collection tube.

5. Washing II

Repeat the washing with 600 µl Wash Buffer as described in step 4.

6. Drying (Important step! Do not reduce the centrifugation time!)

Place the column in the empty collection tube and dry the column completely by centrifugation for 2 minutes at maximum speed.

7. Elution

Place the HiBind® column in a new 1.5 ml tube and pipette 200 µl Elution Buffer (pre-warmed to 70 °C!) directly onto the membrane. Incubate for 3 minutes at room temperature, then centrifuge for 1 minute at 8.000 x g.

Repeat the elution step once more with other 200 µl of the pre-warmed Elution Buffer.

The yields can be slightly improved by incubating the column at 70 °C instead of room temperature before centrifuging. When using the eluate of the first round of elution for the second round, the final yield can be increased together with the concentration of the DNA. But note that the yield is about 30 % decreased compared to a second elution with a fresh aliquot of Elution Buffer. Depending on the type of tissue or cell, between 8 and 30 µg of genomic DNA can be expected to be isolated from 30 mg of tissue material or 1×10^7 cells.

B. Paraffin-embedded Tissue

Required materials to be supplied by the user:

- ! Xylol
- ! RNase A (25 mg/ml in 10 mM Tris-HCl, pH 8.0)
- ! 100 % ethanol
- ! Sterile pipette tips and centrifuge tubes

1. Removal of Paraffin

Place up to 30 mg of tissue (ca. 2 to 3 mm³) in a 2 ml centrifuge tube and remove the paraffin by vortexing thoroughly in 1 ml Xylol, followed by a centrifugation for 10 minutes at 10.000 x g. Discard the supernatant carefully without destroying the tissue pellet.

2. Washing of the tissue pellet

Wash the tissue pellet with 1 ml of absolute ethanol to remove the Xylol completely. Centrifuge for 5 minutes at 10.000 x g. Discard the supernatant containing ethanol without destroying the tissue pellet. Repeat this washing step once with another 1 ml of ethanol. Air-dry the tissue-pellet for 15 minutes at 37 °C.

3. Lysis of the tissue pellet

Add 200 µl TL Buffer and 25 µl OB™ Protease to the tissue pellet and resuspend completely by vortexing.

Incubate the preparation at 55 °C in a shaking water bath. The time required for the lysis depends on the type and amount of tissue to be prepared and is usually less than 3 hours.

The time required for the lysis can be longer than for fresh tissue material.

If no shaking water bath is available, mix the solutions every 20 to 30 minutes by vortexing. The incubation can also last over night.

Use a maximum of 30 mg of tissue per HiBind® DNA column to be prepared, otherwise the column will be overloaded. If you need to isolate higher amounts of DNA, divide the lysate on more than one column. Adapt the volume of buffers and solutions on the bigger sample volume. For example add 400 µl TL Buffer and 50 µl OB™ Protease to 60 mg of tissue material. Also adapt the volumes of BL Buffer and absolute ethanol in the following steps.

Continue the preparation as described under step 2 of protocol A. Elute with 2 x 100 µl Elution Buffer pre-warmed to 70 °C instead of 2 x 200 µl.

The yield and the quality of the extracted DNA depend on the size and the age of the starting material. Tissue fixed with paraformaldehyd leads to a degraded DNA and RNA. Although the degree of degradation of the nucleic acids depends on the fixative, the isolated DNA is usually smaller than 500 bp.

C. Dried Blood

The following protocol enables the isolation of genomic DNA spotted on filter paper. We recommend the usage of special Forensic Filter Paper that is especially suited for this purpose, because it is easily lysed in water-containing buffers and allows therefore efficient isolation of genomic DNA.

Required materials to be supplied by the user:

- ! RNase A (25 mg/ml in 10 mM Tris-HCl, pH 8.0)
- ! 100 % ethanol
- ! Sterile pipette tips and centrifuge tubes

1. Homogenisation and Lysis

Cut or pull a filter paper with maximum 200 µl of dried blood in small pieces and transfer the paper in a 1.5 ml centrifuge tube. Add 250 µl TL Buffer and incubate for 1 to 2 hours at 95 °C. Vortex every 20 minutes. Cool down the lysate to ca. 60 °C, add 25 µl OB™ Protease and mix thoroughly by vortexing. Incubate for 30 minutes at 60 °C and mix from time to time.

