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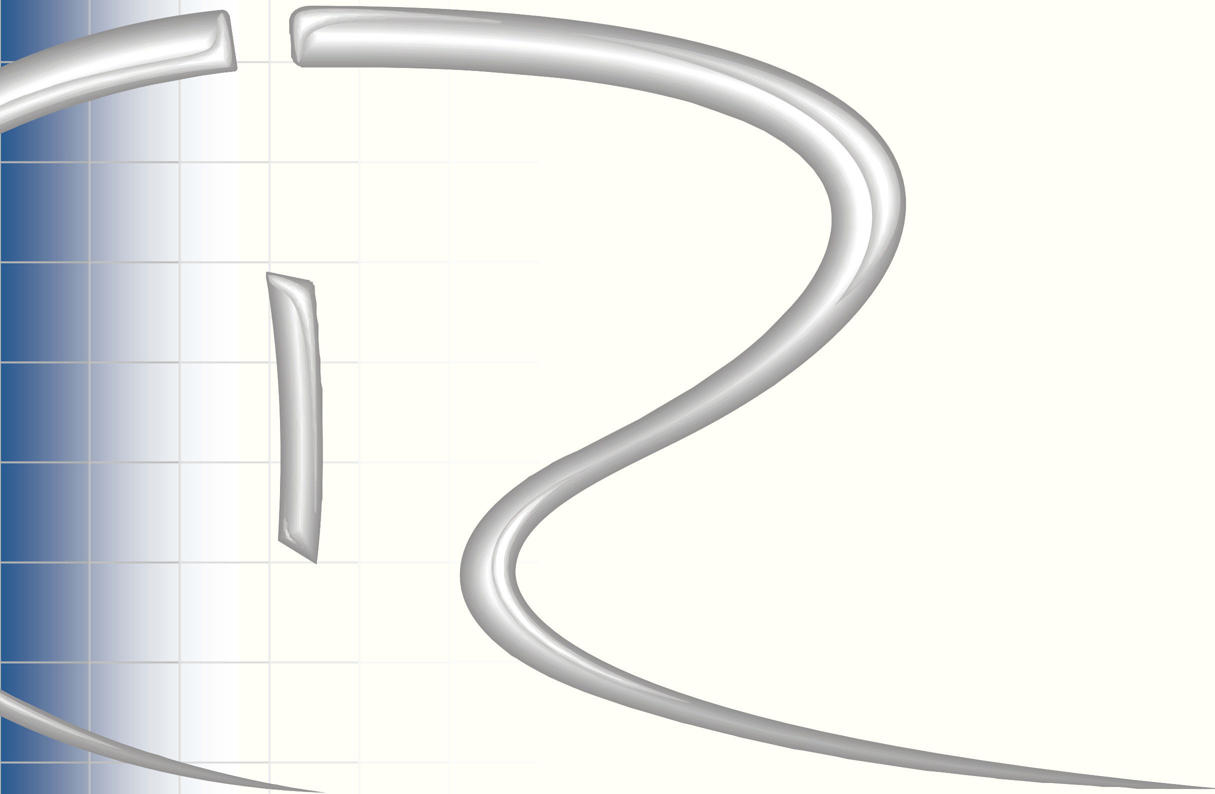

Genomic DNA Extraction Protocol with MACHEREY-NAGEL NucleoSpin® 96 Tissue kit

Cat#740 740.X (8 Well Strip System)

Cat#740 741.X (96 Well Plate)

X-tractor Gene™

MN
MACHEREY-NAGEL

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Protocol Description

Introduction
The genomic DNA Extraction Protocol described here is designed for walk-away automated preparation of DNA from a variety of tissue derived samples (for example mouse tails, mouse embryos, tissue, cultured cells, etc). Final extracted DNA is of high quality and suitable for a wide variety of downstream applications.

Purification kits are available in 96-well plates (**NucleoSpin® 96 Tissue**) or in a flexible 8-well strip format **NucleoSpin® 8 Tissue**).

With the **NucleoSpin® 8/96 Tissue** method, genomic DNA is isolated after lysis achieved by incubation of the samples in a solution containing SDS and Proteinase K. Appropriate conditions for binding of DNA to the silica membrane in the NucleoSpin® 8/96 Tissue columns are created by addition of large amounts of chaotropic salt (buffer BQ1) and ethanol to the lysate. The binding process is reversible and specific to nucleic acids. Contaminations are removed by washing with two different buffers. Pure genomic DNA is finally eluted under low ionic strength conditions in a slightly alkaline elution buffer.

Sample size
For trouble-free operation, samples must be as consistent as possible. After lysis of tissue a lysate free of particulates and debris is ideal for further processing. In addition, processing too much sample will cause the silica matrix to block and the extraction to fail. The protocol is suitable for up to 20 mg of tissue (eg. mouse tail clippings (2 x 0.5 cm) or up to 2 x10^6 cells).

The **NucleoSpin® 8/96 Tissue** kit provides reagents and consumables for purification of up to 40 µg (maximum binding capacity of each column) of pure genomic DNA from up to 20 mg tissue samples with an $A_{260/280}$ ratio between 1.80 and 1.90 and a typical concentration of 100-200 ng/µL.

Typical results:

Sample	Sample amount	Typical yields
Mouse tails	20 mg	7.5-10 µg
Pork liver	20 mg	6-10 µg

Processing time
Total time required to complete the DNA extraction procedure depends on the number of samples processed. Following lysis incubation typically, a single column of 8 samples requires 45 minutes to complete. Each additional column of 8 samples adds a further 5 minutes to the total processing time. Thus a full 96-well plate requires about 95 minutes to complete.

IMPORTANT

Wear gloves and a laboratory coat throughout procedure.

Take care to avoid cross-contamination of samples and reagents.

Make sure reagent tubs, tubes and plates are clearly labelled and clean.

Protocol Validation

Verification Testing
Genomic DNA extraction protocol was functionally tested on the Corbett Robotics X-tractor Gene™ Automated Extraction System using **NucleoSpin® 96 Tissue** kit provided reagents and consumables. Typical results for the extraction of genomic DNA from mouse tails are shown below. Actual results will vary depending upon sample age, quality, type, and species of subject.

Samples
Mouse tail clippings of 0.5 cm / sample were used for each extraction. For investigation of reproducibility and consistency of extraction lysates were combined, mixed and split following the lysis incubation in order to get a homogenous lysate.

