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PRODUCT INFORMATION

Thermo Scientific GeneJET Genomic DNA Purification Kit #K0721, #K0722

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Read Storage information (p. 2) before first use!

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For Research Use Only. Not for use in diagnostic procedures.

#_
Lot _
Exp. _

CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Genomic DNA Purification Kit is qualified by isolating genomic DNA from 200 µL of blood and 5 mg of mammalian tissue following described protocols. The purified genomic DNA has an $A_{260/280}$ ratio of ≥1.7. A single band of more than 30 kb is seen after agarose gel electrophoresis and ethidium bromide staining. Functional quality of genomic DNA is evaluated by PCR amplification of a single-copy gene and digestion with restriction enzymes.

Quality authorized by:  Jurgita Zilinskiene

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COMPONENTS OF THE KIT

GeneJET Genomic DNA Purification Kit	#K0721 50 preps	#K0722 250 preps
Proteinase K Solution	1.2 mL	5 × 1.2 mL
RNase A Solution	1 mL	5 × 1 mL
Digestion Solution	11 mL	55 mL
Lysis Solution	24 mL	2 × 60 mL
Wash Buffer I (concentrated)	10 mL	40 mL
Wash Buffer II (concentrated)	10 mL	40 mL
Elution Buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA)	30 mL	150 mL
GeneJET Genomic DNA Purification Columns pre-assembled with Collection Tubes	50	250
Collection Tubes	50	250

STORAGE

Proteinase K and RNase A solutions are stable at room temperature as long as not opened. After being opened they should be stored at -20 °C. Other components of the kit should be stored at room temperature (15-25 °C).

Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!

DESCRIPTION

The GeneJET™ Genomic DNA Purification Kit is designed for rapid and efficient purification of high quality genomic DNA from various mammalian cell culture and tissue samples, whole blood, bacteria and yeast. The kit utilizes silica-based membrane technology in the form of a convenient spin column, eliminating the need for expensive resins, toxic phenol-chloroform extractions, or time-consuming alcohol precipitation. The standard procedure takes less than 20 minutes following cell lysis and yields purified DNA of more than 30 kb in size. Isolated DNA can be used directly in PCR, Southern blotting and enzymatic reactions. *See* Table 1 for typical genomic DNA yields from various sources.

PRINCIPLE

Depending on the starting material, samples are digested with Proteinase K in either the supplied Digestion or Lysis Solution. RNA is removed by treating the samples with RNase A. The lysate is then mixed with ethanol and loaded on the purification column where the DNA binds to the silica membrane. Impurities are effectively removed by washing the column with the prepared wash buffers. Genomic DNA is then eluted under low ionic strength conditions with the Elution Buffer.

Table 1. Typical genomic DNA yields from various sources.

Source	Quantity	Yield, µg
Mammalian blood	200 µL	4-6
Mouse heart	10 mg	10-15
Mouse tail	0.5 cm	8-10
Rat liver	10 mg	10-20
Rat spleen	5 mg	20-30
Rat kidney	10 mg	25-30
Rabbit ear	20 mg	5-10
Bacillus pumilis cells	2×10^9 cells	10-15
Escherichia coli cells	2×10^9 cells	10-15
HeLa cells	2×10^6 cells	15-20
Jurkat cells	5×10^6 cells	25-30
Saccharomyces cerevisiae cells	1×10^8 cells	3-5

IMPORTANT NOTES

- To minimize DNA degradation, avoid repeated freeze/thaw cycles of the samples and perform extractions from fresh material or material that has been immediately frozen and stored at -20 °C or -70 °C.
- Add the indicated volume of ethanol (96-100%) to **Wash Buffer I** (concentrated) and **Wash Buffer II** (concentrated) prior to first use:

	#K0721 50 preps		**#K0722** 250 preps	
	Wash Buffer I	**Wash Buffer II**	**Wash Buffer I**	**Wash Buffer II**
Concentrated wash solution	10 mL	10 mL	40 mL	40 mL
Ethanol (96-100%)	**30 mL**	**30 mL**	**120 mL**	**120 mL**
Total volume:	40 mL	40 mL	160 mL	160 mL

After the ethanol has been added, mark the check box on the bottle's cap to indicate the completed step.

- Check the **Digestion Solution** and **Lysis Solution** for salt precipitation before each use. Re-dissolve any precipitate by warming the solution at 37 °C, then cool back down to 25 °C before use.
- Wear gloves when handling the **Lysis Solution and Wash Buffer I** as these reagents contain irritants.

ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- Pipets and pipet tips
- Vortex
- Ethanol (96-100%)
- 1.5 mL microcentrifuge tubes
- Microcentrifuge
- Thermomixer, shaking water bath or rocking platform capable of heating up to 56 °C
- Disposable gloves

Buffers

For mammalian cell lysate preparation:

- PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)
- TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA)

For gram-positive bacteria lysate preparation

- Gram-positive bacteria lysis buffer (20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100, add lysozyme to 20 mg/mL immediately before use)

For yeast lysate preparation:

- Yeast lysis buffer (5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA)

GENOMIC DNA PURIFICATION PROTOCOLS

Protocols for genomic DNA purification from mammalian tissue and rodent tail, cultured mammalian cells, mammalian blood, gram-negative, gram-positive bacteria, yeast and buccal swabs are described on p.4-11.

