Bionano Prep SP Tissue and Tumor DNA Isolation Protocol

Document Number: 30339

Document Revision: A

For Research Use Only. Not for use in diagnostic procedures.
Copyright © 2020 Bionano Genomics Inc. All Rights Reserved

Table of Contents

Legal Notice

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

This material is protected by United States Copyright Law and International Treaties. Unauthorized use of this material is prohibited. No part of the publication may be copied, reproduced, distributed, translated, reverse-engineered or transmitted in any form or by any media, or by any means, whether now known or unknown, without the express prior permission in writing from Bionano Genomics. Copying, under the law, includes translating into another language or format. The technical data contained herein is intended for ultimate destinations permitted by U.S. law. Diversion contrary to U. S. law prohibited. This publication represents the latest information available at the time of release. Due to continuous efforts to improve the product, technical changes may occur that are not reflected in this document. Bionano Genomics reserves the right to make changes in specifications and other information contained in this publication at any time and without prior notice. Please contact Bionano Genomics Customer Support for the latest information.

BIONANO GENOMICS DISCLAIMS ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. TO THE FULLEST EXTENT ALLOWED BY LAW, IN NO EVENT SHALL BIONANO GENOMICS BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF, WHETHER OR NOT FORESEEABLE AND WHETHER OR NOT BIONANO GENOMICS IS ADVISED OF THE POSSIBILITY OF SUCH DAMAGES.

Patents

Products of Bionano Genomics® may be covered by one or more U.S. or foreign patents.

Trademarks

The Bionano Genomics logo and names of Bionano Genomics products or services are registered trademarks or trademarks owned by Bionano Genomics in the United States and certain other countries.

Bionano Genomics®, Irys®, IrysView®, IrysChip®, IrysPrep®, IrysSolve®, Saphyr®, Saphyr Chip®, Bionano Access®,and Bionano EnFocus™ are trademarks of Bionano Genomics, Inc. All other trademarks are the sole property of their respective owners.

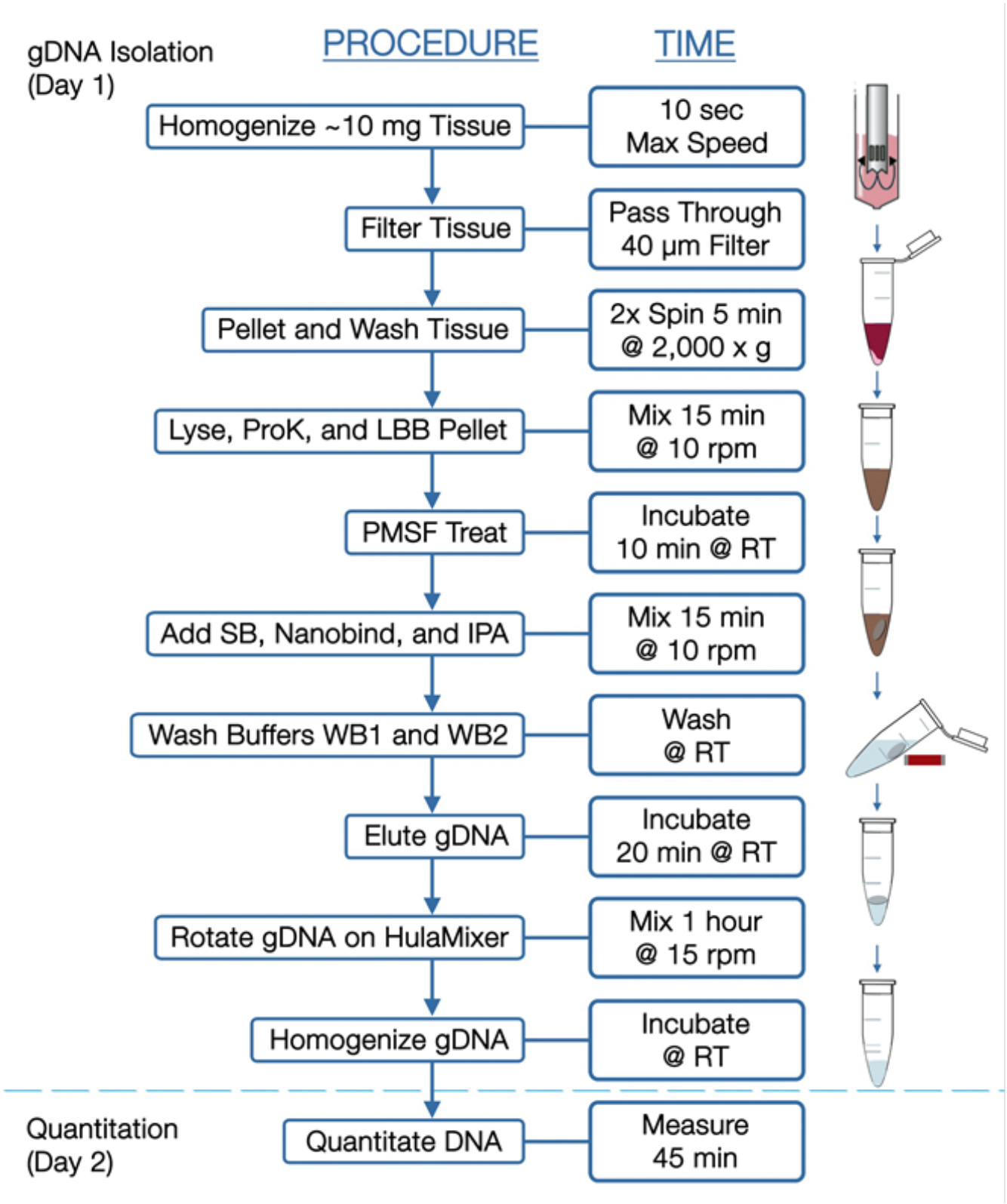
No license to use any trademarks of Bionano Genomics is given or implied. Users are not permitted to use these trademarks without the prior written consent of Bionano Genomics. The use of these trademarks or any other materials, except as permitted herein, is expressly prohibited and may be in violation of federal or other applicable laws.