Cross Contamination Test
To maximise the detection of any potential contamination event, 48 positive and 48 no template controls (NTCs) were arranged in alternating wells (in a "checkerboard" pattern – see *fig. 1*). PCR analysis was done on NTCs targeting the *Mus musculus* cytoplasmic aconitase exon (*acol*) gene in a 40 cycle PCR reaction as well as in a LightCycler™ PCR using SYBR green™ detection. No amplification of NTCs was observed indicating no cross-contamination (see fig. 2 and fig. 3).

	1	2	3	4	5	6	7	8	9	10	11
A											
B											
C											
D											
E											
F											
G											
H											

Sample**(+)**

NTC**(-)**

Fig.1 Illustration of the 'Checkerboard Pattern' utilised for the cross contamination analysis test.

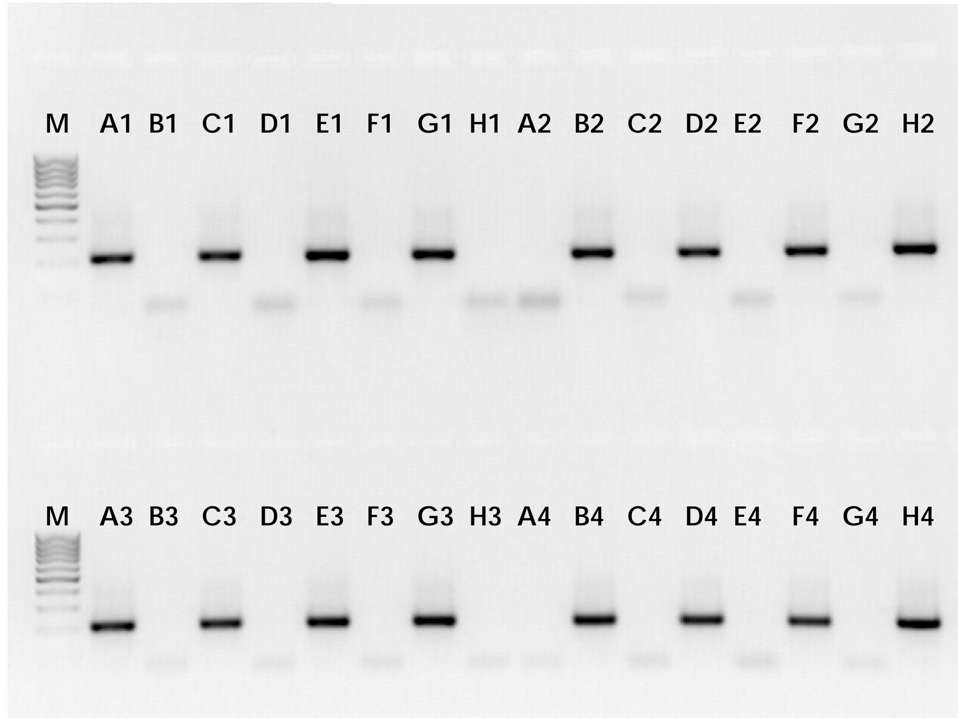


Fig. 2. Cross-contamination analysis. 2 µL of indicated samples and NTCs were analysed by 40 cycle PCR targeting the *Mus musculus* cytoplasmic aconitase exon (*acol*) gene. Lacking the expected 212 bp PCR fragment was observed in eluates derived from NTCs.

Yield Reproducibility

Yield, purity and reproducibility from all samples was measured by UV spectroscopy (see next page). Furthermore, aliquots of the purified DNA was analysed by real time PCR using SYBR green™ detection. Results are summarized as follows:

Reproducibility of DNA yield from 16 randomly selected out 48 samples was measured as threshold cycle (C_T) values from real-time PCR analysis. Sample DNA aliquots were amplified in a LightCycler™ instrument system using SYBR green™ chemistry. Results (Fig. 3) are summarised as follows:

Maximum C_T	26.51
Minimum C_T	26.90
Average C_T	26.71
C_T Std. Deviation	0.44

PCR Inhibitor Test

Reproducibility of real-time PCR C_T values and end-point product yield analysis showed no evidence of PCR inhibitors in any of the extracted DNA samples.

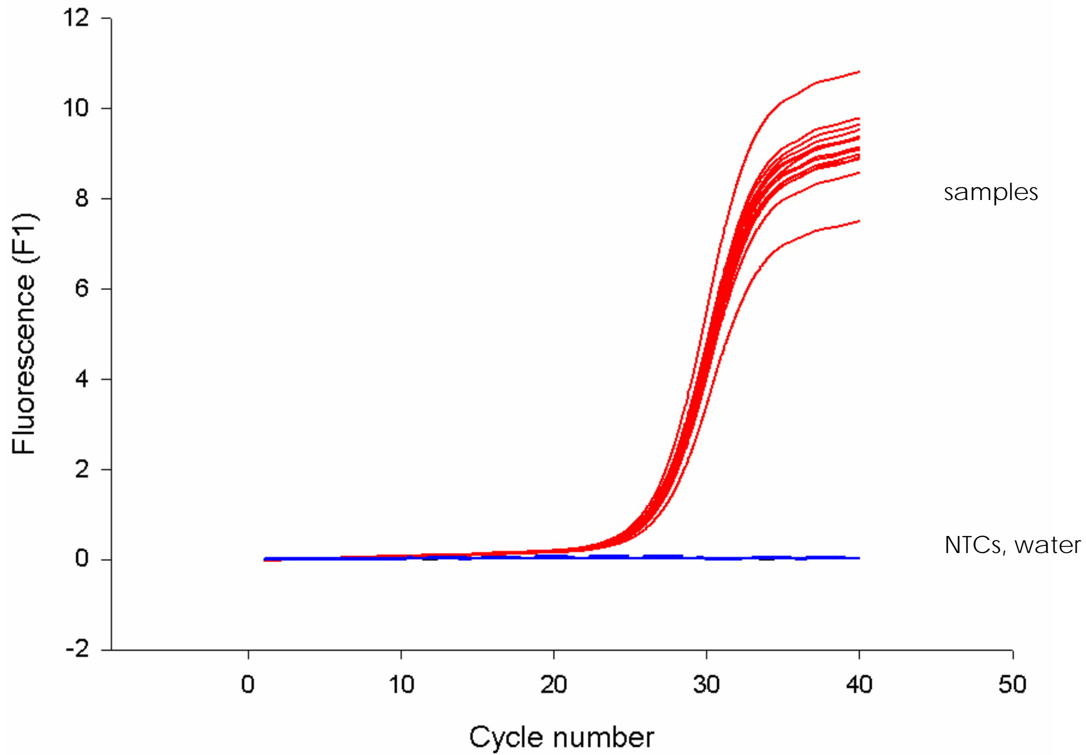


Fig. 3 Real-time PCR analysis of DNA extracted from mouse tails. *16 randomly selected samples were analysed on a LightCycler™ real-time PCR instrument system using primers targeting the Mus musculus cytoplasmic aconitase exon (acol) gene over 40 PCR cycles. Results show strong and reproducible amplification, no evidence of PCR inhibitors.*