A. Mammalian Tissue and Rodent Tail Genomic DNA Purification Protocol

<table>
<tr><th>Step</th><th>Procedure</th></tr>
<tr><td>1</td><td>Grind up to 20 mg of mammalian tissue (use up to 10 mg of spleen tissue), 0.6 cm (rat) or 0.5 cm (mouse) tail clip in liquid nitrogen using a mortar and pestle. Alternatively, cut the tissue into small pieces or disrupt it using a homogenizer.</td></tr>
<tr><td>2</td><td>Collect the material into a 1.5 mL microcentrifuge tube (not provided) and resuspend in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.</td></tr>
<tr><td>3</td><td>Incubate the sample at 56 °C until the tissue is completely lysed and no particles remain. During incubation vortex the vial occasionally or use a shaking water bath, rocking platform or thermomixer.
Suggested incubation times:
<table>
<tr><th>Quantity</th><th>Suggested incubation time</th></tr>
<tr><td>5 mg of tissue (except spleen)</td><td>1 hour</td></tr>
<tr><td>10 mg of tissue (except spleen)</td><td>2 hours</td></tr>
<tr><td>20 mg of tissue (except spleen)</td><td>3 hours</td></tr>
<tr><td>5 mg of spleen tissue</td><td>2 hours</td></tr>
<tr><td>10 mg of spleen tissue</td><td>3 hours</td></tr>
<tr><td>Mouse tail (0.5 cm), rat tail (0.6 cm)</td><td>6 hours</td></tr>
</table>
Note. Lysis time varies on the type and amount of tissue processed. In some cases incubation time should be prolonged to 6-8 hours or overnight (for rodent tail) until complete lysis occurs.</td></tr>
<tr><td>4</td><td>Add 20 µL of RNase A Solution, mix by vortexing then incubate for 10 min at room temperature.</td></tr>
<tr><td>5</td><td>Add 200 µL of Lysis Solution. Mix thoroughly by vortexing for 15 s until a homogeneous mixture is obtained.</td></tr>
<tr><td>6</td><td>Add 400 µL of 50% ethanol and mix by pipetting or vortexing.</td></tr>
<tr><td>7</td><td>Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included).
Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!</td></tr>
<tr><td>8</td><td>Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.</td></tr>
</table>

Step	Procedure
9	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., <5 mg of tissue) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

B. Cultured Mammalian Cells Genomic DNA Purification Protocol

Step	Procedure
1	a) Suspension cells Collect up to 5×10^6 cells in a centrifuge tube. Pellet cells by centrifugation for 5 min at 250 × g. Discard the supernatant. Rinse cells once with PBS to remove residual medium and repeat the centrifugation step. Discard the supernatant. b) Adherent cells Remove the growth medium from a culture plate containing up to 2×10^6 cells. Rinse cells once with PBS to remove residual medium. Discard PBS. Detach the cells from the culture plate by scraping in an appropriate volume of PBS or by trypsinization. Transfer the cells to a microcentrifuge tube and pellet them by centrifugation for 5 minutes at 250 × g. Discard supernatant.
2	Resuspend the cells collected in step 1a or 1b in 200 µL of TE buffer or PBS. Add 200 µL of Lysis Solution and 20 µL of Proteinase K Solution to the cell pellet. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (10 min).
4	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
5	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
6	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
7	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
8	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
9	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., $\leq1\times10^6$ of cultured mammalian cells) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
10	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

C. Mammalian Blood Genomic DNA Purification Protocol

Step	Procedure
1	Add 400 µL of Lysis Solution and 20 µL of Proteinase K Solution to 200 µL of whole blood, mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
2	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (10 min).
3	Add 200 µL of ethanol (96-100%) and mix by pipetting or vortexing.
4	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
5	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
6	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
7	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material (e.g., 50 µL) the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
8	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

D. Gram-Negative Bacteria Genomic DNA Purification Protocol

Step	Procedure
1	Harvest up to 2×10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 10 min at 5000 × g. Discard the supernatant.
2	Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
3	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~30 min).
4	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
5	Add 200 µL of Lysis Solution to the sample. Mix thoroughly by vortexing for about 15 s until a homogeneous mixture is obtained.
6	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
7	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
8	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
9	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

E. Gram-Positive Bacteria Genomic DNA Purification Protocol

Before starting

Prepare Gram-positive bacteria lysis buffer: 20 mM Tris-HCl, pH 8.0, 2 mM EDTA, 1.2% Triton X-100, add lysozyme to 20 mg/mL immediately before use.