© Copyright 2020 Bionano Genomics, Inc. All rights reserved.

Revision History

Revision	**Release Date**	Notes
1		Beta Release
2		Early Access Release
A	7/13/2020	Commercial Release

Workflow Overview



SP Tissue and Tumor DNA Isolation Kit and User-Supplied Materials

Table 1: Bionano Prep SP Tissue and Tumor DNA Isolation Kit Contents (Part # 80038, 10 preps)

Item	Amount	Part Number	Storage
4 mm Nanobind Disks	10 disks	20402	Room Temp (18-25°C)
Protein LoBind Microcentrifuge Tubes, 1.5 ml	10 tubes	20380	Room Temp (18-25°C)
Microcentrifuge Tubes, 2 ml	10 tubes	20396	Room Temp (18-25°C)
40 µm Cell Strainer	10 each	20403	Room Temp (18-25°C)
Detergent	150 µl	20405	Room Temp (18-25°C)
Salting Buffer	1.1 ml	20404	Room Temp (18-25°C)
Homogenization Buffer	96 ml	20406	Room Temp (18-25°C)
Wash Buffer A	12 ml	20407	Room Temp (18-25°C)
Proteinase K	0.5 ml	20372	Room Temp (18-25°C)
Lysis and Binding Buffer (LBB)*	2.5 ml	20375	Room Temp (18-25°C)
Wash Buffer 1 Concentrate (2.5X) (WB1)*	2 x 3.25 ml	20376	Room Temp (18-25°C)
Wash Buffer 2 Concentrate (2.5X) (WB2)	5 ml	20377	Room Temp (18-25°C)
Elution Buffer (EB)	1.1 ml	20378	Room Temp (18-25°C)
SP Sheaths	10 each	20381	Room Temp (18-25°C)

* See Important Notes Section for hazardous waste information

Table 2: User-Supplied Materials

Item	Supplier	Catalog #
Day 1 – Tissue disruption, Pelleting, gDNA Isolation and Homogenization		
Bionano Prep SP Magnetic Retriever (2 pack)	Bionano Genomics	80031
Rotor-stator: TissueRuptor (version I or II)	QIAGEN or Equivalent	9002755
Power Strip (recommended)	General Lab Supplier	
Disposable Probes for TissueRuptor	QIAGEN or Equivalent	990890
Ring Stand & Three Prong Clamp (e.g. VWR 76293-368)	General Lab Supplier	
DynaMag-2 Magnetic Tube Rack	Thermo Fisher	12321D
HulaMixer Sample Mixer	Thermo Fisher	15920D
Weigh Boats	General Lab Supplier	
Precision Scale	General Lab Supplier	
Microcentrifuge Tubes, 1.5 ml, Nuclease Free	VWR	87003-294
Screw Cap Tubes with O-Ring, 1.5 ml	General Lab Supplier	
Razor Blade	General Lab Supplier	
Biosafety Cabinet (optional)	General Lab Supplier	
Dry Ice (optional)	General Lab Supplier	
Aluminum Block	General Lab Supplier	
Metal Spatula	VWR	82027-530
Phenylmethylsulfonyl Fluoride Solution (PMSF),100 mM	Sigma-Aldrich	93482
Ethanol, 200 Proof, Molecular Biology Grade	Sigma-Aldrich	E7023
Isopropanol (IPA), ≥ 99.5%, Molecular Biology Grade	Fisher Scientific	A461-212
Disinfectant Concentrate, TexQ TX651	Texwipe	TX651
Conical Centrifuge Tubes, 50 ml, PP	Thermo Fisher or Equivalent	14-432-22
Conical Centrifuge Tubes, 15 ml, PP	Thermo Fisher or Equivalent	05-539-12
Refrigerated Centrifuge with 1.5/2.0 ml Tube Rotor (2,000 x g spin)	General Lab Supplier	
Refrigerated Centrifuge, Swinging Bucket Rotor for 15 ml Conical Tubes	General Lab Supplier	
Ice Bucket and Ice	General Lab Supplier	
Sterile 5 and 10 ml Disposable Pipettes (TD+)	General Lab Supplier	
Mini Benchtop Microcentrifuge (2,000 x g spin)	Labnet	C1301B
Pointed Forceps	Electron Microscopy Sciences, or Equivalent	78141-01
Wide-Bore Pipette Tips, Filtered, Aerosol, 200 µl	VWR or Rainin Equivalent	46620-642
Extra Long 1000 µl Tips, Sterile	VWR or Rainin Equivalent	16466-008

Pipettes (10, 20, 200, and 1,000 µl) and Nuclease Free, Filtered Pipette Tips	General Lab Supplier	
Day 2 - Quantitation		
Benchtop Vortexer	General Lab Supplier	
Bath Sonicator (optional)	Branson or Equivalent	CPX 952-119R
Fluorometer, Qubit	Thermo Fisher or Equivalent	Q33216
Qubit® BR (Broad Range) dsDNA Assay Kit	Thermo Fisher or Equivalent	Q32853
Qubit Assay Tubes	Thermo Fisher	Q32856
Positive-Displacement Pipette MR-10 (optional)	Rainin or Equivalent	17008575
Pipette Tips, 10 µl, C-10 for Pos. Displ. Pipette (optional)	Rainin or Equivalent	17008604