Use a maximum of 200 µl dried blood per HiBind® DNA column, otherwise the column will be overloaded. If a higher amount of blood needs to be processed, divide the lysate on more than one column. Adapt the volume of buffers and solutions on the bigger sample volume. For example, add 500 µl TL Buffer and 50 µl OB™ Protease to 400 µl dried blood. Also adapt the volumes of BL Buffer and absolute ethanol in the following steps.

2. RNA Digest (optional!)

Some types of tissue or cells contain a high amount of cellular RNA, which is isolated together with the DNA when using the peqGOLD Tissue DNA Mini Kit. Depending on the downstream application, it can disturb the application more (standard enzymatic reactions) or less (PCR), and should therefore be digested with 20 µl RNase A (25 mg/ml) per 200 µl of dried blood for 2 minutes at room temperature if required.

3. Loading and Binding

Centrifuge the solutions for 1 Minute at 10.000 x g. Fill the supernatant in a new 1.5 ml centrifuge tube, add first 1 volume of BL Buffer, then 1 volume of isopropyl (e.g. 200 µl BL Buffer and 200 µl isopropyl per 200 µl supernatant) and mix thoroughly by vortexing.

After addition of the BL Buffer and isopropyl a precipitate can form, but which has no influences on the DNA isolation.

Place a HiBind® DNA column in a 2 ml collection tube and load the whole preparation (inclusive all precipitates) on the column. Centrifuge the column with the collection tube for 1 Minute at 8.000 x g. Discard the flow-through and the collection tube. Continue the preparation as described in step 4 of protocol A.

Blood drops from finger tips contain usually not more than 50 µl of blood and allow for DNA yields between 500 ng and 1 µg. These amounts are sufficient for PCR analyses. To improve the yields, elute the DNA with 50 µl Elution Buffer pre-warmed to 70 °C and repeat this step once with the first eluate.

D. Mouse tail snips

Required materials to be supplied by the user:

- ! RNase A (25 mg/ml in 10 mM Tris-HCl, pH 8.0)
- ! 100 % ethanol
- ! Sterile pipette tips and centrifuge tubes

1. Homogenisation and Lysis

Cut 0.2 to 0.5 cm long pieces of a mouse tail, place them in a 1.5 ml centrifuge tube and add first 180 µl TL Buffer, then 25 µl OB™ Protease.

The mice should not be older than 6 weeks, otherwise the lysis will be difficult and the DNA yields will be decreased. The best results have been shown with samples from 2 to 4 week old mice. These samples can be stored at -70 °C until the DNA isolation is done. The wound of the mouse should be treated correctly and the mouse be marked adequately.

Mix the lysate by vortexing thoroughly and incubate 1 to 4 hours or until the tails are completely lysed at 55 °C in a shaking water bath. If no shaking water bath is available, mix the solutions every 20 to 30 minutes by vortexing.

Incomplete lysis of the mouse tail can clog the HiBind® column and can result in low DNA yields.

The correct incubation time depends on the length of the mouse tail snip and the age of the animal. 0.5 cm long snips from 2-week-old mice usually lyse in about 2 hours. For elder animals, incubation over night can significantly increase the yield of the isolated DNA. Please note that hair and bone do not lyse.

Centrifuge the lysed solution for 5 minutes at 10.000 x g to pellet down unlysable debris. Transfer the supernatant carefully in a new 1.5 ml tube without carrying-over parts of the debris pellet.

Do not use more than 1 cm of mouse tail snip per HiBind® column for the preparation of genomic DNA, otherwise the column will be overloaded. If you need to isolate higher amounts of DNA, divide the lysate on more than one column. Adapt the volume of buffers and solutions on the bigger sample volume. Use for example 360 µl of TL Buffer and 50 µl of OB™ Protease for 2 cm mouse tail. Also adapt the volumes of BL Buffer and absolute ethanol in the following steps.

