INSTRUCTION MANUAL

Monarch® Genomic DNA Purification Kit

NEB #T3010S/L

50/150 preps

Version 4.0_03/24

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Kit Components

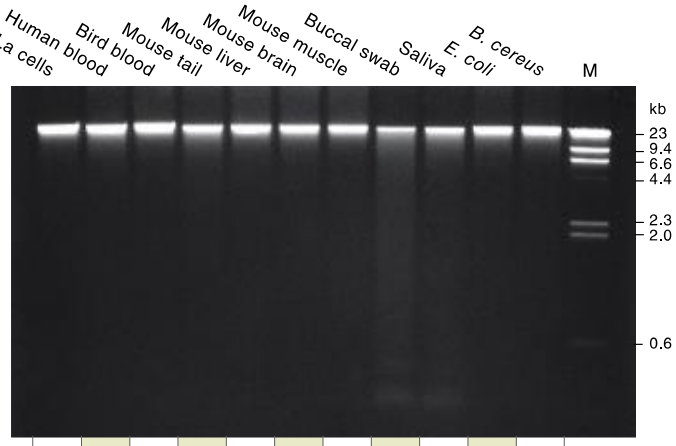
The kit should be stored at room temperature. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag. For information regarding the composition of buffers, please consult the Safety Data Sheets available on our website (http://www.neb.com/T3010). Proper laboratory safety practices should be employed, including the use of lab coats, gloves and eye protection.

NEB #		T3010S 50 preps	T3010L 150 preps	STORAGE TEMPERATURE
T3017	Monarch gDNA Purification Columns	50 columns	150 columns	25°C
T2018	Monarch Collection Tubes II	100 tubes	300 tubes	25°C
T3011	Monarch gDNA Tissue Lysis Buffer	12 ml	34 ml	25°C
T3012	Monarch gDNA Cell Lysis Buffer	6 ml	20 ml	25°C
T3013	Monarch gDNA Blood Lysis Buffer	6 ml	20 ml	25°C
T3014	Monarch gDNA Binding Buffer	24 ml	65 ml	25°C
T3015	Monarch gDNA Wash Buffer	18 ml	60 ml	25°C
T3016	Monarch gDNA Elution Buffer	14 ml	34 ml	25°C
T3018	Monarch RNase A	170 µl	500 µl	-20°C after opening
P8200	Proteinase K, Molecular Biology Grade	0.6 ml	1.8 ml	-20°C after opening

Introduction

The Monarch Genomic DNA Purification Kit is a comprehensive solution for cell lysis, RNA removal, and purification of intact genomic DNA (gDNA) from a wide variety of biological samples, including cultured cells, blood, and mammalian tissues. Additionally, bacteria and yeast can be processed with extra steps to enhance lysis in these tough-to-lyse samples. Protocols are also included to enable purification from clinically-relevant samples such as saliva and cheek swabs as well as rapid cleanup of previously extracted gDNA. Purified gDNA has high quality metrics, including $A_{260/A280}$ > 1.8 and $A_{260/A230}$ > 2.0, high DIN scores and minimal residual RNA. The purified gDNA is suitable for downstream applications such as end-point PCR, qPCR and library prep for NGS sequencing. It typically has a peak size of 50–70 kb, making this kit an excellent choice upstream of long-read sequencing platforms.

Figure 1: The Monarch Genomic DNA Purification Kit efficiently purifies high-quality, high molecular weight gDNA from a variety of sample types.

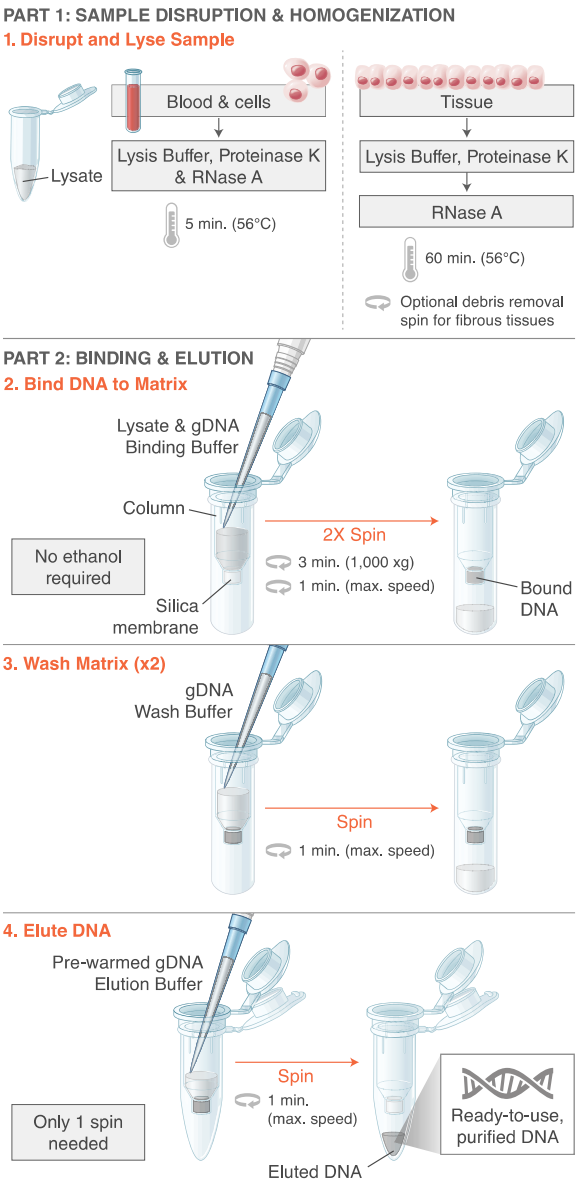


Peak size (kb)	>60	54	>60	>60	>60	>60	>60	39	45	53	>60
DIN	9.3	9.0	9.2	8.7	9.4	9.3	9.2	6.6	7.4	8.7	8.9
A_{260}/A_{280}	1.86	1.85	1.87	1.85	1.85	1.87	1.87	1.85	1.86	1.86	1.88
A_{260}/A_{230}	2.30	2.20	2.40	2.36	2.34	2.47	2.40	2.00	2.17	2.33	2.04

100 ng of genomic DNA from each sample was loaded on a 0.75% agarose gel. gDNA was isolated following the standard protocols for blood, cultured cells and tissue, and the supplemental protocols for buccal swabs, saliva, Gram– and Gram+ bacteria. Starting material used: 1 x 10^6 HeLa cells, 100 µl human blood, 10 µl bird blood, 10 mg frozen tissue powder, 1 buccal swab, 500 µl saliva and ~1 x 10^9 bacterial cells. Lambda DNA-Hind III digest (NEB #N3012) was used as a marker in the last lane (M). Purified gDNA samples were analyzed using a Genomic DNA ScreenTape® on an Agilent Technologies® 4200 TapeStation®. Samples typically yield peak sizes 50–70 kb and DINs of ~9. The cell fractions processed in the buccal swab and saliva preps contain dead cells, as expected, causing a smear like pattern with typical low molecular weight apoptotic bands.

Specifications

Input	Cultured mammalian cells: up to 5 x 10^6 cells Mammalian whole blood: 100 µl Tissue: up to 25 mg, depending on tissue type Bacteria: up to 2 x 10^9 Yeast: up to 5 x 10^7 Saliva: up to 500 µl Buccal swabs Genomic DNA requiring cleanup
Binding Capacity	30 µg genomic DNA
Yield	Varies depending on sample type, see “Choosing Input Amounts”
Genomic DNA Size	Peak size > 50 kb for most sample types; may be lower for saliva and buccal swabs
RNA Content	< 1% (with included RNase A treatment)
Purity	$A_{260/280} \geq 1.8$ $A_{260/230} \geq 2.0$



General Principles of the Monarch Genomic DNA Purification Kit

Lysis

A single lysis buffer cannot address all requirements to reach optimal yields and purity in multiple starting materials. Accordingly, the Monarch Genomic DNA Purification Kit contains 3 unique lysis buffers, optimized to enable maximal yield and purity when preparing genomic DNA from a variety of sample types. The Blood Lysis Buffer contains a strong protective component against the high nuclease activity in blood samples and supports rapid degradation of hemoglobin and other protein components. The Cell Lysis Buffer supplies mild lysis conditions that help to reduce the viscosity that is common in cell samples. The Tissue Lysis Buffer supplies intermediate lysis conditions that enable rapid digestion of the tissue pieces while simultaneously ensuring the genomic DNA fragment length is optimal for binding and elution. Coupled with optimized Proteinase K digestion conditions, the tissue lysis system in the Monarch Kit provides above-average yields for all common animal tissue types, including brain and muscle, tissues that prove difficult for many other commercial kits.

Binding and Washing

By employing a chaotropic salt-based binding buffer with low alcohol content, the Monarch Genomic DNA Purification Kit allows for specific binding of gDNA with very minimal RNA binding. By not employing a precipitation approach favored by many other kits, reproducible results with excellent yields are achieved, often 25–30% higher than other kits. The binding of gDNA to the column takes place at a low speed, maximizing binding time and thus enhancing efficiency. This low-speed spin is followed immediately by a spin at maximum speed to efficiently clear the membrane of lysate components such as proteins, salts and carbohydrates. This ensures that two brief washes are sufficient to provide eluted DNA of excellent purity. Additionally, inversion of the column with wash buffer effectively removes any contaminating chaotropic salt that may be inside the column reservoir.