Introduction and Important Notes

Introduction

This Bionano Prep SP Tissue and Tumor DNA Isolation Protocol can provide ultra-high molecular weight (UHMW) gDNA in less than 6 hours from a batch of up to 8 samples (up to 4 recommended for novice users) with approximately 10 mg fresh or fresh frozen tissue or tumor. It utilizes a homogenization, lyse, bind, wash, and elute procedure that is common for silica-based gDNA extraction technologies in combination with a novel paramagnetic disk. Unlike magnetic beads and silica spin columns, which shear large gDNA, the Nanobind Disk binds and releases gDNA with significantly less fragmentation, resulting in UHMW gDNA. High gDNA binding capacity is the result of a novel nano structured silica on the outside of the thermoplastic paramagnetic disk. This protocol has been used to process liver, lung, kidney, colon, ovary, prostate, testes and uterus tissues from Brown Norway rats, and human bladder, lung, liver, kidney, colon, breast, prostate, brain, thyroid and ovary tumors. This protocol was fully validated in replicate by multiple users by testing human tumors from liver, lung, and breast, and normal rat kidney. gDNA prepared using this protocol has been tested with DLS labeling. See videos for tissue Homogenization and Filtration and SP gDNA Isolation Training Video for critical steps and troubleshooting; the steps mentioned in the video correspond to the Bionano Prep SP Frozen Human Blood DNA Isolation Protocol (30246), but are the same processes as here.

Overview

After homogenization in a buffer containing ethanol, tissue lysis and Proteinase K digestion occurs in a chaotropic buffer and the released gDNA binds to the Nanobind Disk upon the addition of salting buffer and isopropanol. After four wash steps, the disk is transferred to a fresh tube and the gDNA is eluted from the disk. The recovered UHMW gDNA is subjected to limited shearing to make the UHMW gDNA more homogeneous. The gDNA is then mixed and equilibrated overnight at room temperature to facilitate DNA homogeneity and the concentration is determined. Typical gDNA size range is from 50 Kbp to ≥ 1 Mbp.

Important Notes

DNA Homogeneity

Recovered gDNA is subjected to pipette mixing with a 200 µl standard pipet tip to increase homogeneity, ensuring consistent DNA sampling for labeling.

gDNA Quantitation

gDNA quantitation is used to measure concentration and serves as a gauge of UHMW gDNA homogeneity. Invitrogen™ Qubit™ quantitation is used instead of other quantitation methods since it can also be used for measuring gDNA concentration of the labeling reaction and is more accurate than spectrophotometer readings for our samples. The Qubit Broad Range (BR) dsDNA Assay measures gDNA concentration after isolation, while the High Sensitivity (HS) dsDNA Assay measures gDNA concentration after labeling.

To gauge gDNA homogeneity, it is essential to measure the concentration of gDNA at multiple positions in the solution. Since viscous gDNA is difficult to pipet, follow guidelines in the Important Notes and gDNA Quantitation sections below for accurate pipetting. Standard assays for quantification of gDNA concentration will not provide accurate measurements of long gDNA due to its viscous nature.

- Effective fragmentation of sampled gDNA via sonication or extensive vortexing is necessary for accurate quantitation.
- The coefficient of variation (CV) from three unique samplings should be less than 0.30.
- Typical gDNA concentration is 50-300 ng/µl.

Pipetting Viscous Genomic DNA (gDNA)

To draw viscous gDNA, hold the stock tube for close-up visualization, depress the pipette plunger until the first stop, submerge the pipette tip and carefully and slowly release the plunger to start drawing the viscous gDNA into the tip while carefully monitoring uptake. Keep the tip submerged even after the viscous solution stops moving upward and levels off. Be patient. Viscous gDNA can take a few seconds to fill up to 2 µl. Releasing the plunger too fast can produce a bubble in the tip leading to under-sampling (start over if this occurs). After the solution in the tip has leveled off and while the tip is still submerged in the gDNA solution, scrape the tip against the bottom of the tube 3-5 times using a circular motion. Remove the tip from the gDNA solution and visually inspect to confirm that it is filled to 2 µl. Removing the pipette tip from the gDNA solution too early, or ineffectively scraping the tip to break gDNA strands from the tip, can produce a bubble at the tip of the pipette tip indicating under-sampling (start over if this happens).

gDNA Handling

- Mixing of recovered gDNA is always carried out with a wide bore pipette tip to prevent shearing.
- Recovered gDNA should never be frozen or vortexed.
- Pipetting of recovered gDNA for accurate sampling is always carried out with a standard tip or positive displacement pipette.

Characteristics of High Quality gDNA for Bionano Mapping

- A clear gDNA solution is ideal, but an unclear solution does not always correlate with poor sample quality.
- Recovered gDNA in solution is viscous.
- Presence of mega base size gDNA is measured by pulsed field gel electrophoresis (PFGE).

- Recovered gDNA is homogenous as measured with Qubit gDNA quantitation assay with CV < 0.30.