2. RNA Digest (optional!)

Together with genomic DNA, RNA is isolated from the mouse tails. Depending on the downstream application, it can disturb the application more (standard enzymatic reactions) or less (PCR), and should therefore be digested with 15 µl RNase A (25 mg/ml) per 1 cm mouse tail for 2 minutes at room temperature if required.

3. Loading and Binding

Mix lysate by vortexing thoroughly with 410 µl of a BL Buffer/ethanol mixture (200 µl BL Buffer with 210 µl absolute Ethanol, vortexed) per 180 µl of TL Buffer in the sample. Incubate mixture for 10 minutes at 70 °C.

Prepare the BL Buffer/ethanol mixture freshly or prepare it once and store it at room temperature for maximum one month. Thoroughly mixing is very important for this preparation step and the whole isolation procedure. After addition of the BL Buffer/ethanol mixture a precipitate can form, but which has no influences on the DNA isolation.

Place a HiBind® DNA column in a 2 ml collection tube and load the whole preparation (inclusive all precipitates) on the column. Centrifuge the column with the collection tube for 1 Minute at 8.000 x g. Discard the flow-through and the collection tube.

4. Washing I

Place the column in a new 2 ml collection tube and add 600 µl of the diluted DNA Wash Buffer (Buffer concentrate plus 1.5 volume absolute ethanol) on the column. Centrifuge the column with the collection tube for 1 Minute at 8.000 x g. Discard the flow-through, keep the collection tube.

5. Washing II

Repeat the washing with 600 µl Wash Buffer as described in step 4.

6. Drying (Important step! Do not reduce the centrifugation time!)

Place the column in the empty collection tube and dry the column completely by centrifugation for 2 minutes at maximum speed.

7. Elution

Place the HiBind® column in a new 1.5 ml tube and pipette 200 µl Elution Buffer (prewarmed to 70 °C!) directly onto the membrane. Incubate for 3 minutes at room temperature, then centrifuge for 1 minute at 8.000 x g. Repeat the elution step once more with other 200 µl of the pre-warmed Elution Buffer.

In every round of elution with 200 µl Elution Buffer, up to 70 % of the bound DNA can be eluted. Two rounds of elution allow therefore a recovery of about 90 %. Please note that more than one round of elution increase the absolute yield, but decrease the concentration of the eluted DNA. To receive highly concentrated DNA, the elution can be performed with 50 µl to 100 µl of Elution Buffer, taking into account a slightly decreased yield. Elution volume below 50 µl reduce the yields drastically. The yields can be slightly improved by incubating the column at 70 °C instead of room temperature before centrifuging. When using the eluate of the first round of elution for the second round, the final yield can be increased together with the concentration of the DNA. But note that the yield is about 30 % decreased compared to a second elution with a fresh aliquot of Elution Buffer.

Concentrating the DNA

Genomic DNA purified with the peqGOLD Tissue DNA Mini Kits can be further concentrated if required. Add NaCl to an end concentration of 0.1 M and 2 sample volumes of absolute ethanol. Incubate thoroughly by vortexing and incubate for 10 minutes at -20°C . Centrifuge for 15 minutes at $10.000 \times g$ and discard the supernatant. Add 700 μl of 80 % ethanol and centrifuge for 2 minutes at $10.000 \times g$. Discard the supernatant, air-dry the pellet for 2 minutes and dissolve the DNA in 20 μl of sterile, deionised water or 10 mM Tris-HCl, pH 8.0.

Quantification and Storage of the DNA

To determine the concentration and the purity of a DNA-containing solution, the absorbance of a 10- to 50-fold diluted aliquot is measured at 260 nm and 280 nm in a spectrophotometer. One A_{260} unity equals to 50 μg of DNA/ml. The concentration determines as follows:

DNA concentration ($\mu\text{g}/\text{ml}$) = absorbance₂₆₀ \times 50 \times dilution factor

The $A_{260/280}$ ratio of pure nucleic acids is 2.0. The ratio of 1.7 to 1.9 after isolation of genomic DNA with the peqGOLD Tissue DNA Mini Kits corresponds to a purity of 85 % up to 95 %.