RNA Removal

Co-purification of RNA during gDNA extractions is a common problem that leads users to overestimate the total yield of DNA. Many commercial kits utilize binding buffers with high alcohol content or containing PEG, which leads to significant percentages of RNA being co-purified (30% to 90% in some cases). In these cases, an additional RNase A digestion step is imperative to reduce RNA levels. The low-alcohol binding conditions employed in the Monarch Genomic DNA Purification Kit, however, optimize binding of gDNA alone. As such, even if the optional RNase A digestion is *not included*, the amount of RNA that is co-purified is extremely low: 1% for blood, up to 10% for cells and 1–4% for tissue. For many applications, it may not be necessary to further reduce the RNA content, and the RNase A step can be skipped. Regardless of whether it is used or not, an RNase A treatment option is included in each protocol and RNase A is supplied in the kit. Inclusion of the RNase A digestion results in best-in-class residual RNA levels of 0–1%, levels so low, they require more sophisticated detection methods (e.g., LC-MS).

Considerations for Elution & Storage

The elution buffer provided in the kit is 10 mM Tris-HCl, pH 9.0, 0.1 mM EDTA and is suitable for long term storage of gDNA. Nucleases are inactivated both by the inclusion of EDTA and the pH of the solution, which suppresses any nuclease activity. Alternatively, any low salt buffer or nuclease-free water can be used for elution.

Temperature

Elution with the Monarch Genomic DNA Kit is carried out with elution buffer preheated to 60°C; this significantly improves elution efficiency, especially for larger DNA molecules, which bind more tightly to silica. Using elution temperatures > 60°C is not recommended, however, as hot elution under low-salt conditions may result in partial and irreversible denaturation of the eluted gDNA.

Efficiency

Elution with 100 µl at 60°C will result in 80–85% recovery in the first elution. A second elution step can be carried out with another aliquot of 100 µl of preheated elution buffer and may result in 10–15% increase in yield. Alternatively, if high DNA concentration is required, a second elution with the first eluate may increase recovery by approximately 10%.

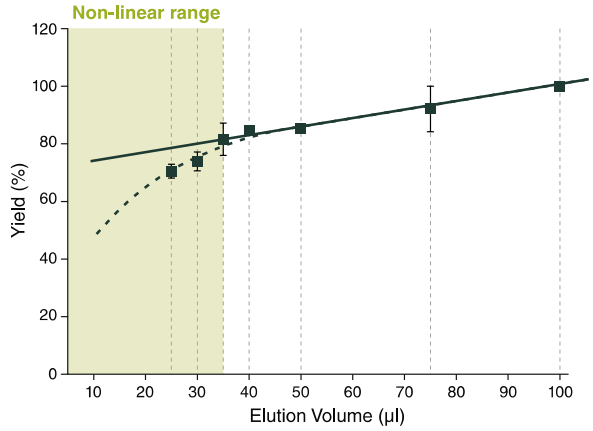
Volume

The recommended elution volume is 100 µl. However, for more concentrated gDNA, the elution volume can be reduced as low as 35 µl; but yields will be reduced by approximately 20% overall.

Typically, when using a volume of 100 µl, only 95 µl will elute, as some buffer will remain on the membrane. If less than 35 µl is used for elution, a larger fraction of elution buffer is retained, resulting in a loss in the linear correlation between elution volume and yield (Figure 2).

Please note that after repeated pipetting steps with pre-heated elution buffer using the same tip, the actual volume of liquid transferred by the pipette may be up to 15% higher than the set volume due to heating of the tip. If this is a problem, change pipette tips between samples.

Figure 2: Recovery of Genomic DNA using Various elution volumes with the Monarch Genomic DNA Purification Kit.



Optimal elution volume range is 35–100 µl. A lysate pool was prepared by using 10 mg RNAlater®-stabilized rat liver samples and following the Monarch tissue protocol. Elution volumes of 25, 30, 35, 40, 50, 75 and 100 µl were used on triplicate samples.

The average yield obtained with 100 µl elution volume was 19.1 µg, which was considered 100% yield. When using 35 µl to elute, the average yield was 81.6%; 25 µl and 20 µl yielded 7.4% and 70.6%, respectively.

For volume ≥ 35 µl, the recovered volume after elution was ~5 µl lower than the elution volume added, and for < 30 µl the recovered volume was ~ 10 µl lower. Tests performed with other starting materials showed similar results with a 20–25% reduction in overall yield when elution volumes were reduced from 100 to 35 µl.

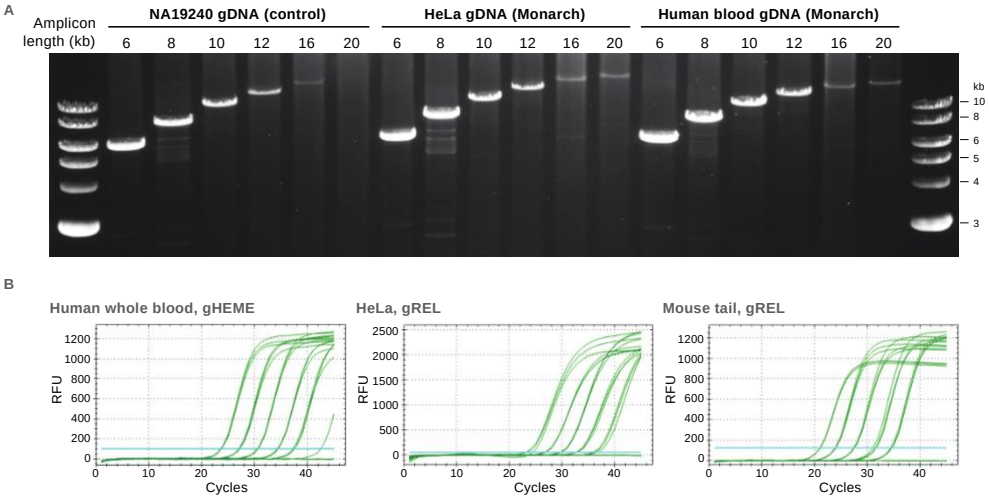
Storage of gDNA Samples

Genomic DNA eluted in the supplied elution buffer can be safely stored at 4–8°C for weeks to months. For long term storage, keeping samples at -20°C is recommended. If possible, repeated freeze thawing should be avoided, since it may lead to a reduction of the overall size of the isolated gDNA.

Performance Data/Downstream Applications

The performance of Monarch purified genomic DNA from various sample types has been tested in several demanding downstream applications such as long-range PCR, qPCR, and Next Generation Sequencing (NGS). In all cases, Monarch-purified gDNA performed well. Additionally, Monarch-purified gDNA works well for long-read sequencing platforms (see “Compatibility with Long Read Sequencing Platforms”).

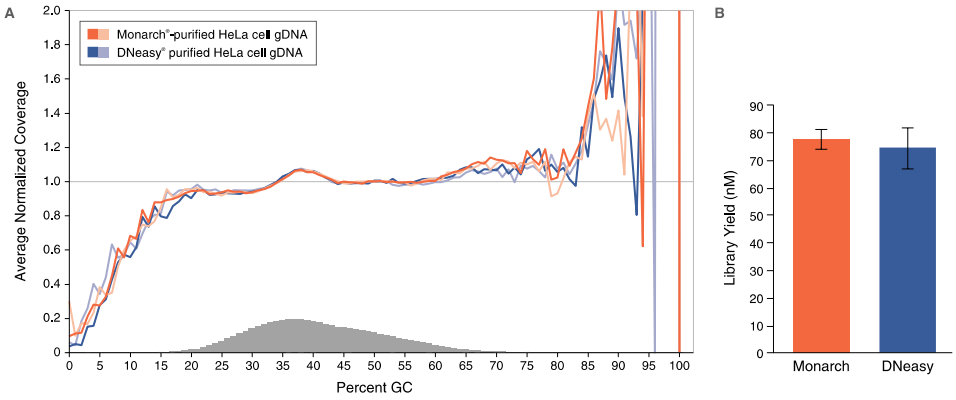
Figure 3: The Monarch Genomic DNA Purification Kit generates high quality genomic DNA suitable for sensitive applications like long range PCR and qPCR.



A. Amplification reactions were set up with primer pairs specific for 6, 8, 10, 12, 16, 20 kb amplicons from human DNA. LongAmp® Hot Start Taq 2X Master Mix (NEB #M0533) was used and 25 ng template DNA was added to each sample. PCR reactions were carried out on an Applied Biosystems® 2720 Thermal Cycler. Monarch-purified gDNA isolated from HeLa cells and human blood were compared to commercially available reference DNA from the human cell line NA19240 F11. 10 µl was loaded on a 1.5% agarose gel, using the 1 kb DNA Ladder (NEB #N3232) as a marker. Results indicated DNA was of high-integrity and suitable for long range PCR.

B. Monarch-purified gDNA from human whole blood, HeLa cells and mouse tail was diluted to produce a five log range of input template concentrations. The results were generated using primers targeting gHEME (human whole blood) and gREL (HeLa, mouse tail) for qPCR assays with the Luna® Universal qPCR Master Mix (NEB #M3003) and cycled on a Bio-Rad® CFX Touch qPCR thermal cycler. Results indicated that DNA is highly pure and free from inhibitors, optimal for qPCR.

Figure 4: The Monarch Genomic DNA Purification Kit generates excellent input material for NGS library preparation with NEBNext® kits for Illumina®.



A. Duplicate libraries were made from 100 ng HeLa cell gDNA purified with Monarch (orange) or Qiagen® DNeasy® Mini Kit (blue) using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (NEB #E7805). Libraries were sequenced on an Illumina MiSeq®. Reads were mapped using Bowtie 2.2.4 and GC coverage was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100 bp regions at each %GC is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library. Monarch GC coverage matched Qiagen DNeasy results.

B. High yield libraries are achieved from Monarch-purified gDNA. Library yields of the samples described above were assessed on an Agilent Technologies 2100 BioAnalyzer® using a High Sensitivity DNA Kit.