Using the Bionano Prep SP Magnetic Retriever

a. Hold a plastic sheath on the sides near the top and insert the Bionano Prep SP Magnetic Retriever into the sheath, positioning it such that it is sitting at the bottom of the sheath.
b. Insert the sheathed retriever into the Protein LoBind microfuge tube to attract the Nanobind Disk to the retriever in the sheath.
c. Carefully lift the sheathed retriever with the bound disk out of tube and insert the sheathed retriever into a new Protein LoBind microfuge tube.
d. Holding the sheath on the side near the top, with one hand pull the retriever up until the Nanobind Disk disassociates from the sheath and drops into the new tube.
e. Change sheath for each new sample.

Suggested Input (mg Tissue) and Tissue Quality

- We recommend starting with 10 mg, where as low as 5 mg and 20 mg or more may be used depending upon the nuclei content of the tissue relative to the tissue mass.
- If possible, we strongly recommend preparing ~ 10 mg tissue pieces before the tissue is frozen. Ideally pre-weigh the fresh tissue before snap freeze and storage at -80˚C.
- We recommend using fresh or snap frozen tissues that are free of bone debris, blood clots and extensive necrosis.
- Surgical specimens should be stored on wet ice or in ice-cold PBS as soon as possible to minimize DNA degradation before cutting and/or snap freezing.
- Compromised tissues (not properly handled or stored) or tissues that have gone through freeze-thaw cycles should be avoided for UHMW DNA isolation.
- The internal success rate of UHMW DNA isolated from tumor samples with high RIN (RNA Integrity Number) ≥ 9.0 was > 90%, whereas the success rate of UHMW DNA isolated from a limited number of tumor samples with significantly lower RIN (5-6) was lower (< 50%).

Batch Size

- We recommend processing up to 8 samples at a time (up to 4 at a time for novice users).

Hazardous Waste Disposal

All biohazardous waste, including plasticware, should be disposed of in accordance with local regulations.

Buffers LBB and WB1 contain guanidine hydrochloride (GuHCl). GuHCl is harmful if swallowed or inhaled and causes skin and eye irritation. DO NOT mix with bleach or acidic reagents. Liquid waste containing GuHCl should be safely decontaminated with a quaternary ammonium disinfectant before disposal in a hazardous waste stream. We recommend bleach for decontamination of pellet supernatant and TexQ for decontamination of all solutions

mixed with GuHCl. This conforms to disposal requirements in the state of California, US, but may be different for your location. Please consult local requirement for decontamination and disposal.

Bionano Prep SP Tissue and Tumor DNA Isolation Protocol

Preparation for gDNA Isolation from Fresh or Fresh Frozen Tissue and Tumor

Note: For best results, we encourage preparing tissue as described in the Appendix.

Before First Use

- Verify access to refrigerated centrifuge with swinging bucket rotor that can accommodate 15 ml polypropylene conical tubes to pellet the homogenate.
- Verify mini benchtop refrigerated microcentrifuge spin speed is 2,000 x g.
- PMSF decomposes rapidly in aqueous solutions. Create aliquots of 120 µl in 1.5 ml screw cap tubes and store stock and aliquots protected from light at 4°C. Each aliquot will be sufficient for ten gDNA isolations.
- Add 125 µl Bionano Prep Detergent to Lysis and Binding Buffer (LBB) tube and invert 10 times to mix. Mark LBB tube that detergent was added.
- Add 100% Ethanol to Homogenization Buffer and Wash Buffers (WB1 and WB2), invert 10 times to mix, and check “Ethanol Added” boxes:
    - Add 96 ml of 100% Ethanol to Homogenization Buffer for a final volume of 192 ml.
    - Add 5 ml of 100% Ethanol to Wash Buffer 1 (WB1) for a final volume of 8.25 ml.
    - Add 7.5 ml of 100% Ethanol to Wash Buffer 2 (WB2) for a final volume of 12.5 ml.

Set Up

- Set refrigerated swinging bucket centrifuge and refrigerated microcentrifuge to 4°C.
- Immobilize the TissueRuptor (Qiagen) on a vertical stand. Connect TissueRuptor to a power strip switch.
- Gather materials (see “User Supplied Material” section above). Pre-chill Homogenization Buffer, Wash Buffer A, and TissueRuptor probe(s).
- For waste disposal, prepare 50 ml conical tubes (1 tube for every 2 samples) with 100 µl TexQ decontaminant per sample (to be disposed as hazardous waste).
- For each sample, label two 15 ml conical tubes and one 50 ml conical tube, and set on ice. Set a 40 µm Cell Strainer (Bionano) into the 50 ml conical tube.
- For each sample, label two 1.5 ml Protein LoBind Tubes (Bionano) and one 2.0 ml microfuge tube (Bionano). Set one of the labeled 1.5 ml Protein LoBind Tubes on ice.
- Invert tubes of PMSF and Proteinase K (Bionano) three times to mix, pulse spin briefly. Place PMSF on ice.
- For convenience, all buffers used at 4°C in this protocol can be stored long term at 4°C.

gDNA Isolation (4.5 hours)

Note: For important instructions on preparing Frozen Tissue, please refer to Appendix.