As an alternative, the approximate yield and the quality of the isolated DNA can be determined by agarose gel electrophoresis followed by staining of the gel with ethidium bromide and comparison of the amount of DNA with known DNA samples.

Genomic DNA isolated with the peqGOLD Tissue DNA Mini Kits can be stored in Elution Buffer, 10 mM Tris-HCl (pH 8.0) or sterile, deionised water for several years at -20°C . Avoid frequent freezing and thawing as this will shear the DNA and result in a reduction of the molecule size.

Ordering information

For the isolation of genomic DNA from cells, fresh or paraffin-embedded tissue, dried blood and mouse tail snips:

peqGOLD Tissue DNA Mini Kit (S-Line)	12-3396-00	5 Preparations
	12-3396-01	50 Preparations
	12-3396-02	200 Preparations
peqGOLD Tissue DNA Mini Kit (C-Line)	12-3496-00	5 Preparations
	12-3496-01	50 Preparations
	12-3496-02	200 Preparations

Trouble Shooting Guide

Problem	Possible Cause	Suggestions
Clogged column	Incomplete lysis	<ul style="list-style-type: none"> Extend incubation time of lysis with Buffer TL and protease. Add the correct volume of Buffer BL and extend incubation time at 70 °C by 10 minutes.
	Sample too large	<ul style="list-style-type: none"> Increase volumes of protease, Buffer TL, Buffer BL and ethanol. Distribute lysate on more than one column.
	Sample too viscous	<ul style="list-style-type: none"> Divide sample into multiple tubes, adjust volume to 250 µl with 10 mM Tris-HCl.
Low DNA yield	Poor elution	<ul style="list-style-type: none"> Repeat elution or increase elution volume. Incubate column at 70 °C for 5 minutes with Elution Buffer before centrifugation.
	Improper washing	<ul style="list-style-type: none"> Wash Buffer concentrate must be diluted with ethanol before use.
	Clogged column	<ul style="list-style-type: none"> See above.

Troubleshooting Guide (continued)

Problem	Possible Cause	Suggestions
No DNA eluted	Poor cell and/or protein lysis in Buffer TL	<ul style="list-style-type: none"> Tissue sample must be cut or minced into small pieces. Increase incubation time at 65 °C with Buffer TL.
	Poor cell lysis due to improper mixing with Buffer BL	<ul style="list-style-type: none"> Mix thoroughly with Buffer BL prior to loading HiBind® column.
	No ethanol added to Buffer BL	<ul style="list-style-type: none"> An Aliquot of Buffer BL/ethanol must be added to sample before applying sample to column.
	No ethanol added to Wash Buffer concentrate	<ul style="list-style-type: none"> Dilute Wash Buffer with the indicated volume of absolute ethanol before use.
Low $A_{260/280}$ ratio	Resin from the column present in the eluate	<ul style="list-style-type: none"> Avoid centrifugation at speeds higher than specified. The material can be removed from the eluate by centrifugation and will not interfere with enzymatic reactions.
	Poor cell lysis due to incomplete mixing with Buffer BL	<ul style="list-style-type: none"> Vortex the sample with Buffer BL immediately and completely.
	Incomplete cell lysis or protein degradation	<ul style="list-style-type: none"> Increase incubation time with Buffer TL and protease. Ensure that no visible pieces of tissue remain.
	Samples are rich in protein	<ul style="list-style-type: none"> After applying to column, wash with 300 µl of a 1:1 mixture of Buffer BL and ethanol and then with DNA Wash Buffer.
Problems with downstream applications	Salt carry-over	<ul style="list-style-type: none"> Ensure that the DNA Wash Buffer is at room temperature.
	Ethanol carry-over	<ul style="list-style-type: none"> Dry the columns after the second washing for at least 2 minutes by centrifugation at maximum speed.