Spectrophotometer Analysis

All 48 positive samples were analysed for yield and purity (Fig. 3, Fig. 4). Results are summarized below.

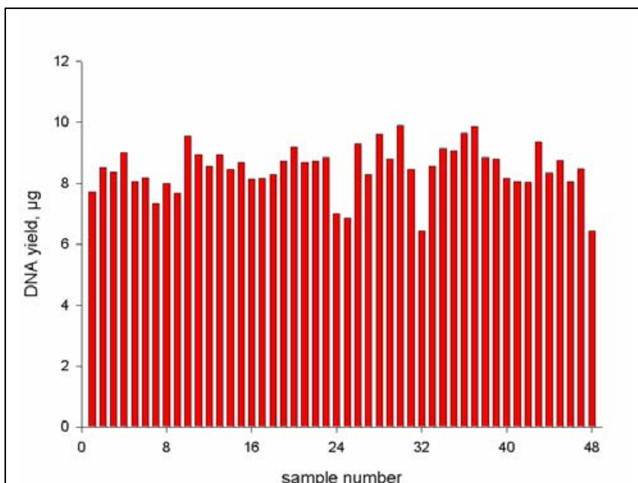
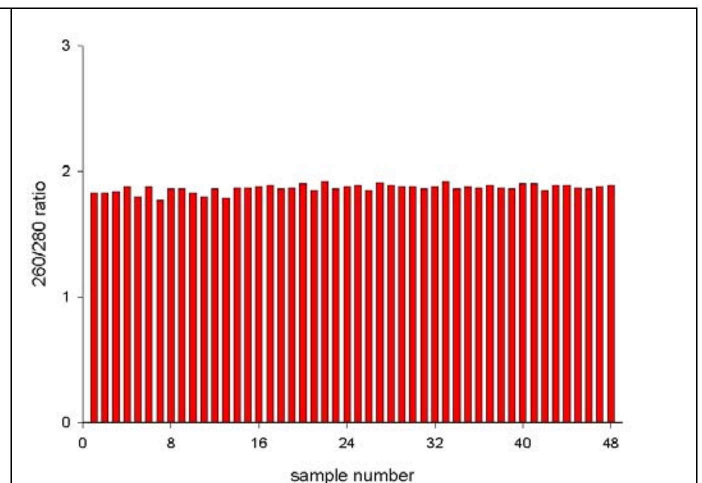
	

Fig. 4 Example DNA yield (µg/20 mg mouse tail tissue)	**Fig 5. Example 260/280 ratios**

Samples 1-48			
	Yield	Concentration	260/280 Ratio
Maximum	9.15 µg	51 ng/µL	1.90
Minimum	6.44 µg	36 ng/µL	1.83
Average	8.48 µg	47 ng/µL	1.87
Standard Deviation	0.7 µg	3.8 ng/µL	0.03

Agarose Gel Electrophoresis Analysis
Agarose gel electrophoresis of raw extracted product (Fig. 6) showed reproducible yields of high quality, intact high molecular weight DNA (>20 kb).

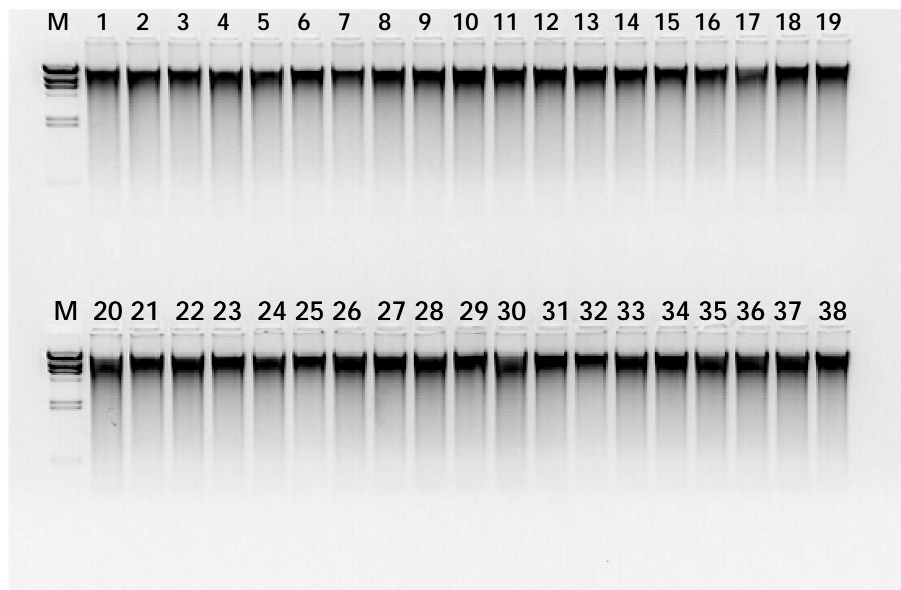


Fig. 6. Agarose gel electrophoresis results for genomic DNA extractions from mouse tails.
10 µL aliquots from the 180 µL total recovered eluate of DNA extract obtained after extraction representing 20 mg of mouse tail tissue were loaded on an agarose gel Electrophoresis: 1.0% agarose gel, 1× TAE running buffer, ethidium bromide stain.
M: Marker λHindIII.