1. For each tissue sample, add 2 ml of chilled Bionano Prep SP Tissue and Tumor Homogenization Buffer to one 15 ml conical tube and keep on ice.
2. Retrieve tissue and prepare ~10 mg (refer to Suggested Input section above) portions:

   Note: Minimize exposure of tissue to room temperature while cutting ~10 mg portions. If a portion is weighed outside the range of 9 - 13 mg, it is recommended to discard that portion and cut a new piece (if more tissue is available).

   a. For **Fresh** Tissue - using a sterilized, ice-chilled aluminum block as your cutting surface, cut tissue with a sterilized razor blade and forceps and weigh ~10 mg in a weigh boat on a precision scale (if weight is not already known).

   b. For **Frozen** Tissue:

      1) If the piece of tissue is no more than 2 x 2 x 4 mm, cut in a weigh boat with a sterilized razor blade and forceps on an ice-chilled aluminum block. Weigh ~10 mg in a different weigh boat and either discard the remainder or refreeze and store at -80°C for isolating DNA that does not have to be UHMW.
      2) If the piece of tissue is larger, and you do not need to sample a specific region of the tissue, place the frozen tissue in a plastic bag that has been cooled on a dry-ice chilled aluminum block, seal the bag and break the tissue into smaller fragments with the aid of a large pestle or hammer. Remove a small piece and treat as above (Step 2.b.1). The remaining fragments can be put back at -80°C for storage.
      3) If the piece of tissue is larger, and you need to sample a specific region of the tissue, place the frozen tissue in a weigh boat on a pre-chilled aluminum block (-20°C) and cut a small piece with a scalpel or razor blade and forceps. Weigh ~10 mg in a different weigh boat and place the remaining large tissue piece back at -80°C for storage.

   Note: For fibrous tissues, it is recommended to cut tissue into small pieces in the weigh boat (1 - 2 mm)
3. Using a sterilized pre-chilled metal spatula, immediately transfer the cut tissue to the labeled 15 ml conical tube containing Homogenization Buffer. Make sure tissue pieces are submerged in the Homogenization Buffer, and the conical tube is set on ice. Transfer pre-chilled probe to conical tube.
4. Repeat steps 2-3 to prepare each additional sample, up to 8 samples total.

   Note: Before continuing to the next step, make sure to place each of tissue or tumor sample in a 15ml conical containing homogenization buffer, and a TissueRuptor probe on ice. See Tissue and Tumor Homogenization and Filtration video for steps 5-11.

5. Remove one conical tube from ice at a time and firmly attach the probe to the TissueRuptor device while holding the conical. Hold the tube in such a way that the tip of the TissueRuptor probe is immersed in the buffer and very close to the bottom of the tube.
6. Pulse the TissueRuptor using the power strip switch by turning it on and off. Check to see if the tissue pellet has been broken apart. If the tissue is particularly dense, pulse an additional 2-3 times in order to break it apart and achieve thorough homogenization.

   Note: Make sure the tissue is not sticking on the tube wall or probe tip. Submerge the tissue in Homogenization Buffer using spatula if needed. For fibrous tissue, we recommend cutting tissue into small pieces before TissueRuptor homogenization.
7. Blend continuously for 10 seconds at maximum speed with the probe tip submerged at all times. Move the tube in an up-down and circular motion during blending to increase the efficiency of homogenization. At the end of the blending, turn off the power strip switch connected to the TissueRuptor, remove the probe from the TissueRuptor, and return the tube (with probe in the tube) to ice.
   Note: The conical tube should be placed back immediately after homogenization to ensure the highest DNA quality.
8. Repeat steps 5-7 for all other samples. Up to 8 samples total.
9. Rinse each probe tip with 6 ml ice-cold Homogenization Buffer using a pipette, collecting in the same conical tube. Dispose of the probe properly (potential biohazard).
10. Decant homogenate through a 40 µm cell strainer on top of the labeled 50 ml conical tube that is set on ice. Retain conical tube for next step.
11. Add additional 6 ml ice-cold Homogenization Buffer to the 15 ml conical tube and swirl to rinse debris off sides of tube, and then pour over the cell strainer, collecting into the same 50 ml conical tube. Discard cell strainer properly (potential biohazard).

    Note: If the homogenate is not passing through the cell strainer, briefly lift and lower the strainer to aid in flow-through.
12. Pipet entire volume 2 times before transferring the homogenate to a new 15 ml conical tube and cap.
13. Centrifuge at 2,000 x g for 5 minutes at 4°C using a swinging bucket rotor (set acceleration and deceleration to 9).
14. Decant supernatant into TexQ waste (potential biohazard) immediately after centrifuge stops, set conical tube on ice for 30 seconds, and use a 1,000 µl pipette to remove as much residual liquid as possible while not disturbing the pellet (leaving < 200 µl volume).
15. Add 300 µl Wash Buffer A, resuspend the pellet with a 1,000 µl tip (set at 300 µl) by slowly pipetting up and down 5 times. Transfer the entire volume of suspension into previously labeled pre-chilled 1.5 ml Protein LoBind tube using a 1,000 µl tip. Retain conical tube for next step.

16. Add additional 700 µl Wash Buffer A to the 15 mL conical tube, gently pipette 2 times to mix, and transfer the entire volume into the same labeled pre-chilled 1.5 ml Protein LoBind tube using a 1,000 µl tip.
17. Centrifuge at 2,000 x g for 5 minutes at 4°C.
18. While not disturbing the pellet, aspirate supernatant to leave behind approximately 40 µl volume. Dispose of supernatant into TexQ waste (potential biohazard). Set tube on ice for 30 seconds.
19. Resuspend pellet in residual volume 10 times using a standard 200 µl tip. Residual volumes may differ.

Lyse and Digest Cells

20. Add 50 µl of Proteinase K to the Protein LoBind tube, cap the tube. DO NOT PIPET MIX.
21. Incubate at room temperature for 3 minutes.
22. Add 225 µl Buffer LBB, containing detergent, to sample with a 1,000 µl tip. Cap and invert tube 15 times to mix.