Choosing Input Amounts

The table included below shows recommended and maximal input amounts for the various sample types that can be processed with the Monarch Genomic DNA Purification Kit. Additionally, typical yields and DIN values are shown. Using input amounts that exceed the recommended amount will lead to a reduction of yield and purity in those samples. If more starting material is required, splitting the sample and processing on multiple columns is recommended. For low input amounts, the use of carrier RNA is recommended (See "Using Carrier RNA for Low Inputs").

The demands on the purification process for tissue samples vary highly between different sample types. Therefore, different input amounts are indicated for individual groups of tissues and additional guidelines are provided in the protocol ensuring that the best possible results are obtained for each tissue type.

SAMPLE TYPE	RECOMMENDED INPUT AMOUNT	TYPICAL YIELD (µg)	DIN	MAXIMUM INPUT AMOUNT
TISSUE*				
Tail (mouse)	10 mg	12–20	8.5–9.5	25 mg
Ear (mouse)	10 mg	18–21	8.5–9.5	10 mg
Liver (mouse and rat)	10 mg	15–30	8.5–9.5	15 mg
Kidney (mouse)	10 mg	10–25	8.5–9.5	10 mg
xSpleen (mouse)	10 mg	30–70	8.5–9.5	10 mg
Heart (mouse)	10 mg	9–10	8.5–9.5	25 mg
Lung (mouse)	10 mg	14–20	8.5–9.5	15 mg
Brain (mouse and rat)	10 mg	4–10	8.5–9.5	12 mg
Muscle (mouse and rat)	10 mg	4–7	8.5–9.5	25 mg
Muscle (deer)	10 mg	5	8.5–9.5	25 mg
BLOOD**				
Human (whole)	100 µl	2.5–4	8.5–9.5	100 µl
Mouse	100 µl	1–3	8.5–9.5	100 µl
Rabbit	100 µl	3–4	8.5–9.5	100 µl
Pig	100 µl	3.5–5	8.5–9.5	100 µl
Guinea pig	100 µl	3–8	8.5–9.5	100 µl
Cow	100 µl	2–3	8.5–9.5	100 µl
Horse	100 µl	4–7	8.5–9.5	100 µl
Dog	100 µl	2–4	8.5–9.5	100 µl
Chicken (nucleated)	10 µl	30–45	8.5–9.5	10 µl
CELLS				
HeLa	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
HEK293	1 x 10^6 cells	7–9	9.0–9.5	5 x 10^6 cells
NIH3T3	1 x 10^6 cells	6–7.5	9.0–9.5	5 x 10^6 cells
BACTERIA				
E. coli (Gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
Rhodobacter sp. (Gram-negative)	2 x 10^9 cells	6–10	8.5–9.0	2 x 10^9 cells
B. cereus (Gram-positive)	2 x 10^9 cells	6–9	8.5–9.0	2 x 10^9 cells
ARCHAEA				
T. kodakarensis	2 x 10^9 cells	3–5	8.5–9.0	2 x 10^9 cells
YEAST				
S. cerevisiae	5 x 10^7 cells	0.5–0.6	8.5–9.0	5 x 10^7 cells
SALIVA/BUCCAL CELLS***				
Saliva (human)	200 µl	2–3	7.0–8.0	500 µl
Buccal swab (human)	1 swab	5–7	6.0–7.0	1 swab

* Tissue gDNA yields are shown for frozen tissue powder, frozen tissue pieces and RNAlater-stabilized tissue pieces. Though frozen tissue powder results in highly-intact gDNA, lower yields can be expected than when using frozen or RNAlater-stabilized tissue pieces. Residual nuclease activity in tissue pieces will cut the gDNA, resulting in a slightly smaller overall size; however, this gDNA is optimal for silica-based purification.

** Human whole blood samples stabilized with various anticoagulants (e.g., EDTA, citrate and heparin) and various counter-ions were evaluated and results were comparable in all cases. Additionally, all indicated blood samples were tested both as fresh and frozen samples, yielding comparable results. Human samples were donated by healthy individuals; yields from unhealthy donors may differ.

*** Buccal swabs and saliva samples partially consist of dead cell material with degraded gDNA. Therefore, the purified gDNA from those samples will naturally have lower DIN values.

Guidelines for Handling Tissue Samples

In general, tissue samples should be processed immediately. If processing of the tissue samples is delayed for several hours, the quality of the isolated gDNA will be lower, particularly for metabolically active organ tissues. In many cases, tissue samples need to be stabilized before genomic DNA purification can be performed. Adequate sample storage can be carried out one of the following ways:

A) Flash frozen tissue samples are stored as whole pieces at -80°C.

B) Flash frozen tissue samples are pulverized under liquid nitrogen and subsequently stored at -80°C as tissue powder.

C) Tissue samples are incubated with stabilizing agents like RNAlater (Thermo Fisher Scientific®) to enable transport at room temperature or on ice, or to enable safe mid-term storage at 4°C or -20°C. Additionally, cutting and preparing aliquots of stabilized samples is significantly more convenient than using fresh or frozen samples.

Below is a list of recommendations for preparing tissue samples from each of the 3 options mentioned above.

Fresh and Frozen Tissue Pieces:

Keep fresh samples on ice and frozen samples frozen (e.g., by storing on dry ice). Label and pre-cool reaction tubes on ice or a cooling block.

- Do not use more tissue than recommended (See “Choosing Input Amounts”).

Fresh Tissue:

- Cut appropriately-sized tissue fragment into small pieces and weigh out the exact amount by transferring small tissue pieces into reaction tube positioned on a micro balance.
- Keep tubes cold and start lysis as soon as possible.

Frozen Tissue:

- Use a clean, frozen cooling block or the bottom side of a frozen metal reaction tube stand for cutting frozen tissue into smallest possible pieces. Samples are most easily cut when they are processed shortly before thawing.
- Weigh the desired amount by transferring small tissue pieces into a pre-chilled reaction tube positioned on a micro balance.
- Keep tubes frozen or on ice, and start lysis as soon as possible. In samples that have been frozen, ice crystals have destroyed cell structures and nucleases have free access to the genomic DNA. Work with the smallest possible tissue pieces to allow for a rapid inactivation of nucleases by Proteinase K. Make sure all tissue pieces are able to move freely in the lysis buffer before immediately starting lysis at 56°C.

Frozen Tissue Powder:

- Label and pre-cool reaction tubes on dry ice. Keep tubes containing tissue powder on dry-ice and use small pre-chilled scoops that allow for the transfer of 5 or 10 mg frozen tissue powder at a time. Tare pre-chilled tube on the micro-balance and transfer appropriate amount of frozen tissue powder to tube for weighing. Work quickly to prevent the tube from warming up on the balance. Keep the aliquoted samples on dry ice to ensure the powder stays frozen.
- When adding Proteinase K and Tissue Lysis Buffer, mix immediately so that the tissue powder is released from the tube wall and dispersed evenly over the lysis buffer. It is important to start lysis at 56°C immediately; add the reaction components to one tube, mix and place at 56°C immediately, then proceed with the next tube. Do not dispense Proteinase K and Tissue Lysis Buffer to all tubes at once.

Stabilized Tissue Samples

If stabilized sample was frozen, thaw first. Remove stabilizing solution from the outside of the tissue sample by blotting on a paper towel or other absorbent paper. Cut the tissue sample into small pieces and weigh the desired amount in a reaction tube (see “Choosing Input Amounts”). Keep tubes cold. Although rapid processing of the samples is recommended, it is not as critical as for fresh or frozen samples because of the presence of the stabilizing agent. Stabilized tissues contain proteins that have an altered fiber structure. These proteins are more difficult for Proteinase K to digest and a fraction of insoluble fiber will remain even if lysis is complete and the lysate looks mostly clear. Since these fibers will block the membrane binding sites when the lysate is spun through, centrifugation of the lysate before loading on the column is recommended for best yield and purity. This is particularly important for brain and fibrous tissue samples (e.g., muscle).

Factors Affecting Genomic DNA Quality

The integrity and length of genomic DNA isolated with silica column-based kits is highly dependent on the quality of starting material. Fresh starting material should result in DNA Integrity Numbers (DINs) of 8.5–9.5, with peak sizes of 50 to > 60 kb, as measured on an Agilent TapeStation. If isolated gDNA samples have lower DINs and peak sizes, it can usually be attributed to sample preparation and storage conditions. Exceptions do apply, for example, buccal swabs and saliva samples consists of dead cells that have undergone apoptotic degradation of the DNA content and will typically result in lower DINs.

The following samples types require special attention:

Whole Blood

The plasma of whole blood is extremely rich in nucleases. As long as the leukocytes in the blood remain intact, these nucleases will not damage the gDNA. When blood samples are stored at 4°C, over time, the leukocytes become increasingly unstable and undergo lysis resulting in the release and activation of nucleases, which will reduce the size of purified gDNA. Therefore, fresh blood samples should not be stored at 4–8°C for longer than a week. On the other hand, same-day blood samples can be more difficult to lyse and the purity of the gDNA from such samples may be less consistent. As such, it is advisable to store fresh blood samples at 4–8°C for 2–3 days before purification.

For archiving samples of whole blood, storage at -80°C is recommended. Frozen blood samples will give excellent quality gDNA but it is essential that samples are not thawed before the purification procedure. During the freezing process, ice crystals damage the leukocyte cell structures and any nucleases released during thawing can rapidly degrade the gDNA. However, if frozen samples are kept frozen during the addition of the lysis components and are then incubated immediately at 56°C, the stringent reaction conditions of the Blood Lysis Buffer will ensure the gDNA is protected and that large gDNA fragments are obtained.