Reagents and Consumables Required

<table>
<tr><th colspan="2">Item</th><th>Requirement for a Full 96-Well or 48 Well Extraction</th><th>Part No.
or Part of (MN)</th><th>Supplier</th></tr>
<tr><td colspan="5">Consumables</td></tr>
<tr><td colspan="2">NucleoSpin® Tissue Binding Plate[1]
(for 96 well plate extractions)</td><td>1 Plate</td><td>740 741.2[2]</td><td rowspan="6">Local MN Supplier</td></tr>
<tr><td colspan="2">NucleoSpin® Starter Set A[3] (Accessory for 8 Strip system)</td><td>1 Column Holder</td><td>740 682</td></tr>
<tr><td colspan="2">NucleoSpin® Tissue Binding Strips (for 8 strip extractions)</td><td>6 strips</td><td>740 740[2]</td></tr>
<tr><td colspan="2">MN Square-well Block[1]</td><td>1 Plate</td><td>740 741.2</td></tr>
<tr><td colspan="2">Round-well Block[1]</td><td>1 Plate</td><td>740 741.2</td></tr>
<tr><td colspan="2">MN Tube Strip Rack[1]</td><td>1 Rack</td><td>740 741.2</td></tr>
<tr><td colspan="2">200 µL Filtered Fine Bore Tips in Robotic Rack sterile</td><td>2 Racks</td><td>2097</td><td rowspan="8">Corbett Office or Distributor</td></tr>
<tr><td colspan="2">Elution Plate with 0.65 mL Cluster Tubes[4]</td><td>1 Plate</td><td>2147</td></tr>
<tr><td colspan="2">Elution Plate Strip Caps (1 bag contains 12 strips of 8) [4]</td><td>1 Bag</td><td>1636</td></tr>
<tr><td rowspan="2">Reagent Tubs</td><td>70 mL Tub</td><td>2 Tubs</td><td>2137 (Disposable)
(2365 Reusable)</td></tr>
<tr><td>170 mL Tub</td><td>2 Tubs</td><td>2136 (Disposable)
(2364 Reusable)</td></tr>
<tr><td rowspan="2">Reagent Tub Lids</td><td>70 mL Tub Lid</td><td>2 Lids</td><td>2505 (For use with disposable tubs)
(2416 Reusable Tubs)</td></tr>
<tr><td>170 mL Tub Lid</td><td>3 Lids</td><td>2504 (For use with disposable tubs)
(2415 Reusable Tubs)</td></tr>
<tr><td colspan="2">Self adhesive PCR/Elisa plate Plastic Sealing Film
(for sealing unused wells of capture plate)</td><td>1 Sheet</td><td>2411</td></tr>
</table>

[1] Part of NucleoSpin® 96 Tissue kit.
[2] Kit is available for different number of preparations. See ordering information of kit protocol.
[3] Required only when using 8 well strips.
[4] not required when using MN Tube Strip Rack supplied with the NucleoSpin® 8/96 Tissue kits.
[4] not required when using MN Tube Strip Rack supplied with the NucleoSpin® 8/96 Tissue kits.

Kits and Reagents		Part No:	Supplier
NucleoSpin® 96 Tissue kit (including buffers, Lysis Block, MN Tube Strips for collection of eluted DNA) Sufficient for 1 x 96, 4 x 96 or 24 x 96 preps	1 kit	740 741.2 740 741.4 740 741.24	MN
NucleoSpin® 8 Tissue kit (including buffers, MN Tube Strips for collection of eluted DNA) Sufficient for 12 x 8 preps or 60 x 8 preps NB: MN Accessory Starter Set A required to use 8-strip System (P/N 740 682)	1 kit	740 740 740 740.5	
Additional reagents to be ordered separately if required			
Lysis buffer T1	25 mL	740940.25	
Buffer BQ1	125 mL	740 923	
Buffer BW	500 mL	740 922.500	
Buffer B5 (concentrate for 500 mL)	100 mL	740 921.100	

Accessories Required

Part No.	Corbett Robotics Description
1675	High Skirt Transfer Carriage
1697 1696	96-well Separator Plate 8-Strip Separator Plate NB: MN Accessory Starter Set A required to use 8-strip system (P/N 740 682)
2443	Elution Riser Block (16.25 mm)[1] Optional not required when using MN tube strip rack supplied with kit. For Corbett Robotics elution tubes only.
2139	Reagent Tub SBS Base plate

[1] Not required when using MN Tube Strip racks supplied with NucleoSpin® 8/96 Tissue kit

Reagent Handling and Storage

HAZARD INFORMATION

Buffers BQ1, BW contain guanidine hydrochloride, alcohols and detergents. Always wear a laboratory coat, disposable gloves, and eye protection when handling solutions containing these chemicals.

Lyophilize Proteinase K: avoid skin contact.
Do not add bleach or acidic solutions directly to solutions containing guanidine or extraction waste.
Guanidine forms reactive compounds and toxic gases when mixed with bleach or acids.
For any items contaminated with these buffers, clean with suitable laboratory detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions
For details refer to the MSDS (material safety data sheet) information available at the following web site:

www.mn-net.com

Reagent storage
Upon receipt of reagents, unpack and store the individual reagents as follows:

Reagent	Store Temp	Storage State
Buffer T1	18-24°C	Not critical
Buffer BQ1	18–24° C	Dark
Buffer BW	18–24° C	Keep bottle tightly closed
Buffer B5	18–24° C	Keep bottle tightly closed
Buffer BE	18–24° C	Not critical
Buffer PB	18–24° C	Not critical
Proteinase K lyophilized or dissolved in buffer PB	4° C	Not critical

Reagent Preparation

Prior To Each Run
Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and re-dissolve any precipitates by warming to 37° C until dissolved. Swirl gently to avoid foaming.

Identifying Required Reagent Volumes For Your Run
The robotics software will calculate for you the exact required volume of each reagent once you have selected the number of columns you will be extracting from.
The X-tractor Gene™ software will display the required volume of each reagent (inclusive of each reagent tub's allocated dead volume) in a hover box when you place the computer's mouse cursor over the reagents designated position.

Proteinase K working solution
Dissolve Proteinase K with buffer PB as indicated on label.

Lysis Reagent
For each sample mix 200 µL of Buffer T1 with 25 µL resuspended Proteinase K solution. Use lysis reagent immediately after preparation. Prepare a small excess of buffer to compensate dead volume of reservoir.
Please refer to the software (see note above) for the calculated required volume.