    Note: Buffer LBB with detergent is a viscous and foamy solution which will adhere to pipette tip. Dispense slowly and change tips between dispensing to ensure accuracy of dispense volume.
23. Rotate sample on HulaMixer for 15 minutes at room temperature at 10 rpm. No shaking/vibration.
24. Pulse spin tube for 2 seconds to collect liquid at the bottom of the tube.
25. Add 10 µl of 100 mM PMSF into the liquid portion of tube. Cap and invert tube 5 times to mix, pulse spin tube for 2 seconds to collect liquid at the bottom of the tube.
26. Incubate at room temperature for 10 minutes.

gDNA Bind, Wash and Elute

27. Add 85 µl Bionano Prep Salting Buffer to the tube, cap and invert 10 times, and pulse spin tube for 2 seconds.
28. Using forceps, carefully transfer a single 4 mm Nanobind Disk to the lysate.

    Note: Disks can sometimes stick together.
29. Add 400 µl 100% isopropanol to tube. Cap and invert tube 5 times to mix.
30. Rotate sample on HulaMixer for 15 minutes at room temperature at 10 rpm. No shaking/vibration.

    Note: Ensure that the Nanobind Disk does not remain in the lid of the tube during initial rotations. If it does, turn off rotator and invert microfuge tube until the Nanobind Disk goes back into the solution. Replace the tube on the HulaMixer and resume mixing.
31. Examine gDNA association with Nanobind Disk and invert to increase binding (See Training Video, 0:25):
    a. Place sample tubes into clear Dynamag tube rack and visually inspect all tubes in rack to ensure that gDNA is tethered to the Nanobind Disk.

b. If gDNA strands are visibly hanging low, quickly invert 180° to bring the gDNA into closer association with the Nanobind Disk.
c. 180° inversions can be done many times until the gDNA association with the Nanobind Disk appears unchanged.

32. Combine clear rack with the magnetic base as outlined below, making sure Nanobind Disk is secured by the magnet near the top of the liquid level. If not, re-rack (See Training Video, 0:50).

Note: The color of liquid in the pictures below was modified for illustrative purposes.

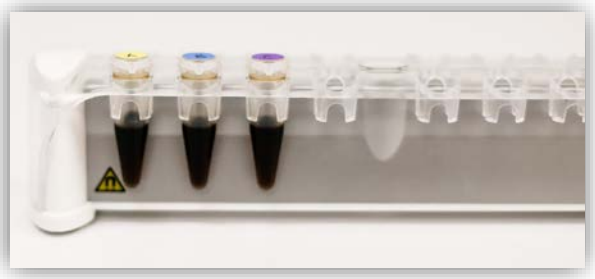
a. Invert clear Dynamag tube rack and place upside down with sample lids touching the work surface. The tubes will be on the same row of the rack, and in the row furthest from you.

b. Invert Dynamag magnetic base and lower onto clear rack.

c. Tilt combined apparatus slowly 90° towards you while it continues to rest on surface. The tubes will now be horizontal and visible to you.

d. Tilt combined apparatus slowly 90° towards you while it continues to rest on surface, so that it stands fully upright and tubes are facing you.

e. Make sure Nanobind Disk is held to the magnet near the top of the liquid level.



33. Set one 1,000 µl pipette to 1,000 µl and a second to 700 µl.
34. Remove supernatant as outlined below, careful not to aspirate the gDNA (See Training Video, 1:15):
    a. Angle entire rack at a 45° angle by holding in one hand (grasping the entire apparatus from below with tubes visible to you and lids towards your other hand).
    b. Wait 2 seconds for gDNA to lay on the Nanobind Disk.
    c. Slowly remove all liquid with a 1,000 µl extra-long tip angled away from the Nanobind Disk and/or gDNA to avoid disruption.
    d. Dispense supernatant into conical tube containing TexQ.

⚠ Ensure that the gDNA is not removed by visually inspecting the tip containing buffer before discarding. If gDNA is accidentally aspirated or becomes unbound from the disk, refer to Troubleshooting section below.

35. Perform Wash WB1 (See Training Video, 2:21):
    a. Dispense 700 µl of Buffer WB1 directly onto the disks in the tubes and cap tubes.
    b. Lift clear tube rack to separate from magnetic base.
    c. Invert clear rack with tubes 180° 4 times to wash.
    d. Re-rack clear tube rack and tubes with magnetic base as described in Step 32.
    e. Remove supernatant as described in Step 34.

⚠ Ensure that the gDNA is not removed by visually inspecting the tip containing buffer before discarding. If gDNA is accidentally aspirated or becomes unbound from the disk refer to Troubleshooting section below.

36. Repeat Wash WB1, Step 35.
37. Set the second pipette to 500 µl (previously at 700 µl).
38. Perform Wash WB2 (See Training Video, 4:10):
    a. Dispense 500 µl of Buffer WB2 directly onto the disks in the tubes and cap.
    b. Lift clear rack to separate from magnetic base.
    c. Invert clear rack 180° 10 times to wash.
    d. Re-rack clear tube rack and tubes with magnetic base as described in Step 32.

e. Remove supernatant as described in Step 34.

⚠ Ensure that the gDNA is not removed by visually inspecting the tip containing buffer before discarding. If gDNA is accidentally aspirated or becomes unbound from the disk refer to Troubleshooting section below.

39. Repeat Wash WB2, Step 38 (See Training Video, 5:50).

    Note: Remove buffer from 2 or 3 tubes at a time and process through Buffer EB incubation step in small batches to prevent the disk/gDNA from drying out.