Tissue Samples

Frozen tissue, previously ground to powder in liquid nitrogen, will be digested within minutes as the protein present in the sample is readily available to digestion by Proteinase K. This highly accessible form of input material will ensure that nucleases can rapidly be degraded, resulting in gDNA of high integrity. However, yields may be slightly lower when using powder as compared with tissue pieces since the intact gDNA may be more difficult to completely elute from the membrane due to the large fragment size.

Tissue pieces, whether frozen or stabilized, require a longer lysis time than tissue powder. It is best to cut tissue into very small pieces, as gDNA in large tissue pieces is prone to nuclease degradation; nucleases present in the interior of the pieces are protected from Proteinase K digestion, allowing them to shear nearby gDNA while the tissue piece slowly disintegrates. If the tissue samples are cut into small enough pieces, this effect is minimized and the yield and quality of gDNA will still be excellent.

Nuclease-rich Tissues

Metabolically-active tissues, often also referred to as soft organ tissues (e.g., liver, kidney, pancreas and intestine), have high nuclease content. Isolating high quality gDNA from such tissues tends to be more challenging than with other samples. However, if samples are stabilized and cut to small pieces (or are processed as frozen tissue powder), good yields can be obtained. Generally, results are best when the input amounts are lower.

Protocol for Extraction and Purification of Genomic DNA from Cells, Blood and Tissues

IMPORTANT NOTES BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Cold PBS (not supplied) is required for processing cultured cells
- Set a thermal mixer (e.g., ThermoMixer® or similar device), or a heating block to 56°C for sample lysis.
- Set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Genomic DNA Purification Consists of Two Stages:

PART 1: Sample Lysis

PART 2: Genomic DNA Binding and Elution

PART 1 SAMPLE LYSIS

Please follow the protocol specific to your starting material:

❑ Cultured Cells

1. **Start with a cell pellet containing 1 x 10^4 – 5 x 10^6 cells (typical starting amount is 1 x 10^6 cells).** If using lower cell inputs, the use of carrier RNA may be beneficial, see "Use of Carrier RNA for Low Input Amounts").
    - **Frozen cell pellets:** thaw pellet slowly on ice and loosen by flicking the tube several times. Add 100 µl cold PBS and resuspend by carefully pipetting up and down 5–10 times. Ensure pellet is resuspended completely.
    - **Fresh cells:** pellet cells by centrifugation at 1,000 x *g* for 1 minute. Remove supernatant and resuspend in 100 µl cold PBS by carefully pipetting up and down 5-10 times. Ensure pellet is resuspended completely.
2. **Add 1 µl Proteinase K and 3 µl RNase A to the resuspended pellet and mix by vortexing briefly to ensure the enzymes are efficiently dispersed.** Do not add the enzymes and the Cell Lysis Buffer simultaneously, as the high viscosity of the lysate will prevent equal distribution of the enzymes. Addition of RNase A can be omitted if a low percentage of co-purified RNA will not affect downstream applications. For greater convenience in pipetting, working aliquots of the Proteinase K stock can be diluted 5X. Determine how much Proteinase K you need for your preps and mix this amount of enzyme with 4 volumes of nuclease-free water or PBS. Do not use EDTA-containing buffers like TE. Add 5 µl of this 5X dilution to the resuspended cells and proceed as indicated above.
3. **Add 100 µl Cell Lysis Buffer and vortex immediately and thoroughly.** The solution will rapidly become viscous.
4. **Incubate for 5 minutes at 56°C in a thermal mixer with agitation at full speed (~1400 rpm**). If an incubator with agitation is not available, use a heating block and vortex once or twice during the incubation. Incubation for longer than 5 minutes is not necessary, but will not negatively affect the quality of the purified gDNA.
5. **Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.**

❑ Mammalian Whole Blood (non-nucleated)

1. **Transfer 100 µl of whole blood to a 1.5 ml microfuge tube.** If processing less than 100 µl of blood, add cold PBS to bring the total volume to 100 µl. For pre-aliquoted frozen samples, do not thaw; add Proteinase K, RNase A and Blood Lysis Buffer to the frozen sample in the following step.
2. **Add 10 µl Proteinase K, 3 µl RNase A and 100 µl of Blood Lysis Buffer to the sample**. **Mix immediately by vortexing.** For frozen samples, do not thaw; add enzymes and lysis buffer directly to frozen sample and proceed immediately to Step 3. When working with multiple samples, prepare a master mix of the three reagents to save pipetting steps. Addition of RNase A can be omitted if a low percentage of co-purified RNA will not affect downstream applications.
3. **Incubate for 5 minutes at 56°C in a thermal mixer with agitation at full speed (~1400 rpm).** If an incubator with agitation is not available, use a heating block and vortex once or twice during the incubation. A longer incubation will not negatively affect the quality of the purified gDNA. For some hemoglobin-rich samples (e.g. horse blood), longer incubation times can be beneficial. Other hemoglobin-rich samples (e.g. guinea pig) can form green precipitates during this incubation that stain and clog the silica membrane. In such cases, lysis time should be shortened to 3 minutes.
4. **Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.**

❑ Nucleated Red Blood Cells (birds, reptiles)

1. **Transfer 10 µl of whole blood to a 1.5 ml microfuge tube.**

2. **Add 90 µl cold PBS and mix by vortexing.**

3. **Add 10 µl Proteinase K and 3 µl RNase A, and mix again by vortexing.** Do not add the enzymes and the Blood Lysis Buffer simultaneously, as the high viscosity of the lysate will prevent equal distribution of the enzymes. Addition of RNase A is only necessary if a low percentage of co-purified RNA will affect downstream applications.

4. **Add 100 µl Blood Lysis Buffer and vortex thoroughly.** The solution will rapidly become viscous.

5. **Incubate for 5 minutes at 56°C in a thermal mixer with agitation at full speed (~1400 rpm).** If an incubator with agitation is not available, use a heating block and vortex once or twice during the incubation. A longer incubation will not negatively affect the quality of the purified gDNA.

6. **Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.**

❑ Animal Tissue

1. **Cut tissue into small pieces to ensure rapid lysis and high yields. Weigh the appropriate tissue amount and place in a 1.5 ml microfuge tube (see table below for recommended input amounts).** Using more than the recommended amounts will not lead to better yields and/or purity. If using more than recommended is required, split the sample into 2 or more preps. Ensure frozen material remains frozen until samples are mixed with lysis buffer and Proteinase K. Stabilized and fresh tissue should be kept cold or on ice during preparation. For more guidance, see “Choosing Input Amounts” and “Guidelines for Handling Tissue Samples”.

STARTING MATERIAL	RECOMMENDED INPUT AMOUNT
Rodent tail	Up to 25 mg
Brain	Up to 12 mg
Fibrous tissue (muscle, heart)	Up to 25 mg
Ear clips, skin	Up to 10 mg
Liver, lung	Up to 15 mg
Spleen, kidney	Up to 10 mg

2. **Add Proteinase K (according to the table below) and 200 µl of Tissue Lysis Buffer to each sample.** Mix immediately by vortexing. Ensure tissue particles are able to move freely in the lysis mix and do not stick to the bottom of the tube. When working with multiple samples, prepare a master mix of Tissue Lysis Buffer and Proteinase K to save pipetting steps.

TISSUE TYPE	PROTEINASE K AMOUNT
Brain, Kidney, Skin, Ear Clips	3 µl
All other tissues	10 µl

3. **Incubate at 56°C in a thermal mixer with agitation at full speed (1400 rpm) until tissue pieces have completely dissolved (typically 30-60 minutes).** If time is not limiting, additional incubation up to 3 hours can further improve yields and decrease residual RNA. If an incubator with agitation is not available, use a tube rotator placed within an incubator, shaking water bath or a heating block (vortex samples every 5-15 minutes to speed up lysis).

4. **Note: The following step can be omitted when working with fresh or frozen (non-stabilized) tissue amounts < 15 mg. Centrifuge for 3 minutes at maximum speed (> 12,000 x *g*) to pellet debris. Transfer the supernatant to a fresh microfuge tube.** This prevents residual debris from clogging the membrane binding sites and helps to reach maximal yield and purity. It is especially important to perform this step if sample appears turbid, contains residual particles, when working with stabilized tissue, or when working with brain or fibrous tissues.

5. **Add 3 µl of RNase A to the lysate, vortex thoroughly and incubate for a minimum of 5 minutes at 56°C with agitation at full speed.** This step can be skipped if a low percentage of co-purified RNA will not affect downstream applications.

6. **Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.**

PART 2 GENOMIC DNA BINDING AND ELUTION

1. **Add 400 µl gDNA Binding Buffer to the sample and mix thoroughly by pulse-vortexing for 5-10 seconds.** Thorough mixing is essential for optimal results.

2. **Transfer the lysate/binding buffer mix (~600 µl) to a gDNA Purification Column pre-inserted into a collection tube, without touching the upper column area.** Proceed immediately to Step 3. Do not reload the same column with more sample; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge. Avoid touching the upper column area with lysate/binding mix and avoid transferring foam that may have formed during lysis. Any material that touches the upper area of the column, including any foam, may lead to salt contamination in the eluate.

3. **Close the cap and centrifuge: first for 3 minutes at 1,000 x *g* to bind gDNA (no need to empty the collection tubes or remove from centrifuge) and then for 1 minute at maximum speed (> 12,000 x *g*) to clear the membrane. Discard the flow-through and the collection tube.** For optimal results, ensure that the spin column is placed in the centrifuge in the same orientation at each spin step (for example, always with the hinge pointing to the outside of the centrifuge); ensuring the liquid follows the same path through the membrane for binding and elution can slightly improve yield and consistency.