Binding Solution
For each sample mix 200 µL of Buffer BQ1 with 200 µL of 96-100% ethanol. Prepare a small excess of buffer to compensate dead volume of reservoir.
Please refer to the software (see note above) for the calculated required volume.

Wash Solutions
Buffer BW: ready to use.
Buffer B5: add 96-100% ethanol as indicated. Close bottle tightly in order to prevent ethanol evaporation.
Please refer to the software (see note above) for the calculated required volume.

Sample Storage

Sample storage
For short time storage (up to 24 hours) tissue samples may be stored at 4° **C**. For long time storage store tissue samples at –20° **C or -80°C**.

Frozen samples should not be thawed more than once. Inappropriate storage of sample may lead to degraded DNA. Repeated freeze-thaw cycles may also lead to poor quality of purified DNA.

Sample Preparation

General Considerations
Sample preparation on the X-tractor Gene™ should be conducted in the same manner as for spin columns. The same issues you address with your samples for processing on spin columns are applicable to samples processed on the X-tractor Gene™.

Sample Digestion
Add up to 20 mg sample (see *Sample Preparation* for advice) to each lysis block well for the columns you wish to extract from.

- Add 225 µL of Lysis Solution (200 µL Buffer T1 + 25 µL Proteinase K) to 20 mg of sample (either manually or using separate X-tractor Gene™ protocol *MN Tissue – Digest preload. CAS4* run file (for automated setup, see Appendix A))
- Incubate at 56° **C** for at least 3-6 hours to overnight.
- To maximise DNA recovery incubate sealed plate with constant agitation.
- Centrifuge at ≥2,500 x g for 10 minutes to pellet remaining debris and precipitates.

Note: A larger volume digest (400 µL) allows a more consistent automated removal of 200 **µL** digested supernatant from the pellet to the Lysis plate for extraction. Please note that additional Lysis Buffer T1(Cat # 740940.25) is required
For small or precious samples the digest volume (225 **µL**) can be maintained as per the original MN protocol, however it is recommended for optimal results that the supernatant (200 **µL**) be manually transferred to the lysis block (Refer to *Appendix A* and *B*).

Preparing Difficult Samples
Avoid transferring material into the lysis block that could cause pipette blockage (e.g. debris or particulate matter).

To maximise nucleic acid recovery from samples (such as mouse tails), non-digestable debris are best removed by centrifugation after the digest step.

Seal and incubate the lysis block after addition of Buffer T1/Proteinase K for at least 3-6 hours or up to overnight (14-18 hours) at 56º **C** with constant agitation.

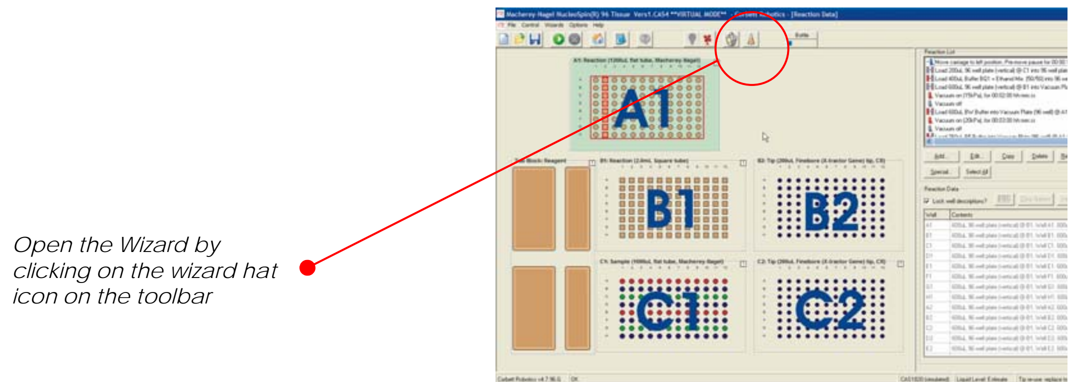
Centrifuge at ≥2,500 x g for 10 minutes to pellet remaining debris and precipitates.

Aspirate 200 µL of the digest supernatant off the pellet and mix with 400 µL of Lysis Buffer as per step 2 of the extraction protocol below.

Blocked Wells
Silica membranes can be blocked by samples with particulate matter or a high viscosity (highly concentrated DNA and DNA/protein lysates are usually very viscous).
DNA overloading of the membrane will also cause blockage, therefore apply no more than 2×10^6 nucleated mammalian cells.
Once a membrane is blocked, buffer flow may halt and the well will require manual removal of most of the subsequent buffers loaded. Alternatively, the membrane can be pierced with a 27-gauge needle. Overloaded or blocked membranes lead to reduced yields and low quality DNA.

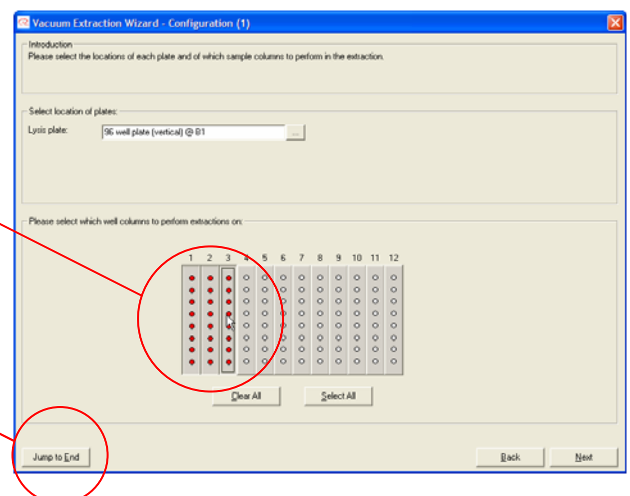
Setting Up to Execute a Run

1. Turn on X-tractor Gene™.
2. Launch software.
3. Open *Macherey-Nagel NucleoSpin(R) 96 Tissue V01.CAS4* run file (for 96-Well Plate)
   Open *Macherey-Nagel NucleoSpin(R) 8 Tissue V01.CAS4* run file (for 8-Well Strip System)
4. Select the number of columns to extract from:



Select the number of columns to extract from

Click Jump To End *button*



5. Once you have selected the required columns, exit the Wizard by clicking on the *Jump to End* button in the bottom left hand corner of the *Configuration* window.
6. This will take you to the *Review Protocol* screen. Confirm the protocol. If required, this screen can be printed to assist in robot setup or for your records. Click *Next*, and then *OK* to exit the Wizard.