40. Open tube lid fully (parallel to lab bench) and lift each tube apart from base.

41. In close proximity to a new Protein LoBind tube, transfer Nanobind Disk to a new Protein LoBind tube using Bionano Prep SP Magnetic Retriever (see Important Notes section for proper usage). Cap tube to prevent disk drying (See Training Video, 7:30).

42. Add 65 µl of Buffer EB to Protein LoBind tube.

43. Pulse spin the tube on benchtop microcentrifuge for 5 seconds.

44. Using a 10 µl standard tip, gently nudge Nanobind Disk towards the bottom of the tube, making sure that it is fully submerged in liquid. The disk should remain parallel to the bench surface (See Training Video, 8:20).

45. Incubate submerged Nanobind Disk in Buffer EB at room temperature for 20 minutes.

46. Collect extracted gDNA by transferring eluate to previously labeled 2.0 ml microfuge tube with a 200 µl standard tip.

47. Pulse spin the tube with the Nanobind Disk on benchtop microcentrifuge for 5 seconds and transfer all of the remaining eluate containing viscous gDNA to the same standard 2.0 ml microfuge tube as in previous step with a standard 200 µl tip. You may remove the disk before aspirating remaining elution buffer.

    Note: Almost all of the viscous gDNA comes off the Nanobind Disk during the pulse spin.

Homogenization of gDNA Solution (70 minutes)

Homogenization of gDNA Solution

48. Slowly pipette the entire gDNA volume into a standard bore 200 µl tip, then slowly dispense the gDNA. Avoid creating bubbles.

    - Repeat this process 3 times for a total of 4 strokes: (1 stroke = 1 aspiration and 1 dispense).

    Note: If gDNA uptake stalls due to high viscosity, it may be necessary to stir gently while slowly releasing the plunger to withdraw the gDNA.

49. Place standard 2.0 ml microfuge tube containing gDNA in rack of HulaMixer and rotate at room temperature for 1 hour at 15 rpm.

Note: During initial rotations, ensure that the gDNA gets drawn from the bottom of the microfuge tube to reside in the lid of the tube during rotations. If the DNA solution remains in the bottom of the tube during initial rotations, turn off HulaMixer and position rack so the microfuge tube is oriented upside down. Gently flick the bottom of the microfuge tube until the gDNA is drawn into the lid and resume mixing.

50. Remove microfuge tube from rack of HulaMixer and pulse spin tube on benchtop microcentrifuge for 2 seconds to bring the gDNA to the bottom of the tube. Allow the gDNA to equilibrate overnight at room temperature (25°C) to homogenize.

    Note: Most samples will become homogenous by the third day (from the start of the protocol), but samples may be labeled as soon as they become homogenous.

gDNA Quantitation (45 minutes)

Qubit Quantitation - BR dsDNA Assay

Refer to the Qubit dsDNA BR Assay Kit user manual for kit details and follow the methods described in the "Pipetting Viscous Genomic DNA" section, to ensure accurate pipetting of viscous gDNA.

1. Equilibrate Qubit BR Assay Kit Standards to room temperature.

   Note: If the gDNA has been stored at 4ºC, equilibrate at room temperature before moving to the next step.

2. Add Qubit BR Buffer to 0.5 ml Qubit Assay Tubes:
   a. For each sample, add 18 µl of Qubit BR Buffer to three separate Qubit Assay Tubes.
   b. For the Qubit Standards, add 10 µl Qubit BR Buffer to two separate Qubit Assay Tubes.
3. Using a 200 µl pipette with a wide bore tip, gently mix the entire gDNA sample volume by pipetting up and down 5 times, being careful not to generate bubbles.
4. Using a fresh standard bore pipette tip or positive displacement pipette tip for each draw:

   Remove 2 µl aliquots from the left side, middle, and right side of each sample and dispense into BR Buffer of corresponding Qubit Assay Tube, rinsing tip when dispensing. Place Assay Tubes in a floating rack and sonicate for 10 minutes. Perform Steps 5 and 6 during sonication.

   Note: If a bath sonicator is not available, vortex for at least 30 seconds at maximum speed, then pulse spin down briefly for 2 seconds.

5. Prepare Working Solution by diluting the Dye Assay Reagent into BR Dilution Buffer (1:200):
   a. 200 µl Working Solution for each of the two standards (400 µl total).
   b. 200 µl Working Solution for each sample aliquot (600 µl for each sample).
6. For the Qubit DNA standards, add 10 µl of Standards 1 and 2 to the Assay Tubes containing BR Buffer from Step 2b.
7. Once sonication is complete, retrieve assay tubes and pulse spin briefly. Vortex tubes for 5 seconds at maximum speed, then pulse spin again.

8. Add 180 µl of Working Solution to each sonicated DNA aliquot and Qubit DNA Standard aliquot. Vortex for 5 seconds, and pulse spin tubes.
9. Incubate samples for at least 2 minutes, then read on the Qubit Fluorometer.
10. Coefficient of Variation (CV = standard deviation/mean) from three readings should be < 0.30.

    Note: If CV > 0.30, gently pipette-mix the entire volume of gDNA with five strokes (1 stroke = 1 up stroke + 1 down stroke) using a wide bore tip. Let the gDNA rest at least overnight at room temperature before repeating quantitation.

    Note: Typical DNA concentrations range from 50-300 ng/µl. If concentration is > 150 ng/µl, refer to Important Notes of Bionano Prep Direct Label and Stain (DLS) Protocol (30206).