4. **Transfer column to a new collection tube and add 500 µl gDNA Wash Buffer. Close the cap and invert a few times so that the wash buffer reaches the cap. Centrifuge immediately for 1 minute at maximum speed and discard the flow through.** The collection tube can be tapped on a paper towel to remove any residual buffer before reusing it in the next step. Inverting the spin column with wash buffer prevents salt contamination in the eluate.

5. **Reinsert the column into the collection tube. Add 500 µl gDNA Wash Buffer and close the cap. Centrifuge immediately for 1 minute at maximum speed and discard the collection tube and flow through.**

6. **Place the gDNA Purification Column in a DNase-free 1.5 ml microfuge tube (not included). Add 35-100 µl preheated (60°C) gDNA Elution Buffer, close the cap and incubate at room temperature for 1 minute.** Elution in 100 µl is recommended, but smaller volumes can be used and will result in more concentrated DNA but a reduced yield (20–25% reduction when using 35 µl). Eluting with preheated elution buffer will increase yields by ~20–40% and eliminates the need for a second elution. For applications in which a high DNA concentration is required, using a small elution volume and then re-eluting with the eluate may increase yield (~10%). The elution buffer (10 mM Tris-Cl, pH 9.0, 0.1 mM EDTA) offers strong protection against enzymatic degradation and is optimal for long term storage of DNA. However, other low-salt buffers or nuclease-free water can be used if preferred. For more details on optimizing elution, please refer to “Considerations for Elution & Storage”.

7. **Centrifuge for 1 minute at maximum speed (> 12,000 x g) to elute the gDNA.**

Supplemental Protocols

Protocol for Genomic DNA Cleanup

There are two protocols provided for the cleanup of genomic DNA. The Desalting/Buffer Exchange Cleanup Protocol is for cleanup of salts and buffer components, including cleanup after extraction with phenol. The Enzymatic Cleanup Protocol should be employed if the removal of proteins and/or RNA is necessary.

BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Set a thermal mixer (e.g., ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- Set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Desalting/Buffer Exchange Cleanup Protocol

1. Add DNA sample to a 1.5 ml reaction tube and bring the volume up to 200 µl with nuclease-free water. Mix well by vortexing. If the total DNA input amount is less than 100 ng add 10 µg/ml of carrier RNA to the gDNA Binding Buffer for quantitative retrieval of the DNA (See "Use of Carrier RNA for Low Input Amounts").
2. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Enzymatic Cleanup Protocol (removal of proteins and/or RNA)

1. Add DNA sample to a 1.5 ml reaction tube and bring the volume up to 200 µl with Tissue Lysis Buffer. Mix well by vortexing.
2. Add 1 µl of Proteinase K and, if RNA needs to be removed, add 1 µl RNase A.
3. Mix briefly by vortexing and incubate at 56°C for 5 minutes.
4. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Genomic DNA Purification from Gram-negative Bacteria

Up to 2 x 10^9 Gram-negative bacteria can be processed using either a quick protocol which employs Lysozyme (not supplied) for bacterial cell wall lysis, or a longer protocol that does not require enzymatic lysis with Lysozyme. Both protocols are available below.

BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Cold PBS or 10 mM Tris-HCl pH 8.0 is required (not supplied).
- Set a thermal mixer (e.g., ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- For Lysozyme-based Lysis:
  - Set a thermal mixer or heating block to 37°C.
  - Prepare or thaw a stock solution of Lysozyme (not supplied) (25 mg/ml in water or 10 mM Tris-Cl, pH 8.0)
- To prepare for elution, set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Rapid Protocol (requires Lysozyme)

1. Harvest a maximum of up to 2 x 10^9 Gram-negative bacteria by centrifugation for 1 minute at > 12,000 x *g*. Discard supernatant.
2. Add 90 µl of cold PBS or 10 mM Tris-Cl pH 8.0 and resuspend bacterial pellet by vortexing or pipetting up and down.
3. Add 10 µl Lysozyme solution (25 mg/ml) and vortex briefly, then add 100 µl Tissue Lysis Buffer and vortex thoroughly.
4. Incubate at 37°C for 5 minutes or until clear. Most lysates will become fully clear, but for some bacteria a slight haze may remain.
5. Add 10 µl Proteinase K, vortex briefly, and incubate at 56°C for a minimum of 30 minutes in a thermal mixer with agitation at full speed.
6. Add 3 µl of RNase A to the lysate, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed (~1400 rpm).
7. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Simplified Protocol (no Lysozyme required)

1. Harvest a maximum of up to 2 x 10^9 Gram-negative bacteria by centrifugation for 1 minute at > 12,000 x *g*. Remove supernatant.
2. Add 100 µl of PBS or 10 mM Tris-Cl pH 8.0 and resuspend bacterial pellet by vortexing or pipetting up and down.
3. Add 10 µl Proteinase K and vortex briefly, then add 100 µl Tissue Lysis Buffer and vortex thoroughly.
4. Incubate at 56°C for 1–4 hours in a thermal mixer with agitation at full speed (~1400 rpm) until the lysate is mostly clear and ceases to change in appearance (lysis is usually complete within 2 hours).
5. Add 3 µl of RNase A to the lysate, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed.
6. Proceed to step 1 of Part 2: Genomic DNA Binding and Elution.

Genomic DNA Purification from Gram-positive Bacteria and Archaea

Up to 2 x 10^9 of Gram-positive bacterial cells can be processed with this protocol.

BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Cold PBS or 10 mM Tris-HCl pH 8.0 is required (not supplied).
- Set a thermal mixer (e.g. ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- Set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- For Lysozyme treatment:
  - Prepare or thaw a stock solution of Lysozyme (not supplied) (25 mg/ml in water or 10 mM Tris-Cl, pH 8.0).
  - Set a thermal mixer or heating block to 37°C.
  - Optional: if additional enzymes like lysostaphin are required for lysis of the bacteria that are used, make sure the respective enzyme is available and ready for use.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Protocol

1. Harvest up to 2 x 10^9 Gram-positive bacteria by centrifugation for 1 minute at > 12,000 rpm. Remove supernatant.
2. Add 80 µl of cold PBS or 10 mM Tris-Cl pH 8.0 and resuspend bacterial pellet by vortexing or pipetting up and down.
3. Add 20 µl Lysozyme solution (25 mg/ml) and vortex briefly, then add 100 µl Tissue Lysis Buffer and vortex thoroughly. Incubate at 37°C for 5–10 minutes or until clear. Other enzymes, like lysostaphin can also be tested if bacteria appear tough to lyse. Add enzymes to resuspended bacteria before adding Tissue Lysis Buffer.
4. Add 10 µl Proteinase K, vortex briefly, and incubate at 56°C for a minimum of 30 minutes in a thermal mixer with agitation at full speed.
5. Add 3 µl of RNase A to the lysate, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed.
6. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Genomic DNA Purification from Yeast

Up to 5 x 10^7 yeast cells can be processed with this protocol. Cell lysis can be accomplished either by enzymatic means or by mechanical disruption; both methods are outlined below. The following protocols have been validated on *S. cerevisiae* but have not been tested for other yeasts and fungi; however, they would be expected to work well.

BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- For Enzymatic Lysis:
  - Set a thermal mixer or heating block to 37°C for enzyme incubation.
  - Ensure that a suitable enzyme and corresponding buffer are available to break down the yeast glycan cell wall and form partial spheroplasts. The protocol describes the use of Zymolyase (we recommend R-Zymolyase from Zymo Research®, which includes RNase A), but other enzymes may be used as well (e.g., lyticase, chitinase or gluculase).
- For Mechanical Lysis
  - Cold PBS or 10 mM Tris-HCl pH 8.0 is required (not supplied).
  - The use of a bead mill is required. Please defer to manufacturer's instructions on bead size, amount and settings.
- Set a thermal mixer (e.g., ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- To prepare for elution, set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Enzymatic Lysis

1. Harvest up to 5 x 10^7 yeast cells by centrifugation for 1 minute at > 12,000 x *g*. Discard supernatant.
2. Add 100 µl of the lytic enzyme's digestion buffer and resuspend yeast cell pellet by vortexing or pipetting up and down.
3. Add lytic enzyme (according to manufacturer's protocol). If the lytic enzyme is not pre-mixed with RNase A, also add 3 µl RNase A.
4. Incubate for 30 min at 37°C or until spheroplast formation is complete.
5. Add 10 µl Proteinase K and vortex briefly.
6. Add 100 µl Tissue Lysis Buffer and vortex thoroughly.
7. Incubate at 56°C for a minimum of 30 minutes in a thermal mixer with agitation at full speed (~1400 rpm).
8. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Mechanical Lysis

1. Harvest up to 5 x 10^7 yeast cells by centrifugation for 1 minute at > 12,000 x *g*. Remove supernatant.
2. Resuspend pellet in 150 µl cold PBS or 10 mM Tris-Cl pH 8.0.
3. Add 150 µl Tissue Lysis Buffer and mix briefly by vortexing.
4. Transfer to a bead-mill tube containing the appropriate amount of beads. Disrupt at the appropriate settings (e.g. 30 seconds at position 6.0 in an MP Biomedical FastPrep®-24).
5. Transfer 200 µl of the homogenized cell lysate to a new tube, taking care not to carry over foam that may have formed.
6. Add 10 µl Proteinase K, vortex briefly, and incubate at 56°C for 30 minutes in a thermal mixer with agitation at full speed (~1400 rpm).
7. Add 3 µl of RNase A, vortex briefly, and incubate for a minimum of 5 minutes at 56°C with agitation at full speed (~1400 rpm).
8. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Genomic DNA Purification from Insects

BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Add 1/10th volume of 0.5 M EDTA to the Tissue Lysis Buffer.
- Set a thermal mixer (e.g., ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- Set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Protocol

1. Grind 10-20 mg of insect material with a microtube pestle.
2. Add 10 ul Proteinase K and 200 µl of the modified Tissue Lysis Buffer (containing EDTA) to each sample. Mix immediately by vortexing. Ensure tissue particles are able to move freely in the lysis mix and do not stick to the bottom of the tube. When working with multiple samples, prepare a master mix of Tissue Lysis Buffer and Proteinase K to save pipetting steps.
3. Incubate at 56°C in a thermal mixer with agitation at full speed (1400 rpm) until tissue pieces have completely dissolved (typically 30-60 minutes). If time is not limiting, additional incubation up to 3 hours can further improve yields and decrease residual RNA. If an incubator with agitation is not available, use a tube rotator placed within an incubator, shaking water bath or a heating block (vortex samples every 5-15 minutes to speed up lysis).
4. Centrifuge for 3 minutes at maximum speed (> 12,000 x *g*) to pellet debris. Transfer the supernatant to a fresh microfuge tube. This prevents residual debris from clogging the membrane binding sites and helps to reach maximal yield and purity. It is especially important to perform this step if sample appears turbid, contains residual particles, when working with stabilized tissue, or when working with brain or fibrous tissues.
5. Add 3 µl of RNase A to the lysate, vortex thoroughly and incubate for a minimum of 5 minutes at 56°C with agitation at full speed. This step can be skipped if a low percentage of co-purified RNA will not affect downstream applications.
6. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Genomic DNA Purification from Saliva

Up to 500 µl saliva can be processed using this kit. Please note that DNA Integrity Numbers (DIN) are typically low for saliva samples, as many dead cells are present in saliva and DNA may be partially degraded.

BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Set a thermal mixer (e.g., ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- Cold PBS (not supplied) and microcentrifuge pre-chilled to 4°C (if available).
- To prepare for elution, set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Protocol

1. Rinse mouth thoroughly with water at least 30 minutes before collection. Do not eat, drink or chew gum in the interim.
2. Collect up to 500 µl of saliva in a 15 ml tube (the typical volume of 1 donation is 200–500 µl) and work on ice.
3. Add 1 ml cold PBS and mix by vortexing. Keep cold and work on ice until lysis buffer is added. Transfer sample to a 1.5 ml reaction tube.
4. Centrifuge for 1 minute at 1000 x *g*, in a centrifuge pre-chilled to 4°C, if available.
5. Remove supernatant without touching pellet. Add 1 ml cold PBS, mix by vortexing briefly.
6. Centrifuge for 1 minute at 1000 x *g*, in a centrifuge pre-chilled to 4°C, if available.
7. Carefully remove most of the supernatant but leave ~100 µl PBS in the tube.

8. Vortex briefly to resuspend.
9. Add 10 µl Proteinase K and 3 µl RNase A, vortex briefly.
10. Add 100 µl Cell Lysis Buffer, vortex briefly and incubate for a minimum of 30 minutes at 56°C in a thermal mixer with agitation at maximum speed.
11. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Genomic DNA Purification from Buccal Swabs

BEFORE YOU BEGIN

- Store RNase A and Proteinase K at -20°C.
- Add ethanol (≥ 95%) to the Monarch gDNA Wash Buffer concentrate as indicated on the bottle label.
- Cold PBS (not supplied) is required.
- If available, a microcentrifuge pre-chilled to 4°C is recommended.
- Set a thermal mixer (e.g., ThermoMixer or similar device), or a heating block to 56°C for sample lysis.
- To prepare for elution, set a heating block to 60°C. Preheat the appropriate volume of elution buffer to 60°C (35–100 µl per sample). Confirm the temperature, as temperatures are often lower than indicated on the device.
- Do not load a single column with the lysed sample more than once; over-exposure of the matrix to the lysed sample can cause the membrane to expand and dislodge.

Protocol

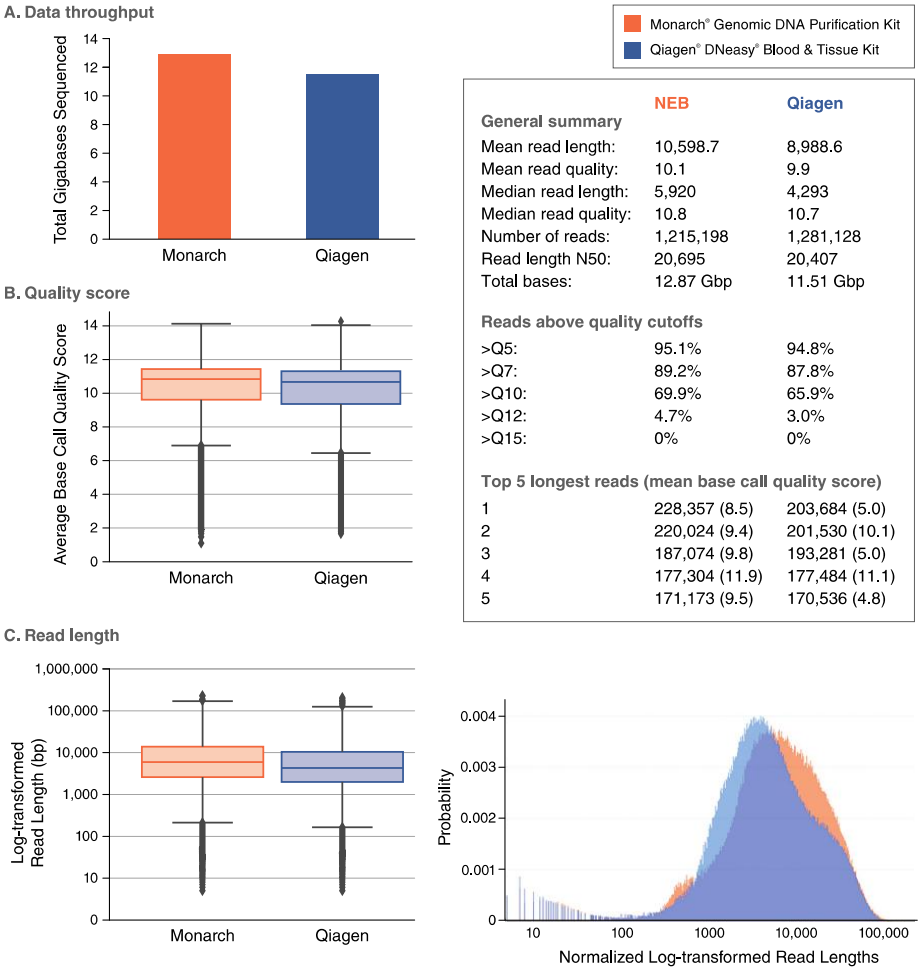
1. Add 1 ml cold PBS to 1.5 ml reaction tube.
2. Scrape inside of cheek with commercial collection device (according to manufacturer's recommendations).
3. Cut off / break off buccal swab cell collection piece and place in the tube with the PBS.
4. Vortex for 10 seconds to release cell material, then remove swab from the PBS.
5. Pellet cellular material by centrifugation for 30 seconds (2000 x *g*) at 4°C if possible.
6. With a pipette, remove most of the PBS, leaving ~100 µl of PBS behind with the cell pellet.
7. Resuspend the pellet by vortexing.
8. Add 10 µl Proteinase K and 3 µl RNase A, and vortex briefly.
9. Add 100 µl Cell Lysis Buffer, vortex briefly, then incubate at 56°C for a minimum of 30 minutes in a thermal mixer with agitation at maximum speed (~1400 rpm).
10. Proceed to Step 1 of Part 2: Genomic DNA Binding and Elution.

Appendices

Compatibility with Long Read Sequencing Platforms

High molecular weight gDNA is routinely isolated using this kit, allowing long sequencing reads to be generated on both the Pacific Biosciences® or Oxford Nanopore Technologies® platforms. Read length will be determined by sample prep reagents and protocols employed, such as sheared versus unsheared input material and vendor- versus user-derived protocols.

Figure 5: Monarch Genomic DNA Kit generates high quality DNA for nanopore sequencing

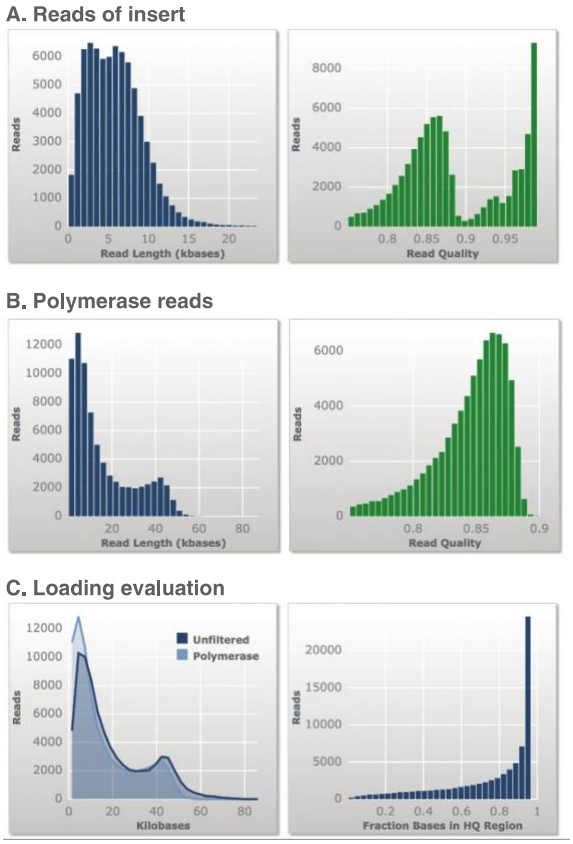


Monarch® Genomic DNA Purification Kit
Qiagen® DNeasy® Blood & Tissue Kit

	NEB	Qiagen
General summary		
Mean read length:	10,598.7	8,988.6
Mean read quality:	10.1	9.9
Median read length:	5,920	4,293
Median read quality:	10.8	10.7
Number of reads:	1,215,198	1,281,128
Read length N50:	20,695	20,407
Total bases:	12.87 Gbp	11.51 Gbp
Reads above quality cutoffs		
>Q5:	95.1%	94.8%
>Q7:	89.2%	87.8%
>Q10:	69.9%	65.9%
>Q12:	4.7%	3.0%
>Q15:	0%	0%
Top 5 longest reads (mean base call quality score)		
1	228,357 (8.5)	203,684 (5.0)
2	220,024 (9.4)	201,530 (10.1)
3	187,074 (9.8)	193,281 (5.0)
4	177,304 (11.9)	177,484 (11.1)
5	171,173 (9.5)	170,536 (4.8)