Step	Procedure
1	Harvest up to 2×10^9 bacterial cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 10 min at 5000 × g. Discard the supernatant.
2	Resuspend the pellet in 180 µL of Gram-positive bacteria lysis buffer. Incubate for 30 min at 37 °C.
3	Add 200 µL of Lysis Solution and 20 µL of Proteinase K. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
4	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~30 min).
5	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
6	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
7	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
8	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
9	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
10	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
11	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

F. Yeast Genomic DNA Purification Protocol

Before starting

Prepare Yeast lysis buffer: 5 mg/mL zymolyase 20T, 1 M sorbitol, 0.1 M EDTA.

Step	Procedure
1	Harvest up to 1×10^8 yeast cells in a 1.5 or 2 mL microcentrifuge tube by centrifugation for 5-10 s at maximum speed ≥12000 × g. Discard the supernatant.
2	Resuspend the pellet in 500 µL of Yeast lysis buffer. Incubate for 1 hour at 37 °C.
3	Centrifuge cells for 10 min at 3000 × g. Discard the supernatant.
4	Resuspend the pellet in 180 µL of Digestion Solution. Add 20 µL of Proteinase K Solution and mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
5	Incubate the sample at 56 °C while vortexing occasionally or use a shaking water bath, rocking platform or thermomixer until the cells are completely lysed (~45 min).
6	Add 20 µL of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
7	Add 200 µL of Lysis Solution. Mix thoroughly by vortexing for 15 s until a homogeneous mixture is obtained.
8	Add 400 µL of 50% ethanol and mix by pipetting or vortexing.
9	Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 × g. Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 mL collection tube (included). **Note. Close the bag with GeneJET Genomic DNA Purification Columns tightly after each use!**
10	Add 500 µL of Wash Buffer I (with ethanol added). Centrifuge for 1 min at 8000 × g. Discard the flow-through and place the purification column back into the collection tube.
11	Add 500 µL of Wash Buffer II (with ethanol added) to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥12000 × g). *Optional*. If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min. at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 mL microcentrifuge tube (not included).
12	Add 200 µL of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 × g. **Note** • For maximum DNA yield, repeat the elution step with additional 200 µL of Elution Buffer. • If more concentrated DNA is required or DNA is isolated from a small amount of starting material the volume of the Elution Buffer added to the column can be reduced to 50-100 µL. Please be aware that smaller volumes of Elution Buffer will result in smaller final quantity of eluted DNA.
13	Discard the purification column. Use the purified DNA immediately in downstream applications or store at -20 °C.

G. DNA Purification from Buccal Swabs

Step	Procedure
1	To collect a sample, scrape the swab 5-6 times against the inside cheek.
2	Swirl the swab for 30-60 s in 200 µL of 1 × PBS.
3	Go to step 1 of the standard Mammalian Blood Genomic DNA Purification Protocol (p. 7).

TROUBLESHOOTING

Problem	Possible cause and solution
Low yield of purified DNA	**Excess sample used during lysate preparation.** Reduce the amount of starting material. Do not use more tissue or cells than indicated in lysis protocols. **Starting material was not completely digested.** Extend the Proteinase K digestion at 56 °C until complete lysis occurs and no particles remain. **Ethanol was not added to the lysate.** Make sure that the ethanol was added to the lysate before applying the sample to the Purification Column. **Ethanol was not mixed with the lysate.** After the addition of ethanol to the lysate mix the sample by vortexing or pipetting. **Ethanol was not added to Wash Buffers.** Make sure that ethanol was added to Wash Buffer I and Wash Buffer II before use. Follow the instructions for Wash Buffer preparation on p.3.
Purified DNA is degraded	**Sample was frozen and thawed repeatedly.** Avoid repeated freeze / thaw cycles of the samples. Use a new sample for DNA isolation. Perform extractions from fresh material when possible. **Inappropriate sample storage conditions.** Store mammalian tissues at -70 °C and bacteria at -20 °C until use. Whole blood can be stored at 4 °C for no longer than 1-2 days. For long term storage blood samples should be aliquoted in 200 µL portions and stored at -20 °C.
RNA contamination	**RNase A treatment was not carried out.** Carry out RNase A treatment step described in the purification procedure.
Column becomes clogged during purification	**Excess sample was used during lysate preparation.** Reduce the amount of starting material. A maximum of 2×10^9 of bacteria cells, $5x10^6$ of suspension cells and 20 mg of mammalian tissue is recommended for lysate preparation. **Tissue was not completely digested.** Extend the Proteinase K digestion at 56 °C until complete lysis occurs and no particles remain.
Inhibition of downstream enzymatic reactions	**Purified DNA contains residual ethanol.** If residual solution is seen in the purification column after washing the column with Wash Buffer II, empty the collection tube and re-spin the column for an additional 1 min. at maximum speed (≥12000 × g). **Purified DNA contains residual salt.** Use the correct order for the Washing Buffers. Always wash the purification column with Wash Buffer I first and then proceed to washing with Wash Buffer II.

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