   The X-tractor Gene™ will now calculate the required volumes of the described reagent for each tub and the number of tips required.

Loading Workspace Prior to a Run

Note: See Appendix B for Sample Introduction Method

1. Load the centrifuged digest block onto the C1 position of the X-tractor Gene™.
2. If you have less than 96 samples to extract you will need to cover the unused portion of the capture plate with a piece of self adhesive sealing film. Covering the unused portion is essential for proper vacuum operation. DO NOT attempt to re-use the unused portion of the plate as repeated handling of the capture plate can result in cross contamination of subsequent extractions.
3. Observing sterile procedure set up the instrument deck with clean accessories, the required consumables and all reagents.
4. Ensure the separator plate is thoroughly clean and dry (see *Cleanup*).

WARNING

Do not dispense the required volumes of reagents into the reagent tubs until just prior to the start of the run.

Keep reagents covered with the provided lids until you are ready to start the run. Leaving reagents in tubs for extended periods will result in evaporation (especially of alcohol solutions) and salt precipitate formation resulting in loss of binding conditions. For this reason reagents left over from a previous run should be disposed of and new or clean tubs loaded onto the deck with fresh regents.

5. Prepare Binding Solution: Pre-mix the Binding Buffer BQ1 with Ethanol (96%-100%) at a ratio of vol/vol 50% Lysis Buffer BQ1 to 50% Ethanol.
6. Finally load Lysis Solution (Buffer BQ1 + Ethanol), Binding Solution, Wash buffers, and Elution Buffer as indicated into their assigned reagent tubs and cover the tubs with the supplied reagent tub lids.

Run Protocol

Pre-Run Checklist

Click the *Start* icon and a pre-run checklist will appear. The checklist may include some of the following:

Please ensure:

- That the columns you wish to extract from are correctly selected and the unused portion of capture plate is sealed with self-adhesive microtiter plate plastic sealing film or PCR foil.
- That the elution plate is in position and its lid removed.
- Sufficient pipette tips are loaded and lids removed.
- Clean reagent tubs are loaded with the required volume of reagent, are in the correct positions, and their covers are on.
- All reagents and samples are at room temperature at the start of the run.

For first time users, the software option of user pause at the end of vacuum steps is enabled as directed; this requires the user to confirm that all the samples have flowed through the capture plate before continuing. This option can be turned off once proper sample preparation has been confirmed.

Start Run

Once you have confirmed that the X-tractor Gene™ workspace is correctly set up as described in the software and observed the pre-run checklist, click *Run* and the protocol will execute as described below.

Sample Transfer

- Aspirate 200 µL digest supernatant off the pellet and transfer to lysis plate at B1.

Load Binding Solution

- Add 400 µL of Lysis solution (Buffer BQ1/Ethanol Mix).

Load Filter Plate

- Load 600 µL of lysate into the capture plate.
- Pre-mix 10 times.
- Apply a vacuum of 15 kPa for 2 minutes and check for slow or blocked wells (user pause on).

Wash Step 1

- Load 600 µL of Wash Buffer BW into the capture plate.
- Apply a vacuum of 20 kPa for 3 minutes (user pause on).

Wash Step 2

- Load 760 µL of Wash buffer B5 into the capture plate.
- Apply a vacuum of 20 kPa for 1 minute.
- Repeat (2 iterations total).

Dry Sample

- Apply a vacuum of 60 kPa for 10 minutes.

Product Removal

- Load 100 µL of BE Buffer into the capture plate.
- Incubate for 5 minutes.
- Apply a vacuum of 40 kPa for 1 minute.
- Repeat (2 iterations total).

Finish

- Recover elution plate with samples.
- Remove and discard used consumables, clean separator plate, sink, tubs and carriage in preparation for the next run.
- (Please refer to Appendix C for nucleic acid storage advice).

IMPORTANT

Samples are always processed in batches of 8 (whole columns).

If you do not use all the wells in a column, make sure the unused wells contain a substitute liquid (such as water or buffer) equal in volume to the sample volume.

Failure to do this can cause undue foaming of a short-filled well during a mix cycle.

Post-Run Cleanup

Disposable Plasticware and Liquid Waste
Dispose of plasticware and liquid waste in accordance with laboratory guidelines for the sample type and reagent hazard.

WARNING

Do not add bleach or acidic solutions directly to solutions containing guanidine or extraction waste. Guanidine forms reactive compounds and toxic gases when mixed with bleach or acids. For any items contaminated with these buffers, clean with general laboratory detergent and water to remove all traces of guanidine before cleaning with bleach or acidic solutions.

You can download reagent MSDS (material safety data sheet) information from the following web sites:

- www.corbettrobotics.com
- www.mn-net.com

Transfer carriages, waste sink and tip chute
Thoroughly rinse under cold tap water and allow to dry.

If further cleaning is desired then soak in 1% Sodium hypochlorite (final concentration of bleach) for >30 minutes. Rinse thoroughly with large amounts of water and allow to dry.

Do not apply hot water, autoclave or heat sterilize these components.

Separator Plates and Non-disposable Reagent Tubs
The separator plate and non disposable reagent tubs must be washed to ensure they are RNA/DNA and RNase/DNase-free. Ensure they are dry before re-using.

Do not autoclave or heat sterilize the separator plate and non disposable reagent tubs (do not exceed 100°C).

When washing the separator plate, scrub lightly with a brush, this will help dislodge air bubbles that can become trapped in the holes and prevent the plate from been cleaned thoroughly. Agitating the plate up and down will also help ensure the holes are properly washed.

1. First rinse with tap water to remove any guanidine salts.
2. Then to clean either soak in 1 % sodium hypochlorite (final concentration of bleach) for >30 minutes, then rinse thoroughly with large amounts of milliQ or Molecular Biology grade RNase-free water.

   Or

   Soak for 1 minute in 0.1 M NaOH, 1 mM EDTA followed by a 1 minute soak in 0.4 M HCl then rinse thoroughly with large amounts of milliQ or Molecular Biology grade RNase-free water.

   Alternatively, the plate may be cleaned with DNA Zap (Ambion Inc, Austin, TX; P/N 9890).