HeLa cell genomic DNA was extracted using either the Monarch Genomic DNA Purification Kit or the Qiagen DNeasy Blood & Tissue Kit. One microgram of purified DNA was used to prepare Oxford Nanopore Technology (ONT®) sequencing libraries following the ONT 1D Ligation Sequencing Kit (SQK-LSK109) protocol without DNA fragmentation. Libraries were loaded on a GridION® (Flow cell R9.4.1) and the data was collected for 48 hrs. Libraries produced using the Monarch gDNA exceeded the Qiagen libraries on common sequencing metrics including: A. total sequencing data collected, B. read quality, and C. read length. Data was generated using NanoComp (Bioinformatics, Volume 34, Issue 15, 1 August 2018, Pages 2666–2669).

Figure 6: Monarch provides excellent quality starting material for Pacific Biosciences (PacBio®) sequencing.



Bacterial gDNA was isolated from 5 x 10^9 E. coli ER2683 cells with pACYC184 plasmid by following the Monarch rapid protocol for Gram⁻ bacteria. 10 µg Monarch-purified gDNA was sheared with a Covaris® g-TUBE™, targeting 10 kb fragment size. 5 µg sheared DNA was used for library preparation according to the standard PacBio protocol (without size selection). Samples were sequenced on a Pacific Biosciences RSII sequencer, using the P6/C4 chemistry, loaded as 10 pM SMRTbell® library and data were collected in a 5 hr movie.

Total bases (yield): 1208 Mb, Average Polymerase Read Length: 16,046, Polymerase Read quality: 0.85. Average Reads of Insert length: 6,060, Reads of Insert quality: 0.89, Longest Read of Insert: 43,680.

A. Insert lengths indicate that the majority of the reads were in the desired insert size range.

B. The polymerase read lengths indicate that the purified DNA was of high quality to allow the enzyme to read the insert several times within the SMRTbell construct.

C. The loading evaluation data further illustrates the purified DNA is high quality.

Use of Carrier RNA for Low Input Amounts

Lower input amounts are easier to process in terms of lysis efficiency and purity of the sample. However, when yields fall below 100 ng (the equivalent of 1 x 10^4 cells), the relative efficiency of gDNA elution decreases. Therefore, when working with very low input amounts, the use of carrier RNA (10 µg/ml in the gDNA Binding Buffer) is recommended (e.g. Sigma-Aldrich® #GE27-4110-02). Prepare a stock solution of 1 µg/µl in Monarch gDNA Elution Buffer or Nuclease-free Water (NEB #B1500) and add 4 µl of the stock solution to each aliquot of 400 µl Binding Buffer before mixing with the lysate. If carrier-RNA is added, the RNase A digestion step should be omitted. The purified gDNA can be quantified by using qPCR-based methods.

DNA Quantitation

Before quantitation, samples should be briefly vortexed to ensure even distribution of the gDNA in the solution. This is particularly important for samples that have been frozen, where gDNA is unevenly distributed upon thawing. A short vortex will not shear gDNA. When measuring thawed samples, allow them to reach room temperature to enable consistent measurements. Spectrophotometric analysis of gDNA eluates can be used for assessing the quantity of the isolated gDNA by measuring the absorbance at 260 nm. Typically, modern micro volume spectrophotometers (e.g. Nanodrop®) automatically calculate the DNA concentration by multiplying the measured absorbance value with the conversion factor, which is 50 for DNA. Concentration measurements at 260 nm can be performed on most micro volume systems down to 1 ng/µl with acceptable accuracy. Below that concentration, the use of fluorescence measurement via Qubit® or similar detection systems is recommended. Please note that the $A_{260/230}$ and $A_{260/280}$ ratios are typically not reliable below 20 ng/µl.

Assessing DNA Purity

Purity of the DNA samples can be assessed using A_{260}/A_{280} and A_{260}/A_{230} ratios.

Samples that have A_{260}/A_{280} and A_{260}/A_{230} values > 1.8 can be considered to be pure. However, when working with gDNA that is below 20 ng/µl, these ratios are no longer reliable. There is also great variation in the ratios depending on the device used to measure them.

A_{260}/A_{280} Ratio

A_{260}/A_{280} values can be used as a general guide for overall purity. For sufficiently concentrated samples (> 20 ng/µl), the following guidelines can be used:

- Mammalian gDNA samples that are very clean will show ratios at or near 1.85–1.87. The range 1.80–1.90 is generally considered clean.
- Values in the range 1.90 to > 2.0 may indicate potential RNA contamination. The higher the value, the greater the contamination level.
- Values < 1.80 may indicate potential protein concentration. The lower the value, the greater the contamination. In cases where the contamination is significant, a shoulder may be observed in the absorbance spectrum around 280 nm. It should be noted that the A_{260}/A_{280} ratio is only a rough indicator of protein contamination; low levels will not be detected as they would if using the A_{260}/A_{230} ratios.

A_{260}/A_{230} Ratios

A_{260}/A_{230} values can be used as an indicator for overall purity. There are many substances that may influence this ratio, therefore, analysis of A_{260}/A_{230} values should be performed with care. Moreover, A_{260}/A_{230} values show a higher coefficient of variation than A_{260} concentration values and A_{260}/A_{280} ratios, and their accuracy diminishes with decreasing analyte concentrations, particularly when measuring dilute samples with DNA concentrations below 20 ng/µl.

For DNA samples > 20 ng/µl, the following guidelines can be used:

- Samples that are very clean will show ratios in the range of 2.20–2.50. The range between 1.80–2.50 is generally considered clean.
- The A_{260}/A_{230} ratio is a more sensitive indicator for protein contamination than the A_{260}/A_{280} ratio. Minor protein contamination will lead to lower A_{260}/A_{230} ratios (e.g. 1.60) but may have no significant effects on the A_{260}/A_{280} ratio.
- Salt contamination resulting from the chaotropic salt guanidine thiocyanate (GTC) will heavily affect this ratio because of its absorbance in the 230 nm range. Even with GTC contamination in the sub-millimolar range, the A_{260}/A_{230} ratio may be as low as 1.0. Meanwhile, the A_{260}/A_{280} ratio may not be affected. Typically, low amounts of GTC will not have an effect on downstream applications.
- The presence of any form of aromatic molecules (like commonly used non-ionic detergents) or molecules with double bonds, (e.g. EDTA) will lower the A_{260}/A_{230} ratio.
- Substances like silica fibers and polysaccharides may also lower the A_{260}/A_{230} ratio.
- Traces of undigested hemoglobin from blood samples will give a specific absorbance peak at 410 nm. However, if measurable amounts of hemoglobin are available in samples stemming from blood material, the A_{260}/A_{230} ratio will also be affected.

Assessing DNA Integrity

Integrity of the genomic DNA can be assessed by gel electrophoresis or using an Agilent Technologies TapeStation. For the former, load approximately 100 ng per sample on an 0.75% agarose gel and compare the size distribution to a suitable marker (e.g. Lambda DNA), either as full length DNA (NEB #N3011) or digested with HindIII (NEB #N3012). Typically, for intact gDNA, the majority of the gDNA signal will be larger than the upper band of the HindIII digest. For the latter, gDNA samples can be run on a TapeStation using a Genomic DNA ScreenTape. This system will provide information on the peak size of the gDNA and the overall DNA integrity via the DIN (DNA Integrity Number). High peak sizes (> 50 kb) and DINs > 8.5 indicate that the DNA is of high quality.

Troubleshooting

Low Yield

Cells:

- Frozen cell pellet was thawed and/or resuspended too abruptly
  - Thaw cell pellets slowly on ice and flick tube several times to release the pellet from the bottom of the tube. Be sure to use cold PBS for resuspension, and resuspend gently by pipetting up and down 5–10 times until a uniformly turbid cell suspension is obtained and the pellet is completely dissolved.

- Cell Lysis Buffer was added concurrently with enzymes
  - Add Proteinase K and RNase A to sample and mix well before adding the Cell Lysis Buffer, otherwise the high viscosity of the lysate will impede proper mixing of the enzymes.

Blood:

- Blood was thawed, allowing for DNase activity
  - Keep frozen blood samples frozen and add Proteinase K, RNase A and Blood Lysis Buffer directly to the frozen samples. Start lysis right away and let the samples thaw upon lysis incubation.

- Blood sample is too old
  - Fresh (unfrozen) whole blood should not be older than a week. Older samples will show a progressive amount of DNA degradation and loss of yield.

- Formation of hemoglobin precipitates
  - Digestion of whole blood samples from some animal species with high hemoglobin content (e.g. guinea pig) may lead to the accumulation of insoluble hemoglobin complexes that stain and clog the membrane, leading to reduced yield and purity. Reduce Proteinase K lysis time from 5 to 3 minutes to prevent the formation of these precipitates.