Sample ID	Left (ng/µl)	Middle (ng/µl)	Right (ng/µl)	Mean (ng/µl)	CV (stdev/mean)

Labeling

DNA is ready for Direct Label and Stain (DLS) labeling. See “Kits and Consumables” section at https://bionanogenomics.com/support/ for applicable kits and protocols.

Troubleshooting

The gDNA comes unbound from the Nanobind Disk.

Evidence: gDNA is aspirated or becomes detached from disk during binding or during washes.

Steps to follow if gDNA is aspirated:

1. Leaving the sample tube racked on the magnet, dispense gDNA-containing liquid back into tube containing disk.
2. Remove racked tube from magnet and invert rack multiple times by hand to re-establish binding.

Alternatively:

1. Leaving the sample tube racked on the magnet, dispense gDNA-containing liquid back into tube containing disk.
2. Aspirate liquid from tube such that a minimal volume (~50 µl) remains above unbound gDNA and discard supernatant leaving the DNA in a minimal volume at bottom of the tube.
3. Carefully aspirate unbound gDNA containing the minimal liquid into pipet tip and pipet directly onto racked disk on magnet to re-establish binding.

Steps to follow if gDNA is visually detached from the Nanobind Disk and not aspirated:

1. Remove the tube from the rack and hold horizontally.
2. Roll the tube between fingers until binding is re-established.

The gDNA is not homogeneous before labeling

Evidence: The gDNA quantitation CV of three measurements (top, middle and bottom) is > 0.30.

Steps to Follow:

1. Aspirate and dispense sample using a wide bore tip for a total of 5 times.
2. Incubate the gDNA at room temperature for 1 to 3 days.
3. After incubation, again aspirate and dispense the sample using a wide bore tip 5 times.
4. Quantitate with Qubit BR Assay.

The gDNA is not viscous

Evidence: Sample consistency is very thin and easily pipetted, but concentration is > 35 ng/µL.

The sample is likely not to have high molecular weight gDNA.

Check sample using pulse field gel electrophoresis before labeling to confirm presence of high molecular weight gDNA.

Evaluate sample prep method and input material quality/age and repeat DNA isolation from biological sample.

Frequently Asked Questions

What tissue types are compatible with this protocol?

- This protocol has been used to successfully process the following tissue types:
    - From Brown Norway Rat: liver, lung, kidney, colon, ovary, prostate, testes, and uterus.
    - From Human: bladder, lung, liver, kidney, colon, breast, prostate, brain, thyroid, and ovary.
    - From Mouse: spleen (2-3 mg input recommended)
- Skin, muscle, and non-vertebrate tissues are not yet fully supported.
- Normal tissue and tumor biopsies are recommended. This protocol has not been tested with fine needle aspirates.

What factors will influence gDNA quality?

- Storage and handling of tissues will influence gDNA quality. The following may negatively affect gDNA quality:
    - Tissues that have gone through a freeze thaw cycle after initial storage at -80C.
    - Frozen tissues exposed to room temperature for extended periods of time during cutting.
    - Necrotic tissue within the tissue sample.
    - Tissues with RIN (RNA Integrity Number) < 9.0.
- Too much starting material will lead to lower gDNA quality.

What factors will influence gDNA yield?

- Nuclei content relative to tissue mass will influence gDNA yield. Tissues that require a large tissue input weight due to low nuclei content may label poorly and have low throughput.

    Note: Tumor samples usually have higher gDNA yield per mg of tissue as compared to normal tissue.
- Tissues with high fat content may have low gDNA yield.

How many samples can be processed?

- This protocol can be used for batch sizes up to 8.
    - It will take up to 6 hours to process a batch of this size.
- Based on typical throughput on the Saphyr Instrument, data can be collected from at least 9 samples per week

What tissue preservatives are compatible with this protocol?

- While we have not currently tested preservatives for compatibility with this product, it is an area of ongoing research and we will update this section with new findings as they become available.

Appendix: Preparing Fresh Tissue or Tumor for Storage

Recommended input: 10 mg fresh tissue or tumor from lung, liver, kidney, uterus, ovary, colon, prostate, thyroid, testes, breast, and bladder. This protocol does not yet fully support muscle tissues.
Note: Tissue with low nuclei content relative to the tissue mass may not produce sufficient gDNA, and too much tissue may produce gDNA that is less pure and more difficult to become homogeneous.

1. Rinse tissue in ice-cold PBS and cut into smaller pieces (~10 mg per piece) on a sterilized ice-cold metal block.
    a. It is recommended to prepare multiple portions of each tissue.
2. Transfer tissue to a 1.5 ml tube with screw-cap, and snap freeze in liquid nitrogen for 3 minutes before transferring to -80ºC freezer for long term storage.
3. The frozen tissue is best used within 6 months storage at -80ºC.
4. If shipping frozen tissue, please follow the guidelines provided in Tissue and Tumor Shipping Instructions (30186).

Technical Assistance

For technical assistance, contact Bionano Genomics Technical Support.

You can retrieve documentation on Bionano products, SDS's, certificates of analysis, frequently asked questions, and other related documents from the Support website or by request through e-mail and telephone.

Type	Contact
Email	support@bionanogenomics.com
Phone	Hours of Operation: Monday through Friday, 9:00 a.m. to 5:00 p.m., PST US: +1 (858) 888-7663
Website	www.bionanogenomics.com/support

Bionano Genomics, Inc.
9540 Towne Centre Drive, Suite 100
San Diego, CA 92121