Troubleshooting

Problem	Cause	Solution
Slow or blocked capture plate wells	Excessive sample	Reduce the amount of starting material. Do not use more than 20 mg of tissue. To preserve the sample you are currently working with, remove it from the well and re-run on a different vacuum plate. Once you have recovered the sample, pierce the bottom of the silica membrane through the nozzle with a 27-gauge needle to allow subsequently loaded buffers to pass through.
	Incomplete lysis of sample material	Increase incubation time and temperature with the Lysis Solution. Increase mixing. See: incomplete digest or lysis of samples (below).
	Particulates and precipitates blocking membrane	Avoid debris carry over when aspirating sample into Lysis block. Pellet particulates (2,500 x g, 10 minutes) and load sample supernatant into a new empty lysis block.
	Vacuum too low	Increase the vacuum or prolong the vacuum time. Ensure the vacuum applied complies with the extraction protocol.
Incomplete digestion or lysis of samples	Proteinase K was not added to the digestion buffer	Add the Proteinase K solution to the buffer T1 to prepare Lysis Solution just prior to the run.
	Proteinase K activity to low	Add Proteinase K solution to Buffer T1 immediately prior to run (Proteinase K should not be in Buffer T1 for not more than 15 minutes/hour before being diluted with sample). Proteinase K tends to self digestion under optimal reaction conditions in Buffer T1 without substrate Freeze-thaw cycles of Proteinase K solution reduce the activity of the Proteinase K; repeat with a freshly prepared Proteinase K solution. Completely mix the Proteinase K solution with the Buffer T1 by inverting tube 10 times.

Troubleshooting

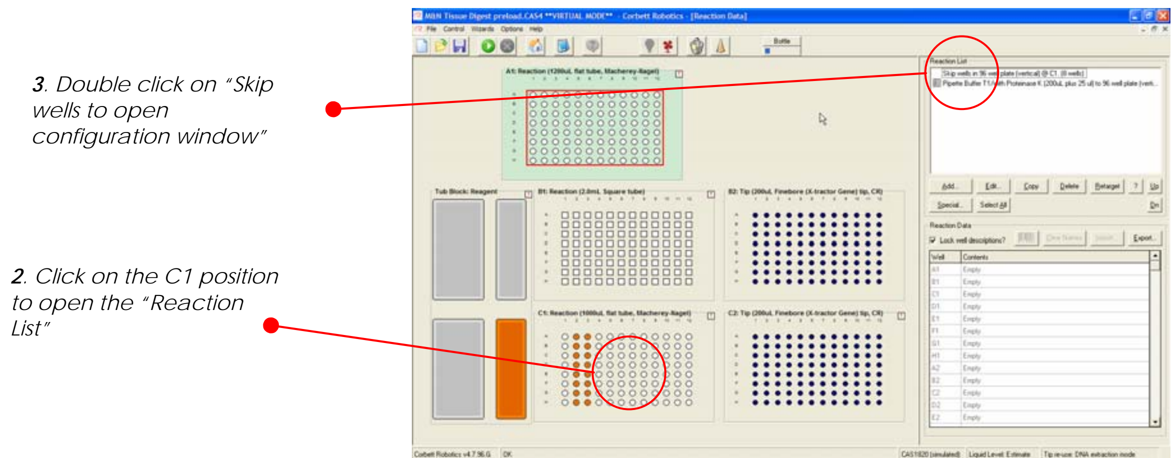
Problem	Cause	Solution
Poor DNA yield	Incorrect type or quality reagent or plasticware	Before continuing, it is essential you use original and fresh MACHEREY-NAGEL reagents and the recommended Corbett consumables (see section *Reagents and Consumables Required*).
	Incomplete digestion or lysis of samples	See: Incomplete digestion or lysis of samples (above).
	Particulates and precipitates and clots blocking membrane	See: Particulates and precipitates and clots blocking membrane (above).
	Incomplete elution	Prolong the incubation time with Elution Buffer, or repeat the elution step again. Incubate the elution buffer at 70°C. Check well was not overloaded. Confirm the DNA was eluted in 200 µL (2x 100 µL). Increase elution vacuum time in preference to increasing vacuum pressure.
	Sample is old or degraded	Collect process and store samples correctly as soon as possible after collection.
	Water was used for elution instead of elution buffer	Elution solution is recommended for optimal yields and storage of the purified DNA. If water is used to elute DNA, confirm that the pH is at least 7.0, as elution efficiency falls dramatically below pH 7.0 and acidic conditions may subject the DNA to acid hydrolysis when stored for long periods of time. Note: stored water can become acidic over time.
	Using lysate stored at -20°C or -70°C	Lysate that has been frozen may have a decreased amount of genomic DNA. For optimal performance, purify the DNA as soon as the lysate is prepared
	Samples have undergone multiple freeze-thaw cycles	Samples that have been frozen and thawed repeatedly will eventually experience DNA degradation. Use fresh samples where possible.

Troubleshooting

Problem	Cause	Solution
Poor DNA yield	Excessive evaporation of reagents	The required volumes of reagents should not be dispensed into the reagent tubs until just prior to the start of the run. Leaving reagents in open tubs for extended periods will result in evaporation of water and alcohols. This will result in salt precipitates and loss of poor binding conditions
	Steps not followed correctly or wrong reagents used	This protocol requires that the correct volumes of reagents are used in a specific order. When done correctly the DNA will bind and remain bound to the membrane during the purification.
Low DNA purity (A260/280 ratio too high)	Genomic DNA is contaminated with RNA	Include an RNase step in future isolations.
Inhibition with downstream applications	Incorrect type or quality reagent or plasticware	Before continuing, it is essential to ensure you use original and fresh MACHEREY-NAGEL reagents and the recommended Corbett consumables (see section *Reagents and Consumables Required*). Low quality chemicals may cause inhibition effects, as can inhibitors leached from incorrect plasticware.
	Salt carryover during elution	Check Wash Buffer for salt precipitates. If there are any precipitates, carefully warm until they dissolve.
	Ethanol carry over during elution	Increase drying time for ethanol removal step. Dry plate at 50°C for 10 minutes.
	Reduced sensitivity	Determine the maximum volume of eluate suitable for your amplification reaction. Reduce or increase the amount of your eluate added accordingly.
	Elution cluster rack tubes autoclaved before elution	Do not autoclave elution cluster rack tubes. This may leach chemicals from the tubes, which may inhibit enzymatic reactions. Repeat the purification with a new set of elution cluster rack tubes. The Corbett Robotics or MACHEREY-NAGEL cluster rack tubes are RNase/DNase and RNA/DNA free.