Tissue:

- Tissue pieces are too large:
  - Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will destroy the DNA before the Proteinase K can lyse the tissue and release the DNA.

- Membrane is clogged with tissue fibers:
  - Proteinase K digestion of fibrous tissues (e.g. muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers that often gives the lysate a turbid appearance. These fibers will block the binding sites of the silica membrane reducing yield and causing protein contamination. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material, otherwise the fiber removal will not be complete.

- Sample was not stored properly:
  - Samples that are stored for long periods of time at room temperature, 4°C or -20°C will show degradation and loss of the gDNA content over time. Flash freeze tissue samples with liquid nitrogen or dry ice and store them at -80°C. Alternatively, use stabilizing reagents to protect the gDNA and enable storage for longer periods of time at 4°C or -20°C.

- Genomic DNA was degraded (common in DNase-rich tissues):
  - Organ tissues like pancreas, intestine, kidney and liver contain significant amounts of nucleases. They should be treated with extreme care and stored properly to prevent DNA degradation. Keep frozen and on ice during sample preparation. Refer to the protocol for the recommended amount of starting material and Proteinase K to use.

- Column is overloaded with DNA:
  - Some organ tissues (e.g. spleen, kidney, liver) are extremely rich in genomic DNA. Attempting to process quantities larger than the recommended input amounts will result in the formation of clouds of tangled, long-fragment gDNA that cannot be eluted from the silica membrane. Reduce the amount of input material to get a higher yield (see “Choosing Input Amounts”).

- Incorrect amount of Proteinase K added:
  - Most samples are digested with 10 µl Proteinase K, but for brain, kidney and ear clips, using 3 µl will provide better yields.

DNA Degradation

Tissue:

- Sample was not stored properly:
  - Samples that are stored for long periods of time at room temperature, 4°C or -20°C will show degradation and loss of the gDNA content over time. Shock freeze tissue samples with liquid nitrogen or dry ice and store them at -80°C. Alternatively, use stabilizing reagents such as RNAlater to protect the gDNA and enable storage for longer periods of time at 4°C or -20°C.

- Tissue pieces are too large:
  - Cut starting material to the smallest possible pieces or grind with liquid nitrogen. In large tissue pieces, nucleases will degrade the DNA before the Proteinase K can lyse the tissue and release the DNA.

- High DNase content of soft organ tissue:
  - Organ tissues like pancreas, intestine, kidney and liver have a very high nuclease content. They should be treated with extreme care (see ‘Sample was not stored properly’ section above) to prevent DNA degradation. Keep frozen and on ice during sample preparation.

Blood:

- Blood sample is too old:
  - Fresh (unfrozen) whole blood should not be older than a week. Older samples will show a progressive amount of DNA degradation and loss of yield.

- Blood sample was thawed, allowing for DNase activity:
  - Thawing frozen blood samples releases DNase, causing degradation. Keep frozen blood samples frozen and add enzymes and lysis buffer directly to the frozen samples. Start lysis right away and let the samples thaw upon lysis incubation.

Salt Contamination

- Guanidine salt was carried over into the eluate:

  The binding buffer contains guanidine thiocyanate (GTC), which shows a very strong absorbance at 220–230 nm. The most common way that salt is introduced into the eluate is by allowing the buffer/lysate mixture to contact the upper column area. To prevent this:
  - When transferring the lysate/binding buffer mix, avoid touching the upper column area with the pipet tip; always pipet carefully onto the silica membrane.
  - Avoid transferring any foam that may have been present in the lysate; foam can enter into the cap area of the silica spin column.
  - Take care to close the caps gently to avoid splashing the mixture into the upper cap area.
  - Do not move the samples too abruptly when transferring in and out of the centrifuge.

    If salt contamination is a concern, invert the columns a few times with gDNA Wash Buffer as indicated in the protocol.

Protein Contamination

Tissue:

- Incomplete digestion of the tissue sample:
  - Cut samples to the smallest possible pieces for rapid and efficient lysis. Allow the sample to remain in the lysis buffer for an extra 30 minutes to 3 hours after dissolving so that any remaining protein complexes are degraded and can be more easily removed during binding and washing.

- Membrane is clogged with tissue fibers:
  - Proteinase K digestion of fibrous tissues (e.g. muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues lead to the release of small, indigestible protein fibers that often give the lysate a turbid appearance. These fibers will block the binding sites of the silica membrane reducing yield and causing protein contamination. To remove fibers, centrifuge the lysate at maximum speed for 3 minutes as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material, otherwise the fiber removal will not be complete.

Blood:

- High hemoglobin content:
  - Some blood samples (e.g. horse) are rich in hemoglobin, evidenced by their dark red color. On occasion, these samples will still appear red after the 5-minute lysis incubation (when in fact, they should be green). Extend lysis time by 3–5 minutes for best purity results.

- Formation of hemoglobin precipitates:
  - Digestion of whole blood samples from some animal species with high hemoglobin content (e.g. guinea pig) may lead to the accumulation of insoluble hemoglobin complexes that stain and clog the membrane, leading to reduced yield and purity. Reduce Proteinase K lysis time from 5 to 3 minutes to prevent the formation of these precipitates.

RNA Contamination

Tissue:

- Too much input material:
  - DNA-rich tissues (e.g. soft organ tissue such as spleen, liver and kidney) will become very viscous during lysis and this may inhibit RNase A activity. Do not use more than the recommended input amount.

- Lysis time was insufficient:
  - Tissue samples benefit from extending the lysis time by 30 minutes to 3 hours after the tissue piece has completely dissolved. Not only may a slightly higher yield be expected, additionally, the efficiency of the subsequent RNase A digestion is significantly higher.

Tissue Digestion Takes Too Long

- Tissue pieces too large:
  - Cut tissue pieces to the smallest possible size or grind with liquid nitrogen before starting lysis.

- Tissue pieces are stuck to bottom of tube:
  - Vortex to release pieces from the tube bottom. Vortex immediately after adding Proteinase K and Tissue Lysis Buffer to the tissue sample. Make sure that all tissue pieces can float freely.

- Too much starting material:
  - Use input amount indicated in the protocol for best results.

Tissue Lysate Appears Turbid

- Formation of indigestible fibers:
  - Proteinase K digestion of fibrous tissues (e.g. muscle, heart, skin, ear clips), brain tissue and all RNAlater-stabilized tissues leads to the release of small indigestible protein fibers that often gives the lysate a turbid appearance. These fibers will block the binding sites of the silica membrane reducing yield and causing protein contamination. To remove fibers, centrifuge lysate at maximum speed for 3 minutes, as indicated in the protocol. For ear clips and brain tissue, use no more than 12–15 mg input material, otherwise the fiber removal will not be complete.

Ratio A_{260}/A_{230} > 2.5

- Slight variations in EDTA concentration in eluates:
  - If the EDTA available in the elution buffer complexes with magnesium or calcium cations, which may be associated with the isolated genomic DNA in small amounts, this will lead to small differences in the free EDTA concentration in the eluate. At NEB, we have observed EDTA has a strong influence on the 230 nm absorbance and a minute concentration reduction of free EDTA may lead to a higher than usual A_{260}/A_{230} ratio. In some cases, this ratio exceeds a value of 3.0 and is consistent with highly pure samples. In these cases, the elevated value does not have any negative effect on downstream applications.

Ordering Information

PRODUCT	NEB #	SIZE
Monarch Genomic DNA Purification Kit	T3010S/L	50/150 preps
Monarch gDNA Purification Columns	T3017L	100 columns + 200 collection tubes
Monarch Collection Tubes II	T2018L	100 collection tubes
Monarch gDNA Tissue Lysis Buffer	T3011L	34 ml
Monarch gDNA Cell Lysis Buffer	T3012L	20 ml
Monarch gDNA Blood Lysis Buffer	T3013L	20 ml
Monarch gDNA Binding Buffer	T3014L	65 ml
Monarch gDNA Wash Buffer	T3015L	60 ml
Monarch gDNA Elution Buffer	T3016L	34 ml
Monarch RNase A	T3018L	1 ml
Proteinase K, Molecular Biology Grade	P8107S	2 ml

Revision History

Revision #	Description	DATE
1.0	N/A	
2.0	Updated guidance for wash step.	7/19
2.1	Adjusted table page 7.	10/19
3.0	Added insect protocol. Added note to prevent double-loading of columns.	2/20
3.1	Updated hyperlink in Kit Components.	10/23
4.0	Updated kit components table with new component number. Updated formatting, header and footer. Updated legal.	3/24

How to Recycle Monarch Kit Components*

Component	Recycling Notes**
Kit Box (paper)	For the greatest environmental benefit, please reuse this box. It is fully recyclable in paper recycling. The small magnets do not prohibit recycling.
Columns and Collection Tubes (hard plastic)	Columns and collection tubes are made from polypropylene 5 and are recyclable. After use, please refer to your institutional policies for proper disposal, especially when working with biohazardous materials.
Plastic Bottles (hard plastic)	Bottles are made from high-density polyethylene 2, and caps are polypropylene 5. Please rinse before recycling.
Plastic Bags (plastic film)	Bags are made from low-density polyethylene 4 and can be recycled with other plastic bags and films.
Protocol Card (paper)	Recycle with mixed paper, or keep in your lab notebook for reference. The finish on this card does not prohibit recycling.

* Information as of November 2015. Please visit NEBMonarchPackaging.com for updates.
** Please defer to your institutional policies for proper disposal of this kit and its components.
Consult with your local and institutional authorities to learn how to maximize your landfill diversion and materials recovery.

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