Digest Preload Protocol

1. Open the Robotics4 software and open the *MN Tissue – Digest preload. CAS4* run file.
2. Click on the reaction plate at workspace position C1. This will open a *reaction list* on the right hand side of the screen.
3. To skip columns, highlight the *Skip wells in 96 well plate* option and then select *Edit*. In the *Reaction Configuration* window,



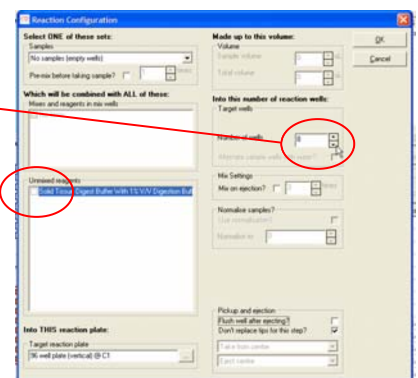
<u>To skip wells</u>

4. Select the number of wells (8 wells equals 1 column) you want to skip in the *skip well* option.
5. Ensure that in the *Unmixed reagents* pane, that *Buffer T1 (with Proteinase K)* is **<u>Not</u>** selected.
6. Click Ok.

<u>To Skip Wells</u>

4. Select the number of wells you wish to skip. 8 wells equals a single column.

5. Ensure that no reagents are selected.



To Load Required wells with Digest Buffer

7. To select the number of columns you want to load with Digest Buffer, highlight the *Pipette Buffer T1 (with Proteinase K)* option then double click (as per step 3).
8. In the *reaction configuration* window, enter the number of *target wells* you wish to load.
9. Ensure that in the *Unmixed reagents* pane, that *Buffer T1 (with Proteinase K)* is selected.
10. Ensure that the total volume in the *volume* pane displays 220 µL.
11. Ensure that the target reaction plate is the 96 well plate in position C1.
12. Click *Ok*.

10 check the load volume is 220 uL.

8. Select the number of wells you wish to load Buffer T1 (With Proteinase K) into. 8 wells equals a single column

9. Ensure that Buffer T1 (with Proteinase K) is selected.

11. Ensure that the target plate is position C1.

> *WARNING*
>
> **Do not add the Proteinase K to the Buffer T1 until just before you start the digest.**

Load wells with Digest Buffer

13. Ensure the reagent tub described within the software has been loaded with the required amount of Buffer T1 (and that the Proteinase K has been added).
14. Click the *Start* icon and a pre-run checklist will appear. Select *Check all* once all messages have been acknowledged and addressed.
15. Click *Ok*.

Once the Buffer T1 has been aliquoted into the wells of the plate, the tissue sample can be added (if not added previously).

Sample Digest Volume

Note: A larger volume digest (400 µL) allows a more consistent automated removal of 200 **µL** digested supernatant from the pellet to the Lysis plate for extraction. Please note that additional Lysis Buffer T1(Cat # 740940.25) is required
For small or precious samples the digest volume (225 **µL)** can be maintained as per the original MN protocol, however it is recommended for optimal results that the supernatant (200 µL) be manually transferred to the lysis block.

Choosing Sample Introduction Method

Selecting the correct method for introduction of digested sample into the extraction run.
There are two options for introduction of the digested sample into the X-tractor Gene™.

1. Have the X-tractor Gene™ transfer 200 µL. of digest supernatant from the pelleted "digest plate" to the "lysis plate". The Lysis Buffer is then added to the digest supernatant. This is the **default** run file option.

   To set up:
   a. Open the *Macherey-Nagel NucleoSpin(R) 96 Tissue V01.CAS4* run file.
   b. Open the Wizard and scroll back two screens to the first page.
   Ensure the "I'd like the samples to be automatically pipetted into the lysis plate from a sample plate" option is selected, and the associated "premix sample" option is unchecked.
   c. Scroll to the end of the wizard and confirm the protocol in the post wizard setup screen.
   d. Place the round well 96-well block of digested material in the C1 position. Use a round well 96-well plate and within the plate configuration window, select the supernatant alternative of the plate.
   e. Manually height calibrate the plate to an appropriate height so that the pellet will not be disturbed during sample aspiration.
   f. Under Options>Run Settings, configure the sample pipetting speed to 60 µL/sec (**Default**)

2. Manually load the required 200 µL of digested material directly into the lysis plate and then place the lysis plate into the B1 position.

   For this option you will need to change the run file from its default settings, therefore:
   a. Open the *Macherey-Nagel NucleoSpin(R) 96 Tissue V01.CAS4* run file.
   b. Open the Wizard and scroll back two screens to the first page. Ensure the "My samples are preloaded into an empty lysis block" option is selected.
   c. Place the square well 96-well lysis block with 200 **µL** of digested material per well in the B1 position.
   d. Scroll to the end of the wizard and confirm the protocol in the post wizard setup screen.

2. Option 2 Manually load samples into lysis block

1. *Option 1* ***Default***
The X-tractor Gene™ automatically transfers the required volume of digested supernatant off the digest pellet from the digest plate at position C1 into the lysis plate at position B1.

Nucleic Acid Storage

A working stock of DNA can be stored at 2 – 4 °C for several weeks. For long term storage DNA should be stored at -20 °C.

RNA should be stored at -80 °C at all times (and held at 2- 4 °C during use).

Note that the solution in which the nucleic acid is eluted in will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Please note that the elution buffer BE supplied within the NucleoSpin® 8/96 Tissue kits contains 5 mM Tris pH 8.5.

Repeated freeze thaw cycles should be avoided as this can shear the DNA.

Disclaimers

Protocol Use
It is the user's responsibility to validate performance of this protocol for any particular application, since performance characteristics of this protocol and its product have not been validated for any specific application.

This protocol is for *in vitro* research use only.

It is not intended to identify any specific organism or for clinical use.

Liability
Corbett Robotics is in no way liable for claims for any damages, whether direct, incidental, foreseeable, consequential, or special (including but not limited to loss of use, revenue or profit), whether based upon warranty, contract, tort (including negligence) or strict liability arising in connection with the sale or the failure of Corbett Robotics products to perform in accordance with the stated specifications.

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